MANGANESE IN SOIL AND PLANT

A Thesis submitted to the
University of Glasgow for the
Degree of Doctor of Philosophy
in the Faculty of Science

by

ERNEST RONALD PAGE

The West of Scotland Agricultural College,
Auchincruive,
Ayr.

April, 1961.

ISSUE LABEL

DURATION OF LOAN—Not later than the latest date stamped below.

13/1/62		
20/8/64		

DEDICATED TO MY WIFE

ACKNOWLEDGEMENTS

I am grateful to the Governors and the Principal of the West of Scotland Agricultural College for the opportunity and facilities to carry out this study. I thank Professor Nicol, in whose department most of the work was performed, for his interest and help, and Dr. McGregor and Mr. Schofield-Palmer of the Plant Nutrition Laboratory for their co-operation and suggestions, particularly the former for suggesting the importance of water-soluble manganese, and the latter for proposing the investigation of the effect of volume ratio on extractable manganese. Without their planning, and the field work of Mr. Wilson, that part of this study which has to do with field investigations would have been impossible.

I thank Professor Robertson of the University of Glasgow for his supervision of this work, and Dr. Rees for the invaluable help rendered.

Thanks are due also to Dr. Finney of Aberdeen University for advice on statistical techniques, and to Dr. Sylvie of Glasgow University for other statistical advice, and for checking the methods used. I am most grateful to Dr. Sim of Glasgow University for taking the X-ray diffraction photographs shown in Figure 32. Mr. Waterson of the Crop Husbandry Department, Auchincruive, kindly supplied oats for use in the experiments recorded in Section V.

I thank Professor Fletcher of the Botany Department of the College for facilities provided for autoclaving and microscopy, and Mr. Alcorn, Dr. Kirkwood and other members of the Botany Department for their co-operation and help.

I am deeply grateful to Dr. Dainty of the Biophysics Department of Edinburgh University for the invitation to work with him in September 1960 using radio-active isotopes of manganese, and to Dr. Smith of the Edinburgh School of Agriculture who provided laboratory accommodation for us. I wish to record thanks to all those members of the Chemistry Department of the School of Agriculture who went out of their way to be helpful during my visit, and who are too numerous to mention individually. Dr. King of the Chemistry Department of Edinburgh University kindly determined the uronic acid content of a sample of oat roots (p 285).

The competent technical assistance of Mr. Smith, Miss Caldwell, and Miss McCann in various parts of this work should also be acknowledged.

I thank Mr. Schofield-Palmer for his critical reading of the script.

Finally I am grateful for assistance from the Colin Thomson Research Fund.

CONTENTS

I. INTRODUCTION

Six elements only are required in large quantities for the normal growth of plants. These are the major plant nutrients; phosphorus, nitrogen, potassium, calcium, magnesium and sulphur, and a heavy crop removes some hundreds of pounds of each from an acre of soil in the course of a year of growth.

Other elements are required by plants in very much smaller quantities, a few parts per million or even a fraction of a part per million being sufficient in culture solutions to give normal healthy growth. Although such small amounts are involved, these "micro-nutrients," as they are called, are essential, and the plant will cease to grow, or will die, without them. These micro-nutrients, or trace elements, are more numerous than the major nutrients. Most of them are absorbed as cations, as in the cases of zinc, copper, cobalt and manganese, but others equally as important are taken up as anions; boron and molybdenum may be cited as examples.

The mere presence of an element in a plant does not of course imply its essentiality, it may be taken up by the plant without being useful. This appears to be the case with aluminium, indeed anything more than a mere trace of aluminium in plants causes symptoms of toxicity. Until recently chlorine was regarded as

such an element. Normally all plants contain considerable quantities of chloride, but it was regarded as in-essential; now it is established as an essential micro-nutrient (Broyer, Carlton, Johnson & Stout 1954).

Manganese was first shown to occur in plants by Scheele in 1785, when he reported its presence in aniseed (quoted by Jacks and Scherbatoff 1940). The first mention of its beneficial action was made as early as 1799 by G. Carradori, who wrote "Il manganese, come io ed altri abbiamo osservato, accelera la germinazione delle sementi; e mi e parso che porti vigore anche alla vegetazione della pianta" (quoted by Verona 1953). A translation reads "Manganese, as I and others have observed, hastens germination of seeds; and it rather seems to me that it also makes the growth of the plant more vigorous"*. This appears to be the earliest reference to the stimulating effect of any element which was to be recognised later as a micro-nutrient.

Manganese was not shown to be essential for normal growth of plants for more than a hundred years after this early observa-

* I am indebted to Professor Hugh Nicol for drawing my attention to this quotation and for providing the translation.

tion. The first clear recognition of its essentiality was by McHargue (1922) and the fact was established beyond doubt by McHargue (1923) and Samuel and Piper (1929).

Manganese deficiency diseases of plants

This is not to say that diseases due to manganese deficiency had not been familiar to farmers and research workers long before; indeed the discovery had been made in 1909 by Sjollema and Hudig that the most common of these diseases, "grey speck" of oats, could be cured by the application of manganese sulphate. But Hudig (1911) had been unable to decide whether the manganese acted by stimulating plant growth or by neutralising some unknown injurious factor in the soil. The first suggestion that "grey speck" was in fact a disease due to manganese deficiency was made by Schagen (1914). Among the many papers published on "grey speck" disease was an important contribution by Hiltner (1924) in which he showed that the disease occurred not only in oats grown on neutral or alkaline soils but also in those grown in acid nutrient solutions which were very low in manganese. But Hiltner regarded the manganese only as causing an increase in carbon dioxide assimilation; it had been found that an increased supply of carbon dioxide cured the disease, and Hiltner regarded this as more important.

Another contemporary theory of the cause of "grey speck" was that put forward by Arrhenius (1923 and 1924) in which it was suggested that the disease was caused by an excess of calcium ions in the soil solution.

It was finally clearly demonstrated by Samuel and Piper (1929) that "grey speck" was a disease due to manganese deficiency, and this is now generally accepted. It should be borne in mind, however, that the etiology of the disease may include other factors; it was pointed out by Gisiger and Hasler (1948) that the absolute content of manganese in the plant is less important than the ratio of manganese to other elements taken up. They produced evidence to show that addition of boron decreases the incidence of "grey speck" and that increased boron fertilisation leads to increased manganese uptake. Somers and Shive (1942) took the view that what is important is the ratio of iron to manganese, not the absolute quantities taken up by the plant. Working with soy-beans they equated manganese deficiency with iron toxicity and vice versa. Other workers have disagreed; in particular Morris and Pierre (1949) have separated and distinguished manganese deficiency from iron toxicity in soy beans, and Hewitt (1946) for cauliflowers and runner-beans.

Another disease due to manganese deficiency is "Marsh

Spot" which affects peas. The plants may appear quite healthy in a condition of mild deficiency, and only when the two cotyledons of the seed are separated can the dark brown speck or brown area be seen on the flat internal surfaces. An illustration showing this is given by Wallace (1951). With more severe deficiency the foliage becomes chlorotic. The disease was shown to be due to manganese deficiency by Pethybridge (1936), who noticed that it occurred in peas growing near oats affected by "grey speck", and independently in the same year by Lohnis. For a review of the literature concerning "Marsh Spot" see Reynolds (1955).

Sugar beet grown with an inadequate supply of manganese suffer from "Speckled Yellows", and a similar condition occurs with other root crops, with interveinal chlorosis of the leaves. Sugar cane suffers from "Pahala blight" (Lee and McHargue 1928).

Many other examples of manganese deficiency diseases are given by Jacks and Scherbatoff (1940), Wallace (1951), and Mulder and Gerretsen (1952); crops affected include potatoes, tomatoes, tobacco, brassicas, fruit trees, raspberries etc. Recently tulips grown on calcareous sandy soil in the Isle of Tiree have been found to be suffering from manganese deficiency (Voss, unpublished 1959).

Manganese toxicity in plants

Excess manganese can cause damage to plants as serious as that due to shortage of the element, and indeed manganese toxicity is one of the main factors in the damage inflicted on plants by extreme soil acidity. Soil acidity may also cause aluminium toxicity, or lead to lack of calcium, magnesium, phosphorus and perhaps molybdenum. Acidity injury may be the result of the operation of some or all of these factors, depending on circumstances. It may be due to the fact that "pure" manganese toxicity seldom occurs naturally that it was not recognised as early as the deficiency disease, except in the special case of plants growing on highly manganiferous soils such as those found in certain parts of Hawaii. Manganese contents as high as 9.7% Mn_3O_4 have been reported for these soils (Wilcox and Kelley 1912) and pineapples grown on them develop a disease known as "Pineapple Yellows." The chlorosis appears to be caused by the excess manganese reducing the availability of iron in the soil (Johnstone 1924), so that it is questionable whether "Pineapple Yellows" is truly a manganese toxicity disease.

Tobacco grown on acid soils in Connecticut was shown by Jacobson and Swanback (1932) to be suffering from manganese toxicity and Bortner (1935) found similar evidence in Kentucky. Neal and Lovett (1938) indicated that "Crinkle leaf" of cotton

was due to manganese toxicity, and final proof was given by Adams and Weir (1957). Van der Merwe and Andersson (1937) reported from the Transvaal that in citrus excess manganese causes "greening" or arrested development.

Manganese toxicity in potatoes was first reported by Hunter and McGregor (1946) and later by Berger and Gerloff (1947).

In 1945 a paper was published by Wallace, Hewitt, and Nicholas dealing with factors injurious to plants in acid soils, which described manganese toxicity symptoms for runner beans (Phaseolus multiflorus) and cauliflower. More details were given by Hewitt (1946). This was followed by a monumental series of investigations in the course of which the workers at Long Ashton Research Station described the manganese toxicity symptoms for swedes, mustard, red clover, potato (Hewitt 1947) sugar beet, kale, oats (Hewitt 1948) tomatoes, lucerne, sprouts, carrots, linseed, celery, mangolds and alsike (Hewitt 1949).

Lohnis (1951) confirmed many of these findings and stressed the differences in susceptibility of various crops; finding vetch and lucerne particularly susceptible. She had independently in the Netherlands during the war found the same symptoms of manganese toxicity in beans as had the Long Ashton Workers (Lohnis 1950).

A number of other examples of toxicity described in the literature are cited in the review by Mulder and Gerretsen (1952).

Levels of manganese in plants

There is a very wide variation in the manganese content of plants grown under natural conditions. Some of the factors on which the availability of the manganese in the soil depends will be examined subsequently, but these are not the only ones to be considered, since different species of plants vary greatly in their ability to take up manganese, even from the same soil. For example, Beeson, Gray and Adams (1947) grew seventeen grasses on a fine sandy loam, and examined the plants for their content of several micro-nutrients including manganese. They were able to divide the grasses between three groups; high, medium, and low in manganese. The highest manganese content on a dry matter basis, was in Agrostis alba with 815.5 p.p.m. $\pm$ 79.09 (mean and standard error) the lowest in Panicum barbinode, with 95.8 p.p.m. $\pm$ 5.80, while the grasses in the medium manganese group has contents varying between 130 and 215 p.p.m.

Even in a single species it is very difficult, if not impossible, to say exactly how much manganese is required to ensure healthy development. It is generally agreed that in oats deficiency symptoms occur if the manganese content falls below about 15 p.p.m. on a dry matter basis, (Samuel and Piper 1929, Piper 1931, Leeper 1935) but plants have been observed with typical deficiency symptoms which have proved on analysis

to contain more than this. On the other hand Gerretson (1937) grew apparently healthy plants whose manganese content was only 5 to 10 p.p.m. These were, however, grown in culture media under sterile conditions, and may not be comparable with plants grown under more normal conditions.

These conditions are, of course, what would be expected if the ratio of manganese to other elements taken up by the plant is more important than the absolute level. This has been suggested, particularly in relation to the manganese/iron ratio by Somers and Shive (1942).

Crops vary in their susceptibility to manganese deficiency, and were classified by Gilbert (1934) as having high sensitivity (oats, beets and spinach) medium sensitivity (maize, lettuce, onions, mangolds, grasses) and low sensitivity (potatoes, beans). Different varieties of the same crop also vary in susceptibility as was shown for thirty two varieties of oats, wheat, barley and rye by Gallagher and Walsh (1943).

The resistance to deficiency disease may be due to a low requirement for the element, for example Jones (1957) grew oats, vetch and rye on the same soil, and found that the oats showed symptoms of "grey speck" when its manganese content was 16 p.p.m., while the rye had only 10 p.p.m. and appeared healthy. The vetch, however, which also was healthy, had a manganese

content was 16 p.p.m., while the rye had only 10 p.p.m. and appeared healthy. The vetch, however, which also was healthy, had a manganese content of 21 p.p.m., and presumably had a more efficient uptake mechanism than either of the other plants. Common grasses are more resistant than oats to manganese deficiency yet Hasler (1951) found that Festuca pratensis with 44 p.p.m. manganese showed symptoms of deficiency.

The manganese levels at which toxicity symptoms appear are equally varied. Lohnis (1950) found that in beans injury symptoms became evident when the content exceeded 1210 p.p.m. of dry matter. She noted that potatoes tolerate a much higher content of manganese without injury.

Just as varietal differences are found in susceptibility to manganese deficiency, so with resistance to toxicity, as has been shown for alfalfa by Beaureaux and Ouellette (1958).

It was suggested by Lohnis (1951) that the tolerance to a high level of available manganese may be due either to weak absorption as in the case of oats, or to a high degree of tolerance within the plant, as for example, with tobacco. Thus oats when grown on a certain acid soil were found to contain between 301 and 370 p.p.m. of manganese, and tobacco 2936 p.p.m., neither sort of plant showing symptoms of toxicity. Tobacco shows symptoms of toxicity at higher levels of manganese content;

among the analyses of plants showing injury symptoms given by Jacobson and Swanback (1932) are figures of 6470 p.p.m., 5500, 8750 and 11,670 p.p.m. According to Chenery (1955) tea bushes remain healthy even when manganese contents of the leaves rise as high as 10,000 p.p.m.; he believes that the large amounts of aluminium also absorbed give protection in some way.

It seems evident from various published figures that in general a more efficient means of uptake for manganese is accompanied by a higher degree of internal tolerance. This is illustrated by the results of Morris and Pierre (1949) for soy beans and cow peas. When both plants were grown in culture solutions with 2.5 p.p.m. manganese the soy beans contained 266 p.p.m. and the cow peas 615 p.p.m., in both cases about 25% of the leaves showing toxicity symptoms. All leaves were affected when the solution contained 10 p.p.m. manganese; the tissue manganese contents were now 2,168 and 4,212 p.p.m. respectively. Efficient mechanisms of uptake are not invariably accompanied by high internal tolerance, as vetch (Vicia sativa) and lucerne (Medicago sativa) are both very susceptible to injury from excess of manganese. Lobnis (1951) found that in 1948 the toxicity level for vetch was 500 p.p.m. and in 1949 about 1,000 p.p.m., and approximately the same in each year for lucerne. Beans did not show this striking alteration from one year to the

next, and no explanation was ventured for the phenomenon. Sutton and Hallsworth (1958) suggested that the explanation lies in the connection between light intensity and manganese toxicity. They found that manganese toxicity symptoms are more marked at high light intensities. They do not, however, suggest why the effect should vary as between different plant species. This problem of the connection between intensity of illumination and manganese toxicity will be referred to again in the section on the functions of manganese within the plant.

Temperature may also affect the uptake of manganese by plants. Medeiski and Wilson (1955) grew soy beans in pots and kept them at different temperatures. The higher temperature resulted in greater uptake, but as the authors pointed out, this might be due either to an increased rate of plant metabolism, or to an increase in availability of soil manganese. There is an obvious need for a series of solution culture experiments to elucidate this point.

The distribution of manganese in the plant is not uniform. Bolle-Jones (1955) found that the concentration was usually highest in older leaves, but that there was little variation in the stems and petioles. This indicates a tendency towards accumulation in the leaves, and mobility

in stems and petioles. It was suggested that potassium enhances the mobility of manganese within the plant.

The Role of Manganese in the Metabolism of the Plant

It is evident from the very small quantities of manganese required by plants that it has no gross structural function to compare with that of, say nitrogen in proteins, nucleic acids, etc. Its essentiallity as a micro-nutrient points to a key catalytic role or roles in a situation where it cannot be replaced by another element, and it is usually assumed that it functions as a co-factor in various enzymatic reactions (Mulder and Gerretsen 1952). Nevertheless it should be borne in mind that the manganous ion has been found to function non-enzymatically in certain decarboxylations (Steinberger and Westheimer 1949) and at least one transphosphorylation (Lowenstein 1957) so that it may have catalytic activity on its own account.

McElroy and Mason (1954) list twenty-nine enzymes which are activated by manganese, but only one of these, prolidase, has a specific requirement. Most of the others are also activated by magnesium, and many also by other ions such as zinc, cobalt, nickel, etc.

Since McElroy and Mason's review appeared, a number of important enzyme systems have been found to require manganese,

including hydroxylamine reductase (Nicholas 1957 a and b, 1959); phosphoenolpyruvic acid carboxylase, (Tchen and Vennesland 1955 and Walker 1957); the enzyme responsible for the condensation of phosphopyruvate and carbon dioxide to give oxalacetate, (Utter and Kurahashi 1954); and those active in the formation from mevalonic acid of squalene, (Amdur, Rilling and Bloch 1957) and cholesterol (Gosselin and Popjak 1958).

Not all these enzymes have been demonstrated in plants, but it is usually assumed that a given enzyme functions in both plants and animals until evidence appears to the contrary.

A large number of the enzymes activated by manganese are concerned with carbohydrate metabolism; work in this field was reviewed by Mulder and Gerretsen (1952). In nearly every case magnesium may act as an alternative ion. This makes it unlikely that one of these enzymes is of primary importance in the production of typical deficiency symptoms, since under conditions of lime induced manganese deficiency magnesium supply is likely to be improved. Similarly in most of the enzymes involved in the tricarboxylic acid cycle (Krebs cycle) magnesium is an alternative ion.

In this connection the observation of Iljin (1951) that citric acid accumulates in the leaves of lupins suffering from lime induced chlorosis (that is, presumably, from manganese

deficiency,) is significant, since Anderson and Evans (1956) found that manganese deficient snap beans (Phaseolus vulgaris) were able to form the apoenzyme of iso-citric dehydrogenase, but manganese had to be added before the enzyme could function. This also occurred with plants grown in cultures with an ample supply of magnesium, in spite of the report by Adler, von Euler, Günther and Plass (1939) that magnesium ions can also activate the enzyme.

The part played by manganese in the growth regulating process of plants may be connected with its activity in the enzymatic oxidation of indolylacetic acid. This oxidation is brought about by manganic ions, (MacLachlan and Waygood 1956a) generated by a manganese-phenol-peroxidase system (Kenten and Mann 1950). A similar effect on β - (3-indolyl)-n-propionic acid and γ- (3-indolyl) - n-butyric acid was noted by Kenten (1955).

The mechanism of the reaction has been formulated by MacLachlan and Waygood (1956b) as follows:-

The indolyl acetic acid ICH_2COOH is oxidised by manganic ions to form a radical which reacts with oxygen after losing carbon dioxide.

$$ICH_2COOH + Mn^{3+} \longrightarrow ICH_2COO\cdot + Mn^{2+} + H^+$$

$$ICH_2COO\cdot \longrightarrow ICH_2\cdot + CO_2$$

$$ICH_2\cdot + O_2 \longrightarrow ICH_2OO\cdot$$

The oxidised product is able to oxidise phenol in the presence of peroxidase, forming an aryl radical RO., which is a mono-phenol oxidation product known to be capable of reversible oxidation and reduction.

$$ICH_2OO\cdot + ROH \xrightarrow{\text{peroxidase}} ICH_2OOH + RO\cdot$$

$$RO\cdot + Mn^{2+} + H^{+} \longrightarrow ROH + Mn^{3+}$$

Manganic ions have been described in the literature as enhancing, inhibiting, or as having no effect on the oxidation of indoleacetic acid, but the discrepancies in the reports were explained, at least partially, by Hillman and Galston (1956) who were able to show that the effects described could be accounted for by variations in the levels of phenolic components in the different systems involved. They found that manganese had an inhibiting effect at low concentrations of added 2, 4 - dichlorophenol, and a stimulating effect at high concentrations. The situation in this field is complex and much work remains to be done; the position was reviewed by Hewitt (1957).

The equilibrium of this system is disturbed by increase in intensity of illumination. Gerretsen (1950a) showed that oxidation of manganese takes place in illuminated chloroplast preparations, and Kenton and Mann (1955) showed that trivalent manganic ions are generated. A good deal of evidence points to the

significance of these changes in connection with the process of photosynthesis as well as in the oxidation of growth regulating substances. It has already been noted that Hiltner (1924) had observed the connection between carbon dioxide assimilation and supply of manganese. In 1949 Gerretsen showed that in manganese deficient oat plants carbon dioxide assimilation is depressed to 25 to 40 per cent of the normal rate, and in the next year he began to investigate the function of manganese in the process of photosynthesis, using suspensions of chloroplasts obtained by grinding oat leaves with sharp sand, centrifuging and suspending in water. The redox potential of these suspensions, which also contained grana, stroma, and cytoplasm, was measured in the dark and while exposed to illumination.

High potentials were developed with illuminated suspensions only when manganese was added (Gerretsen 1950a) and this was interpreted as activation of oxygen as a stage in peroxide formation. It was suggested that the first step might be a light induced electron transfer from manganese to iron,

$$Mn^{2+} + Fe^{3+} \xrightarrow{h\nu} Mn^{3+} + Fe^{2+}$$

followed by photo-oxidation of water by trivalent manganese:-

$$Mn^{3+}(H_2O) \xrightarrow{h\nu} Mn^{2+}(H_2O)^{+}$$

$$Mn^{2+}(H_2O)^{+} + OH^{-} \longrightarrow Mn^{2+} + H_2O + \underline{OH}$$

$$2\,\underline{OH} \longrightarrow H_2O_2$$

where $\underline{OH}$ represents a free radical in an extremely reactive state. This may be the essential part of the "Hill reaction" in which isolated chloroplasts produce oxygen without direct dependence on carbon dioxide assimilation, and which requires manganese ions.

More recent work (Tanner, Brown, Eyster and Treharne, 1960) has linked chloride and manganese and shown their joint functioning in the Hill reaction. According to these workers, the mechanism of the Hill reaction is possibly:-

$$TPNH + H^{+} + HCO_3^{-} + MnCl^{+} \longrightarrow [CHO] + TPN^{+} + MnCl(OH)_2$$

depending on spontaneous alkaline oxidation of $MnCl^{+}$ to $MnCl^{2+}$ due to liberation of OH^{-} when HCO_3^{-} is reduced to a neutral intermediate, followed by:-

$$H^{+} + MnCl(OH)_2 \longrightarrow MnCl^{+} + 1\tfrac{1}{2}\,H_2O + \tfrac{1}{4}\,O_2$$

which would occur spontaneously at some more acid site, perhaps where aerobic oxidation is proceeding.

The authors of this scheme do not rule out other possibilities; in particular, they believe that hydrogen peroxide may play a part in the process, so that their views are not incompatible with, but rather a development of,

Gerretsen's (1950a) suggestion.

Gerretsen (1950b) holds the view that when manganese is in excess the high redox potentials attained lead to destruction of protective proteins surrounding the chloroplasts, which consequently become bleached themselves, and give rise to the chlorosis characteristic of manganese toxicity. Support for this view was cited by Mulder and Gerretsen (1952) in reports that injury due to excess of manganese is more severe under intense illumination (McCool 1935, Hopkins, Pagan and Ramires-Silva 1944, and Morris and Pierre 1949). The same effect was noted by Sutton and Hallsworth (1958).

In algae manganese seems to be essential only for photosynthesis (Pirson 1955) but in higher plants there are almost certainly other processes for which manganese is indispensable.

The effect of the intensity of illumination may also have some bearing on the conflicting reports on the relationship between ascorbic acid content of the plant and its manganese status (Richardson 1954). Rudra (1939) found that the levels of ascorbic acid in several sorts of plant were higher when the germinating seeds were provided with dilute solutions of manganese salts. Hester (1941) similarly noted an increased content of ascorbic acid in fruit from plants supplied with manganese. But Lyon, Beeson and Ellis (1943) found tomatoes

grown in solution cultures had similar ascorbic acid contents whether supplied with manganese or not, and their results were confirmed by Gum, Brown and Burrell (1945). Eaton (1947) found the same lack of effect in tomatoes, but marked increase in ascorbic acid content in sunflowers and tobacco supplied with manganese. Hiron, Doty and Quackenbush (1951) found no effect in soy beans. Lyon and Beeson (1948) found that the toxic levels of manganese decreased the ascorbic acid content of tomato plants and turnips by 20 to 25%.

The contents of various other vitamins in plants has been found to be influenced by manganese supply. Lyon and Beeson (1948) found that in turnip greens riboflavin was increased by increasing manganese supply in culture solution, but that there was no effect on thiamine. Burger and Hauge (1951), working with soy beans, maize, wheat and oats reported that carotene and tocopherol levels were lower in plants grown on manganese deficient soil. Gum, Brown and Burrell (1945) had found a similar but smaller effect in tomato plants.

A number of enzymes concerned in the nitrogen metabolism of plants are activated by manganese, though usually other ions will perform the same function. For example among the peptidases, glycylglycine dipeptidase is activated by manganese, but more efficiently by cobalt (Smith 1951). Arginase requires

either manganese, cobalt, nickel or ferrous iron (Greenberg 1951). An enzyme which functions with either magnesium or manganese is L-leucine-amino-exopeptidase (Smith and Bergmann 1944), which recalls the numerous examples of enzymes concerned with carbohydrate metabolism and other phosphorylations, and with the citric acid cycle, where these two metals are interchangeable. In glutamyl tranferase, on the other hand, manganese is not replaceable by magnesium, zinc, copper, ferrous iron, aluminium or cobalt. (Stumpf and Loomis 1950).

The effect of a number of micro-nutrients on the free amino acid and amide content of tomato plants was investigated by Possingham (1956). He found that manganese had little influence on the amide content, but that there was a general increase in the level of free amino acids.

In manganese deficient cultures the tomato plants lacked histidine and lysine, but the substance pipecolinic acid appeared, although it was absent under all other conditions except those of iron deficiency. Schutte and Schendel (1958) found that manganese deficiency had little influence on the composition of protein obtained from beans, though they did not report the content of histidine or lysine in their hydrolysates, and in any case their method of determination was only semi-quantitative. They found low levels of valine, and rather

higher levels of methionine as compared with the controls.

The observations by Burstrom (1939, 1940) that manganese increased the rate of nitrate assimilation by plants has been explained by the resolution of the reductive sequence of nitrate into the steps nitrate ⟶ nitrite ⟶ hyponitrite ⟶ hydroxylamine ⟶ ammonia, each step being mediated by the appropriate enzyme, (Nicholas 1957 a and b) and the demonstration that the flavoprotein enzyme hydroxylamine reductase requires manganese ions. Nicholas (1957a) has stated that he and Medina found this in 1956 for the enzyme from Neurospora crassa, although the evidence was not presented until later (Nicholas 1959). Spencer, Takahashi and Mason (1957) found a specific requirement for manganese for the enzyme from Azotobacter agile.

Inter-relations of other nutrients with manganese

Iron - manganese interaction

It has already been noted in connection with manganese deficiency that the level of other elements in the plant may decide the effect of a given manganese content. This relationship to another element is most marked in the case of iron. Somers and Shive (1942), and Somers, Gilbert and Shive (1942) reported that the ratio of iron to manganese in culture solutions should be between 1.5 and 2.5 to ensure normal healthy growth; higher ratios

resulted in iron toxicity, which they equated with manganese deficiency, and lower ratios gave iron deficiency or manganese toxicity.

Their soy beans remained healthy even though absolute concentrations of iron and manganese were increased up to 600 and 1,000 times respectively, so long as this correct ratio was maintained. Since the amount of both elements taken up by the plant was proportional to the concentration in the nutrient solution, it was concluded that iron and manganese have some reciprocal functional activity within the plant.

Evidence that chlorotic pineapples grown on magniferous soil respond to sprays of ferrous salt solutions has been produced from Hawaii, (Johnson 1924), and Puerto Rico, (Hopkins, Pagan and Ramirez Silva 1944), and has been quoted as support for the view of Somers and Shive that manganese toxicity and iron deficiency are identical, although to show that iron deficiency is one of the results of manganese toxicity, even if it is the most obvious result, proves nothing of the kind. Numerous other workers have been able to separate and distinguish manganese and iron effects. This has been done for runner beans and cauliflowers (Hewitt 1946), lespedeza (Morris and Pierre 1947) potatoes (Berger and Gerloff

1947) and numerous field and garden crops by Lohnis (1951). Nevertheless Lohnis reported two cases where beans grown at high temperatures in the greenhouse showed manganese toxicity symptoms identical with iron deficiency , and responded to ferrous sulphate solution (1%) applied to the leaves. Morris and Pierre (1949) found no evidence that the two effects were the same in soy beans, in contrast to the findings of Somers and Shive.

Although symptoms of manganese toxicity usually do differ from the effects of iron deficiency it is still possibly for an interaction to occur between the two metals, as was recognised by Hewitt (1947). Support for this point of view was provided by Millikan (1948, 1949) and Warington (1951) working with flax in Australia. Sutton and Hallsworth (1958) believe that the interaction between iron and manganese is more pronounced under conditions of intense illumination, and point out that those investigators who have found the more extreme interactions have all worked at lower latitudes. Bennet (1945) concluded that an antagonism existed between manganese and iron in uptake by tomato plants from culture solutions, and Carlson and Olson (1950) found that the iron content of sorghum plants grown on their medium iron solutions was higher in the low and medium manganese treatment, though they rather surprisingly concluded

that there was no reciprocal relationship between iron and manganese. They had found previously (Olsen and Carlson 1949) that the ratio of extractable iron to manganese in soils producing chlorotic plants was significantly less than in healthy soils, but that absolute levels of iron were more important.

Beeson (1954) was of the opinion that there are two distinct nutritional disturbances attributed to the iron-manganese inter-relationship, one being due to an antagonistic effect of manganese on iron resulting in an "iron chlorosis", the other being an uncomplicated manganese toxicity.

Taper and Leach (1957) found that the optimum value of the iron-manganese ratio in beans was affected by the level of calcium in the culture solution. With 143 p.p.m. calcium in the solution the plants were healthy only if the iron-manganese ratio was 2.15; with 42 p.p.m. calcium any ratio between 0.6 and 3.72 gave normal growth.

Calcium and Manganese

The belief of Arrhenius (1923 and 1924) that "grey speck" disease of oats was caused by excess of calcium ions in the soil solution has been referred to previously. There has been some confusion in the earlier literature between the effect of calcium ions and the effect of liming, with its resulting increase

of soil pH. The aspect of the effects of pH will be considered in the subsequent section on soil manganese. Similarly the effects of calcium added as calcium sulphate to soils on the uptake of manganese from those soils is considered in a later section as being an indirect effect and not a simple interaction.

Swanback (1939) reported that calcium reduced the absorption of manganese from nutrient solutions, and the same sort of effect was noted by Hewitt (1946) who found that increasing calcium supply reduced the severity of manganese toxicity symptoms of cauliflowers and runner beans in sand cultures. This was quite independent of any pH effect. These observations were extended by Hewitt in subsequent years (1947, 1948), and by Lohnis (1960).

Copper

Mulder and Gerretsen (1952) quote several reports of the effect of copper in promoting the occurrence of manganese deficiency. They believe that this is the result of the activation of the biological oxidation of manganous compounds by fungi and perhaps by root cells, causing the manganese to become unavailable. Heintze (1956) found that steam treatment or the application of manganese to cure grey speck in oats caused copper deficiency in a soil which had not previously been known to be copper deficient.

The application of copper sulphate with lime to an acid

clay soil was reported by Sherman, McHargue and Hodgkiss (1942b) to prevent the development of the manganese deficiency in oats which occurred when limestone alone was used. They attributed this to retardation of the oxidation of the manganese ion, which would cause non-availability, but copper is known to release manganese from organic complexes (Hemstock and Low 1953) and this is considered to be a more likely explanation.

The effect of nitrogen compounds

There are numerous reports that nitrates as the source of plant nitrogen, rather than ammonium compounds, improve the uptake of manganese from the nutrient solution. Olsen (1934) found that barley growing in solutions containing the same amount of manganese sulphate took up 90 p.p.m. manganese in the leaves from nitrate solutions and only 23 p.p.m. from ammonium salt solutions. Arnon (1939) has noted a similar effect, as have Millikan (1950) and Lohnis (1951). Harney, Fletcher and Street (1959) reported that tolerance of excess manganese was greater when ammonium ions were the source of nitrogen rather than nitrate.

Other interactions

An interaction between manganese and molybdenum was reported by Millikan (1950). The severity of the manganese toxicity in flax was reduced by an increase in the supply of

molybdenum. Hewitt (1948, 1954) and Warington (1951) found precisely the opposite effect. A recent paper by Kirsch, Harward and Peterson (1960) reconciles these findings by showing that at lower iron and manganese levels added molybdenum had little effect on manganese uptake; at low iron and high manganese levels molybdenum decreased manganese uptake, while at higher lower manganese levels molybdenum increased manganese uptake. It was concluded that the manganese to molybdenum relationship was manifested indirectly through iron by the iron-manganese and iron-molybdenum interactions.

Gisiger and Hasler (1948) found that they were able, in Switzerland during the war years, to prevent the occurrence of "grey speck" by the application of boron. It is not clear, however, from their results whether this is direct facilitation of manganese uptake by boron, ~~or whether this is direct facilitation of manganese uptake by boron~~, or whether the effect is indirect, caused by the influence of the boron, applied as boric acid, on the soil. The quantities of boric acid applied would not measureably affect the pH of the soil, but it seems possible that there is some other influence on the soil, since Mellrath, de Bruyn and Skok (1960) found that boron levels in solution culture had no influence on the uptake of manganese.

Magnesium is able to reduce manganese toxicity in some

plants (legumes), according to Lohnis (1954), though not in others. Extending her observations to deal with several species of plant grown on culture solutions and in the field, and taking into account calcium as well as magnesium, Lohnis (1960) concludes that both magnesium and calcium may influence the uptake of manganese strongly, but emphasises that crop plants vary widely in this respect. Her results for the solution culture experiments show that both ions depress the uptake of manganese, except in the case of lucerne, where magnesium had no effect, but the results from field experiments were much more variable. Evidently the question of these inter-relationships is not a simple one.

The effect of phosphate on manganese uptake is not clear. It has been reported as causing an increase, (with oranges; Reuther, Gardner, Smith and Roy, 1949), a decrease, (with soy beans; Beeson, Gray and Hamner, 1948) and having no effect (with forage crops; Burriel and Suares 1951).

Snider (1943) found that superphosphate, but not rock phosphate, tended to increase the manganese content of grasses. Bingham, Martin and Chastain (1958) also investigating manganese uptake by citrus trees, agreed with Reuther et al (1949) that the absorption is likely to be increased with heavy application of phosphate. The effect may depend on the species of plant

concerned as well as the form of the phosphate, or on some unrecorded factors (compare the iron-manganese ratio controversy and the possible effect of illumination).

The availability of soil manganese to plants

The total content of manganese in soils is very variable. It has been reported that the manganiferous soils of Hawaii contain up to 70,000 p.p.m., but even 1,800 p.p.m. is considered high in the United States (Fujimoto and Sherman 1948). An average figure for Scottish Agricultural Soils would be somewhere in the region of 600 p.p.m. The total content of manganese in soils however, has little bearing on the ability of the soils to make manganese available to the plant, as measured by the actual uptake of the vegetation. Leeper (1947) dismissed total manganese as irrelevent to the problem of the deficiency diseases of "grey speck" of oats and "marsh spot" of peas.

The forms in which manganese exists in soil have not yet been separated and characterised. Such fractionation of the total manganese as has been attempted has been on an empirical basis, using rule of thumb methods.

Exchangeable manganese

Early attempts to show a connection between exchangeable manganese and the incidence of deficiency were disappointing,

although there was evidently a somewhat closer relationship between availability and the level of exchangeable manganese than could be established for total manganese. Extraction of the soil with neutral normal ammonium acetate gives one measure of exchangeable manganese, but the amount extracted varies considerably with the nature of the extracting cation; half molar magnesium nitrate (Steenbjerg 1933, 1934) or half molar calcium nitrate (Heintze 1938) bring much more manganese into solution. Leeper (1947) considers that the term "exchangeable manganese" has little meaning unless the nature of the replacing ion is defined. It is better to use the term "extractable" manganese, and to define the extracting solution.

Steenbjerg's method (1935) for determining extractable manganese involved leaching the soil on a Buchner funnel with half molar magnesium nitrate solution, collecting successive 100 ml. leachates and measuring the manganese in each leachate. If y was the manganese (in mg.) per 100 g. soil, and x was the number of the leachate (in 100 ml. units), then

$$y = \frac{xs}{x + qs} \qquad \text{or} \qquad \frac{1}{y} = \frac{1}{s} + \frac{q}{x}$$

where s was the estimate obtained of the total extractable manganese, and q was a measure of the firmness with which the manganese was held, so that low q values denoted slow extraction

of the manganese, and implied low availability.

This method was used officially in Denmark from 1935, but has recently been replaced by Meintse's method (1958) using 200 ml. of molar magnesium nitrate shaken with 20 g. soil for one hour (Boken 1958). Within certain limits the level of extractable manganese serves as a fairly reliable guide to the liklihood of a given soil causing deficiency disease in oats, according to Schachtschabel (1956) who used half molar magnesium sulphate as extractant, but as a general guide it is useless, at least if neutral normal ammonium acetate is used (Stemuit, Piot and Boon 1956).

Easily reducible manganese

A series of investigations extending over six years into the so called "University" soil, which gives rise to "grey speck" in oats, and is found in Melbourne, Australia, was reported by Leeper (1935). In this report Leeper suggested that the best test to distinguish a deficient soil was to determine the "easily reducible" manganese of the soil, which may be done by extracting the soil with a solution of normal neutral ammonium acetate containing 0.2% hydro quinone. It was argued that this measured not only the exchangeable manganese but also the active higher oxides which also become available to the plant as growth proceeds. Leeper stated

the case as follows, - "Quinol is a chemical metaphor for the plant roots in much the same style as has been suggested for dilute citric acid in the problem of phosphate availability." He suggested that about 25 to 30 p.p.m. easily reducible manganese was needed to escape deficiency. Investigations carried out for a further five years confirmed this suggestion (Leeper 1939). Sherman, McHargue and Hodgkiss (1942a) adopted Leeper's method and recommended in addition the determination of water soluble manganese, and manganese extractable by neutral normal ammonium acetate. They agreed with Leeper that with less than 25 p.p.m. easily reducible manganese a soil is liable to deficiency diseases, and stated that most productive soils have at least 100 p.p.m. Mulder and Gerretsen (1952) considered that this latter figure is necessary to support adequate plant growth on alkaline soils.

But while this method may be useful as a guide to deficient soils for cereals (Sherman and Harmer 1942) it does not necessarily apply for other plants. Heintze (1946) was unable to distinguish soils giving "Marsh spot" of peas from healthy soils by this method, nor by Steenbjerg's (1933) method for exchangeable manganese.

Extractable manganese has normally been taken as a measure of exchangeable manganese, and it had been assumed

that higher oxides of manganese, usually written as "MnO_2" were more or less unavailable to plants. In an attempt to discover which compounds of manganese were brought into solution by 0.2% hydroquinone, or by hydroxylamine, in normal ammonium acetate solution Dion, Mann and Heintze (1947) examined pyrolusite MnO_2, manganite MnO(OH), hausmannite $Mn^{2+}Mn_2^{3+}O_4$, and a preparation of "manganic hydroxide," all added to soil. Of these pyrolusite and manganic hydroxide dissolved appreciably, the others did not. The authors concluded that if the "MnO_2" in soil, usually considered to be unavailable to plants, behaves like pyrolusite, to which it probably corresponds, "easily reducible" manganese is likely to be of little value in diagnosing deficiency.

These results stand in contrast to those of Jones and Leeper (1951a), who found that manganite, hausmannite and pyrolusite were soluble each to approximately the same extent in ammonium acetate and quinol, yet manganite and pyrolusite were available to plants, whilst hausmannite was not. They found an aqueous 0.05% solution of quinol was more satisfactory in that it did not dissolve hausmannite, but did dissolve the other two oxides. Jones and Leeper believe that the "manganic hydroxide" of Dion, Mann and Heintze (1947) was the same as their hausmannite, thus reducing the

area of disagreement, and suggest that different degrees of order of the crystal lattice, which varies according to the history of the sample, may be responsible for the discrepancy in the manganite results.

In an attempt to find a more satisfactory test for distinguishing soils likely to show deficiency Neff and Nederski (1958) used composite soil samples from 2 acre sites on twenty five fields in Ohio and examined them by eight different techniques. The manganese content of the crop (soy beans) was found to correlate significantly with the soil results for all methods at the 1% level except for total soil manganese, which was significant only at the 5% level. The highest correlation coefficient (0.899) and the lowest variance was shown between crop manganese and manganese extracted by 10% ammonium di-hydrogen phosphate solution, and the next best extractant was alcoholic hydroquinone (modified after Jones and Leeper 1951b) (correlation coefficient 0.860).

Influence of micro-organisms on the availability of manganese

Sohngen (1914) showed that many micro-organisms were capable of oxidising soluble manganous compounds to insoluble oxides, or reducing manganese dioxide, depending on the conditions. Gerretsen (1936, 1937) related this to the

problem of availability of manganese in agriculture by demonstrating that precipitation of manganese occurred between pH 6.3 and 7.8, which is the range of soil pH in which manganese becomes unavailable. Leeper and Swaby (1940) confirmed these results using a similar soil agar technique, and found the oxidation would take place at even lower pH's, down to 4.8. Broadfield and Skerman (1950) criticised the technique used, and succeeded, by a different method, in isolating two bacteria which were unable separately to oxidise manganous compounds, but could do so in association.

Mann and Quastel (1946) used a soil perfusion technique to show that manganese was removed from manganous sulphate solution percolated through neutral or slightly alkaline soil, and that "MnO_2" was produced, although they admitted that their manometric method, using hydroxylamine, was unable to distinguish between manganese dioxide and trivalent manganese. This precipitation of "MnO_2" was accomplished by micro-organisms, since it was prevented by biological poisons. They found that reduction of oxides could also be carried out biologically, and proposed a manganese cycle in soils which implied almost complete dependence on biological mechanisms.

Barker and Broyer (1942) grew squash plants in water culture solutions under sterile conditions and with a soil inoculation. Less manganese was taken up by the non-sterile plants, and yields were lower, but whether this was due to precipitation of insoluble oxides is extremely doubtful; it could also be explained by increase in pH of the inoculated medium, by increased carbon dioxide production in the presence of the micro-organisms, as pointed out by the authors, or to simple competition for nutrients between plants and the bacteria and fungi.

Aldrich and Martin (1952) demonstrated that soil sterilisation with a variety of fumigants reduced the rate of decline of water soluble and extractable manganese (using N ammonium acetate) when soils were stored at room temperature and at 50% moisture capacity for up to 250 days, while Timonin and Giles (1952) were able to show that fumigation with cyanogas prevented the development of manganese deficiency on the treated soil, and that there was a negative correlation between the level of extractable manganese and the number of manganese oxidising organisms found.

The general impression given by these papers is that micro-organisms play a key role, perhaps even the most

important part, in the production of manganese deficiency in soils by converting the divalent ion into insoluble higher oxides which are not available to the plant. This was the view of Leeper (1947) and of Quastel (1954), who stated in the Leeuwenhoek Lecture to the Royal Society that non-biological oxidation of manganese was unimportant and almost confined to alkaline soils. Rather less weight was given in the review by Fujimoto and Sherman (1948) to biological oxidations and reductions, and by 1952 Leeper also attached less overwhelming importance to this aspect of the manganese cycles in the soil.

The reasons for the shift in emphasis become apparent in considering the work done on the higher oxides of manganese in connection with their availability to plants.

Higher oxides of manganese and the problem of availability

The literature on soil manganese abounds with references to the non-availability of higher oxides, and indeed the general impression is gained that the terms "non-available" manganese and "higher oxide" are almost synonymous. Most of the leading workers have contributed to this impression in original papers, (Piper 1931, Leeper 1935, 1939, Steenbjerg 1933, Sherman and Harmer 1942, Sherman, McHargue and Hageman 1943, Lohnis 1951), or in reviews (Leeper 1947, Fujimoto and

Sherman 1948, Mulder and Gerretsen 1952, Quastel 1954).

The formula of these higher oxides has usually been written arbitrarily as "MnO_2", although as Leeper (1947) emphasised, compounds with the formula Mn_2O_3 and Mn_3O_4 must also exist in soils. Manganese dioxide itself may exist in several crystalline forms, for each of which the actual composition may vary, as is usual for ionic oxides. Cole, Wadsley and Walkley (1947) examined various samples of manganese "dioxide" and found the α oxide (cryptomelane) to be $MnO_{1.83}$, the β oxide (pyrolusite) to be $MnO_{2.00}$ and $MnO_{1.96}$, and numerous samples of the γ oxide to lie between $MnO_{1.83}$ and $MnO_{1.99}$.

When higher oxides of manganese are added to the soil one would not expect a marked response if soil manganese existing in the same form is unavailable to plants. Yet when the trial has been made an increase in manganese uptake has usually been found, (though not invariably, for example, Sherman and Harmer (1941) found pyrolusite ineffective against grey speck). Boken (1956b) quotes numerous cases where pyrolusite fertilisation has improved manganese nutrition of wheat, maize, mustard, oats, sugar beet, peas and tomatoes. She found that pyrolusite was a source of manganese for oats, but more became available when ferrous sulphate was added with the pyrolusite.

The improvement was ascribed to the reducing action of the ferrous sulphate on higher oxides of manganese (this having been the explanation of the observation previously made that ferrous sulphate increased the availability of manganese in the soil, (Boken 1955 and 1956a). Steenbjerg (1934) found that manganese dioxide served only temporarily as a source of manganese for oats, and suggested that it might even aggravate deficiency in subsequent years, and indeed, according to Piper (1931), Arrhenius in 1924 found that MnO_2 made grey speck worse rather than better. Leeper (1947) reported that manganese dioxide cured grey speck on Mount Gambier soil, and the effect lasted for ten years. Leeper's view was that the higher oxides of manganese exist partly in an inactive form, and partly in a highly active form which may become available to plants. Extraction with quinol gives a measure of the active form, as has been described above. Jones and Leeper (1951a) found that manganite (Mn_2O_3) and pyrolusite were available to plants, but that hausmannite (Mn_3O_4) was not. They ascribed this to the degree of order of the crystal lattice, and suggested that what is important for the health of plants is the degree of <u>surface</u> disorder of the crystal, which is not revealed by X-ray examination. Unfortunately no method is known for

checking this theory.

Heintze (1956) found that pyrolusite, hausmannite and manganite were available to varying extents to timothy grass, and was able to relate the manganese taken up to the reducibility and to the size of the ultimate particles of the oxides, thus following the explanation given by Jones and Leeper (1951a). Fiskel and Mourkides (1955) also found that MnO_2 was a source of manganese for tomato plants in pot culture experiments with marl, peat, and sandy soils.

It is apparent that there is ample evidence in the literature to show that the manganese in higher oxides is available to plants, provided that the unlikely hypothesis that oxides behave differently in the soil under field conditions is rejected. The insoluble higher oxides must be brought into solution for this to happen, since no evidence has been brought forward to support Comber's (1922) contention that uptake could occur by direct absorption of colloidal particles. Söhngen (1914) demonstrated that brown manganese dioxide was dissolved by hydroxy-acids produced by micro-organisms, but not by fatty acids such as acetic, propionic, or butyric acids. Heintze and Mann (1947) showed that citrate, tartrate, and malate were able to dissolve hausmannite and "manganic hydroxide" but not manganese dioxide, where - as malonate, succinate, and oxalate were ineffective. Broomfield (1957) in an examination of the iron sources used in nutrient solutions found

that tartrate, citrate and E.D.T.A. solutions, as well as ferrous ions, were able to make manganese available to plants from γMnO_2 and from a higher oxide of manganese prepared by his microbiological method (Broomfield 1956). He was able to show (1958a) that root washings of oat plants contained a substance or substances able to dissolve the biologically formed oxide, and later (Broomfield 1958b) determined that γMnO_2 was also dissolved by the washings of vetch roots. Contrary to expectation, since vetches take up manganese more readily from a given soil than oats, it was found that oats produce more of the dissolving substance. The substance liberated by the roots was not identified, but it seems likely that it could be a complex phosphate, perhaps a nucleotide, since Heintze and Mann (1949) showed that pyrophosphate is an extremely efficient extractant of soil manganese, and Lundegårdh and Stenlid (1944) reported nucleotides in the exudates of wheat and pea roots.

Quite simple reactions may suffice to render soluble the manganese from manganese dioxide. The action of MnO_2 on carbohydrates was reported by Bose, Foster, Stacey and Webber (1959). These reactions, all quite likely to take place in the soil, would result in manganous ions being produced.

In the light of all this accumulated evidence it is hard to maintain the convention that higher oxides of manganese are

unavailable to plants. It would seem that the hypothesis was suggested in the first place on circumstantial evidence. Under those conditions where manganese becomes non-available in soil, - high pH, and oxidising conditions in particular, - one might expect higher oxides to be found. But there is no logical necessity for non-availability to be a result of higher oxide formation, it is quite possible for non-availability and higher oxide formation to be concurrent, but independent.

The influence of organic matter on manganese availability

The majority of observers of "grey speck" of oats and "marsh spot" of peas have noted that the trouble occurs especially on organic soils of high pH. (For example, Morley Davies 1939, Wallace 1940, Sherman and Harmer 1941, Heintze 1946). It had indeed been stated that for manganese deficiency to occur it is necessary to have either "high organic matter" (Wain, Silk and Wills 1943), "appreciable organic matter (Heintze 1946) or "a certain amount of organic matter" (Mulder and Gerretsen 1952).

It is therefore rather surprising that the amount of experimental work on the influence of soil organic matter on manganese availability should be so meagre.

In 1938 Heintze made the important suggestion that in some alkaline soils manganese might be held in an insoluble

form by combination with organic matter. The evidence for this was not published, and in her many subsequent papers Heintze has unfortunately never stated what was the nature of the work which led her to this original conclusion.

In 1946 Mann and Quastel noted that a Rothamstead soil with little exchangeable manganese to N calcium nitrate gave with a phosphate buffer an extract which formed a strong blue colouration with benzidine acetate. On the basis of this observation Dion and Mann (1946) were able to show that significant amounts of trivalent manganese exist in soils and that this could be extracted by pyrophosphate which was present as an impurity in the phosphate buffer used by Mann and Quastel. Heintze and Mann (1947) found that manganic manganese was also extracted by organic acids such as citric, tartaric and malic, which were capable of forming complexes with manganic or other similar trivalent metallic ions.

It was noted that there was a close parallel between the colour of the extract and its manganese content; this led on to work by Bremner and Lees to explore the potentialities of pyrophosphate as an extractant for soil organic matter. As a result a very important step forward was made when Bremner, Heintze, Mann and Lees (1946) found evidence that metals such as manganese exist in soil as insoluble metallo-organic complexes, and that if the metal is removed by an agent such

as pyrophosphate the organic matter becomes soluble. They found that if the extract was dialysed to remove the pyrophosphate and the various metals the organic matter remained soluble, but could be re-precipitated by addition of Mn, Cu, or Fe.

Heintze and Mann (1946) showed that the manganese extracted by alkaline pyrophosphate was in the divalent form, since it gave no blue colouration with benzidine acetate, and concluded that divalent manganese can also exist in soil under alkaline conditions in the form of co-ordination complexes with organic matter.

Mattson, Eriksson and Vahtras (1948) were able to verify many of the results obtained by the Rothamsted workers, and presented further evidence for the existence and stability of the manganic ion in the form of complexes in the soil. They suggested that the problem of availability of manganese in the soil might be greatly influenced by these complexes, and pointed out that compounds of the same classes as those used by the Rothamsted workers were known to be exuded by plant roots. Lundegårdh and Stenlid (1944) had identified adenosine monophosphate, and possibly adenosine triphosphate, and flavanone, which contains a phenol hydroxyl group and is strongly reducing, in the exudate of wheat and pea roots.

Further work by Heintze and Mann (1949) led them to the hypothesis that in neutral or alkaline organic soils the divalent manganese is held mainly by the organic matter. They suggested that manganese deficiency on such soils is caused by the formation of complexes of divalent manganese with the organic matter which are dissociated so little that the manganese in the soil solution is insufficient for the needs of the plant. While the emphasis in this paper was on divalent manganese being held by organic complexes, the authors had to admit that there was no certainty about the form of the manganese in the soil, since the reversible dismutation reaction occurs which they resprosented as:-

$$2\,Mn^{3+} + 2H_2O \rightleftharpoons Mn^{2+} + MnO_2 + 4H^+$$

In reviewing advances in knowledge of the factors influencing the availability of micro-nutrients in the soil Leeper (1952) devoted considerable attention to this work on manganese. He emphasised how analysis for "exchangeable" heavy metal ions has too often assumed that these ions behave like simple "rare gas" ions, held by electrostatic attraction, and pointed out that they can be much more tightly absorbed, with the formation of covalent links and chelate compounds. This could also explain how the heavy metals become less mobile as the soil pH increases. This review marked an important turning point; previously Leeper (1935, 1939, 1947) had been of the opinion that "higher oxides" of manganese in the soil represented that fraction of the metal which is not available to plants.

A paper published in 1951 by Heintze and Mann was not included in Leeper's review (1952). It showed that the "easily reducible" manganese, (determined by extracting the soil with neutral normal ammonium acetate containing 0.2 per cent hydroquinone), decreased with increase of soil organic matter. "Easily reducible manganese" had been regarded as that part of the manganese which could become available to the plant from the non-available higher oxides. This paper showed that manganese freed by the reduction of insoluble oxides could be retained, at least in part, by soil organic matter, and suggested that the part of the manganese not extracted by hydroquinone or hydrosulphite represented not unreactive higher oxides of manganese, but manganese firmly bound by organic matter.

Hemstock and Low (1953) examined the ways in which manganese might be retained by clay particles. They found that with a pure Wyoming bentonite manganese retention could not be explained by isomorphous substitution in the clay lattice, but could be explained by oxidation and subsequent precipitation. When they examined a sandy loam soil they found that manganese was retained in an entirely different way, and were able to show that it was held by the soil organic matter in the form of a chelate complex. They supported

their case by showing that copper salts were ineffective in dissolving manganese from various oxides, but were extremely efficient in extracting manganese from the organic soil, just as would be expected if the copper were replacing the manganese held in an organic complex.

Beckwith (1955b) carried out more extensive investigations on the release by copper of manganese from organic soils. He found that the addition of copper to an extractant, as in the technique of Hemstock and Low (1953), caused a marked drop of pH, by the release of hydrogen ions in the formation of copper chelates with the soil organic matter. This would tend to release manganese by the well known (though unexplained) pH effect. But Beckwith was able to demonstrate that even when the pH was controlled, the addition of copper still led to a release of manganese, and he concluded that manganese must be held by soil organic matter in the form of a complex. Beckwith (1955a) had previously shown that the divalent manganese of soils could be extracted efficiently with disodium calcium versenate (E.D.T.A.) without fear that reduction of higher oxides of manganese by the reverse disputation reaction described by Heintze and Mann (1949) would give falsely high estimates. He did not examine (1955b) whether the manganese released from the organic complexes by copper was held in the divalent or trivalent

form; he observes that the manganous complex would be weak, and merely states that there is no evidence to favour or exclude the possibility of complexes of trivalent manganese.

Heintze (1957) confirmed Beckwith's finding that divalent manganese is held in alkaline organic soils in the form of a complex, and is extractable with calcium disodium versenate solution. She also demonstrated by means of paper electrophoresis that the manganese in water extracts of organic soils always migrated anionically together with organic matter, and that about 90 per cent of the manganese content of a water extract of such a soil was non-dialysable.

If organic matter in the soil is responsible for holding manganese in a form unavailable to plants it may be possible to provide an explanation for the increase in availability which occurs when soil is steam-sterilised. This increase has been reported by Wain, Silk and Wills (1943), Fujimoto and Sherman (1945 and 1948), Aldrich and Martin (1952), Forsee (1954), and Davies (1957). Hurwitz (1948) showed that the increase in exchangeable manganese was a logarithmic function of temperature above 30°C, and Boken (1952) also reported a great increase in exchangeable manganese when soil had been oven-dried. All these facts could be explained if the manganese-organic matter complex is heat-labile, so that the

manganese could be set free by the destruction or re-arrangement of the organic complexing groups.

It is possible also that the increase in exchangeable manganese which occurs on storage (Boken 1952, Zende 1954) is caused by the breakdown of the organic matter and the release of its bound manganese. Zende (1954) noted that the increase correlated with the organic matter content of the four soils he investigated.

Manganese availability in relation to soil

Some of the earliest observers of "grey speck" and other manganese deficiency diseases noted that they occurred only on neutral or alkaline soils (McHargue 1923, Hiltner 1924), Hiltner (1924) was, however, able to show that the same symptoms would develop in oats grown in acid culture solutions if the manganese level were low enough, so that evidently the effect was caused by a decrease in availability of manganese resulting from high pH values of the soil.

Piper (1931) would seem to be the first to have shown a quantitative relationship between soil pH and manganese taken up by plants, which in this case were oats. His graph showed a small increase in uptake from two soils between pH 8 and 7, and thereafter a rapidly accelerating uptake between pH 7 and pH 6 as the soils were acidified.

Steenbjerg (1933) plotted results from experiments with four crops (oats, rye, swedes and potatoes,) grown in the same soil limed to different pH values. Although actual uptakes differed considerably, the general form of the curves was in each case similar to those obtained by Piper (1931). Steenbjerg also demonstrated that the manganese extracted from the soils by molar sodium nitrate solution varied in the same way with pH. Two years later a much more extended study of the effect of pH on extractable manganese was published (Steenbjerg 1935). Half molar calcium nitrate solution was used as the extractant and if figures from Steenbjerg's (1935) Table 4 and 5 are plotted similar figures may again be obtained. Steenbjerg found that the total manganese extracted by successive leachings could be expressed as a function of the change in pH by the expression

$$T_{Mn} = T_{Mn_o} e^{-kpH}$$

in which T_{Mn_o} was the value of total extractable manganese of the unlimed plot, and pH represents the change in pH consequent on liming.

This equation may be put in the form

$$\ln T_{Mn} = \ln T_{Mn_o} - kpH$$

or alternatively

$$\log_{10} T_{Mn} = \log_{10} T_{Mn_o} - k_1 pH$$

Steenbjerg found that an average figure for k_1 was about 0.48.

Similarly shaped curves were obtained by Olsen (1934) for the relationship between the uptake of manganese by several different species of plants from soil of varying pH, but he was able to show that the pH response of plants grown in solution culture was entirely different. A number of plants exhibited their maximum uptake from solutions at pH values of about 6.5. Although the amount taken up varied a great deal from one species to another, all the plants tested showed a similar optimum pH with a more or less rapid decrease in uptake above or below this value. The "cocked hat" shape of the response curve was markedly similar to that shown in the activity curves of many enzymes, although Olsen rather surprisingly does not comment on this.

Rather different results were obtained by Heintze (1946). She incubated a clay loam for a week with varying amounts of lime, then determined pH and exchangeable manganese (using calcium nitrate). The resulting values, if plotted, present a marked contrast with the results of previous workers. Exchangeable manganese still decreases as pH rises, but the curve is now convex upwards, where as all the other curves were concave. Similar curves were reported by Dion, Mann and Heintze (1947)

for a Rothamsted and a Romney Marsh soil, although in these cases a reducing agent had been added to the soil and extracting solution before shaking. It is not clear why this should alter the pH response of the soils, nor is any other explanation evident for the difference between the results of the Rothamsted workers and others.

No one else has reported relationships of this sort, but de Mairedy (1956) stated in the discussion at the Sixth International Soil Science Congress that he knew of tropical forest soils where exchangeable manganese was greater at higher pH values.

The orthodox relationship, if it may be so termed, has also been reported from America by Gavey and Barber (1952). In the east of Scotland many examples were collected by Smith (1959) of values of manganese contents of oats grown in various soils of differing pH values; these were plotted and once more gave the usual type of graph. Naturally the individual values were scattered, since many soils were involved, but the general pattern is clearly evident, as it is in a recent paper by McGregor, Schofield-Palmer and Wilson (1960).

This well known increase of manganese uptake consequent to increase in the acidity of the soil has frequently been applied as a cure for manganese deficiency. Acidification

has most often been accomplished by the application of powdered sulphur, which becomes oxidised, presumably by microbiological process, and gives rise to sulphuric acid. This method has been especially popular in America (Sherman and Harmer 1941, Tisdale and Bertramson 1949).

Gavey and Barber (1952) were able to show that the sulphur had an effect greater than that produced by the change in pH alone; they attributed this to additional reduction of the manganese oxides in the soil, the oxygen being transferred to the sulphate produced. Essentially the same view was held by Vavra and Frederick (1952) who showed that in soil the reduction of manganese dioxide could be mediated by Thiobacillus thio-oxidans.

Tobia and Pollard (1958) compared the duration of the acidification produced by treatment of soil with sulphuric acid, aluminium sulphate, and ferrous sulphate. They found aluminium sulphate most, and sulphuric acid least effective. The interpretation of their results for manganese released in a leaching experiment would be difficult, and they did not attempt to find any quantitive relationship between pH and manganese in the leachate. Their results do show, however, that it should be possible to control "grey speck" and similar diseases by the application of aluminium sulphate.

The very important question of the mechanism of the retention of manganese by soil was investigated by Hemstock and Low (1955). In part of the work they used a pure clay of the montmorillonite-beidellite type. In an experiment to examine the effect of pH a manganese saturated clay suspension was used with the pH adjusted to various levels between 8.1 and 2.4. The samples were digested for fourteen days at 50°C and then manganese extractable by normal calcium chloride solution was determined. Hemstock and Low reasoned that with a reaction of the type

$$a\,Mn^{++} + bO_2 + c\,H_2O \rightleftharpoons d\,Mn_wOx(OH)y + eH^+$$

the approximate equation

$$\log Z = B - \frac{e}{a} pH_f$$

could be obtained, where Z was the final manganese concentration retained by the clay as a fraction of the original concentration, and pH_f was the final pH value of the suspension.

It will be appreciated that this is an alternative form of Steenbjerg's (1935) equation, and that e/a is the same coefficient as Steenbjerg's k_1.

But Hemstock and Low obtained a figure very different from Steenbjerg's average figure of 0.48.

They obtained the equation

$$\log Z = 26.375 - 4.26\,pH_f$$

They were unable to offer an explanation for the co-efficient 4.26. As they pointed out, in the production from divalent manganese of MnO, MnOOH, Mn_2O_3, MnO_2 or Mn_3O_4, the value of e/a would be 2. It would seem that this ruled out any simple explanation of manganese retention by the precipitation of these oxides.

It is possible that an explanation might be found on the basis of the disumtation equation given by Heintze and Mann (1949)

$$2\,Mn^{+++} + 2H_2O \rightleftharpoons Mn^{++} + MnO_2 + 4H^+$$

If the equilibrium is far over to the right, which it would be under the circumstances of the experiment, practically all the manganese would be in the form either of Mn^{2+} or MnO_2. The MnO_2 only would be retained, while all the Mn^{2+} would be extracted by the calcium chloride solution. Then the equation

$$\text{Log } Z = B - 4\,pH_f$$

would apply. But for this explanation to hold, the manganese added in the form of Mn^{2+} would have to be oxidised to Mn^{3+} without the involvement of hydrogen ions in the process. From Hemstock and Low's other experiments in the same paper (1953) it is clear that manganese is readily oxidised when exposed to the atmosphere, but this would appear from their results to result in the release of hydrogen ions. It is clear that the

reaction is not a simple one, but the chief significance of the results obtained by Hemstock and Low is that the process investigated by them in their pure clay is not the process responsible for the retention of manganese in soil in the field, or, at least, if their process does occur, it plays only a minor role. Yet their process was certainly one of precipitation of an insoluble oxide, which has been so widely regarded as the important process in fixing manganese in an unavailable form. Doubt has been thrown on that viewpoint already; here is additional evidence against it.

Essentially the same approach to the question of pH relationships as that adopted above was shown by Eriksson (1952) in a theoretical paper. Unfortunately all his reasoning was based on the assumption that only Mn^{++} ions and the various higher oxides are important. He believed that essentially Mn^{++} ions are converted into MnO_2 in the soil, and as a result was led to say "It may be safe to conclude that in freely drained soils most probably

$$p\,Mn^{2+} - 2pH = 0."$$

(where $p\,Mn^{2+}$ was the negative logarithm of the manganous ion concentration, analogous to pH).

Eriksson did not appear to be aware that figures existed in the literature to disprove his conclusion. A simple

transformation of Steenbjerg's (1935) relationship gives

$$p\,Mn^{2+} - 0.48\,pH = \text{constant.}$$

and, as has been seen, most other workers have produced similar results. The anomalous results of Heintze (1946) and Dion, Mann and Heintze (1947) do not accord with Eriksson's equation either. The only explanation would seem to be that Eriksson's basic assumption about the behaviour of manganese in soil was at fault, and that the availability of manganese to the plant is controlled by some other mechanism.

N O T E

The above review of the manganese literature is a selective one and is not intended to be comprehensive. It is hoped that the more important papers have been included up to October 1960 but many have had to go without mention, and inevitably less than justice has been done to many able workers in this very crowded field.

II. METHODS

Review of methods of manganese determination

1. The most commonly used methods depend on the oxidation of manganese to its highest valency state to give highly coloured permanganate ions whose concentration is then determined either volumetrically or more usually absorptiometrically. The absorption characteristics of the solution have been investigated by Mehlig (1939). He showed that Beer's Law is obeyed, and reported the absorption spectrum, which has an absorption peak at 522 m μ. A useful list of interfering ions and ways of overcoming interference was included in the paper.

The oxidation can be carried out in numerous ways, of which the following are the most important:-

(a) Lead dioxide and concentrated nitric acid.

If a manganese compound is boiled with lead dioxide and concentrated nitric acid the characteristic purple colouration of permanganate is produced (Crum 1845). The procedure is used nowadays only for the qualitative detection of manganese.

(b) Bismuthate.

In the presence of nitric acid, sodium bismuthate similarly oxidises manganese to permanganate (Gortner and Rost 1912). The use of bismuthate seems to be favoured in the steel industry, but it has also been used in the determination of manganese in

soils. (Iyer and Rajagopalan 1936, and A.O.A.C. "Official Methods of Analysis" 8th Ed. (1955) p. 32).

(c) Ammonium persulphate.

The use of ammonium persulphate as the oxidising agent became widespread after the publication of a paper by Marshall (1901). Silver ions must be present in small quantities; their catalytic action may be explicable in terms of the intermediate formation of argentic oxide, which has been used recently to carry out directly the oxidation of the manganese to permanganate (Lingane and Davis 1956).

Ammonium persulphate is the oxidant preferred by workers in agricultural chemistry on the continent of Europe, and has been used by Boken (1952, 1955, 1956 and 1958), Schachtschabel (1956), Steenbjerg (1933, 1934, and 1935), Olsen (1934), Mydahl (1949 and 1951) and many others.

(d) Periodate.

Most workers in Britain and the United States use sodium or potassium periodate, carrying out the oxidation under acid conditions, more or less following the procedure of Willard and Greathouse (1917) which was worked out for the determination of the manganese content of steels. The material is dissolved in acid (usually nitric or sulphuric acid is used), and the solution heated with periodate until the purple colour

of the permanganate ions reaches its greatest intensity. Care must be taken that development of the colour has proceeded to the maximum extent. The presence of phosphate seems to assist the development in some way which has not yet been explained.

Numerous workers have investigated the technique and improvements have been made, or adaptations suggested for particular circumstances. As in all methods which rely on the determination of the permanganate ion, the absence of chloride is essential. Usually all chloride is driven off during the preliminary treatment of the material at the acid digestion stage. Recommendations were made by Richards (1950) that the final concentration of sulphuric acid in the solution used in the absorptiometer should be 5 to 6%. The procedure given for achieving this seems rather doubtful and experience in our laboratory does not support her findings. In fact development of colour proceeds smoothly under widely different conditions, though at greatly varying speeds. It is indeed quite safe to develop the colour under alkaline condition. This is the procedure used in a method for manganese given by Mehlich (1957). To 5 ml. aliquots of solution containg about 30 p.p.m. manganese was added 0.5 ml. 5% orthophosphoric acid, about 10 mg. sodium periodate and 5 ml. of 8% sodium hydroxide. After mixing, the solution

was allowed to stand at room temperature for one hour, or longer if necessary, as in the case of some organic soils.

The technique for the application of the method with development of the colour under acid conditions, has been worked out by Cook (1941) for feeding stuffs and for plant material by Piper (1944).

(c) Argentic oxide.

As has already been mentioned, argentic oxide, which has become available commercially recently in the U.S.A., may be used in cold nitric, perchloric or sulphuric acid solutions to oxidise manganese to the septa valent state (Lingane and Davis 1956). This method has not yet been put into extensive use. While the intense colouration of the permanganate ion makes it very suitable for use in absorption spectrophotometry there is still a limit to the quantity of manganese that can be determined by this method. Using a Spekker absorptiometer with 4 cm. cells and a green filter the practical limit of determination is about 0.05 mg. manganese. For certain purposes this is insufficient and one must turn to more sensitive methods, though these are not capable of the same precision.

2. Formaldoxime Method.

Using formaldoxime quantities of manganese as small as 5 μ gm. can be determined. The solution is made alkaline, and

a small quantity of formaldoxime is added. A brown colouration develops which is stable for long periods. The reaction was discovered by Brunner (1919) then rediscovered by Denigès (1932) and subsequently again by Kahane (1935). Details of methods applicable to plant material are given by Kahane and Brard (1934). Iron interferes by the formation of a violet-red colour and in the method of Sideris (1937) for plant material the interference is prevented by complexing the iron as cyanide. A later technique of Sideris (1940) is to remove the interference by precipitating ferric phosphate, but the procedure is not simple. A better method is that of Bradfield (1957) which uses N-hydroxyethyl-ethylenediamine-tri-acetic acid (HEEDTA) to complex the iron. This also prevents the precipitation of calcium and magnesium phosphates which might be troublesome under the alkaline conditions of the determination.

The simplicity of the formaldoxime method makes it attractive, but it is unsuitable in the presence of large quantities of calcium, and so could not be used for many of the estimations in this work.

3. Methane Base Method

The method finally chosen for the determination of very small quantities of manganese was that of Cornfield and Pollard (1950) with slight modifications. This uses 4,4', - tetramethyl-diamino-diphenyl-methane (methane base) in a solution

buffered with sodium acetate/acetic acid. In the presence of manganese the dye is oxidised by potassium periodate. At first a fleeting brilliant blue colouration is produced, which is used as a qualitative test for manganese. Attempts have been made to use this colouration for quantitative estimations (Harry 1931, Nicholas 1946) but it is too fugitive for reasonable accuracy. After a few minutes a fairly stable green colouration develops, and this is measured in a Spekker absorptiometer. The procedure is standardised so that a reading is taken exactly ten minutes after the methane base reagent is added; this exact timing is essential because the colour changes in intensity for several hours and finally a dark precipitate forms. The temperature must be controlled to within $\pm 0.5^{\circ}C$, and all reagents must be made up exactly as specified. The mechanism of the reaction is obscure. It has been suggested (Single 1957) that two reactions occur, the first being a catalytic action by the manganese in the oxidation of the methane base, and the second a removal of manganese from the reaction system by complexing with some colourless oxidation product. This second reaction is favoured by increase in temperature.

No systematic investigation has been made in the course of this work into these questions, but it has been noted that if the coloured oxidation product is extracted with organic

such as chloroform, the manganese remains in the aqueous layer. This would be consistent with Single's suggestions if the colourless oxidation product which complexes the manganese were to remain in the aqueous layer.

The methane base method is extremely sensitive to interference, especially from oxidising or reducing agents. The former act by oxidising the methane base; this oxidation may result in the formation of blue and green products as with manganese and periodate, or violet and red products as with ozone. The production of these colours has been used as a test for chlorine, ozone and hypochlorites (Masterman 1939). This sensitivity to oxidising agents makes it necessary to avoid the use of chromic acid in cleaning glass-ware to be used in the determinations; the slightest traces of chromate produce yellow colours. The reducing agents interfere by consuming the periodate, causing results to be too low.

The restriction imposed by the changing depth of the green colouration that compels readings to be taken after an exact time interval following the addition of the methane base reagent is the greatest disadvantage of the method. Yuen (1958) attempted to overcome this by using leucomalachite green, 4,4'-tetramethyl-diamino-triphenyl-methane, which under rather similar conditions give a stable green colour. While this is

a great advantage, and the method as published almost doubles the sensitivity of the methane base method (this is not an unequivocal advantage, it would be better in many circumstances to have half the sensitivity,-) trials showed that there were more than counterbalancing dis-advantages.

It was found that satisfactory reproducibility is even more difficult to achieve than with methane base.

While this drawback could no doubt be overcome with experience, the most telling argument against the use of leucomalachite green is that with quantities of manganese rising above about 1 microgram there is a <u>reduction</u> in the depth of colour produced. This means that when working with solutions of unknown strength at least two readings, and probably a third, would be required in every case before a reliable figure could be obtained.

4. Other sensitive methods.

Manganese may be determined polarographically as tri-dihydrogenpyrophosphatomanganiate after oxidation of the manganese to Mn (III) by lead dioxide in the presence of excess pyrophosphate (Kolthoff and Watters 1943). Interference may be eliminated by the procedure given in a later paper of Watters and Kolthoff (1944). A colorimetric method has been described by Davis (1959) which employs electrogenerated vanadyl ions.

An interesting colorimetric determination was described by Toursky, Issa and Mewaidy (1957). This makes use of the red colouration formed when freshly formed manganese dioxide dissolves in an alkaline tellurate solution. Another colorimetric method given by Nightingale (1959) employs coloured complexes found by Mn (III) with triethanolamine or hydrogen peroxide.

The spectrographic estimation of manganese is widely used. Techniques are described by Mitchell (1948). The accuracy of the determinations expressed as a coefficient of variation has been found for twenty replicate analysis of plant material to be 7% (Calder and Voss 1957).

A very sensitive method, suitable for quantities of manganese between 0.5 and 20 μ g equivalents has been described by Erdey and Szabadvary (1957) which uses Variamine Blue as a colorimetric reagent.

A possible basis for a colorimetric method is the reaction of manganese with p-anisidine, described by Batalin (1957 as a rapid spot-test for manganese in soils. The sensitivity is given as 1 in 370,000, a violet colour being produced when an alkaline solution containing manganese and p-anisidine is acidified. Batalin used oxalic acid, but trials have shown that acetic, citric, and sulphuric acids are equally effective.

A method suitable for the determination of 0.25 to 8 μg of manganese in 5 ml. of solution is described by Kavanovich (1956) using Manganon IRRA (o-salicylideneamino-phenol), a reagent for manganese discovered by Lukin and Ostrova (1956).

As little as 6.4 $\mu g \times 10^{-3}$ of manganese can be detected by chromatography (Heisig and Pollard 1957) using either diphenylcarbazide, as a spray in ethanol, or quercitin as described by Weiss and Fallab (1954), and developing the spots by exposure to ammonia fumes.

5. Separation and concentration of manganese.

Instead of employing more sensitive methods of determination a number of workers have evolved methods for concentrating the manganese from a large volume of solution.

In an early part of the work described here this approach was employed, and the procedure followed is set out in a later section. In essence the method adopted was to oxidise the manganese to MnOOH, filter this off, and then to employ periodate oxidation to give permanganate. The procedure was time-consuming and tedious as well as unpleasant, and the reproducibility achieved was not good. This method was soon abandoned in favour of the methane base method. A rather similar procedure was followed by Koroleff (1947) but here magnesium sulphate was used to provide a carrier and coagulant, and it was assumed that

atmospheric oxidation of the manganese would be complete under the alkaline conditions of precipitation.

An early essay in the use of ion-exchange resins to recover trace elements from plant digest solutions was described by Riches (1947). He used Amberlite IR - 100, and reported satisfactory recoveries. The same cation exchanger was used by Nydahl (1951) to concentrate elements in lake waters. Anions were absorbed on Amberlite IR 4B.

Manganese together with other elements present in special steels were absorbed from hydrochloric acid solutions on to cellulose columns by Venturello and Ghe (1957). It could then be eluted (along with cobalt) by methyl-n-propyl-ketone in 5% hydrochloric acid.

Manganese can be extracted from aqueous solutions into chloroform as a diethyldithiocarbamate complex. This was the procedure used by Clinch and Guy (1958) to separate manganese from cerium, MnO_2 being precipitated along with cerium in the separation of the latter from the lanthanons.

6. Separation of the valency states of manganese.

This is a problem which resists solution. It is desirable, for example, to fractionate the manganese present in soils into its various forms, but no way is known of doing this. Leeper (1935) introduced the concept of "easily reducible" manganese

but this has not been identified with any one compound nor indeed with any class of compounds of manganese. The equilibrium between the various forms is almost certainly dynamic, and will therefore be upset by the act of separation. Trivalent manganese, for instance, is unstable in vitro except under strongly acid conditions, and in other conditions disputes into a mixture of divalent and quadrivalent forms, and yet it has been shown to exist in soil, (Dion and Mann 1946).

Stevens (1957) has begun to tackle this aspect of the manganese problem, and by paper chromatography has succeeded in separating Mn^{2+} or Mn^{VII} from Mn^{3+}, but Mn^{2+} and Mn^{VII} interacted to give Mn^{3+}, so that their separation is not feasible.

Other workers have been able to attack the problem only along empirical lines, for example Leeper's (1935) extraction with N ammonium acetate solution containing 0.2% hydroquinone to give "easily reducible" manganese by difference with extraction with N ammonium acetate giving "exchangeable" manganese. Other facets of the work in this field have been dealt with in the introduction.

ROUTINE METHODS USED IN THIS WORK

I. Methods used for manganese determinations

(a) Total manganese in soil.

The A.O.A.C. method was rejected as it was considered undesirable to use hydrofluoric acid for the dissolution of the soil with the resulting need for platinum vessels (Official Methods of Analysis 8th Ed. 1955 p. 32). Sodium carbonate fusion, followed by solution of the melt and development of permanganate colour by the use of periodate was also considered and rejected as being less straight-forward than the procedure which follows.

A simple method was evolved which is essentially a nitric-sulphuric-perchloric acids digestion technique, similar in some respects to the method of Wain (1938) but differing in that the development of permanganate colour was carried out in the presence of the soil, and solid matter separated finally be centrifugation.

Reagents:-

Nitric acid conc. A.R.

Sulphuric acid conc. A.R.

Perchloric acid A.R.

Phosphoric acid A.R.

Potassium periodate A.R.

The soil is air dried, and passed through a sieve with 2 mm. holes. The sample must be thoroughly mixed, and is sub-sampled by withdrawing a number of small portions at random. 2 gms. of soil are used in each of the determinations, which are performed in duplicate.

The 2 gm. sub-sample is placed in a 300 ml. Kjeldahl flask, and 20 ml. of concentrated nitric acid, 10 ml. of concentrated sulphuric acid and 2 ml. of perchloric acid are added, in that order.

A glass bead is dropped into the flask, and slow digestion proceeds for about 1½ hours, the final fuming stage being maintained for not less than 20 minutes. Heating should be so gentle that fumes do not escape from the neck of the Kjeldahl flask. At the end of this stage the digest should be cream coloured or grey; (a trace of green is seen quite frequently and may be ignored.)

The flask is allowed to cool, then about 100 ml. of distilled water are added, followed by 2 ml. of phosphoric acid, and approximately 0.3 gm. of potassium periodate. The flask is now warmed gently and maintained at a temperature somewhat below boiling point. After a period of time which varies from a few seconds to about half an hour, the purple permanganate colour develops. The flask is maintained at

the same temperature for about half an hour to ensure complete development. If there is any tendency to fade more periodate may be added, and the development period continued for a further half hour.

After cooling, the contents of the flask are transferred to a 250 ml. volumetric flask, and made up to the mark with distilled water. Most of the solid matter settles rapidly; a portion of the supernatent liquid is centrifuged at 3000 g. for 15 minutes to eliminate the rest before being transferred to a 4 cm. cell in a Spekker absorptiometer. Green No. 5 filters are used. The readings obtained are converted into manganese as parts per million of soil by means of a calibration curve obtained by taking appropriate quantities of manganese as manganese sulphate solution and carrying these through the above procedure as for soil.

A solution of 0.1 mg. of manganese per ml. is obtained by taking 0.3077 gm. of $MnSO_4.H_2O$ and making up to 1 litre. $MnSO_4.H_2O$ may be obtained from higher hydrates, such as the usual commercially available form of manganese sulphate, $MnSO_4.4H_2O$, by heating to between 100°C and 200°C for 12 hours (Křepelka and Rejha 1931).

5 ml. of this solution carried through the procedure above and made up to 250 ml. corresponds to 250 p.p.m. manganese in the

soil. Suitable volumes of solution to give a range of readings up to 1250 p.p.m. manganese are used to consturct the calibration curve.

(b) Manganese in plant material.

Where manganese was present in quantities of about 20 parts per million or more the method used was essentially that of Piper (1944).

5 gms. of dry matter are placed in a 300 ml. Kjeldahl flask, and 20 ml.s of concentrated nitric acid, 10 mls. concentrated sulphuric acid and 4 mls. perchloric acid added, strictly in the order given. Gentle heating is continued for 1½ to 2 hours, more nitric acid being added if necessary to clarify the digest. The final fuming stage is continued for approximately 20 minutes, as for total manganese in soil, until a clean, colourless, digest is obtained.

The flask is cooled and about 60 ml. of distilled water added, then 2 ml. of phosphoric acid and approximately 0.3 gm. of potassium periodate. The temperature is raised nearly to boiling point and maintained there until colour develops and thereafter for about half an hour.

After cooling, the solution is made up to 100 ml. in a

volumetric flask, and allowed to stand overnight. During this time any particles of silica, which in Piper's procedure are removed by filtration, settle to the bottom of the flask, and the clear solution is decanted into 4 cm. Spekker cell and read using green No. 5 filters. If there is any slight opalescence visible on holding up the volumetric flask to the light, the solution must be centrifuged before transferring to the Spekker cell, but in practice this is seldom necessary.

A suitable calibration curve may be obtained by simple modification of the procedure given under total manganese in soils.

(c) Determination of very small (microgram) quantities of manganese.

Methods which rely on colorimetric determination of permanganate ions can be used for very small quantities of manganese only if it is possible to concentrate the solution. This may not be convenient, as in the case of water extracts of soil, where a large volume of extract would be required, or may not be possible, as in the case of the extracts made with some of the stronger solutions of calcium nitrate, which began to crystallise before the pink colour became sufficiently intense to give a reasonable reading on the absorptiometer. Again, minute amounts of manganese in plants may demand

prohibitive amounts of material for determination by the periodate techniques.

In any of these circumstances the methane base method of Cornfield and Pollard (1950) was used, with some small modifications designed to improve the accuracy. This was stated by the authors to be only 10% though the precise meaning of this percentage was not made clear.

Using the procedure given below, twenty replicate determinations were made of the quantity of manganese present in 0.5 ml. samples of a test solution, and the following values were obtained:-

1.76, 1.80, 1.82, 1.78, 1.76, 1.78, 1.75,
1.80, 1.77, 1.84, 1.76, 1.81, 1.78, 1.76,
1.75, 1.78, 1.76, 1.82, 1.79, 1.75 gm. Mn.

Mean value = 1.783 gm.

A percentage coefficient of variation may be calculated from these data using the relations:-

$$s = \text{standard deviation} = \sqrt{\frac{(x - \bar{x})^2}{n - 1}}$$

$$\text{Coefficient of variation} = \frac{s}{\bar{x}} \times 100$$

The coefficient of variation found is 1.49%. This

may be taken to be the normal standard of accuracy of the method as modified for use in these investigations.

Reagents

Sodium acetate-acetic acid reagent. 15.0 g. A.R. sodium hydroxide are dissolved in 180 ml. of distilled water and 58.0 ml. of glacial acetic acid added. The mixture is cooled and diluted to 250 ml.

Phosphate solution. 0.48 gm. of potassium dihydrogen phosphate dissolved in 250 ml. of distilled water.

Periodate solution. 0.5 gm. A.R. potassium periodate dissolved in 250 ml. of distilled water.

Methane base reagent. 0.5 gm. 4 : 4'-tetra-methyl-diamino-diphenyl-methane are dissolved in 6 ml. 2N hydrochloric acid and diluted to 100 ml.

Standard manganese solution. A solution containing 0.2 micrograms manganese per ml. is obtained by diluting to 1 litre 2 ml. of manganese sulphate solution containing 0.1 mg. manganese per ml. The preparation of this stronger manganese sulphate solution has been described above under total manganese in soil.

The periodate solution and the methane base reagent should not be used more than 48 hours after preparation.

In the earlier part of the work reported here the methane

base was re-crystallised from iso-propyl alcohol after purification with sugar charcoal, white feathery crystalline plates being obtained, but at a later stage the brown crude material as supplied by British Drug Houses Ltd. was used without purification, the only effect being to shift the calibration curve a little farther from the ordinate.

150 mm. x 22 mm. dia. tubes, fitted with plastic stoppers and manufactured by Emil Ltd. are used instead of the open boiling tubes employed by Cornfield and Pollard. Into each tube 2 ml. sodium acetate-acetic acid reagent and 1 ml. of phosphate solution are measured from burettes, and an aliquot of the unknown manganese solution containing about 1 microgram of manganese is added. The volume in the tube is made up to 21 ml. by adding a suitable volume of distilled water from a burette.

Each tube is placed for at least 20 minutes in a thermostatically controlled water-bath set at 20°C to allow temperature equilibration to take place.

With each series of tubes in a given set of determinations a similar series of tubes must be set up to provide a calibration curve. Quantities of from 0 to 1.8 micrograms of manganese per tube are added using suitable volumes of the standard manganese solution containing 0.2 micrograms of manganese per ml.

After temperature equilibration, each tube is removed in turn from the bath, with two minute intervals between tubes, and 3 mls. periodate solution added from a micro-burette. The tube is thoroughly shaken, and 1 ml. of methane base reagent added, and the tube shaken again. This takes one minute, the tube is then returned to the water bath. Both the periodate and the methane base solutions should be at approximately 20°C.

Exactly ten minutes later the tube is removed from the water bath, the contents poured into a 1 cm. cell and a reading obtained on a Spekker absorptiometer using OR 2 filters.

Scrupulous attention must be paid to the cleanliness of the tubes used. Cleaning mixture containing chromic acid must never be used, since chromates in very small trace quantities cause serious interference. Concentrated nitric acid is suitable for cleaning, but tubes must be well rinsed after its use. Detergents of any kind are to be avoided.

Distilled water preferably should be from all glass apparatus, though water from a copper still was used with success over a long period. In spite of a report of difficulties arising from the use of water from a deioniser (Single 1957), water from a Deminrolite ion exchange apparatus

was used without trouble in the autumn of 1959.

2. Conductivity measurements

Measurements of conductivity solutions or soil suspensions were made using a dip-type of conductivity cell with platinum electrodes coated with platinum black. (Mullard Conductivity Cell, Type E. 7591/B).

This was immersed in the solution or soil suspension and the specific resistance of the solution obtained by balancing against standard resistances by the usual Wheatstone bridge method. The instrument used was one especially constructed at the Royal Technical College, Glasgow, which has been described and illustrated photographically by Nicol (1958).

The circuit is illustrated schematically in Fig. 1.

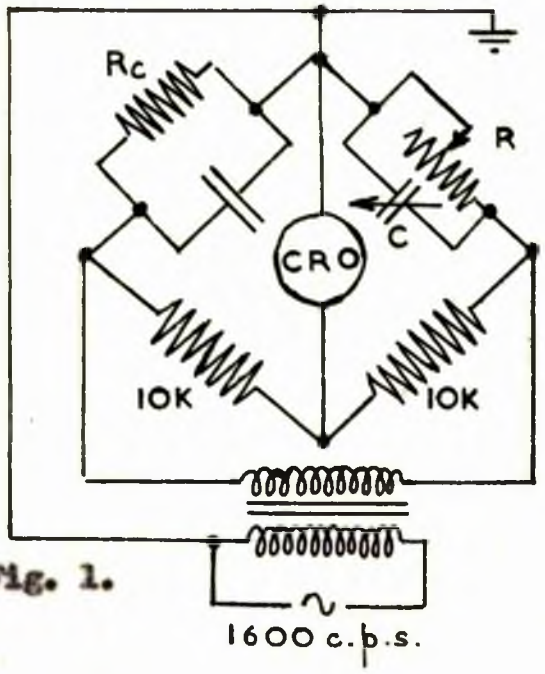


Fig. 1.

The resistance of the solution in the conductivity cell is represented by Rc.

This is balanced against R, representing decades of resistances in eight banks, the first bank consisting of resistances between 0.01 and

0.1 ohms and the last of resistance between 100,000 and 1,000,000 ohms. Each bank also includes a zero resistance position.

A fixed capacity condenser is connected in parallel across Rc, while across R in parallel are five banks of variable condensers, one continuously variable with capacitance from 0 to 1000 micro-microfarads, and four with capacitances in decades of 1 to 10 microfarads down to 0.001 to 0.01 microfarads, represented in Fig. 1 by the variable capacitance C.

The two fixed resistances in the bridge are both of 10,000 ohms.

An alternating current at 1600 cps. from an oscillator made by H.W. Sullivan, Ltd. is fed into the circuit through a transformer as show. The point of balance is found when a straight line trace is obtained at maximum amplification on the oscilloscope. The instrument used was a Furzehill Oscilloscope Type 0.120.

The use of this conductivity meter makes possible an extremely accurate determination of the conductivity of electrolytes.

The conductivity cells were standardised against both 0.1 N potassium chloride solution and saturated calcium sulphate at 20°C.

The specific electrical conductivity of a solution may be expressed most conveniently in the form of pC. This is defined as the negative logarithm (common logarithms) of the specific conductivity (in mhos) of the solution. The form is thus analogous to pH as an expression of hydrogen ion activity. (Whittles and Schofield-Palmer 1951), and a pC of 6 corresponds to a specific resistance of one million ohms.

When the pC of soil has been determined in this work the figure quoted refers to the conductivity of a soil suspension in water. The ratio of weight of soil in grams to volume of water in millilitres was either 1:2.5 or 1:4 depending on circumstances, and is specified in the account where necessary.

3. pH Measurements

These were made on a Cambridge Portable pH meter, using a glass electrode and a calomel half cell. The liquid junction was made by a saturated solution of potassium chloride, contact with the solution under investigation being made through a sintered glass plug.

III FIELD EXPERIMENTS

In 1953 the chemistry department of the West of Scotland Agricultural College began a series of long term experiments on the effects in the field of lime and phosphate amendments to the soil. An outline of the project was given by Nicol (1954a). It was decided to conduct these experiments at selected farms distributed throughout the lowland area served by the college, so that most of the conditions representative of the area would be included. Farms with acid soils and low available phosphate were chosen as being likely to show a greater range of response to the additions of lime and phosphate. The selection was made on the basis of routine soil analysis carried out by the usual methods employed in the soils laboratory of the college at Auchincruive.

Some details of the initial conditions at the ten selected farms are shown in Table 1.

The usual approach in experiments involving liming is to attempt to achieve a known soil pH. This is not altogether a satisfactory parameter with which to characterise a soil since it depends very much on the conditions of measurement. As pointed out by Schofield (1949) the pH of a soil suspension

Table I

Centre	Locality	pH	pC	pL	No. of plots
XA	Abington, (Lanarkshire)	5.26	4.30	6.22	30
XB	Gartmore, (Perthshire)	4.71	4.04	5.38	30
XC	Cowie, (Stirlingshire)	5.57	4.35	6.79	30
XD	Methven, (Perthshire)	4.82	4.07	5.57	30
XF	Mauchline, (Ayrshire)	5.28	4.12	6.44	30
XG	Kilmacolm, (Renfrewshire)	5.66	4.01	7.31	36
XJ	Dalbeattie, (Kircudbright-shire)	5.09	4.29	5.89	25
XK	Bearsden, (Dunbartonshire)	5.12	4.22	6.02	25
XL	Lugton, (Ayrshire)	5.24	4.19	6.29	25
XN	Cleland, (Lanarkshire)	5.68	4.09	7.27	25

depends on the salt concentration, whether set up merely as a consequence of shaking the soil sample with water, or produced by the addition of calcium chloride. He showed that the difference of thermodynamic potential expressed by pH - $\frac{1}{2}$ p Ca remains constant over a range of salt concentration up to about N/50.

This function, or better still, pH - $\frac{1}{2}$ p (Ca + Mg), was later called the "lime potential" of the soil, (Schofield and Taylor 1953) and is described in greater detail in a paper by Schofield and Taylor (1955).

The measurement of $\frac{1}{2}$ p (Ca + Mg) for a soil solution is not a simple matter, but the pC of a soil solution may be obtained simply, quickly, and accurately by the method described in an earlier section (page 80).

It was shown by Nicol and Schofield-Palmer (1952) that a combination of pH and pC may be used to give a measure of the lime requirements of soils as measured by the method of Hardy and Lewis (1929).

The equation is

$$2\,pH - pC = pL.$$

For a soil saturated with lime, that is with its lime requirement to pH 7 satisfied, the pL value is 12.45. For any other soil, the amount by which the pL value of a soil

falls short of 12.45 is a measure of the lime requirement. The definitative claim for pL as a "numerical soil characteristic" has been made by Nicol (1954b), and the constancy demonstrated of the pL value of soils over a considerable dilution range, with soil : water ratios extending from 1:2.5 to 1:10.

The application of pL to the problem of the lime requirements of soils has been carried still further by Schofield-Palmer (1956).

In the lime-phosphate project of the West of Scotland Agricultural College a trial has been made in attempting to lime to definite pL levels in contrast to the usual practice of liming to given pH values.

Five levels of pL were aimed at for all centres except XG. These were 7.25, 8.25, 9.25, 10.25, and 11.25, designated A, B, C, D, and E, respectively. For purposes of comparison it may be stated that pH values of 5.5, 6.25, and 7.00 (as determined at Auchincruive by the methods described by Whittles, 1952), correspond approximately to pL values of 9.45, 10.95 and 12.45 respectively. Calcium carbonate was applied as limestone ground to pass an 80 mesh sieve.

Phosphate was applied at these nine centres as superphosphate at the rates of 25, 50, 100, 200, and 400 lbs. P_2O_5 per acre. These treatments are numbered 1 to 5

respectively in the tables in the Appendix.

At each centre experimental plots were set out in the form of Graeco-Latin squares, each plot being one fortieth of an acre. At nine centres the squares were 5 x 5, but at five of these centres there were an extra five plots duplicating certain of the treatments and giving a total of thirty plots (see Table 1, and Tables 1 to 4 and 6 to 9 in the Appendix).

One centre, XG, had thirty six plots set out in the form of a 6 x 6 Latin square. At this centre the treatments were as follows:-

A no phosphate and no gypsum

B 100 lbs./acre P_2O_5, no gypsum

C 200 lbs./acre P_2O_5, no gypsum

D 100 lbs./acre P_2O_5, 3 tons/acre gypsum

E 200 lbs./acre P 0, 3 tons/acre gypsum

F no phosphate, 3 tons/acre gypsum

Centres XG and XK were laid out in 1954, the work on other centres began in 1953.

At the beginning of the experiments in 1953 ground limestone was applied to the plots in quantities calculated to approach the desired levels of pH. A basal dressing of 3 cwts./acre of potato manure was given (6% N, 8% water soluble P_2O_5, 2% insoluble P_2O_5, 12% K_2O). The crop grown was oats.

In 1954 limestone was again applied in quantities calculated to give the required adjustment to the desired pL levels. Phosphate was applied in April. Nitrogen (as 2 cwts. per acre of sulphate of ammonia) and potash (as 1 cwt. per acre sulphate of potash) were applied at all centres to ensure that these would not be limiting factors. In this first year of the main trials the crop sown was swedes.

In 1955 oats were sown, variety "Sun II", at the rate of 5 bushels per acre, the seeds having been dressed with a mercurial dressing. Where necessary lime was applied in March at such rates as required to achieve the desired pL levels, check determinations of pH and pC having been made during the previous months. Phosphate was applied at the same time at the established rate. A basal dressing of 1 cwt. per acre sulphate of ammonia, and 1½ cwt. of sulphate of potash was given.

The results for field experiments quoted in this work refer to 1955 samples.

Sampling

Soil samples were taken from each plot at the same time as the first sampling of the crop was made. This took place in May or June 1955 depending on the centre. Samples were taken at random, using a 6" auger, and a number

of samples from each plot were pooled. The pooled sample was air-dried, passed through a 2mm. sieve and stored in paper bags until analysed.

Samples of plant material were taken at a first sampling in May or June 1955, and later at harvest. Only first sampling results are reported here. Whole plants were gathered by a random technique, and were washed free of soil, cut up into convenient lengths and dried in tin trays in an oven at 80°C. The dried material was milled to a fine powder and stored in glass bottles until analysed.

The author took no part in this sampling, which was carried out under the supervision of Mr. C.C.S. Wilson, who also directed the application of the various fertilisers mentioned above.

Results of field experiments

1. Analysis of effect of lime and phosphate treatments on manganese content of crop

The analysis of the plant material for manganese was begun before the author joined the staff of the West of Scotland Agricultural College, and was carried out under the supervision of Mr. A. McLean. The author took a minor part in the work towards the end of the determinations. The technique was the modified nitric-sulphuric-perchloric acid digestion method of Piper (1944) which has been described on p 74-75.

The air dried soil samples taken in 1955 were examined in 1958 and water soluble manganese, pH and pC were determined by the techniques described in the previous section. The ratio of soil to water employed in these determinations was 1 gm. to 2.5 mls.; the mixture was shaken for 1 hour and the soil separated from the solution by filtration through either Whatman No. 1 or No. 42 filter papers as required to obtain a clear filtrate.

These results, together with manganese analyses of the

plant material, are recorded in Tables I to X inclusive in the Appendix.

The effects of the treatments of the lime-phosphate experimental centres on the manganese content of the crop are analysed in Tables XIa to XIh in the Appendix. Centres XA, XB, XC and XD, which each had thirty plots, have been treated as simple 5 x 5 Graeco-Latin squares, i.e. the extra column of plots has been discarded.

The analyses of variance show that in every case the effect of lime on manganese content of the crop is highly significant, whereas in no single case does the effect of phosphate on manganese content become great enough to be accepted at the 5% level of significance, although at two centres, XD and XJ, the effect is almost enough to meet this criterion. (The ratio of mean squares, F, significant at the 5% level, for degrees of freedom (4, 8) is 3.84, and significant at the 1% level is 7.01) (Table 7, Lindley and Miller's "Statistical Tables" 1953 C.U.P.).

In order to make the investigation of the effect of phosphate fertilisation on manganese uptake more sensitive, an analysis of variance for all the lime-phosphate centres combined is presented in Table 2.

Table 2

Analysis of variance of manganese uptake by plants due to lime and phosphate treatments over eight centres.

Adjustment for mean		3,256,530.642	
Source of variation	d.f.	Sum of squares	Mean square
Centres	7	207,964.948	
Phosphate	4	7,499.729	1,874.932
Lime	4	349,063.615	
Centres x phosphate	28	9,361.692	334.346
Centres x lime	28	54,123.872	
Rows within centres	32	38,204.296	
Columns within centres	32	27,117.556	
Residual error	64	20,772.760	324.574
Total	199	714,108.468	

A comparison of the variation due to centres x phosphate with residual error shows that the effect of phosphate does not differ significantly between centres ($F = 1.04$). The overall phosphate effect on manganese content may now be assessed; the F value is 5.77 which exceeds $F(4,60) = 5.31$ at the 0.1% level.

The more sensitive test reveals clearly that manganese uptake has been significantly increased by phosphate fertilisation. This result is interesting in view of the conflicting reports found in the literature. The effect of phosphate was a small one, and the results for any one centre taken in isolation would not justify the conclusion that manganese uptake was increased by application of phosphate, and would thus appear to support the findings of Burriel and Suarez (1951).

But the overall result supports the findings of the majority of workers (Reuther, Gardner, Smith and Roy 1949; Bingham, Martin and Chastain, 1958; Bingham and Garber 1960). In these cases application of phosphate were in the form of super-phosphate, almost certainly sufficiently heavy enough to lower the soil pH. This lowered pH would results in the release of more manganese to the plant. It has been suggested that the effect of phosphate on manganese uptake is due solely to the lowering of pH by the super-phosphate, especially since Snider (1943) found that manganese content of grasses was increased by manuring with super-phosphate, but not with rock phosphate.

It is possible to use the figures in Tables I to X of the appendix to check this theory. Effect of treatments on

the soil pH have been examined by analysis of variance (Tables XIIa to XIIh in the Appendix). In no case has the application of super-phosphate had a significant effect on soil reaction. But this is of course a parallel situation with the effect on manganese uptake. If all centres are combined a far more sensitive test may be made; this is done in Table 3.

Table 3

Analysis of variance of soil pH due to lime and phosphate treatments over eight centres.

Adjustment for mean		7,224.3806045	
Source of variation	d.f.	Sum of squares	Mean square
Centres	7	3.9521115	
Phosphate	4	0.1722830	0.04307075
Lime	4	51.0321120	
Centres x phosphate	28	0.4697010	0.016779035
Centres x lime	28	1.3006320	
Rows within centres	32	1.4524240	
Columns " "	32	1.0572640	
Residual error	64	1.3035680	0.02036825
Total	199	60.7400955	

F (phosphate) = 2.14

Again the phosphate effect does not differ significantly between centres, so that the effect of phosphate on soil pH may be tested validly.

The F value is 2.14; reference to the tables shows that a value of 2.53 is required for significance at the 5% level, in other words the applications of super-phosphate taken over two hundred plots show no significant pH effects.

It may be, of course, that the manganese uptake by the plants was more sensitive to pH than the means employed to measure the soil pH in the laboratory, but it is rather unlikely that such a marked contrast in effect of super-phosphate on manganese uptake and on soil pH should be found if the effect exerted by super-phosphate were through pH alone.

Since the effect of phosphate fertilisation on uptake of manganese by the plant is significant at the 0.1% level, and the effect on soil pH is not significant even at the 5% level, it seems that there is strong presumptive evidence for a phosphate effect in its own right on manganese uptake, apart from the indirect effect exerted through incidental change of soil reaction.

2. <u>Water soluble and total manganese of soil and their influence on manganese uptake by the plant</u>.

The method used for the determination of total

manganese has been described above. Each individual plot of centres XA, XJ, and XL was examined. Results are given in the Appendix in Tables XIII to XV inclusive.

Co-efficients of variation for these three centres are 4.3, 6.8, and 10.7% respectively. These co-efficients may be compared with the results obtained by McKenzie (1955), in an investigation by spectrographic methods of the variations in concentration of manganese (as well as copper, magnesium, gallium, vanadium and molybdenum) in an Australian soil. He took sixty-eight samples at six inch intervals from an 8 ft. by 3 ft. area, and found that the co-efficient of variation of the true field values for manganese (after eliminating errors due to the spectrographic technique used) was 7.89 per cent.

It was decided that it would be sufficient for the purposes of the investigations in progress in the department to determine the average value of the total manganese contents of the plots at a given centre.

A representative sample of soil was obtained for each centre by taking equal weights of soil from each plot, (random samplings being made to give a small sub-sample of the soil available), and mixing thoroughly. This was done in a large glass jar, in which a sloping wooden baffle was

fixed, by turning slowly in an end-over-end shaker for a minimum of four hours. Trials showed that this gave efficient mixing of a small quantity of potassium dichromate, in a finely powdered crystalline form, throughout a large sample of soil.

Determinations of total manganese contents of these representative soils for each of the remaining centres are reported in Table XVI of the Appendix.

Figs. 2 to 10 show the relationship between water soluble manganese of the soil for each plot and the manganese contents of the oat plants at first sampling, using the values from the tables in the Appendix. No figures for manganese content of the crop were available for centre XF, where there was a crop failure caused by unfavourable weather conditions at the time of germination.

Similar figures have been obtained by plotting the manganese contents of the grain and straw at harvest, but are not reported here (unpublished figures of Plant Nutrition Laboratory, West of Scotland Agricultural College).

It is apparent that the uptake of manganese by the oats is clearly dependent on the level of water soluble manganese in the soil at all centres except XG. Correlation co-efficients and regression equations have been calculated, and are set out in

Fig. 2.

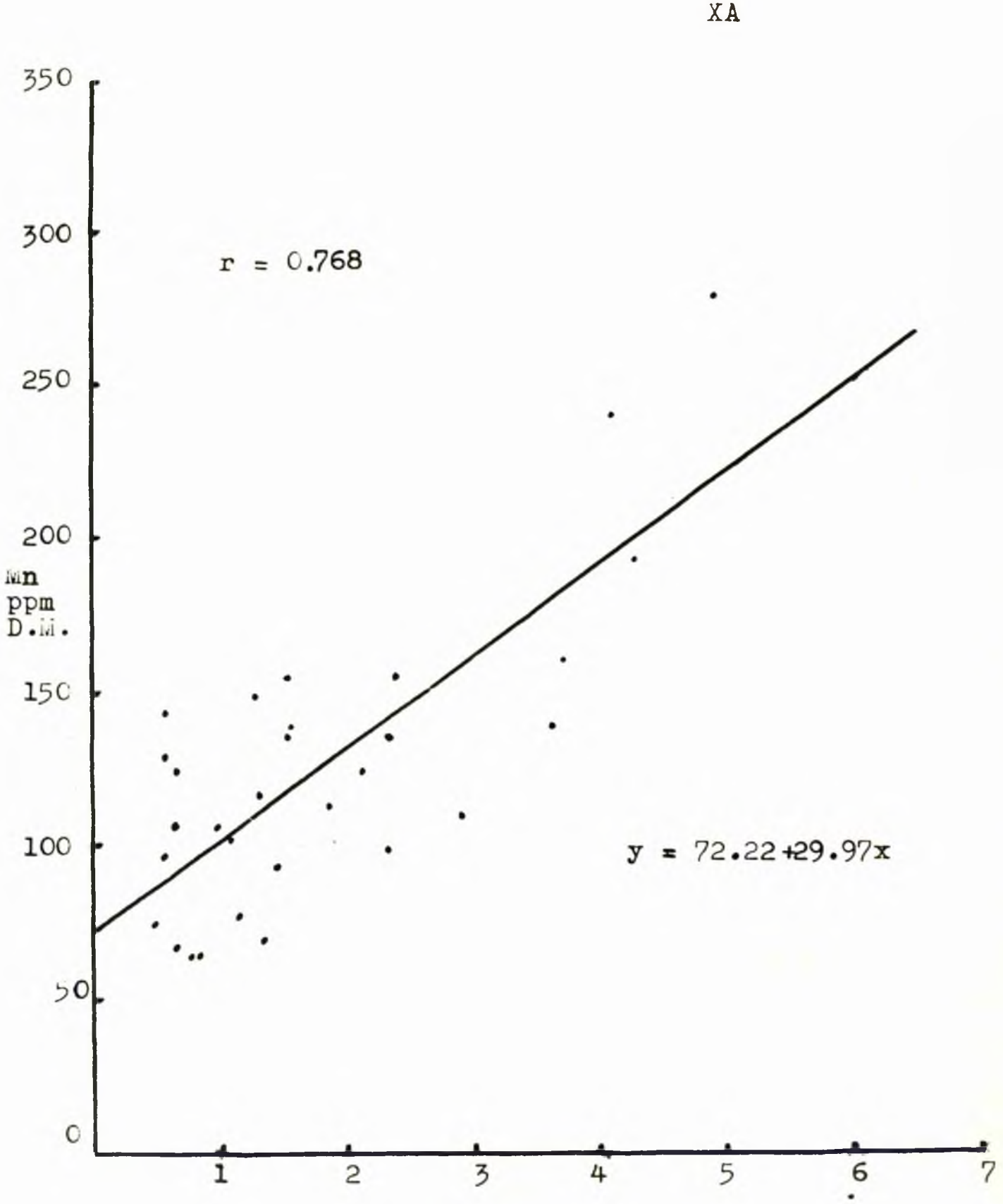

XA
350
300
250
200
150
100
50
0
Mn
ppm
D.M.
r = 0.768
y = 72.22+29.97x
1
2
3
4
5
6
7
W.S. Mn ppm.

Fig. 3.

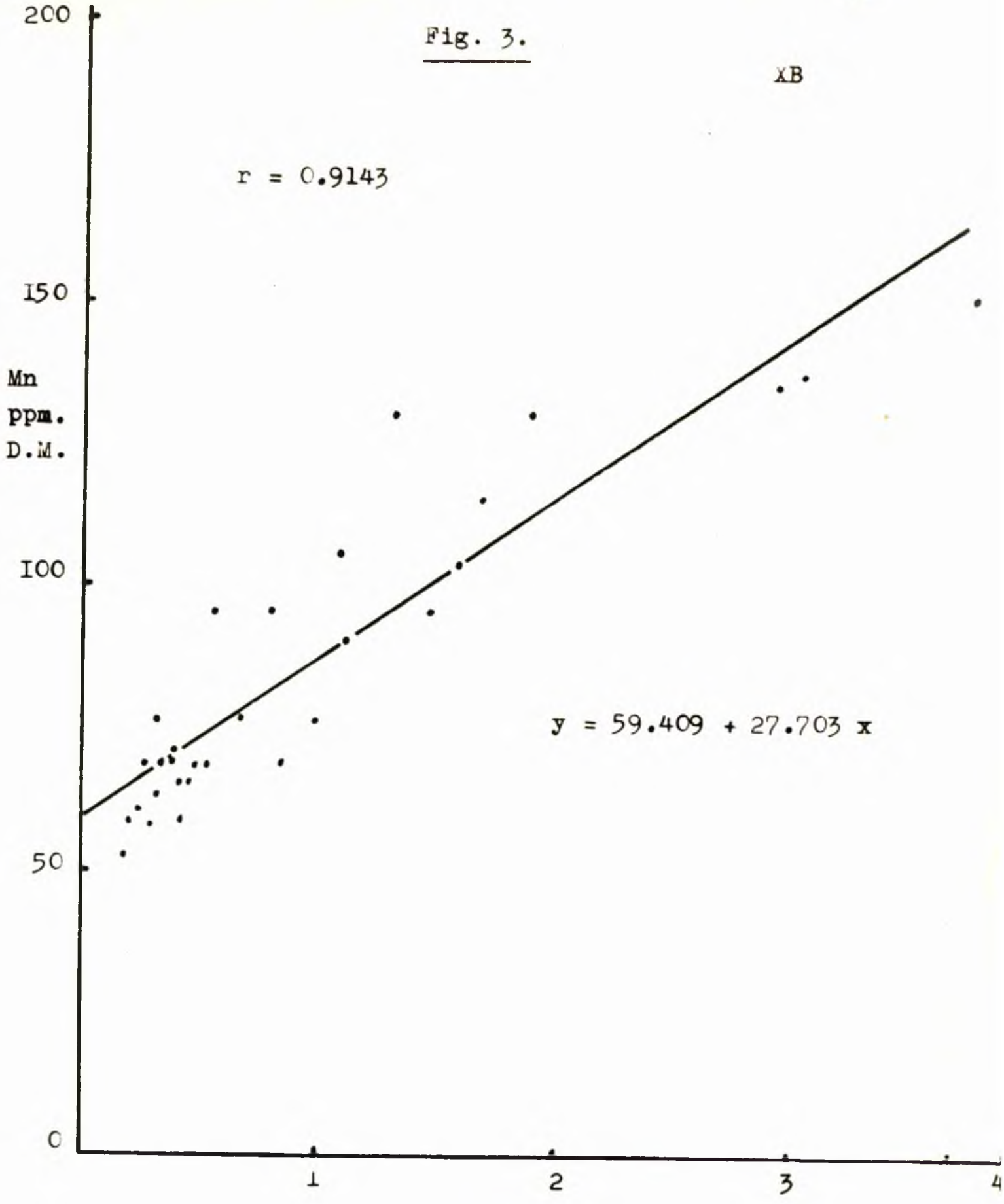

XB
r = 0.9143
y = 59.409 + 27.703 x
200
150
100
50
0
Mn
ppm.
D.M.
1
2
3
4
W.S. Mn ppm.

Fig. 4.

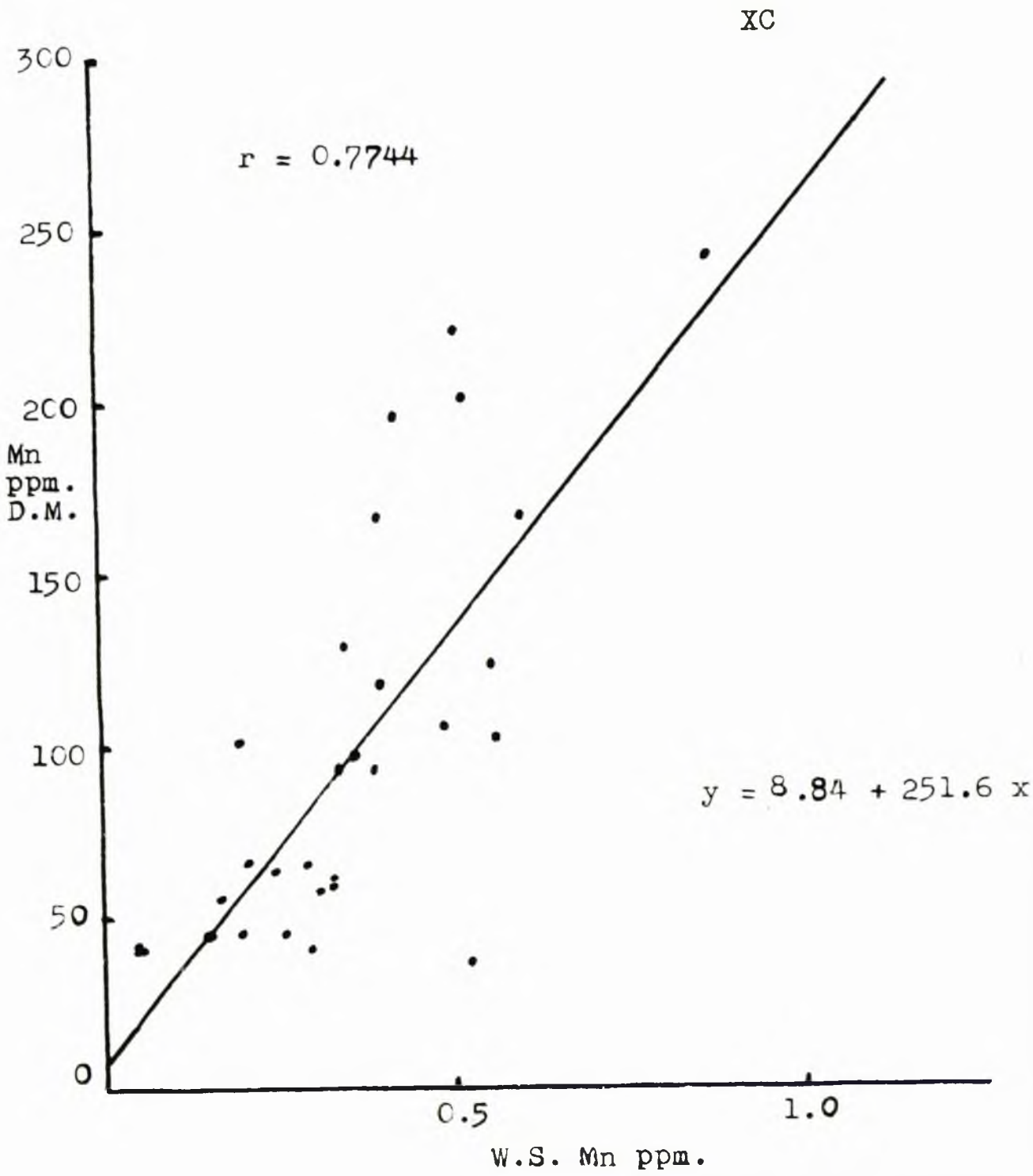

XC
300
250
200
Mn
ppm.
D.M.
150
100
50
0
r = 0.7744
y = 8.84 + 251.6 x
0.5
1.0
W.S. Mn ppm.

Fig. 5.

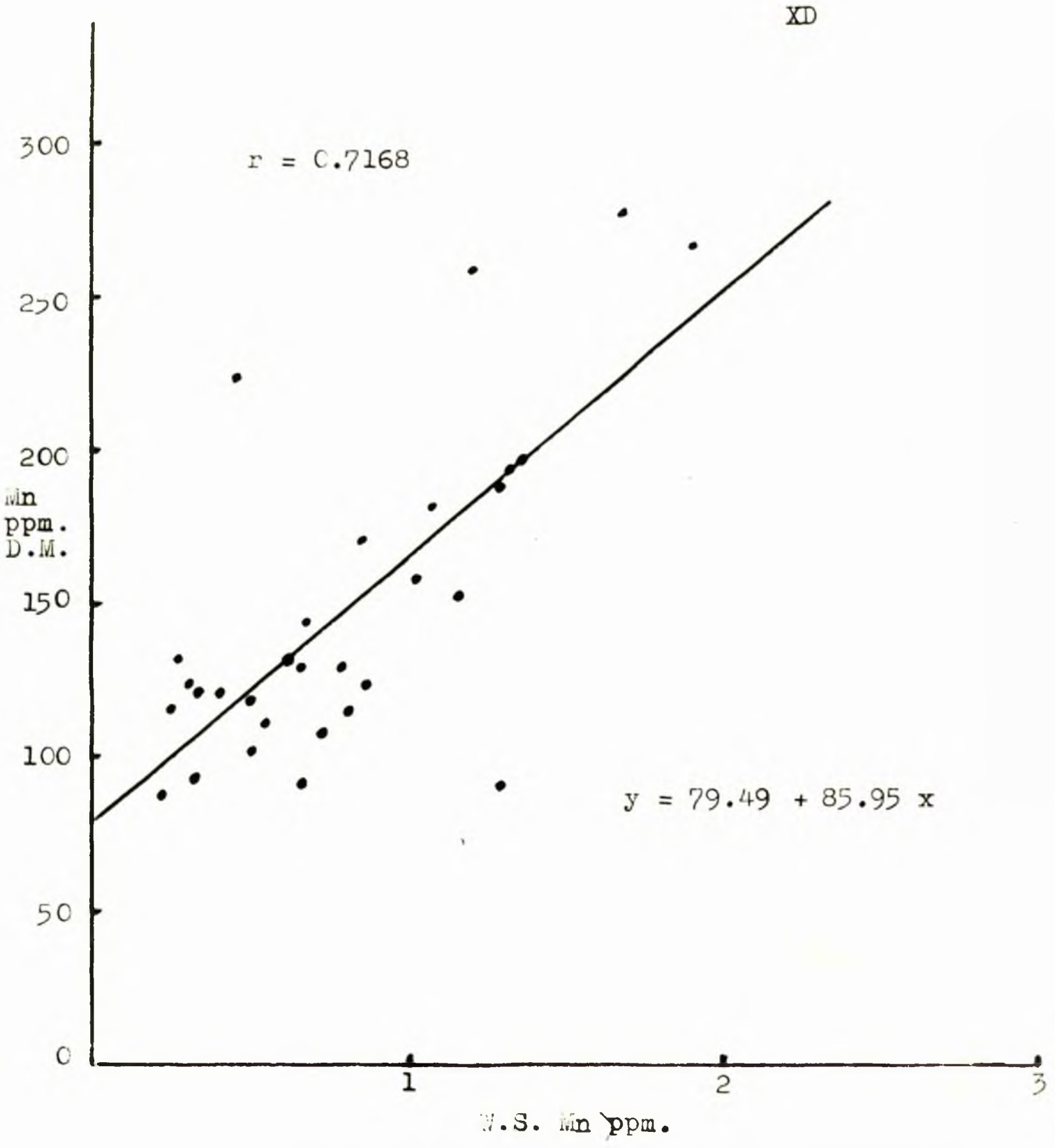

XD
r = 0.7168
y = 79.49 + 85.95 x
300
250
200
150
100
50
0
Mn ppm. D.M.
1
2
3
W.S. Mn ppm.

Fig. 6.

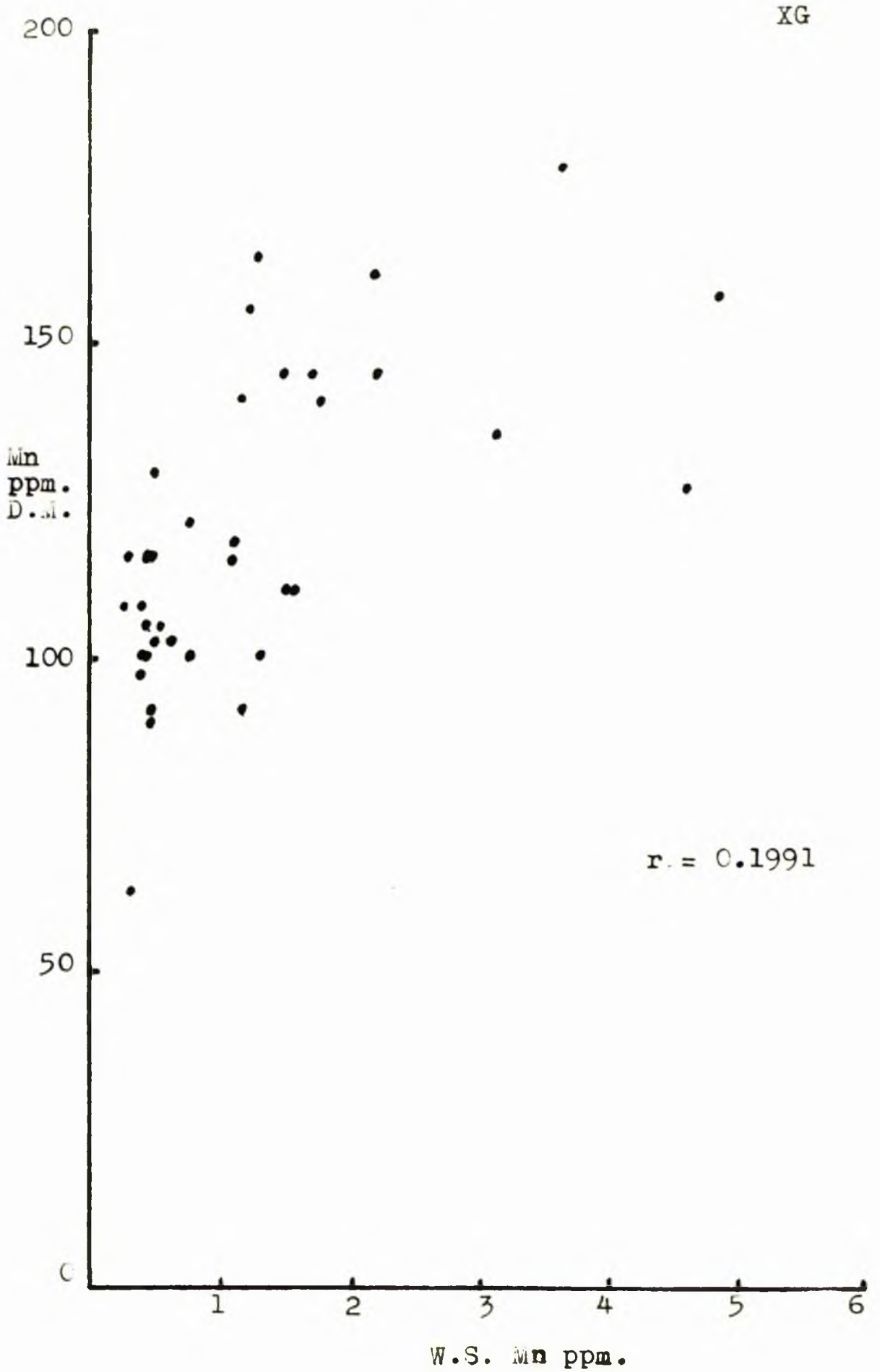
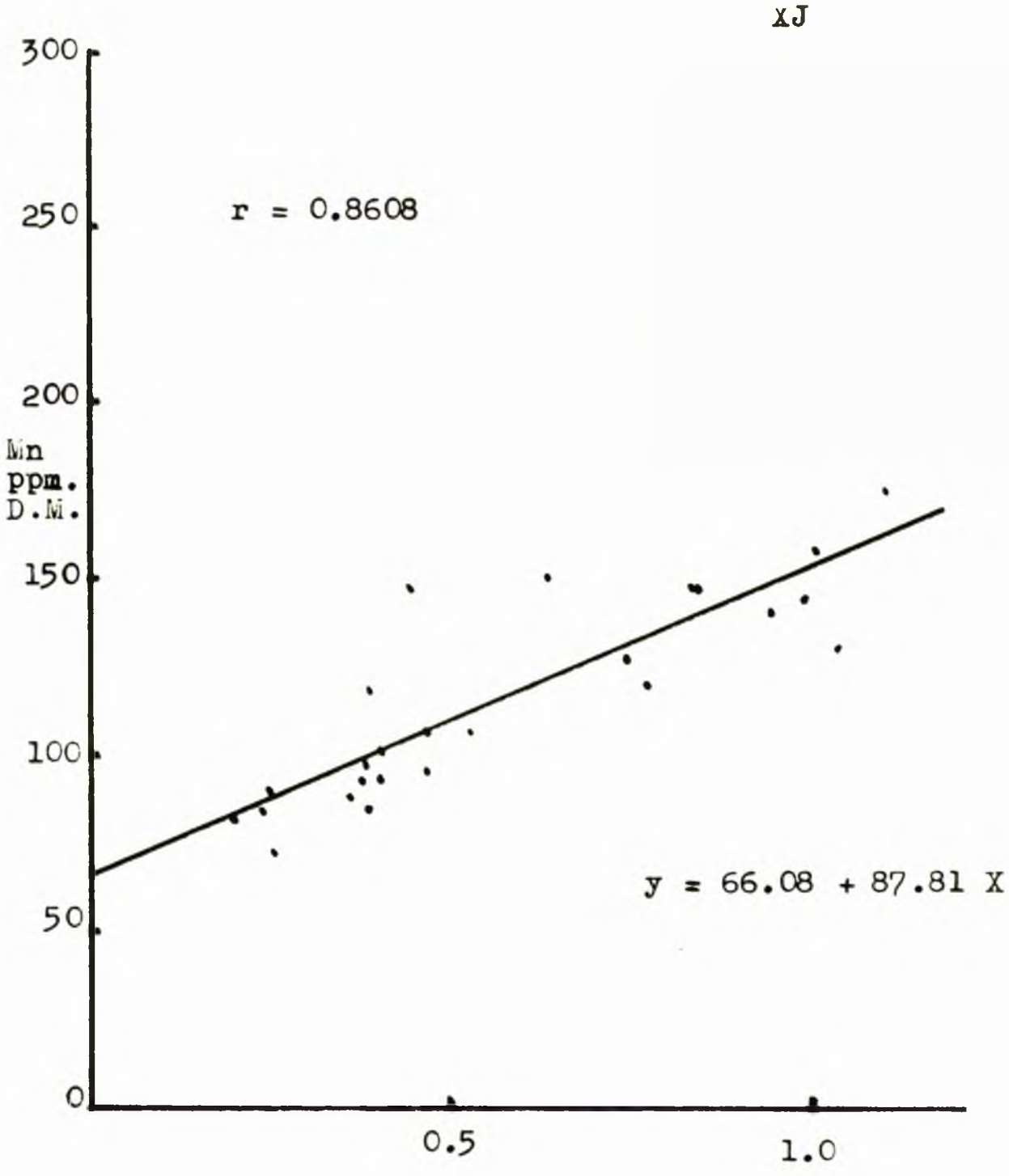


Fig. 7.


XJ
300
250
200
150
100
50
0
Mn
ppm.
D.M.
r = 0.8608
y = 66.08 + 87.81 X
0.5
1.0
N.S. Mn ppm.

XK

Fig. 8.

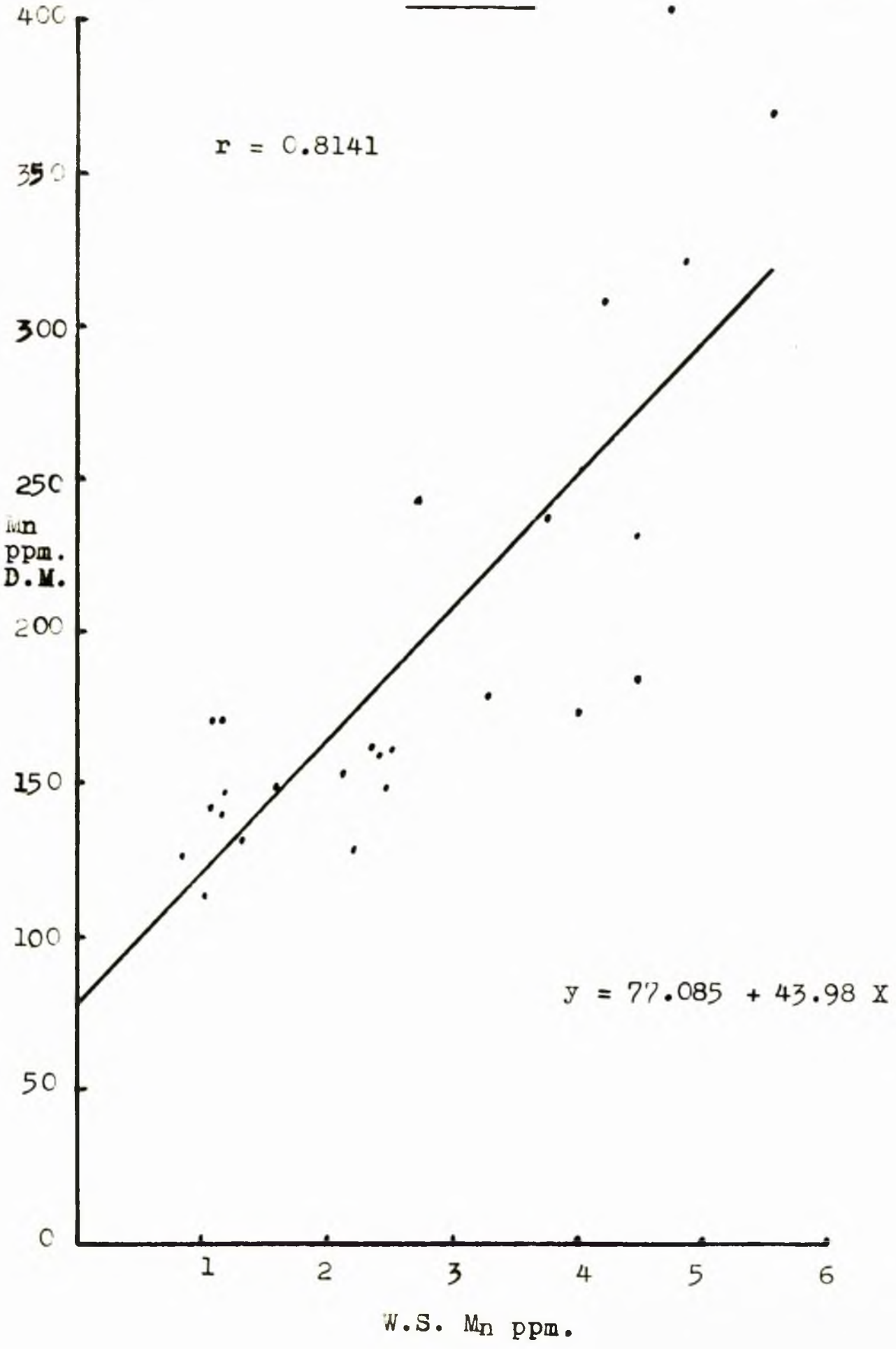

400
350
300
250
200
150
100
50
0
Mn ppm. D.M.
r = 0.8141
y = 77.085 + 43.98 X
1
2
3
4
5
6
W.S. Mn ppm.

Fig. 9.

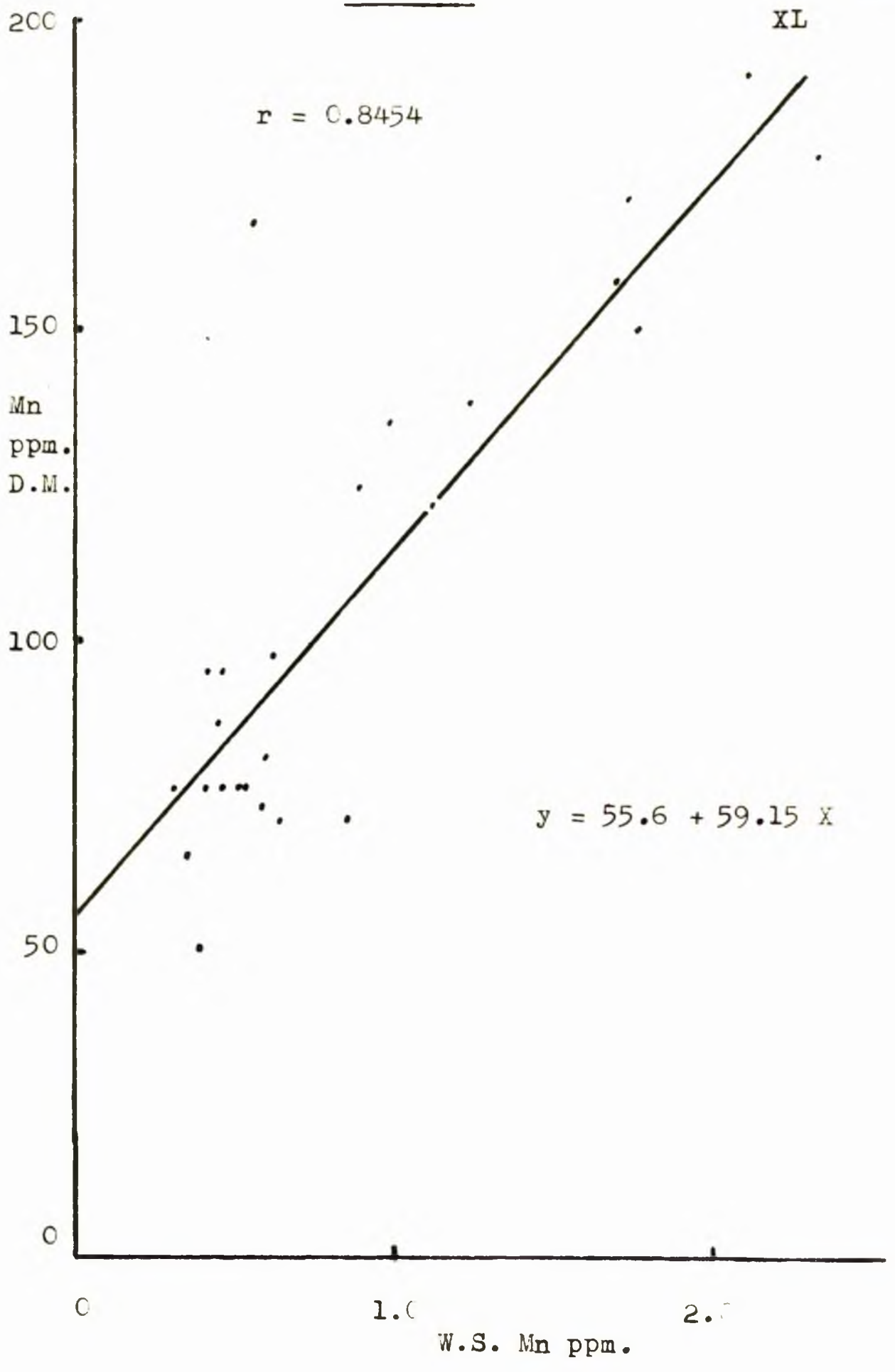


Fig. 10.

XN

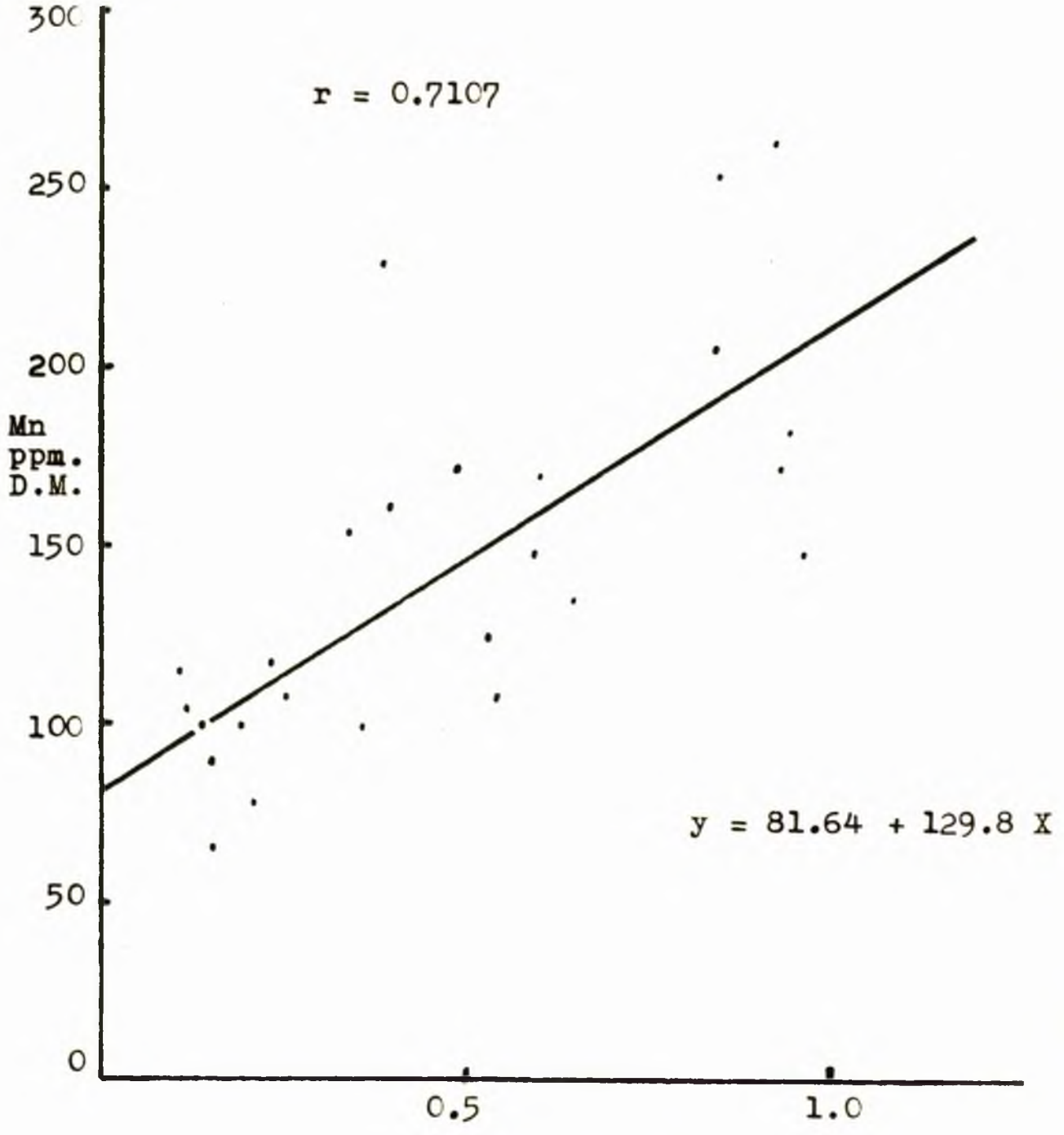

300
250
200
150
100
50
0
r = 0.7107
y = 81.64 + 129.8 X
Mn
ppm.
D.M.
0.5
1.0
.S. Mn ppm.

Table 4. In every case, excepting XG, the value of the correlation co-efficient exceeds that for the 1% level of significance.

Table 4

Centre	Correlation co-efficient	Regression equation
XA	0.7682	y = 72.22 + 29.97 x
XB	0.9143	y = 59.41 + 27.70 x
XC	0.7744	y = 8.84 + 251.60 x
XD	0.7168	y = 79.49 + 85.95 x
XG	0.1991	..... *
XJ	0.8608	y = 66.08 + 87.81 x
XK	0.8141	y = 77.09 + 43.98 x
XL	0.8454	y = 55.60 - 59.15 x
XN	0.7107	y = 81.64 + 129.80 x

where y = manganese content of plant, as p.p.m. dry matter, and x = water soluble manganese, as p.p.m. of soil.

* no regression equation was calculated for XG since the correlation co-efficient was so low.

But if the uptake of manganese by the plant were solely dependent on the level of water soluble manganese, the

intercept value in all the regression equations would be zero, within the limits of experimental error. This is clearly not the case, and it seems unlikely that the explanation could lie in any departure from linearity of the equation at very low levels of water soluble manganese.

The intercept values appear to have some connection with the total manganese content of the soil, as may be seen in Table 5, which sets out the centres in order of total manganese content.

Table 5

Centre	XD	XK	XA	XB	XL	XJ	XC	XN
Total Mn.	817	800	639	630	625	544	350	340
Intercept	79.5	77.1	72.2	59.4	55.6	66.1	8.8	81.6

It cannot of course be expected that the intercept value should be a precise measure, especially when the correlation co-efficient is as low as 0.71 as in the cases of XN and XD; bearing this in mind, one cannot fail to be impressed with the remarkably close connection in rank between the figures, an anomaly being presented only by centre XN.

Discussion

Water soluble manganese has seldom been measured because the extremely small quantities in solution make determination unreliable by techniques which depend on oxidation to permanganate. Attempts have been made to relate the level of water soluble manganese to the onset of deficiency or toxicity symptoms; usually the conclusion has been reached that there is no reliable connection, as in the work of Stenuit, Piet and Boon (1956), who could find no relationship between water soluble manganese of various soils and the occurrence of "grey speck" in oats. On the other hand, Adams and Weir (1957) showed that the development of "crinkle leaf" in cotton (manganese toxicity) was related to the level of water soluble manganese in the soil. Forsee (1954), working with organic soils from the Everglades of Florida found water soluble manganese less reliable as a guide to the amount taken up by the plant than exchangeable manganese, determined with neutral normal ammonium acetate solution. Carlson and Olsen (1950) found that in the growth of sorghum plants in nutrient solutions the manganese content of the plants increased in proportion to the concentration of manganese in the culture solutions.

In the results reported here there is a close correlation,

always significant at the 1% level or better, between water soluble manganese and uptake by the plants growing at a particular centre. If comparisons are made between centres it will be seen in Figs. 2 to 10 that both intercept values and slopes of the lines vary. This means that water soluble manganese alone cannot be used to predict the uptake of manganese by plants growing on different soils, since two soils with the same values of water soluble manganese might have widely differing plant uptakes, exactly as found by Stenuit, Piot and Boon (1956) and others.

Much of the difference between centres can clearly be accounted for by the differences in intercepts, which have been shown to be connected with the total manganese value of the soil. The total manganese is greatly in excess of the water soluble manganese, which appears as will be shown later, to be a fraction of the exchangeable manganese. That fraction of total manganese which is not exchangeable is presumably in the form of various oxides of manganese. These are insoluble in water, and have almost invariably been regarded as being unavailable to the plant. Nevertheless, the evidence here points to the total manganese of the soil being an important factor in the uptake of the plant. These findings are not inconsistent with the investigations of Hoff and Mederski

(1958) who reported that while total manganese of various soils was a very poor guide to uptake by soy beans, there was nevertheless some connection, though this was not significant at the 1% level.

If the total manganese is largely present as higher oxides, insoluble in water, as seems to be established, then the fact that these can be, at least in part available to the plant might be taken to imply that plants can take up nutrients directly from the solid phase. This was the viewpoint of Comber (1922); although he did not refer to manganese, his grounds for suggesting the hypothesis in the case of iron would apply. One of his more telling arguments was that the amount of nutrient in soil solution was so small that impossibly large volumes of liquid would have to be absorbed by the plant. This fails in the face of modern knowledge that uptake of ions by plants is an active metabolic process, not a passive intake of dissolved substances in the water passing through the plant in the transpiration process.

Jenny and Overstreet's (1939) contact exchange theory is a modified form of Comber's theory. They suggested that direct exchange of ions takes place between soil colloids and plant roots. Some of the evidence for this theory has been summarised by Walker (1960).

It is considered that a more likely explanation of the uptake of manganese from the "insoluble" oxides is to be found in the work of Broomfield (1958a and b). He was able to show that roots of oats and vetch (and presumably other plants) exude a substance capable of dissolving oxides of manganese normally present in an insoluble form in soil. He was able (1958b) partially to isolate the substance responsible by washing the roots with 70% ethanol. Oats would therefore be able to take advantage of the stock of manganese represented by at least some fraction of the total manganese present in the soil.

This source of manganese is measured, to some degree of approximation, by the intercept values of the regression equations given in Table 4, and is additional to the source of manganese represented by the level of water soluble manganese found in the soil in the absence of the crop.

The differing slopes of the lines of the regression equations of Table 4 have not yet been explained. It may well be that other ions present in the soil solution interfere with the mechanisms of uptake of the plant roots in such a way that varying proportions of the manganese in solution are taken up by the plants. This problem is investigated in a later section, but no information is available on the ionic composition of the soil solutions of the centres.

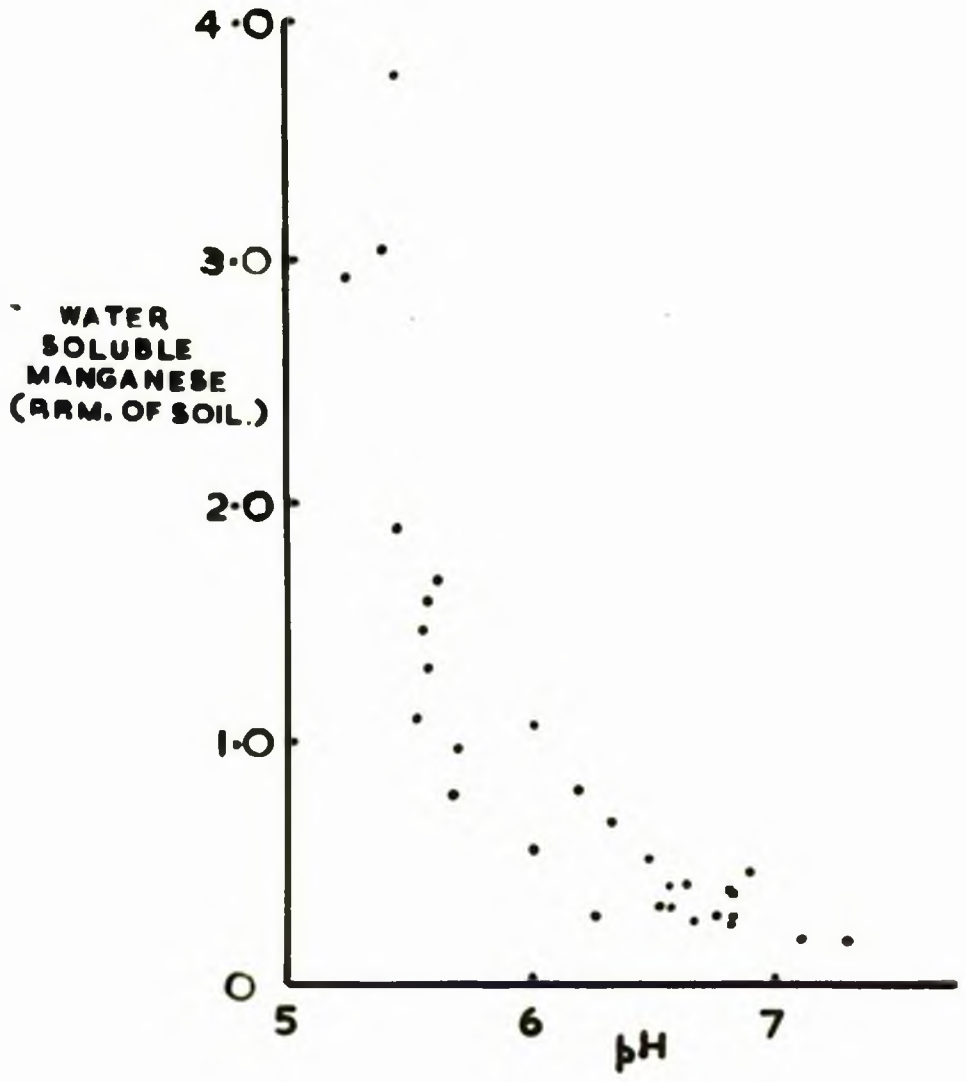


Fig. 11. Water soluble manganese of soil against pH for each plot of centre KB.

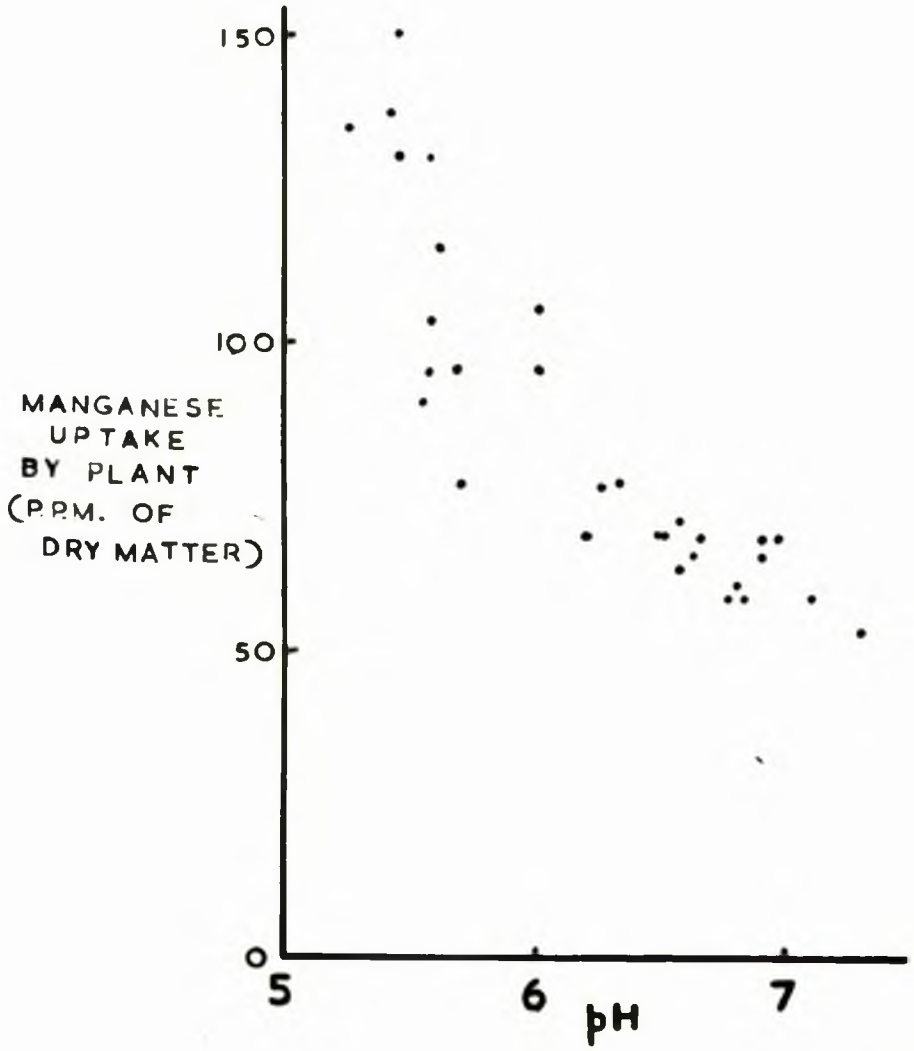


Fig. 12. Manganese uptake of plant against pH for each plot of centre XB.

3. Water soluble manganese in relation to soil pH in the field

It has been shown in Tables XIa to XIh of the Appendix, and is also apparent in Table 2, that the application of lime had a profound effect on manganese uptake by the plant at all centres. Tables XIIa to XIIh and Table 3 show the effect of liming on soil pH. It is usual to assume that the only important results of liming are the consequent changes in soil reaction, and other effects are ignored. It is by no means certain that the improvement in the supply of calcium ions, to take the most obvious aspect of the situation, has no influence on the availability of manganese to the plant, but there is no doubt that the alteration of soil reaction is of paramount importance, and this is the aspect of the experimental results which is examined here.

If the values for pH and water soluble manganese from Tables I to X of the Appendix are plotted figures are obtained similar to that for centre XB shown in Fig. 11. For comparison, Fig. 12 shows the corresponding plot of manganese uptake by the plant against soil pH. It will be apparent that the pH relationship is similar in both cases, as would be expected from the results of the previous section, where the close connection between water soluble manganese and uptake by the plant was demonstrated.

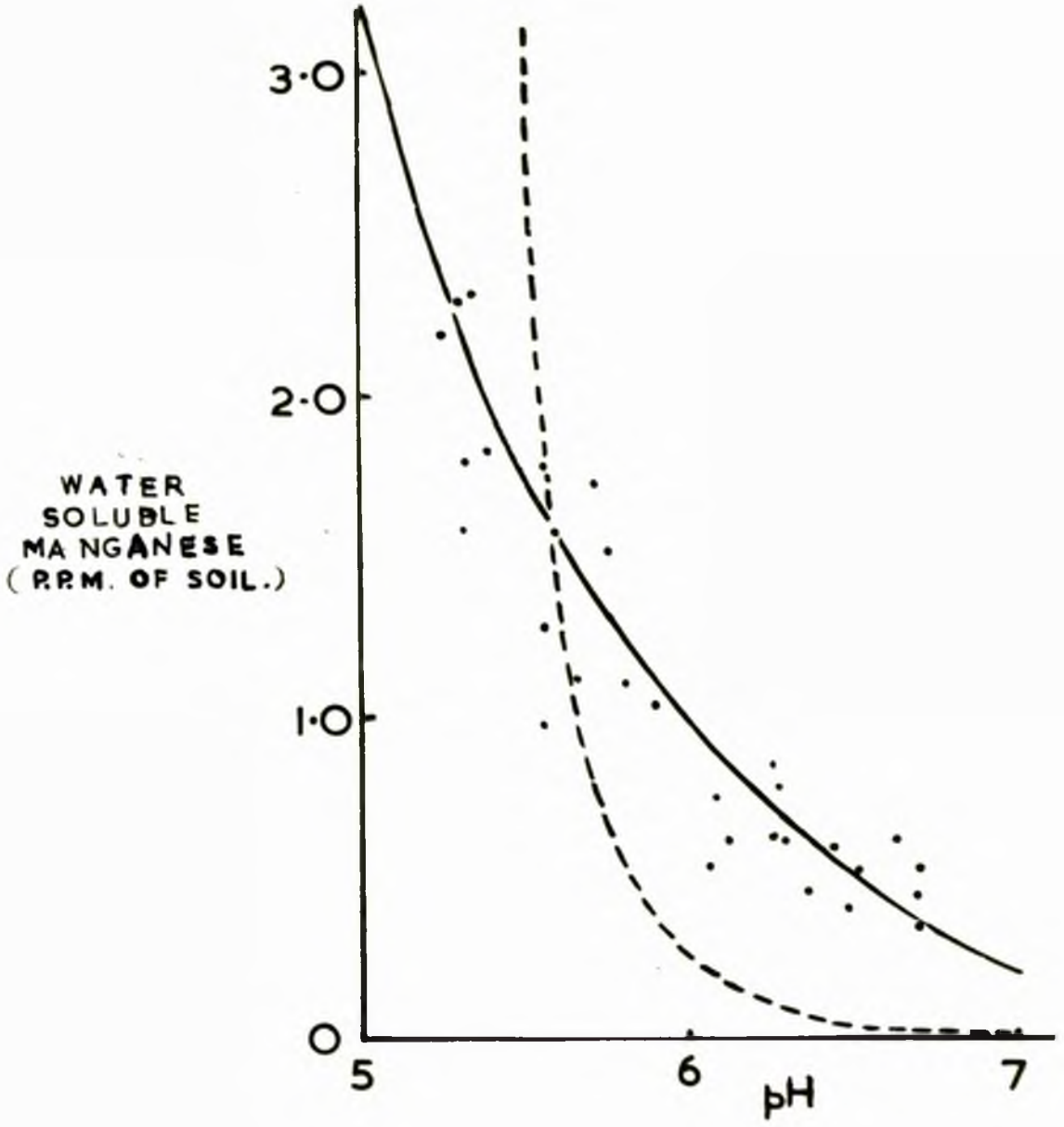


Fig. 13. Water soluble manganese versus pH for each plot at centre XF.
Solid line drawn according to equation
pMn = 0.5 pH - 3
Broken line according to Eriksson's (1952) equation pMn = 2 pH - 11.4

The general shape of the curve is like that obtained by most other workers (Piper 1931; Steenbjerg 1933, 1935; Olsen 1934; Smith 1959). Fig. 13 presents the results for centre XF (Table V in the Appendix) with a solid line superimposed drawn according to the equation

$$-\log Mn = 0.5\ pH - 3$$

- log Mn may be written as p Mn, analogously to pH. It can be seen that this line fits the points quite well, whereas a line, shown broken, drawn according to Eriksson's (1952) predicted formula

$$pMn - 2pH = 0$$

evidently does not.*

In order to reflect more accurately the relationship between water soluble manganese and soil reaction the data contained on Tables I to X of the Appendix were subjected to statistical analysis, the manganese figures first being transformed in the corresponding negative logarithms, and then a regression analysis performed in the usual way. Results are shown in Table 6.

* Actually the equation used to draw the line was

$$pMn = 2pH - 11.4$$

so as to fit into the scales used in Fig. 13. Eriksson could have expressed his formula as

$$p\,Mn = 2pH - \text{constant}.$$

Table 6

Regression equations of pMn on pH for ten field centres.

Centre	Regression equation	r^2	r
XA	pMn = 0.554pH - 3.347	0.8178	0.9043
XB	pMn = 0.571pH - 3.362	0.8475	0.9206
XC	pMn = 0.414pH - 1.941	0.4263	0.6529
XD	pMn = 0.295pH - 1.567	0.3956	0.6290
XF	pMn = 0.488pH - 2.877	0.8668	0.9310
XG	pMn = 1.428pH - 7.961	0.5086	0.7132
XJ	pMn = 0.439pH - 2.417	0.8517	0.9229
XK	pMn = 0.404pH - 2.720	0.9297	0.9642
XL	pMn = 0.353pH - 1.999	0.7233	0.8505
XM	pMn = 0.556pH - 2.913	0.7701	0.8776

(r value for 1% significance level for 23 d.f. = 0.505, 28 d.f. = 0.463, 34 d.f. = 0.423)

Table 7a

Analysis of variance of regressions of pMn on pH for all centres.

	d.f.		Sums of squares	Mean square
Average regression	1		15.220592666	
Differences of "	9		1.672798092	0.1858664544
Separate regressions	10	XA	2.299696556	
		XB	3.435415849	
		XC	1.122652198	
		XD	0.760986746	
		XF	1.617822158	
		XG	2.238014889	
		XJ	0.998681591	
		XK	1.461502179	
		XL	1.189337151	
		XN	1.769281441	
		Total	16.893390758	
Residual error	266		7.481878002	0.0281273609
Total	276		24.575268760	

F = 6.61

F for (9,120) d.f. is 2.56 at 1% level of significance.

It is immediately obvious that centre XG is anomalous; it will be remembered that XG also failed to show the relationship between water soluble manganese and manganese uptake by the crop which was evident at the other centres.

In order to show whether the differences in the regression equations for the separate centres are significant an analysis of variance of regressions was performed for all centres, (Table 7a) and for nine centres omitting XG (Table 7b).

Table 7a shows that the difference between regressions are significantly greater than could reasonably be attributed to sampling and experimental errors. Even if the obviously different centre XG is omitted from the analysis, the difference between centres still remain significant, though now much reduced, as shown in Table 7b.

An examination of Table 6 shows differences in both co-efficients and constants in each equation. One would expect the constant to vary with different soils since the total manganese and the ease of availability of manganese and many other relevant factors vary from soil to soil. The analysis of variance shows that the regression co-efficient also varies from centre to centre.

It is more pertinent to the question of the form in which manganese is held in the soil to investigate the

Table 7b

Analysis of variance of regressions of pHn on pH of nine lime-phosphate centres.

	d.f.	Sums of squares	Mean square
Average regression	1	14.01790889	
Difference of "	8	0.637466979	0.07968337237
Separate regressions	9	14.655375869	
Separate regressions (XA - XF, XJ - XN, figures as in Table 7a)			
Residual error	232	5.31969551	0.02292972202
	241	19.97507138	

F = 3.48

differences between co-efficients. This is possible by applying a t test, and assuming the null hypothesis that the mean value of this co-efficient is 0.5

$$\text{For all centres } t = \frac{0.550 - 0.5}{0.10173} = 0.491$$

t for 9 degrees of freedom is 2.26 at the 5% level

3.25 at the 1% level

If XG is omitted the variance is much reduced; for the remaining centres $t = \frac{0.5 - 0.4526}{0.032284} = 1.468$

t for 8 degrees of freedom is 2.23 at the 5% level

3.17 at the 1% level

In neither case can the possibility be rejected that the mean value of the co-efficient is 0.5, but it is apparent that its value cannot be 2 as predicted by Eriksson (1952); the insertion of the figure 2 in place of 0.5 in the above calculations shows that the difference between the co-efficients found and the value 2 is very highly significant.

Discussion

These results, for the centres where lime and phosphate were applied, fall into place beside the majority of others reported in the literature. The relationship is the same regardless of whether it has been examined between the pH of the soil and uptake by the plant (Piper 1931, Olsen 1934) extractable manganese (Steenbjerg 1933, 1935) or water soluble manganese.

Of these workers, only Steenbjerg (1935) attempted to derive a mathematical expression for his results; this he gave as

$$T_{Mn} = T_{Mn_o} e^{-kpH}$$

where T_{Mn_o} was the manganese level of the extract of the unlimed plot, and pH the change of pH.

This equation may be expressed in the alternative form

$$\log Mn = \log Mn_o - k_1 pH$$

The constant k_1 was found to have values varying between 0.332 and 0.626 for various Danish soils.

Steenbjerg did not attempt to interpret the meaning of this equation, he was content to give simply a mathematical description of his results. Eriksson (1952), on the other hand, when writing a paper which so far as manganese was concerned was purely theoretical, seemed not to be aware of Steenbjerg's work, nor that of the other workers (Piper 1931, Olsen 1934) who obtained similar results without reducing them

to a mathematical form. Eriksson (1952) argued from the almost universally accepted stand-point which took it for granted that non-available manganese in the soil was in the form of higher oxides. If this were so, then it follows that the pH relationship would be governed by the sort of reaction expressed by

$$MnO_2 + 2H^+ + S \longrightarrow Mn^{++} + H_2O + SO$$

where S represents some oxidisable substrate, for example organic matter in the soil.

Eriksson concentrated his attention mainly upon the reaction

$$2\ Mn(OH)_3 + 2H^+ \longrightarrow Mn^{++} + MnO_2 + 4H_2O$$

which is known to occur in soil, and further assumed that the MnO_2 produced by the disnutation reaction remained completely insoluble and unavailable to the plant.

If either of these equations is transformed into the negative logarithmic form one obtains

$$2pH = pMn + constant.$$

But as Table 6 and the t tests on the co-efficients from this table show, this formulation is inconsistent with the results obtained, and is of course also inconsistent with the results of Piper (1931), Olsen (1934), and Steenbjerg (1933 and 1935).

The disnutation reaction cannot therefore be the important

one which governs the effect of pH on the availability of manganese, neither can the reduction of manganese dioxide by soil organic matter.

If the higher oxides of manganese were acted on by hydrogen ions in accordance with the equation

$$MnO_2 + 4H^+ + 2e \rightleftharpoons Mn^{++} + 2H_2O$$

that is if some electron donor were available, such as ferrous ions, the relationship would be

$$4pH = pMn + constant$$

which is almost the relationship found by Heanstock and Low (1953) working with pure clay, low in organic matter, but for which they were unable to provide an explanation. It is clear that their process is even more remote from the behaviour of normal soils under field conditions.

The relationship found, $pMn = 0.5pH - constant$, implies that two manganese ions became available for every hydrogen ion introduced into the system. It follows that the mechanism by which manganese becomes available cannot be one of simple ion exchange; if it were, the same type of equation as that predicted by Eriksson (1952) would apply, since simple ion exchange is governed by Gapon's equation (1933)

$$\frac{[H^+]}{[Mn^{++}]} = k\frac{[H^+]_{ad}}{[Mn^{++}]_{ad}}$$

where $[H^+]$ and $[Mn^{++}]$ are the activities of the hydrogen and manganese ions in solution, and $[H^+]_{ad}$ and $[Mn^{++}]_{ad}$ are hydrogen and manganese ions absorbed on the exchange surfaces. Where changes of concentration are very small so that the amounts held on the exchanger are virtually constant this equation may be put in the form

$$pH - \tfrac{1}{2} pMn = \text{constant}$$

$$\text{or } 2pH = pMn + \text{constant}$$

Evidently both solution of higher oxides and simple ion exchange processes are ruled out by the relationship found in the field. It becomes necessary to consider alternatives, especially the micro-biological oxidation and reduction processes which have been shown to be possible in soil (Mann and Quastel 1946, Starkey 1955, Bromfield 1956) and which might be dependent on pH in quite different ways. This problem is taken up once more in Section IV under pH effects on the level of water soluble manganese found in laboratory experiments.

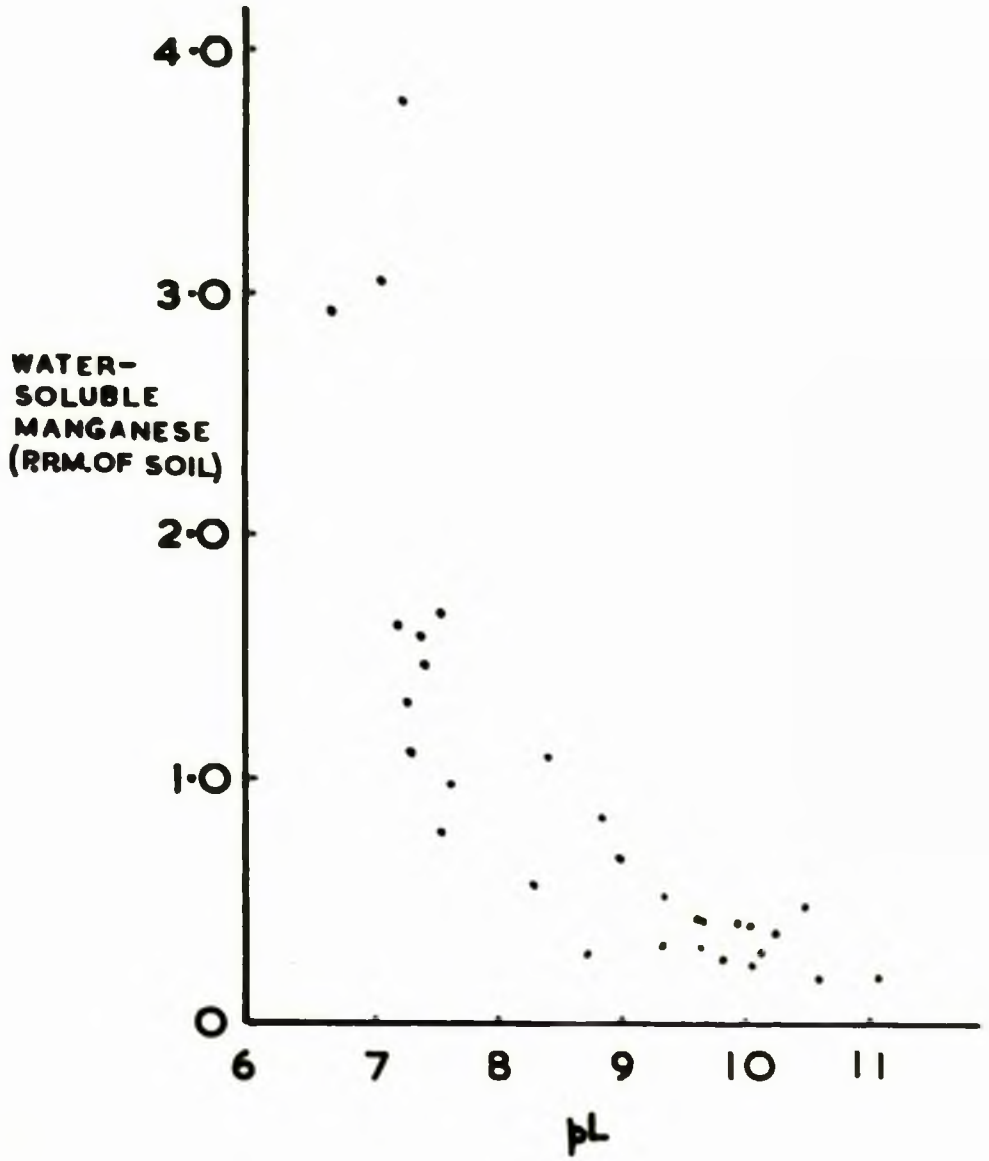


Fig. 14. Water soluble manganese of soil against pL for each plot of centre XB.

4. The effect of pL and pC on water soluble manganese in the field.

The combination of pH and conductivity of a soil solution in the form of pC, placed together in the expression 2pH - pC, and called pL, was claimed to give a characteristic parameter of soils by Nicol (1954). It has been claimed on the basis of records of thousands of measurements of pH and pC and lime requirements that pL provides an accurate means of assessing the lime requirements of soils (Nicol and Schofield-Palmer 1952).

It was therefore thought possible that the pL measurement of a soil might provide a better guide to its level of water soluble manganese than pH alone, especially as pC is a measure in general terms of the activity of the totality of ions in the soil solution, and the higher the total concentration of all ions in the soil solution the more efficient this will be as an extractant of exchangeable manganese.

Fig. 14 shows water soluble manganese of the thirty plots of centre XB plotted against the pL.

A comparison of Fig. 11, where water soluble manganese was plotted against pH for the same centre shows that the graph has the same general form; it is not apparent that the pL has any advantage over pH as a determinant of the level of water soluble

manganese.

In order to make a more thorough investigation of the relationship between pL and water soluble manganese, analyses were performed to obtain the regression of pMn on pL for all centres, using the figures in Tables I to X of the Appendix. The results are given in Table 8.

Table 8

Regression equations of pMn on pL for ten field centres.

Centre	Regression equation	r^2	r
XA	pMn = 0.264pL - 2.227	0.8186	0.9048
XB	pMn = 0.260pL - 2.099	0.8180	0.9045
XC	pMn = 0.193pL - 1.039	0.4198	0.6479
XD	pMn = 0.136pL - 0.983	0.3882	0.6231
XF	pMn = 0.231pL - 1.876	0.8244	0.9079
XG	pMn = 0.174pL - 1.293	0.0169	0.1301
XJ	pMn = 0.196pL - 1.423	0.7990	0.8939
XK	pMn = 0.184pL - 1.832	0.9115	0.9547
XL	pMn = 0.157pL - 1.1901	0.6864	0.8285
XN	pMn = 0.258pL - 1.7429	0.7747	0.8801

Comparison with the values given in Table 6 for regressions of pMn on pH show that pL correlates rather less well with pMn than does pH. Only in centres XA and XN is there any improvement in the correlation co-efficient, and this is too small to

be significant. On the other hand centre XG shows a decidedly poorer correlation; evidently on balance pL is less reliable as a guide to the level of water soluble manganese than is pH alone.

Since it has been suggested (Nicol 1958) that in different circumstances equations similar to 2pH - pC might be found to apply but with different co-efficients it was decided to perform bivariate regression analyses of pMn using both pH and pC.

The bivariate regressions obtained were then compared by means of an analysis of variance with the sample regression equations of pMn on pH to see if any significant improvement had been effected by the inclusion of pC as a second variable. These analyses of variance of the regressions are given in Tables XVIIa to XVIIj in the Appendix.

The F value must exceed 4.21 if the inclusion of pC is to improve the accuracy of the regression equation at the 5% level of significance, or 7.68 at the 1% level of significance.

It will be seen by reference to Tables XVIIa to XVIIj that there is an improvement at the 1% level in centres XB, XF, XG, XJ and XK, and at the 5% level in centre XL, but no significant improvement in the remaining centres.

The actual bivariate regression equations obtained are presented in Table 9.

Table 9

Regression equations for pMn on pH and pC for ten centres

XA	pMn = 0.538 pH - 0.170 pC - 2.636
XB	pMn = 0.727 pH + 0.983 pC - 7.896
XC	pMn = 0.422 pH + 0.070 pC - 2.256
XD	pMn = 0.432 pH + 0.691 pC - 5.024
XF	pMn = 0.555 pH + 1.139 pC - 7.434
XG	pMn = 0.739 pH + 0.629 pC - 6.385
XJ	pMn = 0.533 pH + 0.596 pC - 5.143
XK	pMn = 0.347 pH - 0.989 pC + 1.259
XL	pMn = 0.519 pH + 1.011 pC - 6.685
XN	pMn = 0.644 pH + 0.550 pC - 5.412

This does not mean that pC has no connection with the level of water soluble manganese in the remaining centres; it is more likely that the comparatively large residual error in these cases obscures the small effect accompanying the pC changes.

The pC co-efficient in the regression equations is more variable than the pH coefficient, hence the physical significance underlying the numerical relationship is more difficult to interpret. The data given here are inadequate for drawing any definite conclusions on the relationship between the level of water soluble manganese in the field, and the conductivity, expressed as pC, of the soil suspension.

IV. THE EXTRACTABLE MANGANESE OF THE SOIL

In the previous section it has been shown that in all the centres except XG there is a close correlation between the level of water soluble manganese and the uptake of manganese by oat plants. It has already been mentioned that few investigators have determined water soluble manganese, the great majority having concentrated their attention on exchangeable manganese, which has been found to give a better guide to "sickness" or "health" of soils in connection with the incidence of, for example, "grey speck" in oats (Steenbjerg 1933, 1934 (b)) and "Marsh Spot" in peas (Heintze 1946) than has the total manganese content of the soil.

The extractant most commonly used for the determination of exchangeable cations in soil has been that proposed by Schollenberger in 1927; namely N ammonium acetate adjusted to pH 7. This was recommended by Mitra and Prakash (1957) as compared with N ammonium chloride, N ammonium chloride adjusted to pH 7, N/2 acetic acid and N sodium chloride. It has the great advantage that it can be driven off completely by evaporation of the leachate.

But ammonium ions, and other monovalent ions give lower values for exchangeable cations than do calcium, magnesium, and other divalent ions, and for this reason Steenbjerg (1933)

selected N magnesium nitrate solution for his determinations; he found that calcium nitrate at the same strength gave comparable but slightly lower values. Others, for example Boken (1958), have found calcium nitrate to be a more efficient extractant than magnesium nitrate. Other extractants have been used; Snider (1943) used N/2 ammonium sulphate, and Schachtschabel (1956) used N magnesium sulphate; in general it may be stated that the extracting power of an ion depends on its position in the lyotropic series, which is $Li^{+} < H^{+} < Na^{+} < K^{+} < Mg^{++} < Ca^{++} < Sr^{++} < Ba^{++}$ but the order of a particular ion in the series is subject to some variation when different soils are used, or when the concentration of the extracting solution is varied (Wiklander 1955). These facts about ion exchange in soils have caused Leeper (1947) to emphasise that the term "exchangeable" means nothing unless the replacing ion is defined.

A. Factors involved in the extraction of soil with calcium nitrate.

It was decided to study the extraction of manganese from soil by solutions of calcium nitrate, using essentially the technique of Heintze (1946) but studying the effect of variations of such factors as time of shaking, ratio of volume of extracting solutions to weight of soil, and strength of extracting solution. Before this work was carried out no investigation of these factors had been reported, but since then a paper has appeared by Boken (1958) in which certain of these variables have been studied.

Heintze's method (1946) was chosen rather than that of Steenbjerg (1933) since the latter is more time consuming and was shown by Heintze to possess no advantage. In Steenbjerg's method 100 gm. of air dried soil is placed in a Buchner funnel with a diameter of 9 cms., slightly compressed and leached with N magnesium nitrate. A total of 100 ml. of leachate is collected, in successive portions of 25, 25 and 50 ml. With different soils very different volumes of magnesium nitrate solution are required to give a leachate of 100 mls. As a result of Boken's investigations (1958) she reports the decision in Denmark to abandon Steenbjerg's technique as the official method in favour of Heintze's method.

Materials and Methods.

(a) The standard soil. This was a medium loam, sample number R30647 of which a reasonably large sample was available. It was air-dried, passed through a 2 mm. sieve, and thoroughly mixed.

The water pH was 4.51 and the pC 3.15, both being determined after shaking 30g. of soil with 75 ml. of distilled water for 30 mins., by the standard procedures described previously. The total manganese content was 210 p.p.m.

(b) Calcium nitrate solutions. These were prepared from B.D.H. "Analar" nitric acid and "Analar" calcium carbonate, the latter being in excess, and measured quantities of the acid being used. After the reaction had ceased the mixture was boiled for at least one hour, and then allowed to stand overnight before the excess calcium carbonate was filtered off. The filtrate was made up to volume and the strength of the solution checked by titration with E.D.T.A., by the procedure of Smith and McCallum (1956).

The pH values of the solutions prepared in this way were in the range 6.3 to 7.5, whereas B.D.H. "Analar" calcium nitrate as supplied and dissolved in water gave a pH value of 3.60.

(c) Extraction of soil with calcium nitrate solutions. Weighed quantities of soil were placed in glass bottles with measured volumes of solution and shaken mechanically in a shaker with a reciprocating motion. The time of shaking was one of the

variables studied, in most of the other experiments it was 2 hours. After shaking, the contents of the bottle were filtered through a No. 1 Whatman filter paper; the filtrate was refiltered if necessary using No. 42 papers to achieve clarity.

(d) Determination of manganese in filtrates. At the beginning of the work manganese determinations were made by the periodate oxidation method of Willard and Greathouse (1917) using nitric acid in place of the more usual sulphuric acid (Richards 1930) to avoid precipitation of calcium sulphate. The depth of the permanganate coloration was read on a Spekker absorptiometer, using 4 cm. cells and green No. 5 filters, and compared with a suitable calibration curve.

In some of the investigations, however, when the ratio of volume of extractant to weight of soil was high, and when the extractant was a solution of calcium nitrate of high normality, the colouration of the permanganate was too weak to register with any precision on the Spekker, and concentration of the solution could not be carried sufficiently far without crystallisation.

Attempts to use elution chromatography from cellulose columns by the method of Venturello and Ghe (1957) revealed that considerable modifications would be necessary and an

alternative was sought in a precipitation method.

The manganese was precipitated as MnO.OH by the addition of fresh bromine water to the filtrate and the addition of sufficient N/10 sodium hydroxide solution to bring the solution to pH 10 (as shown by indicator papers). The solution was boiled and allowed to stand overnight; the treatment was then repeated. The precipitate was filtered off using Whatman No. 42 filter papers, and washed with cold distilled water. It was then dissolved off the filter paper with sulphurous acid, made by saturating distilled water with sulphur dioxide from a cylinder.

The solution was boiled to expel sulphur dioxide and the manganese determined as before by the periodate method.

The method was tedious and unpleasant and it was difficult to obtain reproducible results. After quite lengthy trials it was abandoned in favour of the methane base method of Cornfield and Pollard (1950). Their observation that both calcium and nitrate ions interfere to some extent with the development of the measured colour was confirmed, but the effect of the interference was overcome by adding calculated quantities of calcium nitrate solution so that in every case the determinations were made in a final solution which was half molar with respect to calcium nitrate.

The calibration curve remained similar to that obtained in

the absence of calcium nitrate, but colours were proportionally less intense. In other respects the procedure was as given on page 75 under "(c) Determination of very small (microgram) quantities of manganese."

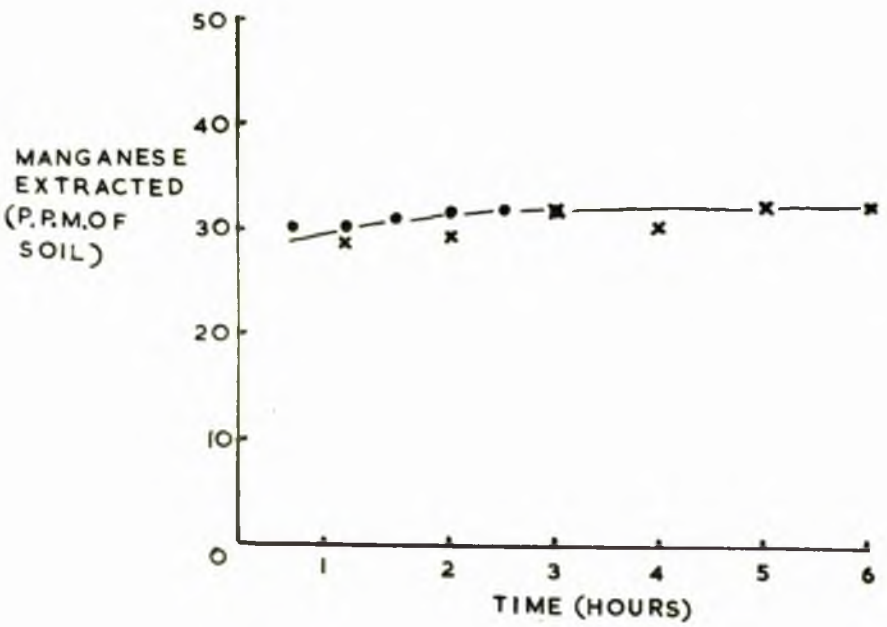


Fig. 15. Effect of time of extraction on manganese extracted from soil H30647 by 2N calcium nitrate solution.

• Experiment 1.

× Experiment 2.

Results

(a) Experiments with soil R 30647

These results are shown in the Appendix, Tables XIX to XXIII.

1. Effect of variation in time of extraction

Table XIX gives the results obtained in the investigation into the effect of time of extraction. These are shown graphically in Fig. 15. The extractant used was 2N calcium nitrate solution. This shows a slight increase in the amount of manganese brought into solution in the period of six hours - something of the order of 7 per cent more as compared with the amount in solution after half an hour. This is less than the increases found by Boken (1958) in any of the soils she examined. The eight soils examined by her showed increases ranging from 29 to 76 per cent in the same time. Her investigations were continued over a twenty four hour period, and by the end of this time even greater increases were apparent. The causes of this increase of extractability of manganese are unknown. Boken was unable to suggest an explanation. It is clear from her results that the increase is not connected with the pH of the soils, nor with their carbon content. Boken concludes that equilibrium is not reached even after twenty four hours, though it is apparent from her Fig. 1 that it is being approached.

In experiments on the extraction of phosphate from soil by acetic acid solutions Schofield-Palmer and McGregor (1955) found a similar increase with time of shaking, but here the maximum was reached after about one and a half hours, and subsequently there was a re-absorption of phosphate by the soil. The time taken to reach the maximum varied with strength of extractant.

It is evident that in any extraction experiments it is necessary to specify the length of time over which extraction has been made. In these experiments the standard time of extraction was fixed at two hours.

In passing, it may be noted that the results shown in this table (Table XIX, Appendix) illustrate clearly the ability of a soil to impress a characteristic pH value on a solution in contact with it. Although the extracting solution had an initial pH of 3.60, all the extracts had pH values in the range 3.97 to 4.00. The same soil shaken with 2N calcium nitrate solution with varying soil to solution ratios showed the same effect up to a soil to solution ratio of 1:20 (see Appendix Table XXI). In this case the initial pH of the solution was either 6.96 or 7.02, the buffering effect of the soil was such as to give a final pH for the extract in the range 3.95 to 4.16. Even when the soil to solution ratio was as extreme as 5gms. of soil shaken with 800 ml. of extracting

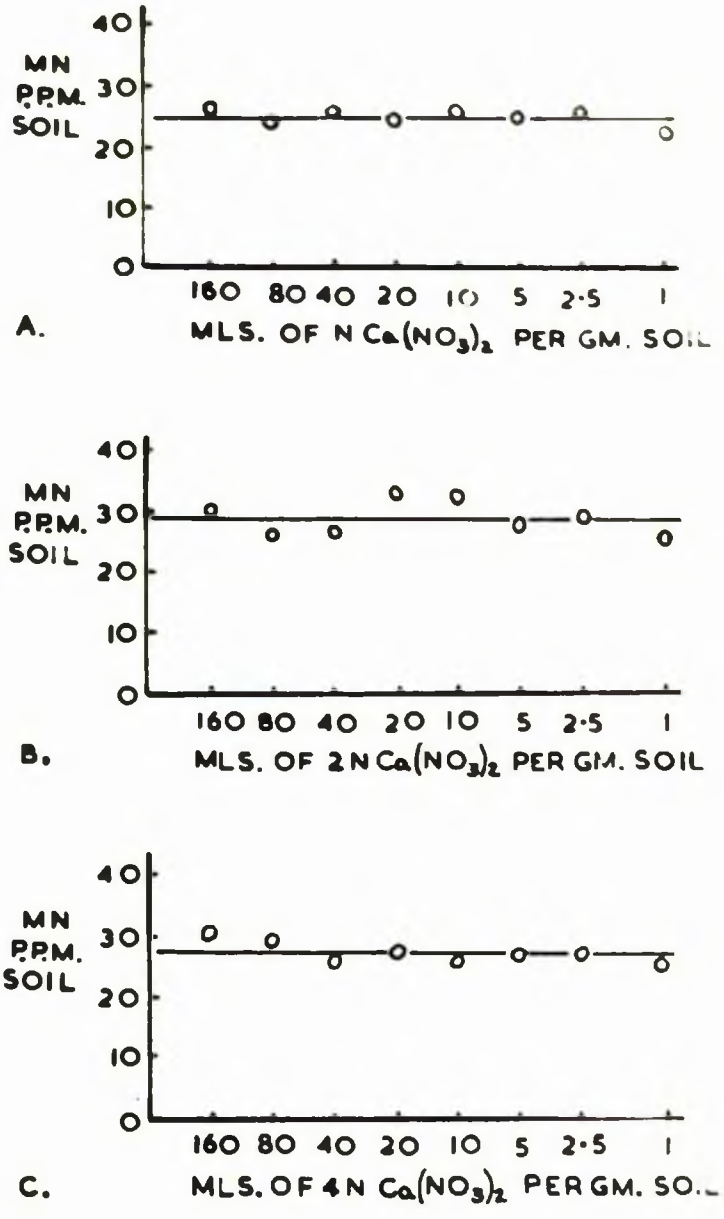


Fig. 16. Effect of variation of volume ratio on extraction of manganese from soil R30647.

solution the soil was able to change an extractant pH of 7.02 into an extract pH of 5.20.

2. Effect of variation of volume ratio.

Tables XX, XXI, and XXII, (in the Appendix) show the effect of shaking the soil for two hours with N, 2N, and 4N solutions of calcium nitrate with wide variation in the ratio of weight of soil to volume of extractant. Boken (1958) investigated the effects of volume ratio between the limits of 1:4 to 1:40, (soil: extractant); here the limits are 1:1 to 1:160.

Figure 16 (a) shows that the manganese extracted from the soil by N calcium nitrate was found to be approximately 25 p.p.m. whatever ratio of soil to extractant was used. With 2N calcium nitrate solutions (Fig. 16 (b)) the values are rather more scattered, but the general indication of the points is that the level of extractable manganese is about 29 p.p.m. whilst with 4N calcium nitrate the level is about 28 p.p.m.

It seems unlikely that there is any significance in the difference between the estimates of extractable manganese obtained by using 2N and 4N solutions, as these are within the limits of the probable experimental error, indeed it will be seen by reference to Table XXIII of the Appendix that determinations with the same soil on a different occasion gave values of 25.6 and 27.7 p.p.m. for the 2N and 4N solutions respectively.

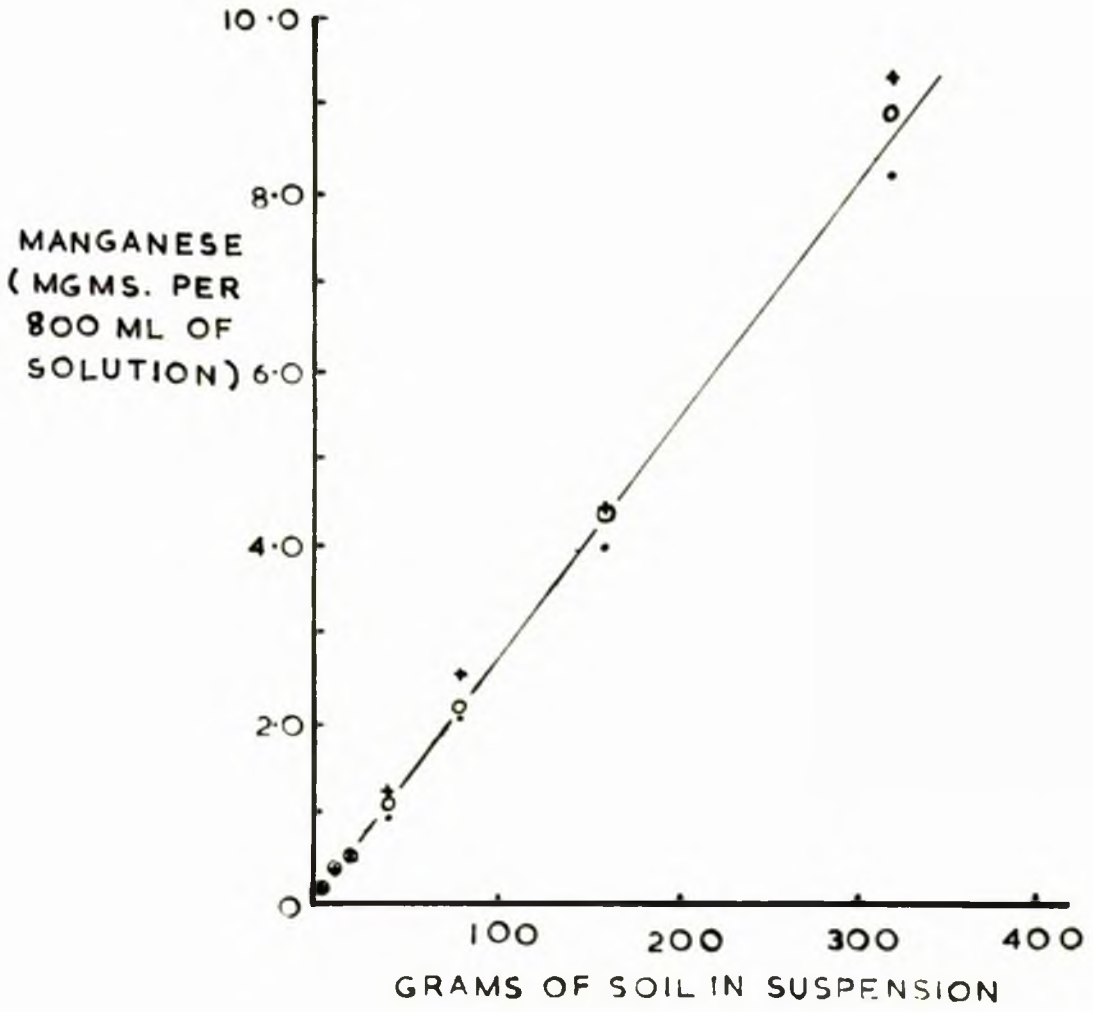


Fig. 17. Effect of volume ratio on manganese extracted from soil R 30647.
Manganese extracted by 800 mls. of solution against weight of soil in suspension.

- o extraction with 4 N calcium nitrate solution
- + extraction with 2 N calcium nitrate solution
- • extraction with N calcium nitrate solution

The amount of manganese extracted by N calcium nitrate is certainly rather lower at 25 p.p.m., and this is borne out by the value of 24.2 in Table XXIII. The question of the relationship between concentration of extractant and amount of extractable manganese will be discussed later in connection with the results in Table XXIII.

If the figures in Tables XX, XXI, and XXII of the Appendix are used to calculate the total amount of manganese in solution in a given volume of extract, and this value is plotted against the weight of soil suspended in the same volume of extract, as in Fig. 17, a straight line is obtained which passes through the origin of the graph. This shows that the concentration of manganese in solution is proportional to the amount of soil in suspension, exactly as would be expected if simple exchange occurs between soil manganese and calcium ions from the solution, with the latter in overwhelming excess. The behaviour of the soil may be represented by the equation:-

$$\boxed{\text{Soil}}\!\!-\text{Mn} + \text{Ca}^{++} \rightleftharpoons \boxed{\text{Soil}}\!\!-\text{Ca} + \text{Mn}^{++}$$

When excess calcium ion is present $[Mn^{++}] \propto [Soil]$ giving precisely the kind of relationship shown in Fig. 17. The amount of extractable manganese found, expressed as p.p.m. of soil, remains constant within the limits of precision of measurement.

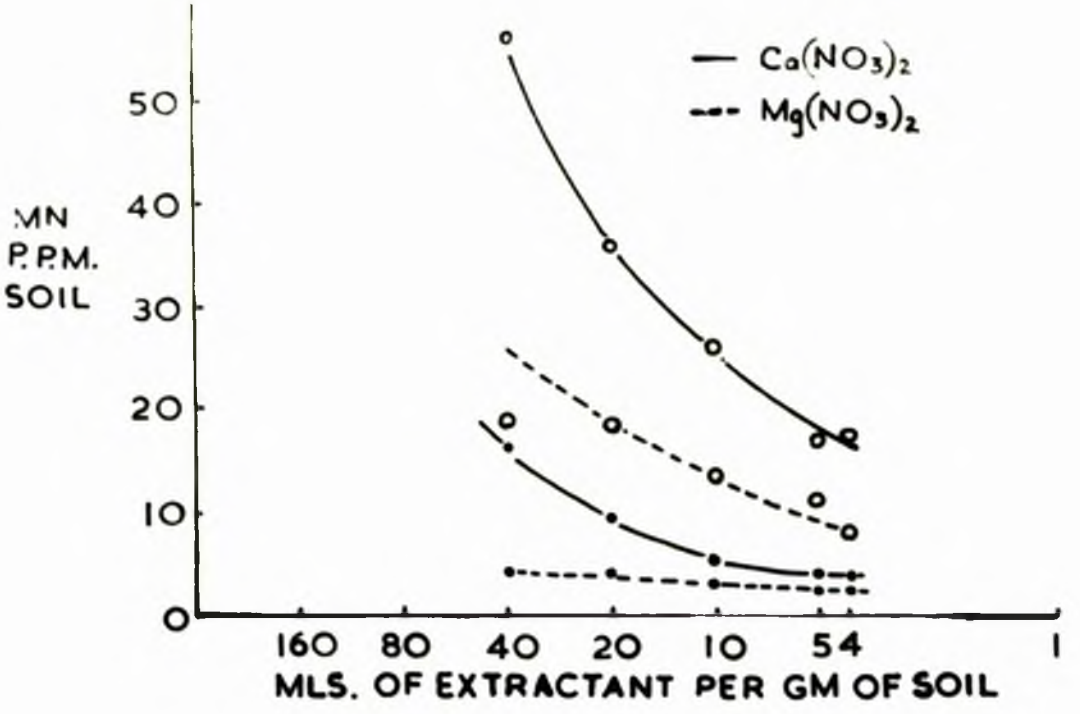


Fig. 18. Effect of variation of volume ratio on extraction of manganese for two soils reported by Boken (1958)

o Soil No. 16

• Soil No. 4

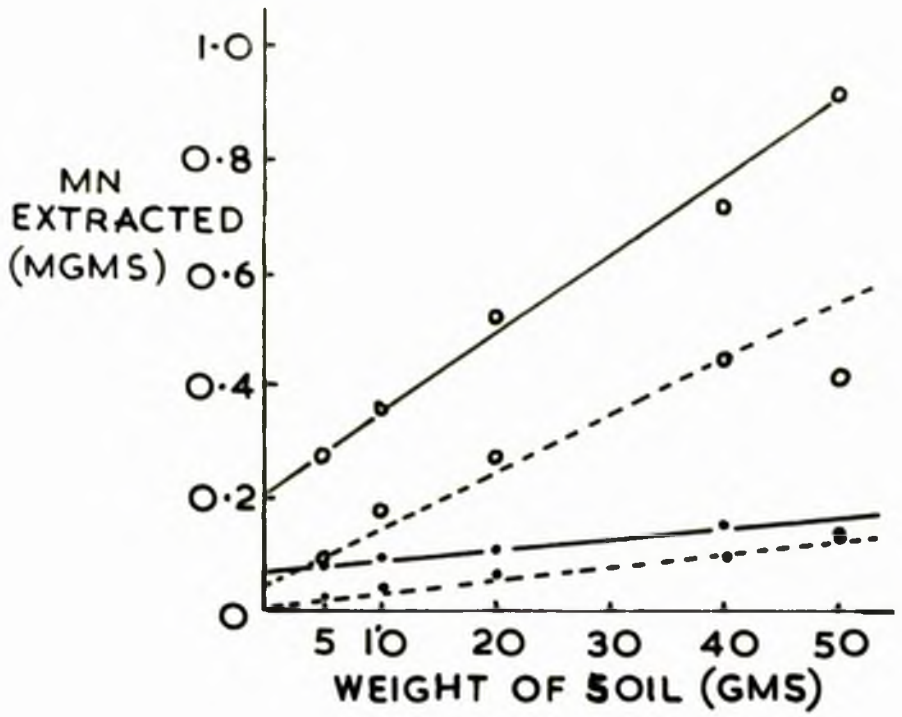


Fig. 19. Volume ratio effect : Manganese extracted from Boken's soils plotted against weight of soil extracted.

o Soil No. 16.

• Soil No. 4.

3. Additional experiments on the effect of variation of volume ratio, using other soils.

When Boken's paper reporting similar work was published (March 1958) a striking contrast was immediately apparent. She found a marked increase in the level of extractable manganese as the ratio of volume of extractant to weight of soil was increased; this happened whether calcium nitrate or magnesium nitrate was used as extractant, but the effect was more marked with calcium nitrate.

Results reported for two soils by Boken are illustrated graphically in Fig. 18, using the same scales and semi-logarithmic plotting as in Fig. 16 to facilitate comparison. Had Boken's investigation been extended over the same range of volume ratios the contrast presumably would have been even more striking. Fig. 19 displays Boken's results for those two soils in the same form as Fig. 17. The majority of Boken's results obtained with magnesium nitrate as the extractant were like those for soil No. 4 in that a plot of this form gave a zero intercept, but all results obtained with calcium nitrate as the extractant were similar to those shown in Fig. 17, with a positive intercept of considerable magnitude.

This would seem to indicate that relatively large amounts of manganese could be obtained as the weight of soil extracted tended towards zero; the most likely explanation would appear

to be that the extracting solutions themselves contained manganese, and that the intercept value represented this contaminating manganese.

This explanation was suggested to Boken by letter, but was thought by her to be unlikely. Her comment on this point ahouldbe quoted in full:-

"..... concerning the manganese content of my soil (No. 14) I am giving you the full particulars of the analytical data (October 1958) in the following:-

g soil	mg Mn in 200 ml. $Mg(NO_3)_2$	p.p.m. Mn of soil	mg Mn in 200 ml. $Mg(NO_3)_2$ + 0.015 mg Mn in $MnSO_4H_2O$
5	0.092	18.4	0.092
10	0.136	13.6	
20	0.208	10.4	0.208
40	0.400	10.0	
50	0.480	9.6	0.480

If one plots those figures (as you did) the result will be seen from the included graph. If we connect the points with a straight line, 200 ml. $\frac{M}{}$ $Mg(NO_3)_2$ might contain 0.047 mg. Mn, which it probably does not, as the content of the 200 ml. $\frac{M}{}$ $Mg(NO_3)_2$ solution used this time was 0.015 mg. Mn according to my analysis.

Further I did what I have done before, that is I added

0.015 mg. Mn in a solution of $MnSO_4H_2O$ to the extracting solution, and as usual it was "absorbed" to the soil - and could not be measured as shown in the last column." (Boken, private communication, October, 1958).

The results illustrated in Figure 17 were obtained by the methane base method; Boken's method for measuring manganese depended on the formation of permanganate by oxidation with ammonium persulphate, and subsequent colorimetric determination. To compare the methane base method, the periodate oxidation method, and Boken's method, a sample soil, No. 70696 was examined by the first two methods using the pure half molar calcium nitrate solutions prepared as already described from calcium carbonate and nitric acid, and was sent to Denmark for analysis by Boken, who used the persulphate oxidation method.

The results are set out in Table 10, the last column being the results obtained by Boken. (Private communication, 1960).

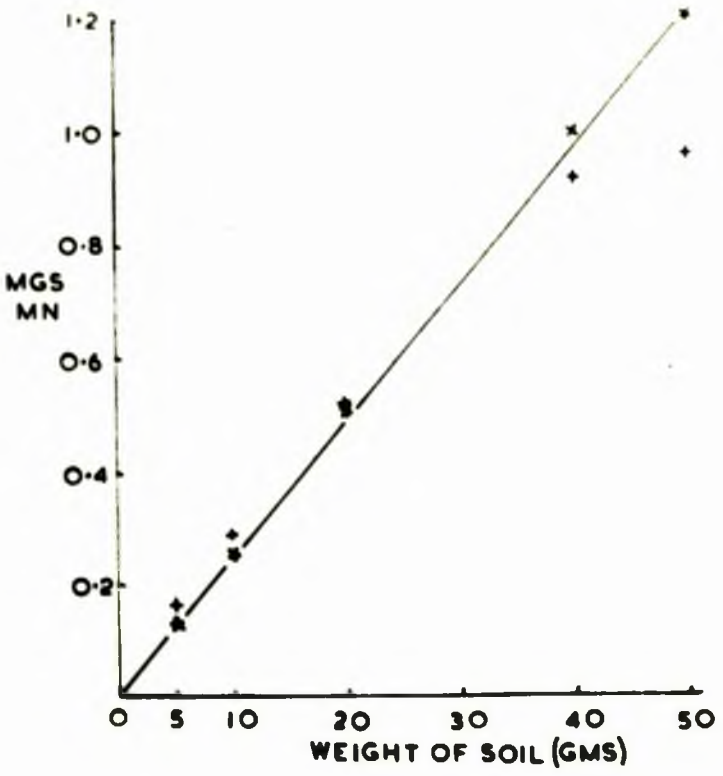


Fig. 20. Effect of volume ratio on extraction of manganese for soil No. 70696.

Manganese extracted by 200 ml of extractant measured by :-

× Methane base method by author.

• Periodate method by author.

\+ Persulphate method by E.Boken.

Table 10

Extractant used	$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Mg(NO_3)_2$
	Mn found as p.p.m. of soil No. 70696		
g soil	Methane Base method	Periodate method	Persulphate method
5	25.9	26.8	32.0
10	22.9	25.0	28.8
20	25.3	25.0	26.0
40	25.0	25.0	23.0
50	24.1	24.0	19.2

The apparent effect of variation of volume ratio of extractant to soil is once again very evident in Boken's results if these are expressed as p.p.m. of soil, but if these same results are plotted on a graph as manganese extracted by 200 ml. of extractant against weight of soil as in Figure 20, it becomes evident that these apparently large differences could be caused by quite a trivial experimental error. A line drawn through the experimental points has a zero intercept, and as a test of the hypothesis that the discrepancy between the constancy of the level of extractable manganese found in this work, and the variability with changing volume ratio found by Boken, is due to contamination of the extracting solution, this experiment is inconclusive.

The effect of using contaminated calcium nitrate, and the effect of adding manganese were investigated in a set of determinations made with another medium loam, soil No. 801560. The calcium nitrate used was analytical reagent grade supplied by May and Baker, and known to be heavily contaminated with manganese. This was used without further purification; a molar solution was made up and the strength adjusted after checking the calcium content by E.D.T.A. titration. No attempt was made to adjust the pH of the solution. Weights of soil as shown were each shaken for two hours with 200 ml. of extractant and manganese was determined on 100 ml. of the clear filtrate using the periodate method of determination. An exactly similar determination was performed on 100 ml. of the extracting solution as a blank. Results are recorded in Table 11. Certain of the determinations were repeated after the addition of 0.49 ml. M/100 manganese sulphate solution to the soil and extracting solution immediately before shaking. This was equivalent to the addition of 0.270 mg. of manganese. These results are also recorded in Table 11. Manganese determinations were by the periodate method.

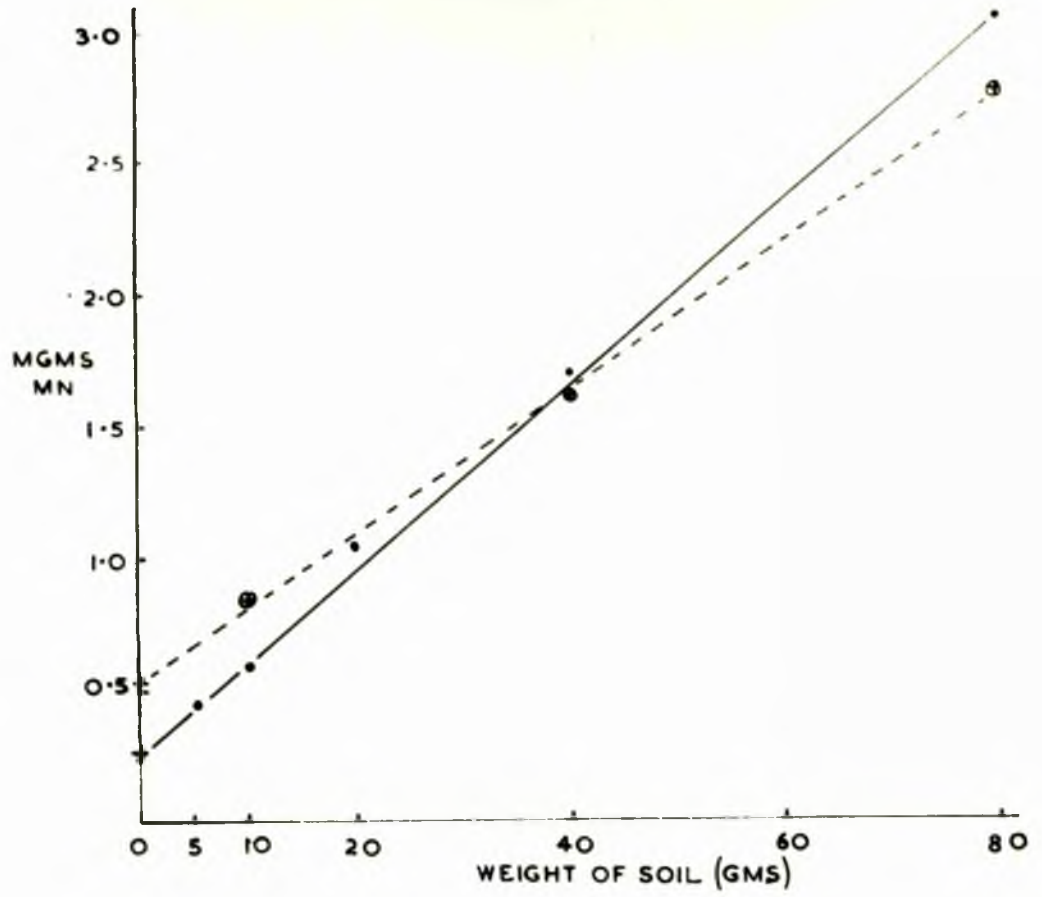


Fig. 21. Effect of volume ratio on extraction of manganese from soil No. 801560.

- • Extraction using 200 ml of M $Ca(NO_3)_2$
- ⊕ Extraction using M $Ca(NO_3)_2$ plus 0.270 mgs manganese.
- \+ Manganese found in 200 ml of extractant

Table 11

Extractable manganese of soil No. 801560

Weight of soil	Extractant			
	200 ml. M $Ca(NO_3)_2$		200 ml. M $Ca(NO_3)_2$ +0.270 mg. Mn	
	Wt. Mn found (mg.)	Mn p.p.m. soil	Wt. Mn found (mg.)	Mn as p.p.m. soil
Blank	0.264	-	-	-
5 g	0.440	88.0	-	-
10	0.585	58.5	0.840	84.0
20	1.025	51.25	-	-
40	1.630	40.8	1.695	42.4
80	3.040	38.0	2.770	34.6

Figure 21 displays the results in graphical form. It can be seen that the extractant contaminated with manganese gives results of the same type as those obtained by Boken, and the line drawn through the points obtained cuts the ordinate precisely at the point expected for the measured amount of contamination. The added manganese was not completely absorbed into the soil, as Boken found; thenew values of extractable manganese when graphed gave a straight line cutting the ordinate close to the point which would be expected if the two levels of contamination were simply additive. (Point marked

with a cross in Figure 21.)

An unexpected, and unexplained, feature of the results is an alteration in the slope of the line by the addition of manganese. To this extent Boken's observation that added manganese is absorbed by the soil is confirmed; the effect of the addition of manganese is to reduce the apparent extractability of the soil manganese. This is seen clearly in the determination using 80 gms. of soil; less manganese was extracted when the contamination of the extracting solution was increased. Other examples of this strange effect are seen in Figure 23.

These results were communicated to Boken. In her reply, maintaining that contamination was not the explanation for her results, Boken quoted the case (private communication 1960) of a soil giving the following figures:-

Table 12

Results obtained by E. Boken for soil No. 47150

Wt. of soil	ml M $Mg(NO_3)_2$	mg Mn in 200 ml. M $Mg(NO_3)_2$	Mn p.p.m. of soil
5	200	0.044	8.8
10	200	0.072	7.2
20	200	0.096	4.8
40	200	0.176	4.4
50	200	0.224	4.5

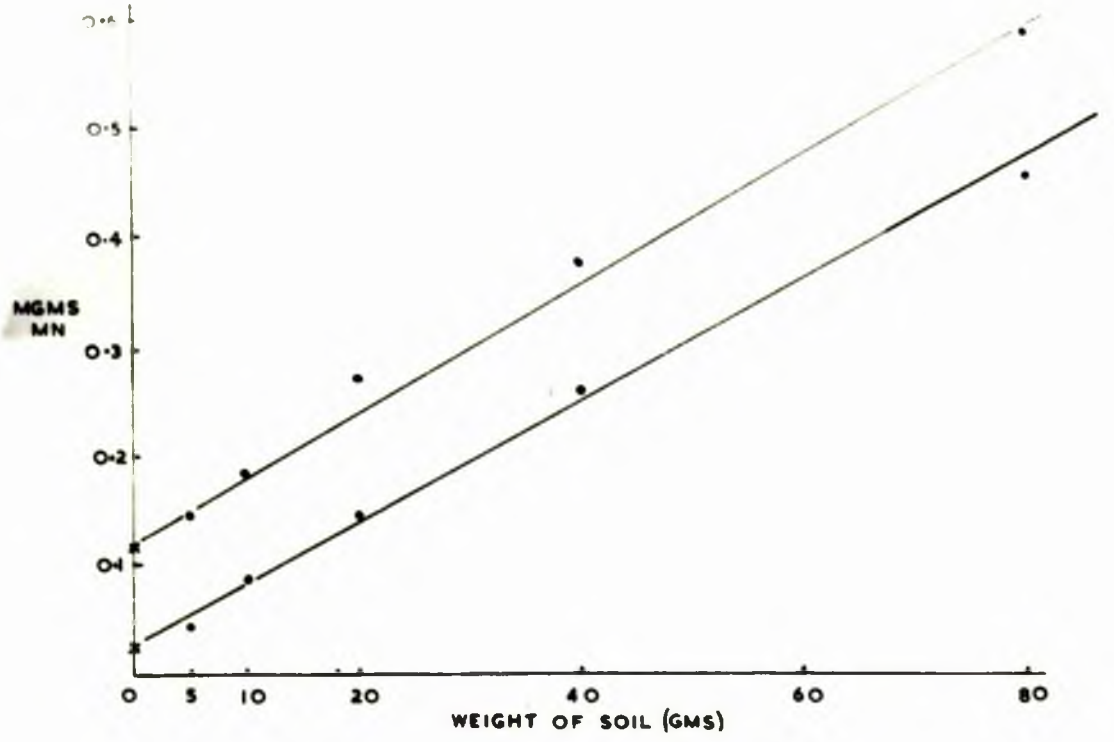


Fig. 22. Effect of volume ratio on extraction of manganese from soil No.47150 (Royal Veterinary and Agricultural College, Copenhagen.)

Lower set of points, extraction using 200 ml M $Ca(NO_3)_2$

Upper set, extraction with 200 ml M $Ca(NO_3)_2$ and contaminated nitric acid.

× Manganese found in 200 ml of extractant with and without contaminated nitric acid.

If these results are plotted (see Figure 23) the value of the intercept indicates that 200 ml. of extractant should contain 0.025 mg. of manganese. But Boken was confident that the magnesium nitrate used (Merck, batch No. 52799) contained no manganese. She supplied a sample of this magnesium nitrate and of soil No. 47150 (Royal Veterinary and Agricultural College number, Copenhagen, Denmark) for examination by the techniques used in the investigations reported here.

This soil was extracted both with molar calcium nitrate solution and with a molar solution prepared from the magnesium nitrate supplied by Boken. The extracts made with calcium nitrate were duplicated, and one set of permanganate colours were developed using nitric acid known to be contaminated with manganese. These results are given in Table 13, and illustrated in Figure 22.

Table 13

Extraction of soil No. 47150 with molar calcium nitrate solution

	200 ml. extractant		200 ml. extractant; contaminated nitric acid	
Weight of soil	Wt. Mn found (mg.)	Mn p.p.m. soil	Wt. Mn found (mg.)	Mn p.p.m. soil
5	0.043	8.6	0.144	28.8
10	0.088	8.8	0.184	18.4
20	0.148	7.4	0.272	13.6
40	0.260	6.5	0.376	9.4
80	0.452	5.7	0.585	7.3
Blank	0.024	-	0.116	-

If lines are drawn through each series of points and extended to the ordinate the intercept is in each case equal to the amount of manganese found in the blank determination. It will be noted that the lines in this instance are parallel, unlike the lines in Figures 21 and 23. This is to be expected, since manganese added at the stage of colour development cannot be adsorbed onto the soil; but nevertheless the fact that the lines run parallel provides a check on the accuracy of the determinations, since the extracts from the larger amounts of soil contain appreciable quantities of organic matter which makes complete development of the permanganate colour difficult. The lower line of the pair gives no indication that contaminating manganese in the calcium nitrate extracting solution is adsorbed onto the soil as claimed by Boken.

Table 14 gives the results obtained by extraction of the same soil with molar magnesium nitrate solution. One set of extracts were made in the usual way, and to a second set manganese was added in the form of manganese sulphate. To each bottle of soil and extractant an amount of manganese was added, before shaking, equal to rather more than twice the amount of contamination suspected from the results obtained by Boken (vide Table 12 and Figure 23).

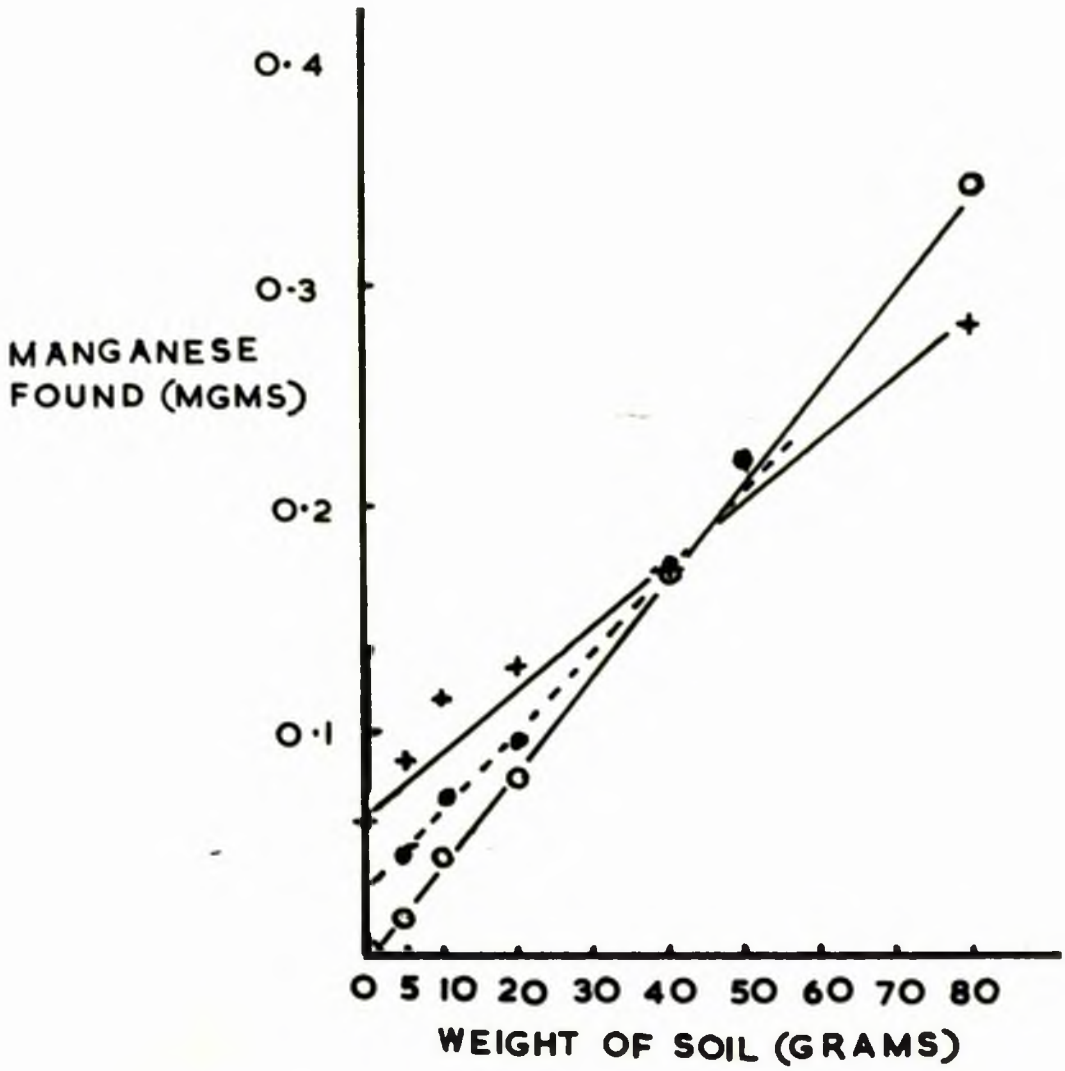


Fig. 23. Extraction of soil No. 47150 with molar magnesium nitrate solution, using varying volume ratios.

- o points obtained using 200 mls of solution
- \+ points obtained using 200 mls of solution + 0.060 mg of manganese added as manganese sulphate
- • points obtained by Boken in Royal Veterinary and Agricultural College, Copenhagen.

Table 14

Extraction of soil No. 47150 with molar magnesium nitrate solution.

Weight of soil	200 ml extractant		200 ml extractant + 0.060 mg Mn.	
	Wt. Mn found (mg.)	Mn p.p.m. soil	Wt. Mn found (mg.)	Mn p.p.m. soil
5	0.016	3.2	0.088	17.6
10	0.044	4.4	0.116	11.6
20	0.080	4.0	0.128	6.4
40	0.172	4.3	0.172	4.3
80	0.344	4.3	0.280	3.5
Blank	0.000	-	0.060	-

These results, together with Boken's results from Table 12, are shown graphically in Figure 23. It may be seen that the extract made with the pure magnesium nitrate does in fact give a line with zero intercept as expected. The line drawn through the points obtained with deliberately added contaminating manganese gives an intercept which is close to the value expected (shown by a cross). The same curious effect on the slope of the line which was found in the case of soil 801560 (Figure 21) is again evident, and in the case of 80 grams of soil extracted with 200 mls. of extractant, the amount of manganese found after addition of manganese to the extractant is less than before.

The line drawn through the points representing Boken's results is shown dotted. Quite clearly it is bracketed by the other two

lines, and approximately bisects the angle between them. This would seem to be adequate proof that the apparent variation in level of extractable manganese found by Boken is due to a quantity of contaminating manganese represented by the size of the intercept.

This explanation would cover the cases reported by Boken (1958) when calcium nitrate was the extractant, especially as Boken has used calcium nitrate solutions known to contain contaminating manganese without attempting to correct for its presence (private communications 1958, 1960). The explanation for the varying values of extractable manganese when expressed as p.p.m. of soil in the case of magnesium nitrate extractions is not so clear. With soil No. 47150, reported in detail here, contamination would appear to provide an explanation, though how it was introduced cannot be explained. It could not have been introduced at the development of colour stage, by the use of contaminated reagents, as was done deliberately in the example recorded in Table 13, since had this been the case the dotted line would have been lifted with unchanged slope in Figure 23, whereas its origin was lifted and its slope decreased, indicating that the manganese was introduced at some stage prior to shaking. Yet the extracting agent was indeed found to be manganese free, as had been claimed by Boken.

In the majority of cases reported by Boken (1958) where magnesium nitrate was the extracting agent, a plot of the points similar to those in Figures 17 and 19 to 23 gives a line almost cutting the origin of the graph. The very small increases in measured manganese for the smaller soil ratios could quite easily be the result of slight turbidity in the solutions which would give instrumental readings recorded as high manganese figures. The consistant trend of Boken's results, in which low soil to extractant ratios always produce higher extractable manganese figures would seem to favour this explanation.

On the other hand, where Boken has used high soil to extractant ratios the level of extractable manganese tends to be low. When the results are graphed in the form used here this becomes evident, as in the case of soil No. 16 extracted with magnesium nitrate in Figure 19, and again for soil No. 70696 in Figure 20. Most of the striking cases are those described by Boken (1958) as peaty soils. This suggests that the potassium persulphate technique used by Boken is unable to give complete oxidation to permanganate when the organic matter content of the solutions is high. Indeed it seems likely that the correlation between the variation in extractable manganese between extremes of volume ratio and carbon content of the soil found by her for the peaty soils can be explained by this artefact.

It is considered that Boken's results do not provide evidence against a simple ion exchange theory for the facts of manganese extraction from soils, as they would seem to do at first sight.

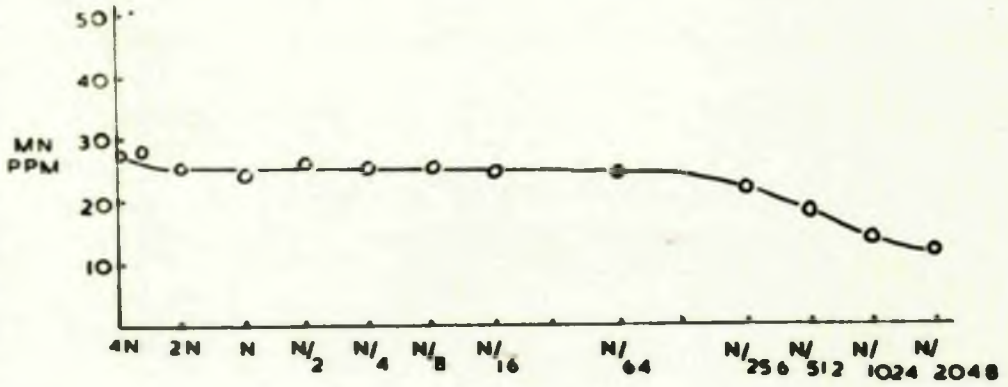


Fig. 24. Effect of variation of concentration of extractant on manganese extracted from soil R30647

10 gm of soil extracted with 200 ml of calcium nitrate solution. Shaking time 2 hrs.

(4) Effect of variation in concentration of extractant.

The extraction of manganese from soil R 30647 by solution of calcium nitrate of various concentrations between 4N and N/2048 was examined, and compared with the water extract. The results are recorded in Table XXIII of the Appendix. A volume ratio of 10 gms. of soil in 200 mls. of calcium nitrate solution was used in all cases in this experiment.

Figure 24 shows the effect of variation in concentration of extractant on the manganese extracted. The normality of the extracting solution is plotted on a logarithmic scale. It is clear that the concentration of the extractant can vary within wide limits without affecting the amount of manganese extracted from a given weight of soil. The slight rise in the level of extractable manganese found with 3N and 4N calcium nitrate was probably due to contaminating manganese in the extracting solutions; these were prepared in separate batches from the calcium nitrate solution used in the other extractions, and which was added to compensate for interference in the methane base determinations.

Only when the concentration of the extracting solution falls below N/64 does a reduction in the level of manganese extracted begin to be evident. In the routine determination of extractable manganese there is evidently little to chose between 2N, N, or 0.1N extracting solutions except on grounds of economy. In all reasonably strong calcium nitrate solutions the concentration of

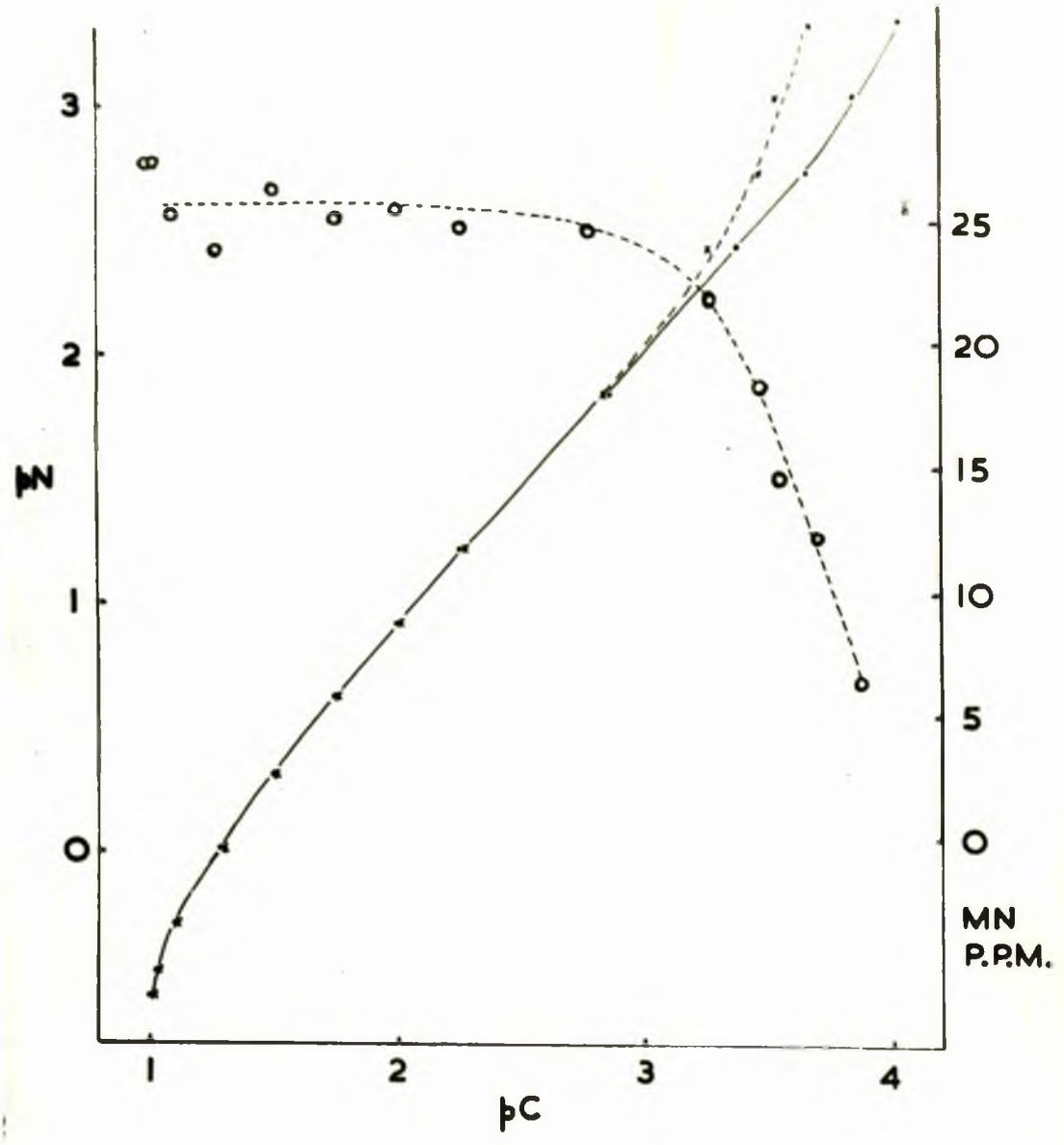


Fig. 25. Effect of variation of concentration of extractant on manganese extracted from soil 13(64)

- o Manganese extracted, plotted against pC of extract.
- × pN of extractant, plotted against pC of extract.
- • pN of extractant, plotted against pC of extractant.

calcium ions so greatly exceeds that of the manganese ions in the extract that extraction is virtually complete.

It is evident from inspection of Figure 25 that the level of extractable manganese begins to fall only when there is a divergence between the concentration of the extractant used and the concentration of the resulting extract. In this figure the normality of the extractant is graphed on a negative logarithmic scale, as pN, against the conductivity, of the extractant and the extract, both expressed on a negative logarithmic scale, that is to say as pC. It is seen that the pC of the extract begins to fall below that of the extractant from about pN = 1.860 upwards, corresponding to a concentration of N/64 downwards, and that this corresponds with the point of inflection of the manganese extraction curve as plotted against pC of extract. (The inflection occurs in the same place, but is less sharply defined if plotted against pC of extractant).

The fact that the pC of the extract is lower than the pC of the extractant indicates that the concentration of ions in the extract is greater than that in the extractant, in other words the soil is yielding up ions to the extracting solution. With extractant solutions of calcium nitrate stronger than N/64 the pC of the extract is higher than that of the extractant, that is to say the soil has gained ions from the solution. Under

these conditions the maximum amount of manganese appears in the extract.

Where the soil is losing ions to the extracting solution less than the maximum extractable manganese is obtained. As the divergence between the strength of extract and strength of extractant becomes greater the amount of manganese extracted steadily decreases. It must be remembered that the manganese ions lost by the soil at any time represent a small fraction only of the ions held by it in exchangeable form. If exchangeable manganese is present to the extent of 28 p.p.m. of the soil as in this case, the amount extracted under the conditions of the experiment represent only 0.0509 m.eq. per litre of extractant, that is the manganese ions represent the concentration that would be found in an N/19,600 solution.

The approximate concentration of ions in the water extract can be judged from the pC figure for the extract. This is 3.8774, which would correspond to a calcium nitrate concentration (see Figure 25) of about N/1000 (pN = 3). Most of the conductivity of the water extract would be provided by calcium ions, and the fact that the anions would not be nitrate ions would not greatly affect the situation. In the water extract 6.4 p.p.m. manganese were found, so that about 1 in 90 of the ions given up to the water by the soil in this case were manganese ions.

It is to be expected that manganese would be released more readily than calcium; Kitchener (1957) reports the lyotropic series $Mn^{++} < Mg^{++} < Zn^{++} < Cu^{++} < Ni^{++} < Co^{++} < Ca^{++}$ for the affinity of ions for an ion exchange resin, and the series for soils are not likely to differ, except in detail, if no chemical reaction occurs capable of fixing some specific ion. Thus when a great excess of calcium ions are available, virtually the whole of the exchangeable manganese is extracted from the soil.

But where the quantity of calcium ions present is not overwhelmingly in excess of the exchangeable ions of the soil it is to be expected, if the soil behaves as a simple exchange system with immobile anions and exchangeable cations, that the laws of Donnan equilibria would be seen to be obeyed. These laws have been applied to soils in some detail by Wiklander (1955); the relevant sections of his treatment are applied here to the behaviour of the manganese and calcium ions in this series of investigations.

For homovalent exchange such as that involving calcium and manganous (Mn^{++}) ions in their relationship with soil, as represented by the exchange:

$$\boxed{\text{Soil}}\text{-Mn} + Ca^{++} \rightleftharpoons \boxed{\text{Soil}}\text{-Ca} + Mn^{++}$$

the equilibrium equation may be written

$$\frac{(Ca^{++})_i \ (Mn^{++})_o}{(Mn^{++})_i \ (Ca^{++})_o} = k_{Mn,\ Ca} \qquad (I)$$

where parentheses represent activity; the subscript i refers to the internal or exchange phase associated with the soil particles, and o refers to the external phase or outer solution: k is the equilibrium constant.

For the case where mono- and di-valent ions are concerned, as with, say, potassium and calcium ions the equation is

$$\frac{(Ca^{++})_i \ (K^{+})_o^{2}}{(K^{+})_i^{2} \ (Ca^{++})_o} = k_{K,\ Ca} \qquad (2)$$

The activity of each diffusible electrolyte must be constant throughout the aqueous phase, so that the equilibria are as follows:-

$$\frac{(Ca^{++})_o}{(Ca^{++})i} = \frac{(Mn^{++})_o}{(Mn^{++})i} = \frac{(K^{+})_o^{2}}{(K^{+})_i^{2}} = \frac{(A')^{2}_i}{(A')^{2}_o} = K_1 \qquad (3)$$

where A is a mono-valent diffusible anion such as the nitrate ion in the experiments reported here. The minutely small proportion of hydroxyl ions present will of course be subject to the same law.

If equation 3 holds, the $k_{Mn,\ Ca}$ of equation one must be equal to unity, that is,

$$\frac{(Ca^{++})i \quad (Mn^{++})o}{(Mn^{++})i \quad (Ca^{++})o} = 1 \qquad (4)$$

It is possible to test the applicability of Donnan theory to the results given in Table XXIII by examining their consistency with equation 4. This can be done only for the lower concentrations since no reliable estimate of $(Ca^{++})i$ can be obtained by the methods used here at concentrations higher than N/64, and in the same range of high concentrations the estimate of $(Mn^{++})_i$ becomes more and more imprecise.

The above equations employ activities of ions; in the following calculations concentrations are used instead, and should of course be multiplied by the appropriate activity coefficient in each case, so that, for example

$$(Ca^{++})_o = [Ca^{++}]_o \times f_{Ca\ o}$$

when square brackets represent concentration, and $f_{Ca\ o}$ is the activity coefficient of the calcium ion.

The activity coefficient is unity only at infinite dilution, for low concentrations it can be obtained from the Debye-Hückel limiting law:-

$$-\log f = 0.5092\ z^2_i \sqrt{I}$$

where I is the ionic strength of the solution and is equal to $\frac{1}{2}\sum m_i z_i^2$, in which m_i is the molality of each ionic species and

Table 15

Concentration of Extractant	pH of extract (from graph)	$[Ca^{++}]_o$ (meq/litre)	$[Mn^{++}]_o$ (μeq/litre)	$[Mn^{++}]_o / [Ca^{++}]_o$ x 1000
Water	3.02	0.952	11.65	12.25
N/2048	2.77	1.698	22.35	13.16
N/1024	2.60	2.571	26.70	10.40
N/512	2.52	3.021	33.45	11.10
N/256	2.30	5.025	40.35	8.02
N/64	1.74	18.215	45.15	2.48

z_i its valency, but this law breaks down for calcium nitrate solutions more concentrated than 0.002 molar (calculated from activity coefficient data given by Latimer (1952)).

However, since both species of ion, manganese and calcium, have the same valency, over the range covered by the Debye-Huckel limiting law both will have the same activity coefficient, since the ionic strength of the solution is the same for both. Hence the correction to be applied would be the same for both, and so long as ratios of the two ions in similar situations are considered it can be ignored.

When the limiting law ceases to be applicable the activity coefficients will probably differ, but are unlikely to be widely divergent for the concentrations considered here, although the differences at higher concentrations might be serious.

The extracted manganese was measured, so that $[Mn^{++}]_o$ is known directly. The concentration of calcium in the extract was markedly different from that of the extracting solution at the lower concentrations, as may be seen from the pC figures in Table XXIII. Figure 25 may be utilised in order to obtain an estimate of the concentration of calcium ions in the extract, the corresponding pH to the pC of the extract may be read off on the graph of pC of extractant against pC.

The figures obtained in this way are presented in Table 15.

It should be noted that equation 3 does not imply that the ratio $[Mn^{++}]_o/[Ca^{++}]_o$ obtained above ought to be constant; equation 3 applies only to a given system, whereas in effect the use of different extractants gives a new system on each occasion. Since the total manganese involved in the Donnan equilibration is constant, while the total calcium is increasing with the use of more concentrated calcium nitrate solutions, it is to be expected that the ratio $[Mn^{++}]_o/[Ca^{++}]_o$ will decrease in the manner found.

An estimate of $[Mn^{++}]_i/[Ca^{++}]_i$ can be obtained by similar methods, although in this case the precision is likely to be lower, since the derivation of the figures is more indirect and subject to greater probable error.

If the total exchangeable manganese is $[Mn^{++}]_T$ it is safe to assume that almost all of this goes into the external solution when the concentration of extractant is high. Figure 25 shows that a reasonable estimate of the total exchangeable manganese is 28 p.p.m. of soil, that is 1.02 μeq per gram. If the amount of manganese extracted from the soil by each extractant is subtracted from the total, what is left is an estimate of $[Mn^{++}]_i$

$$[Mn^{++}]_i = [Mn^{++}]_T - [Mn^{++}]_o \times v$$

where v is the volume of extractant per gram of soil. Values of $[Mn^{++}]_i$ calculated in this way are shown in Table 18.

Similarly an estimate of $[Ca^{++}]_i$ can be obtained by sub-

tracting the calcium removed from the soil from $[Ca^{++}]_T$, the total exchangeable calcium. The calcium removed from the soil in the process of extraction is given by the difference between the calcium content of the extractant and the calcium content of the resulting extract. This latter has already been estimated in Table 15 as $[Ca^{++}]_0$

Hence where v = volume of extract

$$[Ca^{++}]_1 = [Ca^{++}]_T - v\ ([Ca^{++}]_0 - [Ca^{++}])$$

The total exchangeable calcium of the soil was estimated in three ways, by the following procedures:

(a) 10 g of soil were shaken mechanically with five successive lots of 200 ml of 10% hydrochloric acid for one hour each. After filtration through Whatman No. 542 papers the filtrates were combined and evaporated almost to dryness with 10 ml. of 100 vol. hydrogen peroxide to destroy organic matter. The solution was diluted to about 200 ml. and neutralised with N/10 sodium hydroxide solution. A large quantity of ferric hydroxide was precipitated and was filtered off. The filtrate was made up to 500 ml. and titrated with N/50 E.D.T.A. solution, using an EEL photo-electric titrator fitted with a green filter. The procedure was that of Smith and McCallum (1956) and the indicator used was Eriochrome Black, so that the estimate obtained was actually for calcium plus magnesium. Iron interference was overcome by the addition

of hydroxy-ammonium chloride. The estimate of calcium plus magnesium is more appropriate for the calculation employed here than calcium alone, since strictly speaking, it is the total concentration of divalent ions, of which the calcium are by far the most numerous, which have been obtained from the pC measurements.

(b) 10 gms. of soil were shaken with five successive lots of 200 ml. of neutral N ammonium acetate, each shaking proceeding for one hour. The filtrates were combined after filtration through 542 papers as before, and evaporated with 10 mls. of 100 vol. hydrogen peroxide. The ammonium acetate was driven off completely with great ease, leaving a clean dry deposit which was taken up in water and made up to 500 ml. This solution was titrated as before, except that it was not necessary to add hydroxy-ammonium chloride to overcome interference from iron.

(c) 10 gms. of soil were shaken with five successive lots of 200 ml. of N ammonium chloride adjusted to pH 5, each shaking proceeding for one hour. After filtration as before the combined filtrates were evaporated down with 10 ml. 100 vol hydrogen peroxide. The ammonium chloride was much more difficult to get rid of than the ammonium acetate; it was necessary to transfer the crystalline mass which formed to an evaporating dish for strong heating to sublime away the solid ammonium chloride. The

final residue was taken up in water, and the calcium plus magnesium content determined as for the ammonium acetate extract.

The three methods gave different results as shown in Table 16.

Table 16

Exchangeable calcium plus magnesium of soil R 30647

Extractant	m eq per gm. of soil
10% hydrochloric acid	0.0723
N ammonium acetate solution	0.0278
N ammonium chloride solution	0.0355

When such divergent results can be obtained for one and the same soil, doubt must be cast on the efficiency of the established methods in measuring "exchangeable" cations in soils. Mitra and Prakash (1957) recommended N ammonium acetate as an extractant mainly on the grounds that it simplifies procedure, not because it gives a "true" measure of the exchangeable ions of the soil. It has already been stated that ammonium ions give lower values for exchangeable cations than do divalent ions; these resultsshow that the anion of the extractant also has considerable influence possibly through its effect on ionisation.

While ammonium salts almost certainly give an underestimate

for exchangeable divalent ions, it seems likely that the more drastic effect of the 10% hydrochloric acid would result in overestimation, through its effect on the structure of the soil. It is likely that the solution of iron, which has been mentioned already, would effect the release of some small amounts of calcium which are not strictly exchangeable. The soil, of course, was free of calcium carbonate, and by treating it like a simple ion exchange resin to be freed from adsorbed cations it was hoped to obtain an estimate of total exchangeable calcium which would be more accurate than that obtained by the use of ammonium salts.

Evidently with three different estimates of $[Ca^{++}]_T$ there are three possible values for $[Ca^{++}]_i$ in each case. These are shown in Table 17.

These values of $[Ca^{++}]_i$ are used with the corresponding values of $[Mn^{++}]_i$ to obtain the values of the ratio $[Mn^{++}]_i/[Ca^{++}]_i$ in Table 18, and the figures are combined with those for $[Mn^{++}]_o/[Ca^{++}]_o$ from Table 15 to obtain the series of values for

$$K_{Mn,\,Ca} = \frac{[Ca^{++}]_i\,[Mn^{++}]_o}{[Mn^{++}]_i\,[Ca^{++}]_o}$$

which are shown in Table 19.

Table 17

Estimated values of $[Ca^{++}]_i$ for soil R 30647

$[Ca^{++}]_o$ meq/litre	$[Ca^{++}]_{ext}$ meq/litre	$[Ca^{++}]_i$ meq/gm soil when total exchangeable calcium is: 0.0723	0.0278	0.0355
0.952	0 (water)	0.0533	0.0088	0.0165
1.698	0.4883	0.0481	0.0036	0.0113
2.571	0.9766	0.0404	- *	0.0036
5.021	1.9532	0.0509	0.0064	0.0141
5.025	3.9063	0.0499	0.0054	0.0131
18.215	15.6250	0.0205	- *	- *
$[Ca^{++}]_T$ estimated by :—		10% HCl	N NH_4Ac	N NH_4Cl

* negative value.

Table 18

$[Mn^{++}]_1$	$[Mn^{++}]_1/[Ca^{++}]_1$ x 1000		
μ eq/g soil	10% HCl	N NH_4Ac	N NH_4Cl
0.784	14.7	89.0	47.5
0.570	11.8	158.0	50.5
0.484	12.0	-	135.0
0.349	6.9	54.5	24.7
0.211	4.2	39.0	16.1
0.116	5.5	-	-

Table 19

Values of $K_{Mn,\ Ca}$ obtained using values for Tables 15 and 18.

Extractant	Total exchangeable calcium estimated with		
	10% HCl	N NH_4Ac	N NH_4Cl
Water	0.83	0.14	0.26
N/2048 $Ca(NO_3)_2$	1.12	0.08	0.26
N/1024 "	0.87	-	0.08
N/512 "	1.61	0.20	0.45
N/256 "	1.91	0.21.	0.50
N/64 "	0.45	-	-

Assuming that extraction with 10% hydrochloric acid gives a reasonable estimate of exchangeable calcium the results in that column are sufficiently close to unity, bearing in mind the large degree of experimental error likely to be involved in their derivation, to support the claim that the relationship between calcium and manganese ions is in accordance with the conditions of Donnan equilibrium. The average figure for the constant, 1.13 shows good agreement with the law.

This relationship means that manganese in the soil, in this case at least, is behaving as a simple divalent ion, and there is no need to invoke the existance of more complex ions, such as $(MnOH)^+$. Indeed, had such ions existed in any large percentage of the total manganese ions the value for $K_{Mn,\ Ca}$ calculated by the above method would have been widely variable; $K_{Mn,\ Ca}$ would have been constant only if it had been calculated from the expression:

$$\frac{[Ca^{++}]_i [MnOH^+]_o^2}{[MnOH^+]_i^2 [Ca^{++}]_o} = K_{Mn,\ Ca.}$$

This observation is important, since Schofield (1946) and others have suggested that polyvalent ions, particularly of the transition

metals, many form ions of this type, and this may explain some rather puzzling aspects of their behaviour in soil.

In all this discussion the effect of the hydrogen ion has been ignored, yet to do so can only be justified to the extent that its influence here is secondary and reasonably small compared with the effect of the variation in calcium ion concentration. It has been seen in the previous section dealing with field results that the effect of pH on manganese availability is profound, and this will be shown to be the case also for laboratory experiments in the subsequent section. Nevertheless, reference to Table XXIII in the Appendix shows that the pH of the extract for these experiments varied in the range from 5.70 to 4.67, and this would have a marked effect on the level of extractable manganese; it may account for some of the variation of $K_{Mn, Ca}$. It is noticeable that the pH of the extract made with N/256 $Ca(NO_3)_2$ is much lower than the rest, namely 4.67, and this would increase $[Mn^{++}]_0$ at the expense of $[Mn^{++}]_1$; this fact may account for much of the discrepancy in the value of $K_{Mn, Ca}$ calculated.

B. Extraction of soil by solutions of calcium chloride, with adjustment of pH of extraction by means of lime water.

Since the pH of the soil in the field is well known to be of such importance in its effect on the availability of manganese it is desirable to discover by what mechanism it has this effect.

The most widely accepted view is that the pH effect is the result of insoluble higher oxides being brought into solution by increased acidity; if this were so, the relationship between pH and manganese availability would be other than that actually found in the field soils results in Section II. This point has already been discussed at some length in that section.

Another possibility is that manganese availability is determined mainly by bacterial activities; this has been the view of, for example, Mulder and Gerretsen (1952) and Quastel (1954). If this is so, the effect of pH might be exerted indirectly through the bacteria, whose activities vis à vis manganese might be controlled by environmental pH. The field results could shed no light on this problem. But a laboratory experiment should decide the point easily, because if a given soil has its pH adjusted to different levels and the water soluble or extractable manganese immediately takes up appropriate levels in accord with the relationship found in the field results, the effect cannot be due to bacterial mediation, which would require a considerable time to

take effect.

To examine these and other aspects of the problem a series of experiments were performed with another soil, using calcium chloride as the extracting solution, and adjusting the pH of the extraction by means of varying quantities of calcium hydroxide solution.

Materials and Methods

(a) The standard soil. This was an acid loam, No. 801560, obtained from Bellahouston Park, Glasgow, from under long-established turf with no record of any fertiliser treatment for many years. The soil was air-dried, passed through a 2 mm sieve, and thoroughly mixed.

Water pH was 5.06 and pC 4.0477, these values being obtained after shaking 30 g. of the soil with 75 mls. of water for 30 mins. The total manganese content was 440 p.p.m.

(b) Calcium chloride solutions. These were prepared from B.D.H. Analar $CaCl_2$. The solution pH was adjusted by the addition of limewater to near neutrality, the strength of the solution was checked by titration with E.D.T.A. for calcium and in some cases also for chloride with silver nitrate, and adjusted if necessary. On exposure to the atmosphere the solutions always became slightly acid again (pH 5.35 to 6.25, depending on the concentration of calcium chloride) as may be seen by reference to the tables of results in the Appendix.

No attempt was made to interfere with this equilibrium pH.

(c) Adjustment of pH. The calcium chloride solutions of different strengths prepared as above were saturated with calcium hydroxide by adding slight excess of freshly slaked quicklime and shaking mechanically for several hours, then allowing to stand for approximately one week so that equilibrium could be attained. Atmospheric carbon dioxide was excluded by means of a soda-lime trap.

The lime water content of the extracting solution was varied by mixing the extractant solution alone with increasing quantities of extractant solution saturated with calcium hydroxide. This was done in nine steps so that the extractant used varied in its lime water content from zero to one hundred per cent. Thus in the experiment reported in Table XXIV of the Appendix the 560 mls. of N calcium chloride extractant used contained the following amounts of calcium hydroxide in N calcium chloride:- 0, 70, 140, 210, 280, 350, 420, 490, and 560 mls. The pH and pC of the solutions were measured immediately after mixing.

(d) Other solutions used. In one experiment saturated calcium sulphate solution was used, and in other 0.02 N potassium chloride. Analar B.D.H. chemicals were used in both cases.

(e) The saturated calcium hydroxide solutions made up in the solutions of calcium chloride, calcium sulphate, or potassium chloride were titrated with 0.1 N hydrochloric acid to measure

the hydroxyl ion normality. This was done electrometrically, using the pH meter, and the titre to pH 7 read off from the graph of the results obtained.

(f) Experimental procedure. This was based on the method used for the determination of the lime-requirement of soils by Hardy and Lewis (1929) although the procedure was very much modified.

Equal quantities of the air dry soil were weighed out into each of nine wide mouthed bottles of 40 oz. capacity. The nine solutions with varying amounts of lime water were added so that the volume ratio of soil:extractant was 1:4; thus in the above mentioned experiment the weight of soil used in each bottle was 140 grams. The screw caps of the bottles were tightened immediately to allow the minimum access of air, and the nine bottles were shaken on a mechanical end-to-end shaker for two hours.

At the end of two hours the suspensions were filtered through Whatman No. 1 filter papers, the first part of the filtrate, if it was at all cloudy, being returned through the paper. By this procedure it was possible to obtain clear filtrates on each occasion. While filtration was proceeding, the pH and the pC of a portion of the suspension were measured.

When filtration was complete the pH and pC of aliquots of the filtrates were obtained and further aliquots were used for various other determinations. While the only results reported here are those for manganese, determinations were also made of sodium, potassium, calcium, magnesium, ammonium and phosphate, the author being responsible for the sodium, potassium and ammonium determinations, as well as for the general supervision of the conduct of the experiments.

(g) Manganese determinations were made by the methane base method; the amounts of calcium chloride introduced by the small aliquots used were insufficient to make any allowance for interference necessary, except perhaps in the cases of N and 0.4 N calcium chloride extractions.

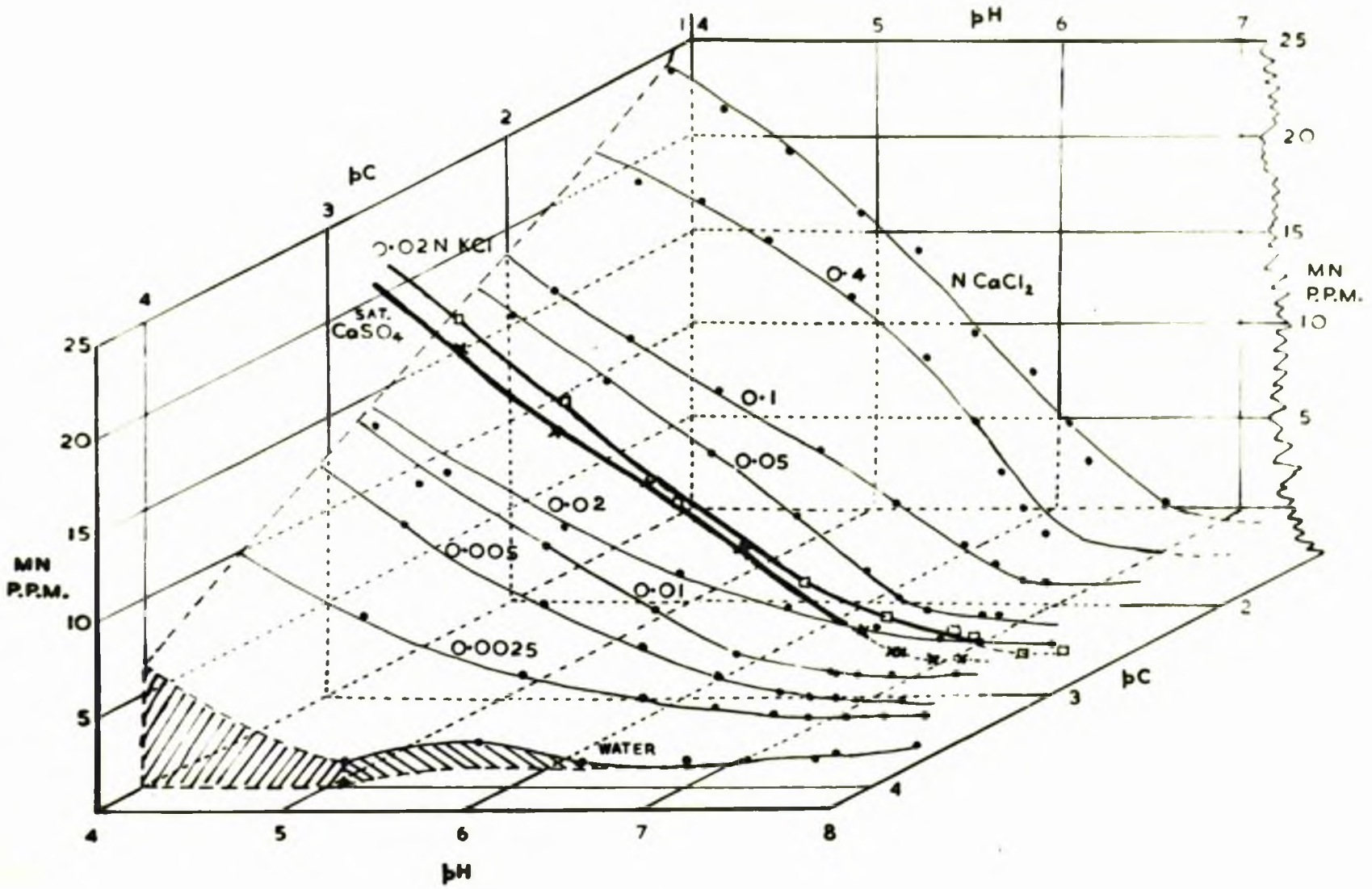


Fig. 26. - see p. 187 for details.

Results and Discussion

The results are given in Tables XXIV to XXXIV of the Appendix.

Figure 26 presents all the results graphed three dimensionally, the three mutually perpendicular axes representing pH, pC and extractable manganese expressed as parts per million of soil.

Clearly the extraction curves form a regular series without abrupt transitions, and this series embraces the whole range of observations from those obtained with N calcium chloride on the one hand to those obtained with water and varying additions of lime water on the other. The set of results obtained with 0.4 N calcium chloride (pC = 1.49) appear rather irregular, probably due to experimental error. It is thought that this set of results and those obtained with N calcium chloride are less reliable than the remainder as the level of calcium was approaching the amount known to cause interference in the methane base determinations of manganese, and no correction had been made to eliminate the effect of contaminating manganese.

A very important point emerges on consideration of Figure 26. If the extraction of manganese by different strengths of calcium chloride operated on one mechanism of retention of manganese by the soil, and the pH effect on another, the form of the contours in the figure would be regular, in such a way that the pH curve would be lifted without distortion as pC decreased; that is to say the effect of pH and the effect of increase in concentration would be additive. But inspection of Figure 26 reveals that this is not the

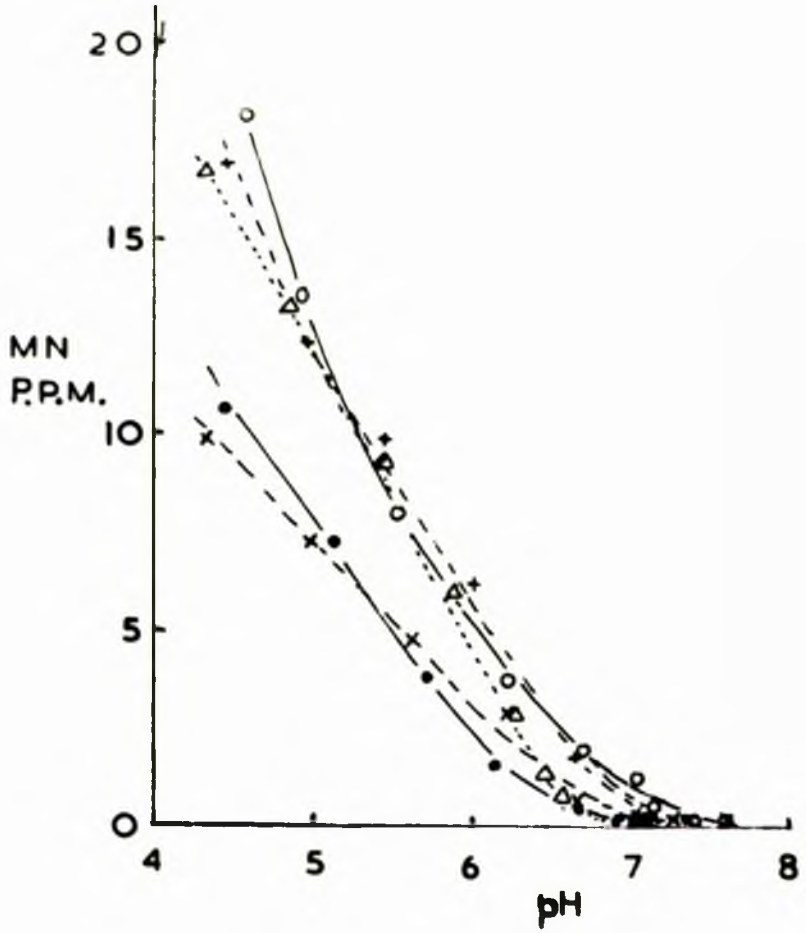


Fig. 27. Manganese extracted from soil 801560 plotted against pH of extract.

- o 0.02 N KCl
- + saturated $CaSO_4$ (0.0297 N)
- △ 0.05 N $CaCl_2$
- × 0.02 N $CaCl_2$
- • 0.01 N $CaCl_2$

case, the effect of pH is not simply in addition to the effect of concentration of extractant, both effects operate together, and the three dimensional figure could not be obtained by stepping up the same pH response curve by an amount porportional to the fall in pC.

The form of Figure 26 indicates clearly that both pH effect and the effect of alteration of extractant concentration operate on one and the same mechanism of manganese retention in soil.

It might seem from a cursory inspection of Figure 26 that the results for saturated calcium sulphate and 0.02 N potassium chloride fall into place in the family of curves, and consequently that the level of extractable manganese could be predicted if the pH and pC of the extracting solution were known. That this is not so can be seen clearly from Figure 27, which is in effect a projection of certain of the lines of Figure 26 on to a plane cutting the pC axis of that figure at right angles. In Figure 27 the manganese extracted by 0.02 N potassium chloride, saturated calcium sulphate, and 0.05 N, 0.02 N and 0.01 N calcium chloride solutions are plotted against pH of extractant. This diagram suggests that the figures obtained for the 0.02 N calcium chloride extract were somewhat underestimated, for they clearly should lie between the figures for the other two calcium chloride extracts. But in any case it is evident that saturated calcium sulphate (normality 0.0297) is a more

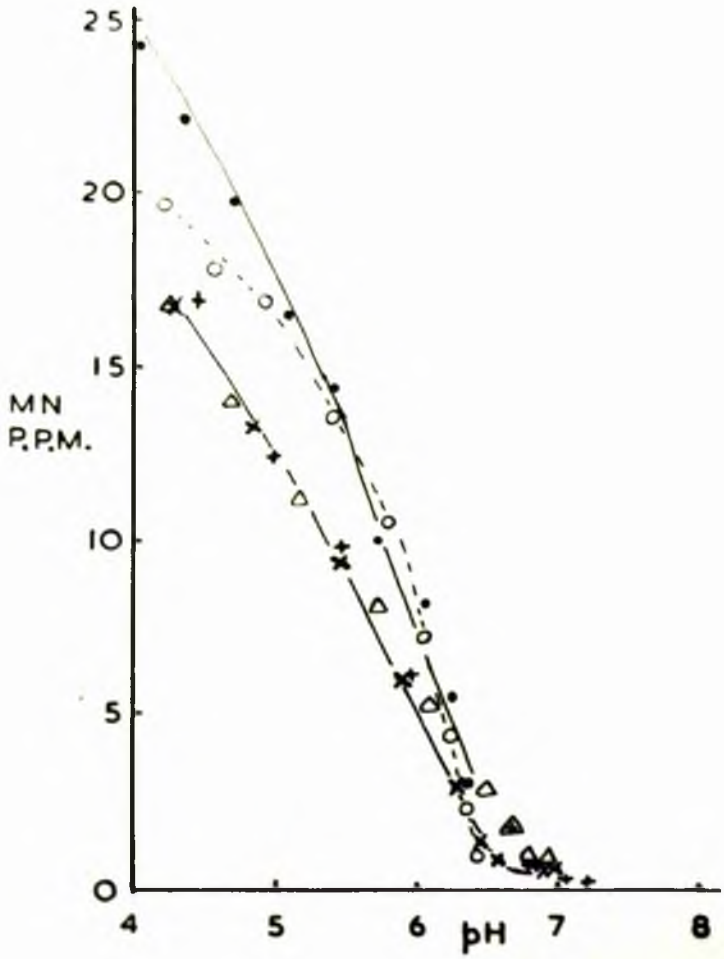


Fig. 28. Manganese extracted from soil 801560 plotted against pH of extract.

+ saturated $CaSO_4$

• N $CaCl_2$

o 0.4 N $CaCl_2$

△ 0.1 N $CaCl_2$

× 0.05 N $CaCl_2$

efficient extractant of manganese than 0.02 N calcium chloride and 0.02 N potassium chloride is about as efficient as the latter. The pC of 0.02 N calcium chloride extract is 2.66, of the saturated calcium sulphate 2.70 and of 0.02 N potassium chloride 2.61, so that the extraction efficiency of the solution is not a simple function of its pC.

If the efficiency of the extraction of manganese is dependent on the activity of the calcium ions in the solutions of calcium salts it is to be expected that saturated calcium sulphate would be more effective than 0.02 N calcium chloride, but it should be less effective than 0.05 N calcium chloride, which was not found to be the case. However comparison of the figures for saturated calcium sulphate, and 0.05 N, 0.1 N, 0.4 N, and N calcium chloride solutions as in Figure 28 suggests that there is no significant difference between the figures for manganese extracted by saturated calcium sulphate, 0.05 N and 0.1 N calcium chloride, and that the apparently higher extraction achieved with 0.4 N and N calcium chloride may well be due to interference by the higher concentrations of calcium chloride with the manganese determinations. It looks then as if the higher concentrations of calcium chloride and the saturated calcium sulphate solution are comparable to the higher concentrations of calcium nitrate used in the previous section of this work, where it was shown (see Figure 24) that at concentrations higher than N/64 no significant increase in extraction of manganese occurred with

increasing strength of extractant.

Potassium chloride could not be expected to extract manganese as efficiently as a solution of calcium chloride of the same strength, since monovalent ions in cation exchange are below divalent ions in the lyotropic series. Yet in this case the extraction achieved with 0.02 N potassium chloride solution is comparable with that achieved by 0.05 N and 0.1 N calcium chloride, and with saturated calcium sulphate. The explanation lies once again in the limited quantity of extractable manganese; it would appear that all of these solutions were strong enough to extract the maximum amount of manganese at any given pH level. The apparently higher quantities of extractable manganese achieved by N and 0.4 N solutions of calcium chloride can be ascribed to contamination and/or interference by the greater quantities of calcium chloride used.

Figures 27 and 28 may be used to obtain values for manganese extracted by the various strengths of calcium chloride solution at any given pH level in the range used. If this is done the figures so obtained may be used to perform the calculation used in the previous section to show that these results are compatible with a simple Donnan equilibrium explanation for the behaviour of exchangeable manganese ions.

If the pH selected is 5.5 the values of manganese in solution are shown (as Mn_o) in Table 20. Values of Ca_o are obtained from

the pC figures for the extracts (see Tables XXVIII to XXXII in the Appendix) read off as pH from Figure 29 and converted to calcium concentrations.

Table 20

Conc. of extractant	$[Ca^{++}]_o$ m eq/litre	$[Mn^{++}]_o$ μ eq/litre	$[Mn^{++}]_o / [Ca^{++}]_o$ x 1000
Water	1.26	11.8	9.35
N/400 $CaCl_2$	3.24	25.0	7.72
N/200 "	5.89	25.4	4.32
N/100 "	11.22	46.4	4.14
N/50 "	21.40	46.4	2.17
N/20 "	53.70	76.5	1.43

If the total exchangeable manganese is estimated to be 10 p.p.m. at this pH (from Figure 28, disregarding the values for N and 0.4 N calcium chloride extractions, for the reasons given previously), $[Mn^{++}]_i$ may be estimated from $[Mn^{++}]_i = [Mn^{++}]_t - [Mn^{++}]_o \times v$, as in Section IV A, (4), p 172.

$[Ca^{++}]_i$ may also be estimated from the difference in concentration of extractant and extract and the value of total exchangeable calcium, as explained in the same section.

As in the previous section, three estimates were obtained for total calcium, by the procedures previously described. These are shown in Table 21. In this instance the pH of the ammonium chloride solution was adjusted to 5.5.

Table 21

Exchangeable calcium plus magnesium of soil No. 801560

Extractant	m eq per gm of soil
10% hydrochloric acid	0.0912
N ammonium acetate solution	0.0231
N ammonium chloride solution	0.0263

With this soil the discrepancy between the results obtained by extraction with acid, and extraction with ammonium salts, is even greater than with R 30647.

Estimates of $[Ca^{++}]_i$ can be obtained as in the previous section (compare Table 17). These are given in Table 22.

Table 22

Estimated values of $[Ca^{++}]_i$ for soil No. 801560

$[Ca^{++}]_o$	$[Ca^{++}]$ ext	$[Ca^{++}]_i$ meq/gm soil when total exchangeable calcium is		
meq/litre	meq/litre	0.0912	0.0231	0.0263
1.26	0 (water)	0.0862	0.0181	0.0213
3.24	2.5	0.0882	0.0201	0.0233
5.89	5.0	0.0876	0.0195	0.0227
11.22	10.0	0.0863	0.0181	0.0213
21.40	20.0	0.0856	0.0175	0.0207
53.70	50.0	0.0764	0.0083	0.0115

The values from Table 22 are used with the corresponding values of $[Mn^{++}]_i$ to obtain the value of $[Mn^{++}]_i/[Ca^{++}]_i$ given in Table 23, and these ratios are used with the corresponding values of $[Mn^{++}]_o/[Ca^{++}]_o$ to obtain

$$K_{Mn,\,Ca} = \frac{[Ca^{++}]_i\,[Mn^{++}]_o}{[Mn^{++}]_i\,[Ca^{++}]_o}$$

(Compare Tables 18 and 19 in Section IV A (4).)

The values of $K_{Mn,\,Ca}$ obtained are shown in Table 24.

Table 23

$[Mn^{++}]_i$	$[Mn^{++}]_i/[Ca^{++}]_i \times 1000$		
μ eq/gm soil	10% HCl	N NH_4Ac	N NH_4Cl
0.316	3.67	17.46	14.85
0.264	3.00	13.13	11.34
0.262	2.99	13.45	11.54
0.178	2.07	9.85	8.35
0.178	2.08	10.18	8.60
0.058	0.76	6.99	5.04

Table 24

Values of $K_{Mn, Ca}$ obtained from values in Tables 22 and 23

Extractant	Total exchangeable calcium estimated with		
	10% HCl	N NH_4Ac	N NH_4Cl
Water	2.54	0.54	0.63
N/400 $CaCl_2$	2.58	0.59	0.68
N/200 "	1.44	0.32	0.37
N/100 "	2.00	0.42	0.50
N/50 "	1.04	0.21	0.25
N/20 "	1.88	0.20	0.28

It will be remembered that according to Wiklander (1955) the value of $K_{Mn, Ca}$ should be unity if the theory of Donnan equilibrium is applied to a simple case of homovalent cation exchange in soil.

The value of unity is exceeded where exchangeable calcium probably has been overestimated, and is not reached where calcium is certainly underestimated, just as was found in the case when soil R 30647 was considered.

Bearing in mind the successive approximations applied in the treatment given here, and especially the difficulty of obtaining a satisfactory estimate of the total exchangeable

calcium in the soil, it is considered that the evidence is at least not inconsistent with the hypothesis that manganese behaves as a simple divalent ion according to the classical theories of ion exchange.

It may be necessary to go beyond Wiklander's (1955) simple theory of ion exchange to explain the processes which occur in soil, indeed, it would be strange if this does not prove to be so. It seems likely for instance that if $K_{Mn,\ Ca}$ falls in value as the extractant becomes stronger in the case where exchangeable calcium has been overestimated, then it should rise in the same circumstances when exchangeable calcium has been underestimated, yet this was not found to be the case. It is, however, unprofitable to speculate on this as the calculations made here are not based entirely on direct determinations and in the indirect derivation of values used to calculate $K_{Mn,\ Ca}$ there has inevitably been a loss of precision. Wiklander's theory cannot be rigorously tested until an accurate means of measurement of individual exchangeable cations is devised. The rule of thumb methods at present accepted, such as extraction with normal ammonium acetate solution, are inadequate for the purpose; it is certain that they underestimate the amounts of divalent exchangeable ions in soil. At present techniques for the measurement of exchangeable ions in soil lag behind exchange theory; further advances in theory must wait on improvements in experimental method.

The effect of pH on manganese extraction

Figures 27 and 28 compared with Figures 11 and 13 in section III show that the effect of changed pH on the level of manganese extracted is similar in the laboratory experiments and in the field results. In the laboratory the manganese in the soil was influenced by the changed pH of the soil for two hours, yet in this time the level of extracted manganese adjusted itself in the same way as it had done over a period of months in the field soils. It is inconceivable that in this short time the bacterial or fungal spores present in the air dry soil should proliferate at room temperature to such a degree that metabolic transformation of manganese into the higher oxides could take place in the manner described by Mann and Quastel (1946). Indeed, in their experiment it took about three days for the level of manganese to fall significantly in the manganese sulphate solution perfused through this soil at 70°F.

The hypothesis that manganese becomes unavailable in soil solely as a result of bacterial activity is therefore quite ruled out by these experiments, and consequently the pH effect cannot be exerted indirectly through its influence on bacterial activities.

In the results from the field experiments the relationship between water-soluble manganese and soil pH was calculated by statistical analysis on the pMn and pH figures for each plot of each centre. In these calculations the relationship between pMn

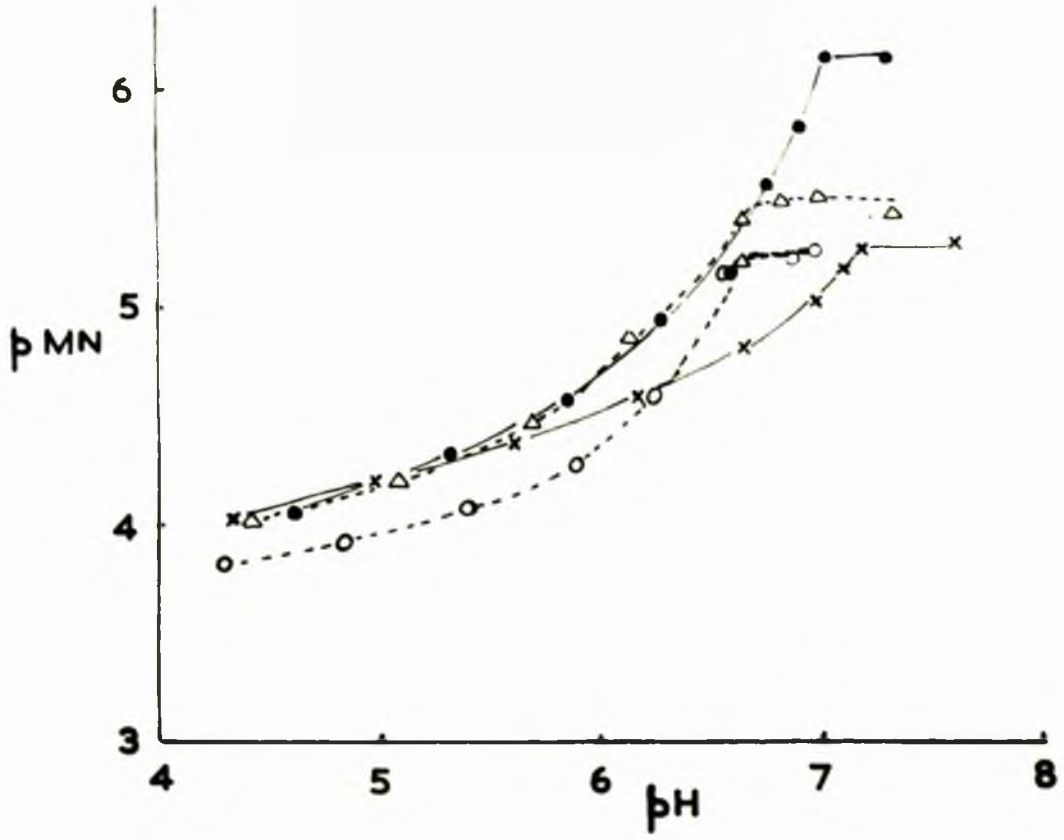


Fig. 29. pH relationship of extracted manganese, expressed as pMn.

- o 0.05 N $CaCl_2$
- × 0.02 N $CaCl_2$
- △ 0.01 N $CaCl_2$
- • 0.005 N $CaCl_2$

and pH was assumed to be linear, an assumption reasonable enough in view of the considerable scatter of the results, which would make it impossible to prove otherwise.

The form of the equation found in nearly every centre (see Table 6) was close to pMn = 0.5 pH - constant, the constant varying considerably from one centre to another.

If similar analyses are performed on the figures given in Tables XXIV to XXXIII in the Appendix, similar equations may be obtained, with pH co-efficients varying between approximately 0.34 and 0.43, with co-efficients of variation better than 0.9. But the random variation inescapable in field experimentation no longer operates in these laboratory experiments, and Figure 29 shows that the relationship between pMn and pH is not in fact linear, so that the slope of each line changes. This means of course that the equations derived for the field soils, and presented in Table 6 can have only a limited validity, and like Steenbjerg's (1935) equations, must be regarded as approximations over the whole pH range used, which are true for part only of the range.

If the theory were true that manganese became available by the solution of insoluble oxides as the soil pH fell, then the relationship would be a linear one as predicted by Eriksson's (1952) equation

$$2\,pH = p\,Mn$$

This has been discussed already in connection with the field results, where it was shown that the oxide solution theory must be

incorrect because the coefficient found for pH is in fact 0.5 instead of 2. The field results could have been consistent with a linear relationship; these more precise laboratory results clearly are not. This is additional proof that Eriksson's basic assumption was wrong. It may be seen in Figure 29 that the pH coefficient is 0.5 for a part only of the curve, it is in fact continually changing, and becomes almost equal to unity near pH 7.

Any satisfactory theory attempting to explain the relationship between pH and manganese availability must take account of these facts, as well as the other factors known to be involved. The following is suggested as a possible mechanism:-

The organic matter present in the soil forms complexes with the exchangeable manganese, and once complexed the manganese ceases to be exchangeable, becoming fixed in the complex. This would be rather similar to the view of complex formation given by by Leeper (1952) and by Bremner, Heintze, Mann and Lees (1946) except that they tended to regard manganese held in organic complex form as separate from, and alternative to, the exchangeable manganese held to the clay colloids by electrostatic forces. If the formation of complexes between the organic matter and manganese is controlled by pH, so that the organic matter is completely dissociated at high pH and not dissociated under

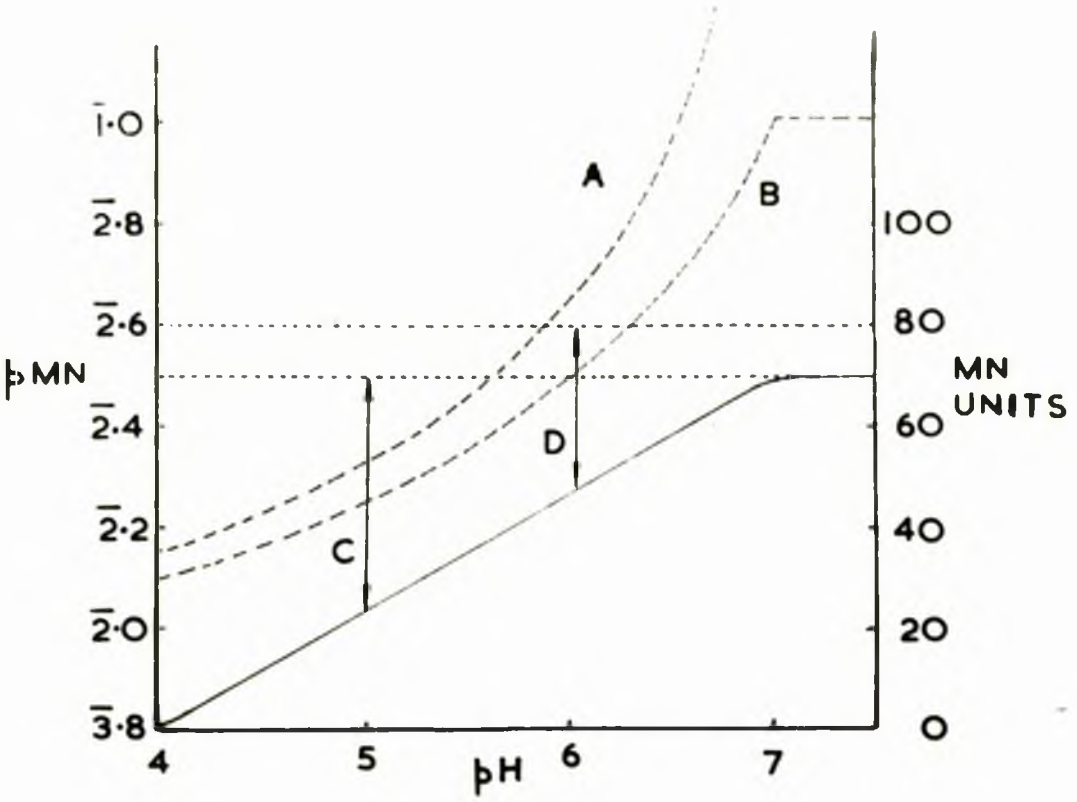


Fig. 30. Manganese fixation by organic complexes.
Full line represents dissociation of organic complexing material, 100% at pH 7.

A Exchangeable manganese, as pMn, computed for total exchangeable manganese of 70 units.

B Exchangeable manganese, as pMn, computed for total exchangeable manganese of 80 units.

C Amount of manganese in solution when total is 70 units. (gives curve A)

D Amount of manganese in solution when total is 80 units. (gives curve B.)

acid conditions, the situation would be as illustrated in Figure 30. The full line represents the percentage dissociation of the organic complexing material, ranging from zero below pH 4 to 100 per cent above pH 7. If the total exchangeable manganese is represented by the dotted lines, the lower of the two lines represents an amount just equal to the capacity of the complexing material, so that at pH 7 there is no manganese at all left in solution, while at pH 4 100% of the exchangeable manganese can be brought into solution. This level of exchangeable manganese has been arbitrarily set at 70 units, for the purpose of drawing the diagram, and the exchangeable manganese has been read off from the graph at various pH levels and computed to the pMn form. These figures are graphed against pH, giving the upper broken line A on the graph.

The upper dotted line represents 80 units of exchangeable manganese, so that the amount of exchangeable manganese left in solution never falls below 10 units. Again computing the p Mn figures for various pH levels, the plot shown in the lower broken line B of the graph is obtained, a comparison of this with Figure 29 shows a close similarity in form, which suggests that this model bears some resemblance to reality.

The mechanism which may exist is illustrated diagrammatically in Figure 31. The organic molecule shown is not necessarily phenolic, although phenolic substances are known to be present in

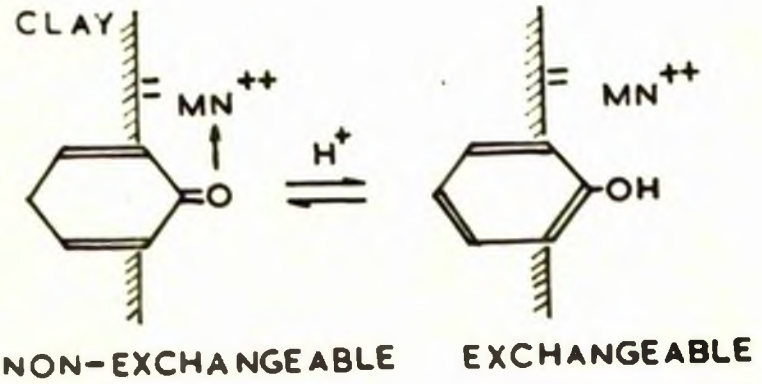


Fig. 31. Possible mechanism of manganese fixation by organic matter under the control of pH.

soil (Coulson, Davies and Lewis 1960), but may be any molecule capable of forming a complex. It could itself behave as an exchangeable cation if it possessed positively charged groups at other points; it would then be closely associated with the clay particles, and this might explain why Heintze and Mann (1949) found that electrolytes able to extract manganese from soil tend to be efficient also in extracting the organic matter which seems to be associated with it.

This theory that non-availability of manganese is caused by the formation of organic matter complexes which are dependent on the soil pH does not imply that oxides of manganese are not present in soil, nor that their formation is unconnected with pH. It does imply, however, that the manganese in such oxides is not wholly unavailable to the plant. The manganese held in the organic complexes, on the other hand, does cease to be available. The proportion of the total manganese in the soil which becomes available to the plant will depend on two factors (1) the amount of organic matter of the type capable of forming complexes with manganese which is present in the soil and (2) the percentage of this organic matter able to complex manganese, this proportion being decided by the pH value of the soil. The percentage of organic matter in the soil responsible for this complexing action need not be large. Assuming that a soil contains 550 p.p.m. manganese and that 1000 gms of organic matter are capable of complexing one equivalent of manganese, then 1 gm of soil contains 20 μ eq of

manganese which would require 0.02 gm of organic matter or 2%. Even with this generous estimate of the weight of organic matter required to complex 1 equivalent of manganese there will be every possibility of a normal soil containing sufficient organic matter to form complexes with all the manganese present.

V. THE UPTAKE OF MANGANESE BY OAT PLANTS

A. Uptake of manganese from various oxides

The theory that non-availability of manganese in the soil is due to the formation of various higher oxides which are not able to act as a source of the element for plants has been reviewed in section I, and it has been pointed out that there are a number of cases recorded in the literature where the addition of oxides under field conditions has lead to an improved uptake of manganese by the crop. (pp 39-41).

Nevertheless little work has been done to demonstrate directly the availability or otherwise of manganese present in higher oxide form, other than field experiments, which must inevitably be subject to greater uncertainty than those conducted under controlled conditions in the laboratory.

Jones and Leeper (1951a) showed that manganite (Mn_2O_3) and pyrolusite (βMnO_2) were available to oats grown in pot cultures in the open air using three different manganese deficient soils, but not hausmannite (Mn_3O_4), while peas were able to obtain manganese only from manganite, except with one soil (Penola) which apparently was able to make manganese available from pyrolusite. Heintze (1956) found that synthetic γMnO_2, hausmannite, and manganite, and mineral pyrolusite were all

available, in differing degrees, to timothy grass (Phleum pratense) grown in pot culture in alkaline sandy loam. Fiskel and Mourikides (1955) found that γ manganese dioxide was available to tomatoes in pot cultures in soil.

Enough has been said in the introductory section of this work to make clear the complexity of the reactions possible between soil and manganese compounds, including the oxides. Any experiments performed in pot cultures with soils must be subject to some doubt in case some soil constituent should be responsible for the reduction of the oxides of manganese, and consequently providing the plants with available manganous ions. It is desirable then to conduct experiments in which plants are grown without soil, but only Broomfield (1958a and b) appears to have done this, and then only with γ MnO_2 and a similar oxide prepared by his microbiological method (Broomfield 1956). He grew his oat plants on agar slopes in 6" x 1" test tubes, as well as in pot cultures with soil or quarts sand, and in culture solution. He found that manganese from the oxides was available under sterile conditions, but rather less so under non-sterile conditions.

It was decided to investigate the availability of manganese from an extended range of oxides to oats, using the same variety, Sun II, as employed in the field experiments reported in section III.

Experimental

(a) Preparation of the oxides

The oxides of manganese are by no means clearly defined, there is, for example, no such compound as "MnO_2". Analysis of any sample of the "dioxide" reveals considerable deviation from the simple formula. Even with samples from different sources which have approximately the same formula, there may be considerable differences in colour, density, and other physical characteristics. X-ray analysis reveals differences in crystalline form, and on this basis it is possible to identify at least three forms of dioxide, α, (cryptomelane), β (pyrolusite) and γ. Details of a number of the dioxides and methods of preparation have been given by Cole, Wadsley and Walkley (1947) in a study of the oxides used in Leclanché cells.

The oxides used in this study were prepared as follows:-

α manganese dioxide (cryptomelane)

51.5 g $MnSO_4 . 4H_2O$ (B.D.H. Analar reagent) were dissolved in 500 mls of water, and acidified with 40 mls of concentrated nitric acid. The solution was boiled, and solid potassium chlorate was dropped in a little at a time until 20 g had been added. A black precipitate formed slowly and was filtered off at intervals over several days. (Method of Vanino, 1925).

β manganese dioxide (pyrolusite)

Manganese nitrate was prepared by dissolving 73 g of manganese carbonate (B.D.H.) in slight excess of 2N nitric acid. A few drops of hydrogen peroxide dispersed the turbidity due to manganese dioxides of unknown composition. The solution was evaporated to dryness on the hot plate, and the manganese nitrate transferred into an oven and maintained at 200°C for about twenty hours. The pyrolusite formed was a metallic steel grey crystalline specimen. (Method of Kelley and Moore 1943).

γ manganese dioxide

24 g potassium permanganate (B.D.H. AnalaR reagent) were dissolved in about 200 mls of water and added drop-wise with stirring to a boiling solution of 50 g manganese sulphate (B.D.H. AnalaR reagent) in a similar quantity of water. When the precipitate settled, more potassium permanganate solution was added to the clear supernatent liquid until this was just pink. The solution was allowed to cool slowly on the hot plate overnight, and the precipitate was filtered off and washed many times until neither permanganate nor sulphate could be detected in the filtrate. The precipitate was a dark brown fine powder. (Method of Dubois 1936).

Manganous manganite (Mn_2O_3?, called MnO_2 by Dubois 1936).

59.3 g potassium permanganate (B.D.H. AnalaR reagent) were dissolved in 500 mls of water, and 10 volume hydrogen peroxide

B.D.H. AnalaR 100 vol. diluted ten times) added drop by drop for several days with continual stirring. A total of 2.7 litres was added before the permanganate colour disappeared. The brown precipitate was filtered off and washed five times by suspension in water and filtration. The material was so fine that some of it passed through No. 42 Whatman filter papers at each filtration. (Method of Dubois 1936).

Manganite (MnO.OH or Mn_2O_3)

51 g $MnSO_4 . 4H_2O$ (B.D.H. AnalaR reagent) were dissolved in 500 ml of water and 5 g sodium hydroxide pellets (B.D.H. AnalaR reagent) added. The solution was cooled in the refrigerator for about 2 hours, then the beaker was placed in an ice bath while 25 mls of 100 volume hydrogen peroxide (B.D.H. AnalaR) were added drop by drop with continuous stirring. A dark brown precipitate formed. The preparation was placed in the refrigerator overnight and then filtered and washed five times. (Method of Feitknecht and Marti 1945).

Hausmannite (Mn_3O_4)

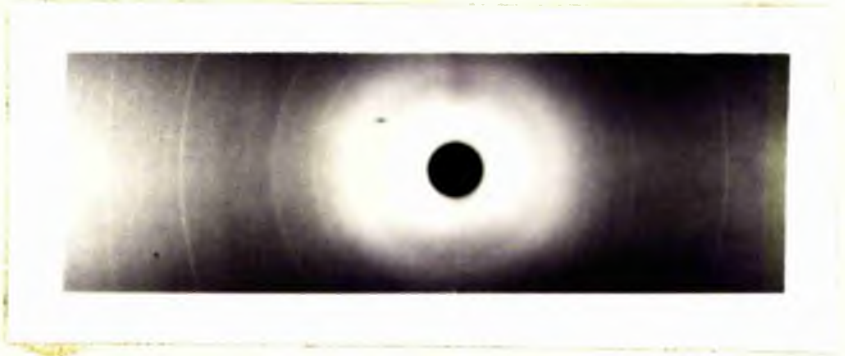
58.5 g $MnSO_4 . 4H_2O$ (B.D.H. AnalaR reagent) was dissolved in 400 mls of water and 218 mls of 1.61 N ammonium hydroxide added. A total of 25 mls of 100 volume hydrogen peroxide was added slowly and the solution allowed to stand for about one hour. An additional 109 mls of 1.61 N ammonia was added and

the whole solution boiled for a short time on the hot plate. The dark brown precipitate was filtered off and washed five times. (Method of Feitknecht and Marti 1945).

These oxides were kindly examined by Dr. Sim of Glasgow University by X-ray power photography. Photographs were taken by the Van Arkel method using copper K_{α} radiation, wavelength = 1.542 Å. A camera of radius 3.00 cm. was used for the photographs of pyrolusite and γMnO_2, and one of radius 5.7415 cm. for the rest.

Contact prints made from the negatives obtained are shown in Figure 32. These show that the six oxides have different crystalline forms.

The formulae of the oxides was ascertained by boiling a known quantity of each oxide with oxalic acid acidified with sulphuric acid, and back titrating with potassium permanganate solution. The formulae obtained are set out in Table 20.



β Manganese dioxide (pyrolusite)

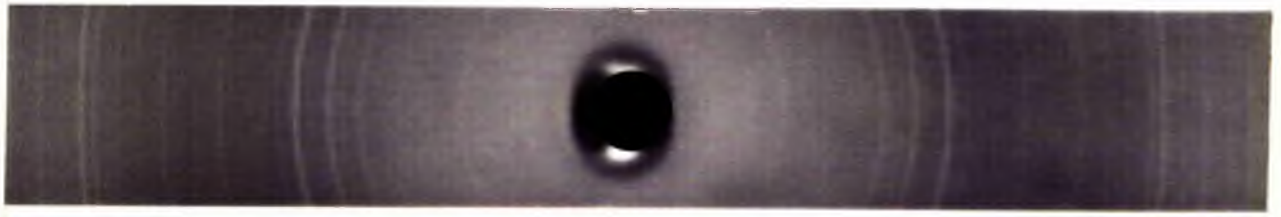
γ Manganese dioxide

Fig. 32 (a) X-ray diffraction photographs (contact prints) taken with a camera of radius 3.00 cm.

α Manganese dioxide (cryptomelane)

Manganous manganite

Manganite



Hausmannite

Fig. 32 (b) X-ray diffraction photographs (contact prints) taken with a camera of radius 5.7415 cm.

Table 20

Oxide of manganese	Theoretical formula*	Formula found
α manganese dioxide (cryptomelane)	MnO_2	$MnO_{1.85}$
β manganese dioxide (pyrolusite)	MnO_2	$MnO_{2.01}$
γ manganese dioxide	MnO_2	$MnO_{1.86}$
Manganous manganite	MnO_2	$MnO_{1.67}$
Manganite	$MnO.OH(Mn_2O_3)$	$Mn_2O_{2.94}$
Hausmannite	Mn_3O_4	$Mn_3O_{4.08}$

(b) Growth of oat plants

The oats were grown under sterile and non-sterile conditions on 1% agar gel made up from Hoagland and Arnonb solution No. 1 (1950) modified by the omission of manganese chloride from the micro-nutrient solution. Iron was added as 0.5% ferric chloride instead of the usual ferric tartrate, since Broomfield (1957) has observed that ferric tartrate causes manganese dioxide to go into solution, and it seems likely that this would also be the case for other oxides. The pH of the nutrient solution was adjusted to about 6 by the addition of 0.1 N sodium hydroxide before preparing the agar solution. A check of the agar when cool always showed a drop in pH; at the end of the period of growth the agar reached a

pH of between 6 and 7.

For growth under non-sterile conditions the oat plants were germinated, and two seedlings were placed on the surface of 400 mls of agar nutrient solution including 1 ml 0.5% ferric chloride solution in 600 ml beakers. The seedlings were protected by placing the top or bottom of a petrie dish over the open beaker until the roots had penetrated the agar. Brown paper sleeves were placed over the outside of the beaker to a height of about half an inch above the agar to protect the roots from the light. As the growth proceeded the agar tended to shrink, and distilled water was added as necessary to counter the effect as far as possible.

In the case where sterile conditions were desired it was thought that Broomfield's (1958a) method using 6" x 1" test tubes gave conditions which were too highly artificial. Trials were made of the method of Wieringa and Bekhuis (1957), who used powdered quarts coated with paraffin wax to maintain sterility, the plants being able to grow through the powder while the roots remained under sterile conditions. Powdered glass was used instead of quarts, and was coated with wax by immersing in a solution of wax in benzene, then allowing the benzene to evaporate. This method was unsuitable, however, because the wax coated glass powder cover collapsed and exposed the surface of the agar below

when shrinkage took place.

It was therefore decided to use what is essentially a modification of Broomfield's (1958a) method, and to grow the oats on agar in 1000 ml bottles designed for use in an end-over-end shaker. 200 mls of nutrient 1% agar (including 1 ml of ferric chloride solution) was placed in each bottle, with appropriate additions of the source of manganese. Each bottle was stoppered with a cotton wool plug and its contents sterilised in an autoclave for twenty minutes at 15 lbs. per square inch pressure.

The oat seedlings were sterilised by shaking for one minute in 70% alcohol, then for two minutes in 0.1% mercuric chloride solution, and finally washed with seven washes of sterilised distilled water. They were then allowed to germinate in sterile petri dishes containing a film of nutrient agar, which maintained appropriately moist conditions.

The sterilisation of oat seeds is difficult and any treatment drastic enough to give a reasonable likelihood of sterility results in the death of a high proportion of the seeds. A large number were sterilised on each occasion, and a number of petri dishes used for germination. Any petri dish showing fungal infection was discarded. When germination was well advanced, two seedlings were transferred to each shaking bottle by the sterilising techniques (flaming etc.) usual in bacteriology.

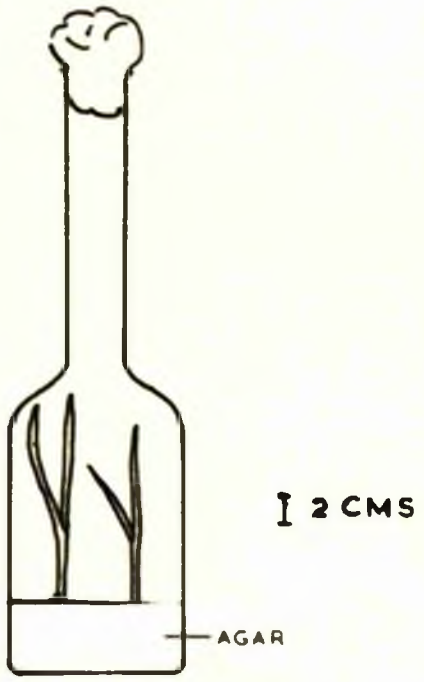


Fig. 32. Oat plants growing in 1000 ml shaking bottles under sterile conditions.

The lower portion of the bottles containing the agar was protected from light, either with a brown paper sleeve as before, or with black paint. The appearance of one of the bottles used for the sterile growth experiments is illustrated in Figure 33.

Each treatment in both sterile and non-sterile conditions was repeated in triplicate.

The growth took place in a greenhouse with minimum temperature controlled at 55°, and minimum day length maintained at 15 hours by means of 400 watt Osram mercury vapour lamps. The beakers or bottles were arranged in a block at random and their positions interchanged at intervals.

Results

In the first experiment pyrolusite and γ manganese dioxide were compared as sources of manganese with manganese sulphate solution. A control without manganese was included, and possible interaction between the sources of manganese was tested by growing non-sterile oats with mixtures of pyrolusite and manganese sulphate and γ manganese dioxide and manganese sulphate, and under sterile conditions by supplying pyrolusite/manganese sulphate, γ manganese dioxide/manganese sulphate, and pyrolusite/γ manganese dioxide mixtures.

The level of manganese supplied was in each case 1.6 mg., whether as sulphate or as oxide, thus representing 4 p.p.m. of the nutrient solution in the non-sterile preparation, or twice as much

(as p.p.m.) in the sterile agar. Where two manganese sources were combined, the amount of each was sufficient to give 1.6 mg of manganese, or a total of 8 p.p.m. in the non-sterile preparations.

The oats were planted in the non-sterile preparations on 25th April, and the above ground portions alone (to avoid possible root contamination by mechanical transfer of oxides) harvested on 28th July. Analysis were performed by the periodate method.

The results obtained are recorded in Table 21.

It is evident by inspection of the table that γMnO_2 is quite readily available to oats, the plants being able to obtain one third to one half as much as from the equivalent amount of manganese sulphate. Even pyrolusite is available to some extent; the point is established more clearly by performing Student's t test on the data, using:-

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad \text{where} \quad S^2 = \frac{\sum(x_1 - \bar{x}_1)^2 + \sum(x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

Employing exact figures for manganese content instead of the rounded figures for p.p.m. as in the table, a value of 8.42 is obtained for t, when the uptake from pyrolusite is compared with

Table 21

Uptake of manganese from higher oxides under non-sterile conditions

Manganese source	Yield (D.M. mgs)	Manganese found (mg)	Manganese P.P.M.
Nil	1.1362	0.007	6
"	0.8120	0.006	7
"	1.2257	0.007	6
Pyrolusite	1.7620	0.020	11
"	1.5021	0.017	11
"	1.6867	0.018	11
γ MnO_2	1.8835	0.164	87
"	2.1034	0.190	90
"	1.7022	0.148	87
$MnSO_4$	2.2490	0.387	173
"	1.5243	0.370	243
"	1.7251	0.365	212
$MnSO_4$ + pyrolusite	2.1246	0.412	194
"	2.2625	0.400	177
"	1.7277	0.328	190
$MnSO_4$ + MnO_2	1.1969	0.318	266
" + "	1.1562	0.315	273
" + "	1.5265	0.345	226

the manganese content in the absence of added manganese. For 4 degrees of freedom t is 2.78 at the 0.05 level of significance, 4.60 at the 0.01 level, and 8.61 at the 0.001 level.

The figures for uptake when both manganese sulphate and one of the oxides are present do not indicate that uptake from the oxides occurs in the presence of manganese sulphate. Indeed the average uptake from pyrolusite + manganese sulphate is less than that from manganese sulphate alone. Performance of the t test, however, gives a value of 0.78, indicating that the difference in uptake is not significant.

Similarly a t test for γMnO_2 + manganese sulphate as compared with manganese sulphate alone gives a value of 1.85, which is still not significant even at the 0.05 level.

It might be argued from these results that uptake from the oxides occurs only when no more readily available source of manganese is present, but it would be injudicious to maintain this without undertaking more extensive replication. In these experiments the variance is so large that uptake from the oxides could easily be obscured.

In the sterile preparations the oats were planted on 1st June, and harvested on 28th July; as with the non-sterile plants above-ground portions only of the plants were used. In the highly

artificial environment provided by the bottles, growth was poorer than in the non-sterile preparations, and yields much lower, hence analyses were performed for manganese by the methane base method, following the method for plant material given by Cornfield and Pollard (1950). In this the plant material was ashed at 500° for 4 hours, and the ash evaporated to dryness twice with dilute hydrochloric acid. The residue from the second evaporation was taken up in water, filtered if necessary, and made up to volume, usually 50 ml. Aliquots of this solution were used in the methane base manganese determinations already described.

Results are shown in Table 22.

In spite of the precautions taken to ensure sterility five of the bottles showed fungal infection; conditions in the bottles were of course favourable for fungal growth but the yield of the plants and the manganese content does not appear to be affected significantly.

Comparison of Table 22 with Table 21 shows that under sterile conditions the uptake of manganese, expressed as p.p.m. dry matter, was always higher than with similar sources of manganese and non-sterile conditions. This shows that the mechanisms of manganese uptake were not affected adversely to the same degree as some other processes, so that the total yield,

Table 22

Uptake of manganese from higher oxides under sterile conditions

Manganese source	Sterile	Yield (D.M. gms)	Manganese found ug	Manganese P.P.M.
Nil	No	0.0904	1.3	14
"	No	0.1030	1.9	18
"	S	0.0807	1.8	22
Pyrolusite	S	0.0856	3.1	36
"	S	0.0831	3.8	46
"	No	0.0989	5.4	54
γMnO_2	No	0.0862	16.9	197
"	S	0.0797	17.6	222
"	S	0.0757	19.3	255
$MnSO_4$	S	0.0597*	32.5*	545
"	S	0.1020	58.5	574
"	S	0.1192	60.5	508
$MnSO_4$ + Pyrolusite	No	0.0759	48.3	635
"	S	0.0823	52.8	641
"	S	0.1054	68.5	649
$MnSO_4$ + γMnO_2	S	0.0783	33.5*	428
"	S	0.1051	66.3	630
"	S	0.1142	71.5	627
Pyrolusite + γMnO_2	S	0.0905	22.5	249
"	S	0.0824	21.5	261
"	S	0.0778	24.0	308

* One plant only survived.

as dry matter, did not keep pace with manganese uptake. A similar effect was noted by Broomfield (1958a), who found that it existed even when his non-sterile plants were grown in tubes under the same conditions as in the sterile preparations. But Broomfield's sterile plants were grown in agar of pH 7.5, while his non-sterile plants were in agar of pH 8.5, so that it seems likely that his differences were due to the effect of pH on the power of the root exudate to bring oxides of manganese into solution, although it must be said that his results demonstrating the effect in vitro showed no solution at all occurring in this pH range.

In the experiments reported here, the initial pH of the agar was 5.58; this rose during the growth of the plants until when measured at the end of the experiment it varied in the different bottles from 6.39 to 7.03; mean value 6.58. There was no apparent effect on pH due to non-sterility in the five infected bottles (6.65, 6.66, 6.40, 6.84 and 6.74).

The behaviour of the oxides as sources of manganese under sterile conditions as shown in the results in Table 22 is similar to that shown in the non-sterile experiment reported in Table 21. The uptake from pyrolusite is significantly better, (at the 0.05 level) than in the absence of added manganese

(t = 4.07); γ MnO_2 is obviously quite a good source.

In this experiment the uptake from pyrolusite plus manganese sulphate is significantly better (t = 5.09) than from manganese sulphate alone, but comparing uptake from γ MnO_2 plus manganese sulphate against manganese sulphate alone shows no significant difference (t = 0.28) due to the very large variance in the γ MnO_2 plus manganese sulphate figures.

The uptake from the following pairs of sources show no significant difference at the 0.05 level:- γ MnO_2 as against γ MnO_2 plus pyrolusite (t = 1.97); manganese sulphate plus pyrolusite as against manganese sulphate plus γ MnO_2 (t = 1.19).

In calculating these t values the figures for the non-sterile bottles have been used, since there was no detectable effect on manganese content which could be attributed to non-sterility, and there could of course have been no effect of bacterial action in making manganese available in the bottles without added manganese.

In the second series of experiments there was no attempt to look for interactions between possible alternative sources of manganese, four additional oxides were tested as sources of manganese, and a comparison made with plants grown without added manganese, and with manganese supplied as manganese sulphate. The quantities of manganese provided were the same as in the first series of experiments, namely 1.6 mg of manganese to each pair of plants.

The growth under non-sterile conditions took place from 23rd August to 3rd October, 1960, except in the case of the three preparations containing manganite, which were grown from 25th November to 10th February, 1961. The plants were harvested as before, dried, and analysed for manganese by the periodate method. The results are presented in Table 23.

It is quite clear that each of the higher oxides used is able to provide oats with manganese, although the availability varies considerably. Hausmannite and manganite are particularly good sources.

A parallel experiment was performed under sterile conditions, the oats being planted on the 10th September and harvested on 7th November. The much smaller yields necessitated analysis by the methane base method. Results are given in Table 24.

The similarity between the results for sterile and non-sterile conditions of growth is strikingly evident, bearing in mind the restrictions on growth provided by the sterile environment. Again it is clear that all the higher oxides are available to the plant; manganite and hausmannite were very readily so.

Taking the results from Tables 21 to 24 inclusive, it is clear that while all higher oxides of manganese are available to oats, the availability varies considerably, the order of availability being pyrolusite < manganous manganite < cryptomelane < γMnO_2 < manganite < hausmannite.

Table 23

Uptake of manganese from higher oxides under non-sterile conditions

Manganese source	Yield (D.M.) gms	Manganese found mgs	Manganese P.P.M.
Nil	1.1500	0.004	4
"	1.2231	0.004	3
"	0.7376*	0.003	4
Cryptomelane	1.1857	0.038	32
"	1.0597	0.032	30
"	0.9226	0.032	35
Manganous manganite	1.3960	0.038	27
"	1.0535	0.026	25
"	1.1659	0.024	21
Manganite #	1.5780	0.196	124
"	1.6932	0.176	104
"	1.6420	0.192	117
Hausmannite	1.1223	0.168	150
"	0.7739*	0.132	171
"	1.2193	0.164	135
$MnSO_4$	1.1536	0.324	282
"	1.1186	0.304	272
"	1.3303	0.292	220

* one plant only

N.B. The manganite experiment was not concurrent with the others.

Table 24

Uptake of manganese from higher oxides under sterile conditions

Manganese source	Sterile	Yield (D.M.) gms	Manganese found ug	Manganese P.P.M.
Nil	No	0.0781	2.5	32
"	No	0.0734	2.6	36
"	S	0.0636	1.6	25
Cryptomelane	S	0.0740	9.4	127
"	S	0.0666	10.9	164
"	No	0.0724	10.1	140
Manganous manganite	S	0.0734	10.3	140
"	S	0.0723	7.2	100
"	S	0.0648	9.0	139
Manganite	S	0.0610	14.4	237
"	S	0.0752	15.7	209
"	S	0.0737	19.1	260
Hausmannite	S	0.0743	18.3	247
"	S	0.0781	20.8	265
"	S	0.0784	21.1	269
$MnSO_4$	S	0.0775	38.0	491
"	S	0.0856	48.4	565
"	S	0.0680*	35.4	522

* one plant only

B. THE UPTAKE OF MANGANESE FROM SOLUTIONS BY EXCISED OAT ROOTS

The absorption of ions by plants is an imperfectly understood subject in which much active research is proceeding; some of the conflicting evidence and differing interpretations are dealt with in recent reviews by Laties (1959a) and Epstein (1960).

An early paper by Stiles and Skelding (1940) reports only two previous references to work on uptake of manganese salts, namely Collander (1939) and Laine (1934), the work of Olsen (1934) apparently being overlooked.

Stiles and Skelding (1940) found that when manganese was absorbed from solutions of the chloride, sulphate, or nitrate by discs of carrot tissue there was an initial rapid uptake complete in a few hours, followed by a slow continuous absorption. In this it resembled potassium and other cations, but not the anions, which were found to lag behind in the phase of initial uptake, but to overtake and pass the cations in the later phase of slower uptake.

The initial rapid uptake of cations was regarded by Robertson (1951) as passage into the "free space" of the cells, and the slower accumulation as uptake into the vacuole. Later (Briggs and Robertson, 1957) the "free space" was divided into "water free space" (W.F.S.) and "Donnan free space" (D.F.S.), the latter being a system with non-diffusible anions, able to

take up cations by an ion exchange mechanism. This stage of uptake is purely physical, in the sense that it depends on the properties of cell and tissue structure, and is not dependent on current expenditure of metabolic energy.

The slower phase of accumulation is generally regarded as the result of active transport against a concentration gradient, and dependent on the expenditure of energy derived from metabolic processes, since it is blocked by metabolic poisons, such as cyanide or azide. Lundegårdh suggested an anion carrier system dependent on cytochrome which would carry cations into the cell in the wake of the actively transported anions. (See Lundegårdh 1955). This view would seem to imply that cation absorption should be non-selective, at least as far as ions of equal valency are concerned, unless the cations can be transported only when attached to specific carriers with differing mobilities. Laties (1959b) has shown that with potato storage tissue the generation of anion (chloride) transport capacity requires the expenditure of metabolic energy, and such generated capacity can be stored for a short time, probably in the form of a carrier precursor.

When Epstein and Hagen (1952) investigated the absorption of alkali cations by barley roots, they found that specific sites were involved, so that ions mutually interfered if they were taken up from one site, but not if different sites were utilized. Epstein (1953)

found a similar site specificity in the absorption of mono-valent anions, and Epstein and Leggett (1954) showed that it operated also for the uptake of alkaline earth cations. Russell and Ayland (1955) criticised Epstein for employing the procedure of Lineweaver and Burk (1934), developed for enzymatic studies, in a situation where its use was not justified, but did not suggest a better alternative.

Rothstein and Hayes (1956) studied the uptake of manganese and calcium by yeast cells, and by comparing the uptake of manganese in a similar period of time from solutions of widely differing concentrations they were able to show that two binding sites were involved for manganese.

Rothstein and Hayes (1956) made no attempt to remove the exchangeable manganese which would be taken up in the D.F.S. of the cell. Fried, Noggle and Hagen (1958) washed their excised barley roots in 10^{-2} M calcium chloride solution before measuring uptake of radio-active calcium, rubidium, and strontium; by applying essentially the same analysis as that used by Rothstein and Hayes they were able to demonstrate that each of the ions they investigated was still taken up at two binding sites. Two sites of uptake were also involved in phosphate uptake (Hagen and Hopkins 1955) and in the uptake of each of the ions K, Rb, Na and Sr by barley roots. (Fried and Noggle 1958).

The difference between the treatment of Rothstein and Hayes (1956) and that of Fried, Noggle and Hagen (1958) was intended to

eliminate the D.F.S. ion uptake. Briggs, Hope and Pitman (1958) showed that the initial rapid phase of uptake of cations by plant cells could be accounted for by the existence of a D.F.S. containing a high concentration of non-diffusible anions. But they considered it necessary to use three changes of 10^{-2} M calcium chloride to wash out exchangeable ions from the D.F.S., so that the procedure of Fried, Noggle and Hagen (1958) was possibly inadequate to eliminate the "Donnan free space" effect, and one of their "sites of uptake" might therefore be the Donnan free space.

Briggs, Hope and Pitman (1958) considered that the D.F.S. was located in the cytoplasm, but Keller and Deuel (1957) showed that the "pectin" content of the cell walls of roots of higher plants was sufficient to account for 70 to 90% of the cation exchange capacity of roots; the un-methylated galacturonic acid groups of "pectin" could therefore provide the D.F.S.

Dainty and Hope (1959) were able to show that in Chara australis the D.F.S. was situated in the cell wall, and that the required exchange capacity was provided by the carboxyl groups of the polyuronic acids (Dainty, Hope and Denby 1960). Chemical analysis indicated that these acids were more complex than those reported by Keller and Deuel (1957) both pectin and hemicellulose fractions of the cell wall were involved, and uronic acids other than galacturonic played a part. Crooke, Knight and MacDonald

(1960a) provided additional evidence from studies on storage tissues that cation exchange capacity is satisfactorily accounted for by the "pectin" content of the tissues, although their method would include all uronic acids, and possibly some other compounds, in the "pectin" fraction.

MacRobbie and Dainty (1958), working with the Characean alga (Nitellopsis obtusa,) identified three kinetic compartments involved in the uptake of ions: an outer compartment with very rapid uptake, situated in the cell wall; a slower compartment comprising the cytoplasm; and the very slow compartment identified as the vacuole. Diamond and Solomon (1959), using Nitella, found similar compartments, and suggested that uptake was in series, cell wall to cytoplasm, then to vacuole, although their evidence refers only to the second step in this series. Nevertheless, since it has been shown by several workers (Elgabaly and Wiklander 1949, Smith and Wallace 1956), that uptake of cations by complete plants is highly correlated with the cation exchange capacity of the roots, it seems likely that the first steps in uptake are in series from solution to cell wall and then from cell wall to cytoplasm.

Most of the work referred to has been performed with single celled algae, yeast cells, or simple storage tissue, to avoid the complexities and difficulties involved in the use of the whole plant. It is important that the study of these simpler organisms

should be carried forward to the more highly differentiated tissues and organised systems of the growing plant, even though results are likely to be less clear cut; this point has been stressed by Epstein (1960). An attempt has been made to apply some of these recent developments in this field to a study of the uptake of manganese by oat roots.

Methods

Oats (variety Sun II) were grown in culture solution free from manganese until the roots were 3-8 cms long. The seeds were soaked in distilled water and when germinated were spread on waxed nylon mesh held taut in a metal frame. The waxed nylon rested on a plastic photographic tray 10½" x 8½" x 2¼" deep. In the early stages of growth the seedlings were covered with blotting paper kept moist by a wick leading down to the distilled water in the tray. When the roots had penetrated through the holes in the nylon mesh and were 1 to 2 cms long, the blotting paper was removed and the water in the tray replaced by Hoagland and Arnon's No. 1 solution (1950) modified by the omission of manganese chloride from the micronutrient solution. Under these conditions the shallowness of the tray made aeration unnecessary. It was found that growth in 2 litre beakers of nutrient solution with nylon mesh stretched over the mouth was unsatisfactory without aeration.

When the roots were long enough they were cut off below the

nylon mesh and rinsed two or three times with distilled water before being used. They were weighed after being gently but firmly blotted dry with filter paper.

Normally 0.5 or 1 gm portions of root material were used in each determination. In the uptake experiments performed with inactive manganese sulphate solutions these portions were ashed separately in small porcelain crucibles for about 4 hours at 500°C. The clean ash was then moistened with water and dilute hydrochloric acid added. The contents of each crucible were evaporated to dryness using 50 ml beakers standing on a hot plate as individual water baths. The procedure was then repeated using a few ml of concentrated hydrochloric acid. The residue was taken up in water and filtered into a 50 ml volumetric flask. Aliquots of the resulting solution were used directly for methane-base determinations of manganese by the method of Cornfield and Pollard (1950) modified as previously described. (pp 75-80).

Mn^{54} was used for the work with radioactive manganese. Its half life is 291 days; it was obtained, as manganous chloride, from the Radiochemical Centre, Amersham. This was diluted with inactive manganous chloride to give suitable specific activities. Counting rates were measured using a liquid counter (Type M6) holding 10 ml of solution and connected to a Panax scaler, (Type D554). Where necessary root material was wet-ashed in micro-Kjeldahl flasks using

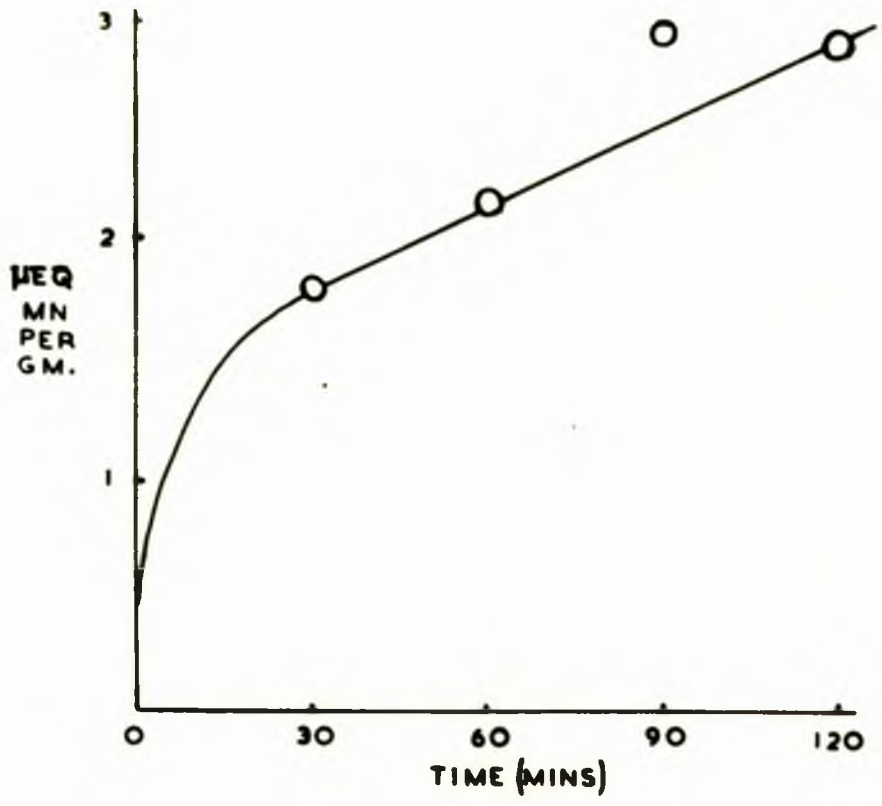


Fig. 34. Time course of uptake of manganese by oat roots from 10^{-4} M manganese sulphate solution.

sulphuric and nitric acids for digestion; the acid digest was made up to volume and a 10 ml aliquot counted, the solution being pipetted directly into the counting tube.

Results

(1) Time Course of Uptake of Manganese by Oat Roots

A representive curve obtained for a time rate of uptake experiment with oat roots is shown in Figure 34. In this, 0.5 gm batches of roots were immersed in 100 ml of 10^{-4}M manganese sulphate solution at room temperatue for varying lengths of time, at the end of which the roots were washed with several changes of distilled water in Buchner funnels before being ashed for manganese analysis.

The uptake curve shows the features typical of cation uptake - there is an initial rapid phase of uptake, which, as will be shown later, is virtually complete within 15 minutes, followed by a steady slower uptake. Similar two phased uptake curves have been described by many workers (see review by Laties, 1959a).

Figure 34 shows another feature which was a constant source of difficulty in this work, namely the lack of uniformity in the batches of root material used. It can be seen that the batch used for the 1½ hour period of uptake had a higher rate of uptake than the rest, and that the extra uptake was too large to be accounted

Fig. 35. Manganese uptake by oat roots.
Dark area indicates region of greater uptake.

for by the degree of experimental error involved in the manganese determination.

It was therefore decided to investigate the roots for possible gross differences in manganese uptake as between different parts of the root.

(2) Location of manganese taken up by the roots

Excised roots were washed and immersed in 10^{-3}M manganese sulphate solution for two hours. They were then washed with distilled water and the area of manganese uptake was located by staining, the technique used being based on a method devised by Batalin (1957) for the detection of manganese in soils.

The roots were irrigated on a microscope slide for 5 minutes with 10% aqueous potassium hydroxide, the excess was removed by blotting and 5% p-anisidine solution was applied. After 5 minutes this was removed and the roots were acidified with dilute sulphuric acid; the presence of manganese was revealed by a purple coloration.

Figure 35 shows that almost all the manganese (the dark area in the photograph) involved in short term uptake is concentrated in the 1 or 2 mms comprising the meristematic zone immediately behind the root cap. Such preparations as these are only temporary as the dye is water-soluble and soon diffuses away.

To exclude the possibility that the area of staining was due to the differential absorption of the dye in this zone, actual

analyses for manganese were performed, using the method of Cornfield and Pollard (1950). After similar immersion in manganese solution, and washing, the tips (about 2 to 3 mms) were separated from the remaining portions of a batch of roots, and the two fractions sucked dry on a sintered glass filter, weighed and analysed. Results are shown in Table 25.

Table 25

	Manganese absorbed by	
	Complete roots	Tips and remainder separated
Tips	400 p.p.m.	260 p.p.m.
Remainder	100 p.p.m.	130 p.p.m.

It is clear that the tips take up about four times as much manganese as the remainder of the root.

The absorption of manganese from 10^{-3}M manganese sulphate solution by separated tips and the remainder of the root was investigated to see if translocation was involved. The two batches of root tips and remaining portions were immersed separately in considerable excess of manganese sulphate solution as before, and analyses for manganese carried out. These results are also shown in Table 25. They can be only approximate, since it is impossible to separate efficiently the meristematic zone from the rest of the root, and the portions of root material represented in the two parts

Fig. 36 a. Root rinsed with distilled water and stained.

Fig. 36 b. Root rinsed with 10^{-2}M calcium chloride solution and stained.

of the table were grown at different times, but it seems possible that some translocation from the upper part of the root down into the meristematic zone is involved, since the figures for the meristematic zone are now significantly lower, and those for the rest of the root higher, than in the previous experiment with complete excised roots.*

The high concentration of manganese found in the meristematic zone is made up, at least for the most part, of exchangeable manganese. This is easily shown by taking the excised roots which have absorbed manganese for two hours as before, and rinsing with 10^{-2} M calcium chloride solutions for 15 minutes before staining. When this is done, no purple coloration develops, as may be seen by the comparison of the photographs in Figure 36; one photograph was taken of a root stained directly after the uptake period, the other was a root from the same batch which was rinsed for 15 minutes with calcium chloride solution and then washed before staining.

Crooke, Knight and MacDonald (1960b) found that in leek roots the cation exchange capacity is considerably greater in the first 5 mms of the root and that the "pectin" content (taking all substances which are decarboxylated by boiling 12% (w/w) hydrochloric acid as "pectin") is also higher, though falling

* This part of the work has been reported in "Nature" (Page, 1961).

short of the cation exchange capacity. Since the unmethylated carboxyl groups of pectin have exchanger properties it seemed likely that the exchangeable manganese of oat roots is taken up by similar "pectins."

An attempt was made to investigate this by measuring the pectin content of the tips and the remaining portions of a batch of oat roots. Some oat roots were grown on waxed nylon mesh over culture solution in the usual way, and cut off below the net. The tips were separated and the two portions dried in a vacuum desiccator over concentrated sulphuric acid. The dry weight of tips was 0.0131 gm and that of the rest of the roots 0.0378 gm. corresponding to a dry matter content of 6%.

A fractionation of the substances likely to be responsible for the cation exchange capacity of the roots was made according to a simplification of the procedure of Ordin, Cleland and Bonner (1955). Protopectins, pectins and soluble sugars were removed by treatment for 1 hour with 0.05 N HCl at 70°C. The roots and tips were filtered off, then treated for 1 hour with 0.5% ammonium oxalate solution at 70°C to remove pectates. Finally hemicelluloses were obtained by treatment with 17.5% sodium hydroxide solution at 25°C for 4 hours. In each treatment 30 ml of solution were used for the tips and 80 ml for the roots.

The uronic acids (galacturonic and glucuronic) in the solutions

were measured by means of the carbazole method of Dische (1947); the detailed procedure used was that described by Stark (1950) for the determination of pectic substances in cotton. By this method quantities of uronic acid in the range 10 to 80 micrograms per ml of solution may be measured. The method is selective for uronic acids, which give a reddish-purple coloration whose intensity can be registered on a Spekker absorptiometer using a green filter.

The extract with 0.05% hydrochloric acid gave a green coloration when the carbazole was added, so that a determination of uronic acids in the pectin and protopecting fraction was impossible. The nature of the interfering substance is unknown, but it was quite evident from the intensity of the green coloration that the proportion of the substance was much greater in the roots than in the tips.

The concentration of pectates was measured in the 0.5% ammonium oxalate solution and was found to be 10.8 peq/gm fresh weight in the roots, and 3.9 peq/gm fresh weight in the tips. Evidently the pectate content of the roots is not responsible for the greater uptake of exchangeable manganese by the root tip.

Hemicellulose uronic acid was measured in the 17.5% sodium hydroxide extract. This was found to be 10.2 peq/gm fresh weight in the roots, and rather more, 12.0 peq/gm in the tips. It seems likely that hemicellulose uronic acids contribute to the cation exchange capacity of the roots, as was found to be the case with the cell walls of Chara australis by Dainty, Hope and Denby (1960), but

the greater exchange capacity of the root tips is not entirely the result of higher hemicellulose content.

It is clear that a considerable amount of investigation is required to determine the substances responsible for the exchange properties of roots, and that new techniques will be required. It is not sufficient to regard all substances which evolve carbon dioxide on heating with 12% hydrochloric acid as "pectins," but existing colorimetric methods are not sufficiently selective, nor free from interference.

It is obvious that in a number of samples of root material the proportion of tip material might vary considerably, and it is believed that the variability of the material found throughout this work is the result of this variation. An attempt was made in later experiments to overcome the variability by cutting up the roots into short lengths and stirring well to mix as thoroughly as possible before taking a sample of root material. This procedure was not conspicuously successful, but seemed to reduce the variability to some extent.

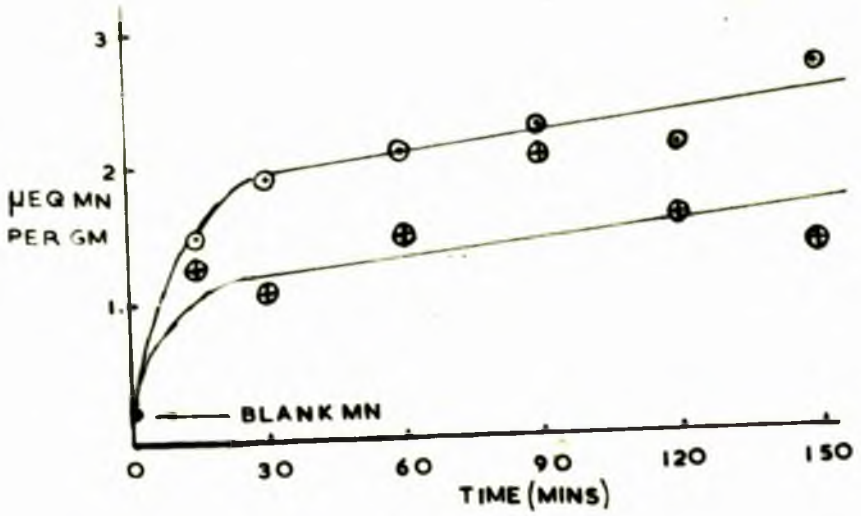


Fig. 37. Interference by calcium ions with manganese uptake.

⊙ Uptake from 10^{-4}M manganese sulphate solution

⊕ Uptake from 10^{-4}M manganese sulphate solution in the presence of 10^{-4}M calcium sulphate.

(3) UPTAKE OF MANGANESE FROM 10^{-4} M MANGANESE SULPHATE, AND INTERFERENCE BY 10^{-4} M CALCIUM SULPHATE.

0.5 gm portions of oat roots were placed for various lengths of time in 100 ml. of either 10^{-4} M manganese sulphate solution, or a solution containing both manganese sulphate and calcium sulphate each at this concentration. Manganese taken up was measured by the methane base method as in the previous section. The time course of uptake curve obtained is shown in Figure 37. The blank value was obtained by a similar determination on fresh roots.

Figure 37 makes it clear that the interference by calcium sulphate takes effect mainly on the uptake in the initial fast phase. The slower so-called metabolic uptake, cannot, on the basis of these results, be shown to be affected; the slopes of the lines showing the course of this uptake are probably the same, although the variability of the material once again makes precision impossible, and it might be that the slow phase of uptake proceeds more rapidly in the case when it is from manganese sulphate solution alone. If the two slow phase uptake rates are the same this would be evidence that the first and second mechanisms of uptake are independent; if the slow phase of uptake proceeds more slowly in the presence of calcium sulphate it would be evidence that the uptake occurs in series, firstly in phase one, then from phase one to phase two, since uptake in phase two would be controlled by the

concentration of manganese reached in phase one, which is clearly less in the presence of calcium sulphate.

Extrapolation backwards of the line of the slow phase of uptake gives intercepts which represent the uptake of manganese in phase one. These are 1.6 μ eq manganese per gm of roots in pure manganese sulphate solution, and 0.9 μ eq in the presence of calcium sulphate.

A second experiment was carried out in which manganese uptake was measured by withdrawing samples of solution at intervals and measuring the manganese remaining in it; uptake by the roots was obtained by comparison with the initial concentration; Three batches of oat roots, each of 10 gm, were used; these were placed in 100 ml of the following solutions - (a) 10^{-3}M manganese sulphate, (b) 10^{-3}M manganese sulphate plus 10^{-3}M calcium sulphate, (c) 10^{-3}M manganese sulphate plus 2×10^{-3}M calcium sulphate.

1 ml portions of each solution were withdrawn at fixed time intervals, and made up to 100 ml. 2 ml. of these solutions were used directly in methano-base manganese determinations by the standard method. Since manganese uptake was obtained by difference it will be plain that small errors in the manganese determinations will appear as large differences of uptake. The difference resulting from an error of 0.02 μg in the actual manganese figure determined (about 1.0 μg) is represented by the lines on the graph - Figure 38 - of the results obtained. All points are the mean of duplicate determinations.

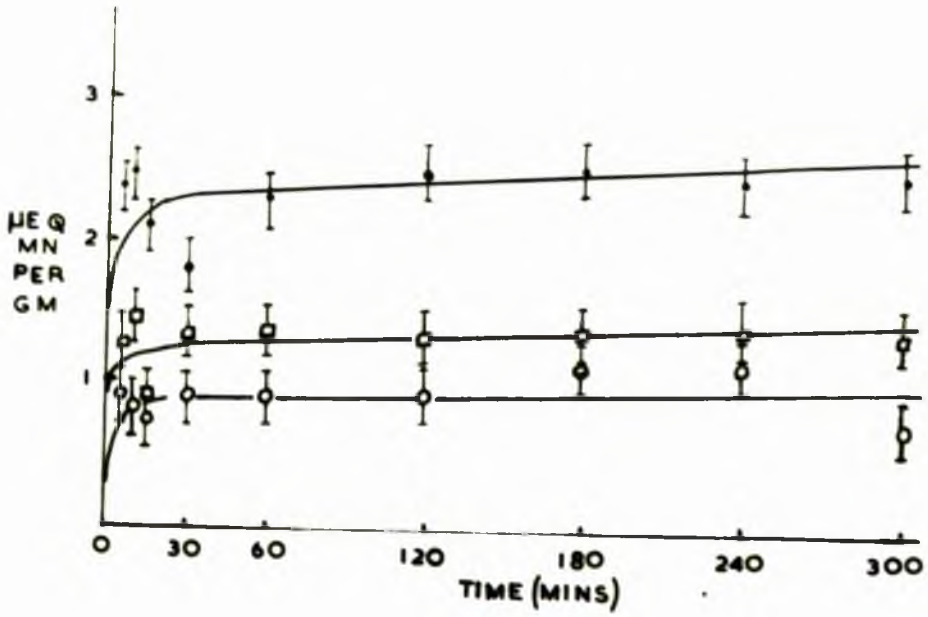


Fig. 38. Interference by calcium ions with manganese uptake.

- Uptake from 10^{-3}M manganese sulphate solution.
- Uptake from 10^{-3}M manganese sulphate plus 10^{-3}M calcium sulphate.
- Uptake from 10^{-3}M manganese sulphate plus 2×10^{-3}M calcium sulphate.

Extrapolation backwards as before gives the uptake of manganese in the first phase. In pure manganese sulphate solution this is 2.31 μ eq manganese per gm of roots; in the presence of an equal concentration of calcium sulphate it is 1.36 μ eq and in the presence of calcium sulphate at twice the concentration of the manganese sulphate it is 0.91 μ eq.

If the only factor responsible for the uptake of manganese in the first phase was the establishment of a Donnan equilibrium in which all divalent ions behaved similarly, the manganese uptake in the presence of calcium sulphate at a concentration equal to that of the manganese sulphate would be one half of that from manganese sulphate alone, and where the calcium sulphate was twice as concentrated as the manganese sulphate, the uptake would be one third as great.

Both experiments indicate that manganese ions are absorbed rather more readily than calcium ions under similar conditions. The immobile anions of the D.F.S. at the beginning of the experiments would be counter-balanced, in the main, by calcium and magnesium ions from the culture solution. The effect of these has been ignored, but would in fact result in slightly less than half the manganese being adsorbed on to the roots in the presence of an equal concentration of calcium sulphate.

(4) THE SLOWER PHASE OF MANGANESE ABSORPTION BY OAT ROOTS

The more rapid phase of cation entry into plant tissues is generally regarded as exchange adsorption which is purely physical, non-selective, and which does not require expenditure of metabolic energy. The second phase, however, has been called that of active absorption, and has been described as requiring energy expenditure derived from aerobic metabolism; (see review by Gauch 1957). It should therefore be susceptible to metabolic poisons, and should be almost completely abolished at low temperatures.

Two treatments were applied to batches of oat roots before the performance of time course of uptake experiments; in the first case the roots were immersed for two hours in 10^{-3}M potassium cyanide, and in the second, the roots were placed in chloroform for one hour. It was found that by this latter treatment 1 gm of roots was reduced in weight to 0.627 gm.

In these experiments radio-active manganese was used. 1 g batches of the roots treated with potassium cyanide were immersed in 10^{-3}M manganese chloride solution for various lengths of time. After rinsing with 15 ml of water and sucking dry on a sintered glass filter, the roots were transferred to 100 ml of 10^{-2}M calcium chloride solution for 15 minutes then washed with 50 ml of water. The combined filtrate of 150 ml was counted. The

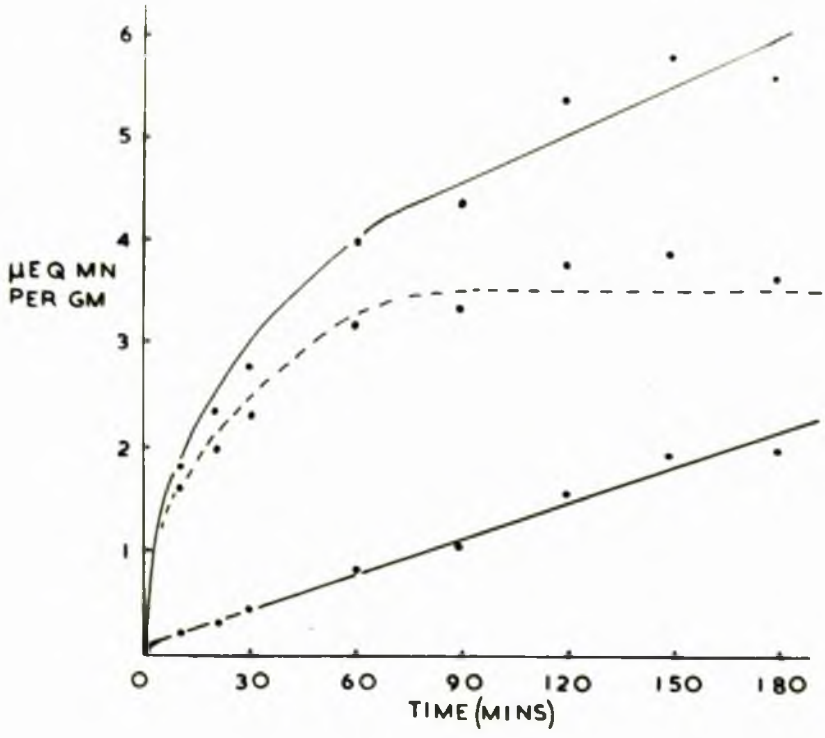


Fig. 39. Effect of potassium cyanide (10^{-3}M) for two hours on manganese uptake by oat roots from 10^{-3}M manganese chloride.

Upper full line - total manganese uptake.

Broken line - exchangeable manganese.

Lower full line - manganese remaining in root.

procedure was then repeated, and the total manganese found in the two 150 ml was taken as "exchangeable" manganese. The non-exchangeable manganese left in the roots was taken to be "absorbed" manganese; this was determined after digestion of the roots in a mixture of nitric and sulphuric acids (5 ml and 2 ml respectively) and dilution of the digest to 25 ml.

The roots treated with chloroform were taken through the same procedure, except that 0.5 gm batches were used and "exchangeable" manganese was determined by washing twice with 50 ml of 10^{-2} M calcium chloride solution followed by two successive washes with 15 ml of water, so that each wash gave a total volume of 80 ml.

Figure 39 shows very clearly that KCN does not abolish the slow phase of uptake, consequently the second phase of manganese uptake does not depend on any cyanide-sensitive step in metabolism. The "exchangeable" manganese, reaches a maximum at the end of about 90 minutes and thereafter remains constant, whereas the manganese "absorbed" by the roots increases steadily with no indication that a maximum has been reached.

Figure 40 shows equally clearly that the second phase of uptake is not affected by treatment for one hour with chloroform, even though this results in the loss of 37% of the weight of the tissues. It seems unlikely that metabolic activity could survive such drastic treatment, since the lipid constituents of the cytoplasm would be removed and profound disorganisation would result. The same

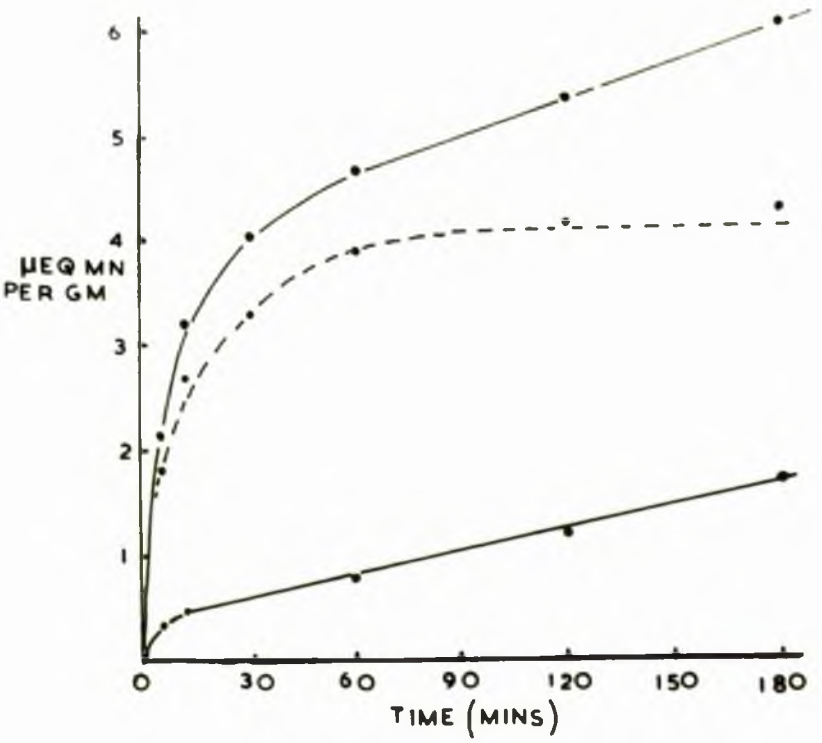


Fig. 40. Effect on manganese uptake from 10^{-3}M manganese chloride of treatment for one hour with chloroform.

Upper full line - total manganese uptake.

Broken line - exchangeable manganese.

Lower full line - manganese remaining in root.

picture emerges as in the cyanide experiment; the two phases of uptake may be separated into a rapid phase, due to exchangeable manganese, complete in about 60 minutes in this case, and a phase of steady accumulation which shows no sign of approaching completion in 180 minutes.

Figures 39 and 40 may be compared with the results for uptake by normal roots at room temperature as shown in Figure 41. In all three cases a similar separation into two phases of uptake may be made.

The phase of steady accumulation does not appear to account for all the non-exchangeable manganese in the root. Figure 40 shows that there is an initial period of less than two minutes in which a small amount of manganese is taken up very rapidly, and only after this does accumulation proceed steadily. This gives non-linear uptake during the first few minutes, so that the resulting graph presents a "shoulder" and the linear uptake does not extrapolate to zero. A similar shoulder is present, but less evident, in Figure 39. The binding of manganese which occurs at this site must be very firm as well as rapid since the manganese is not exchangeable. The quantity involved is not more than 0.25 μ eq of manganese per gm (fresh weight) of root material.

There can be few metabolic processes which would remain unaffected by the cyanide or chloroform treatments, but all should be slowed almost to stopping by low temperatures in the region of

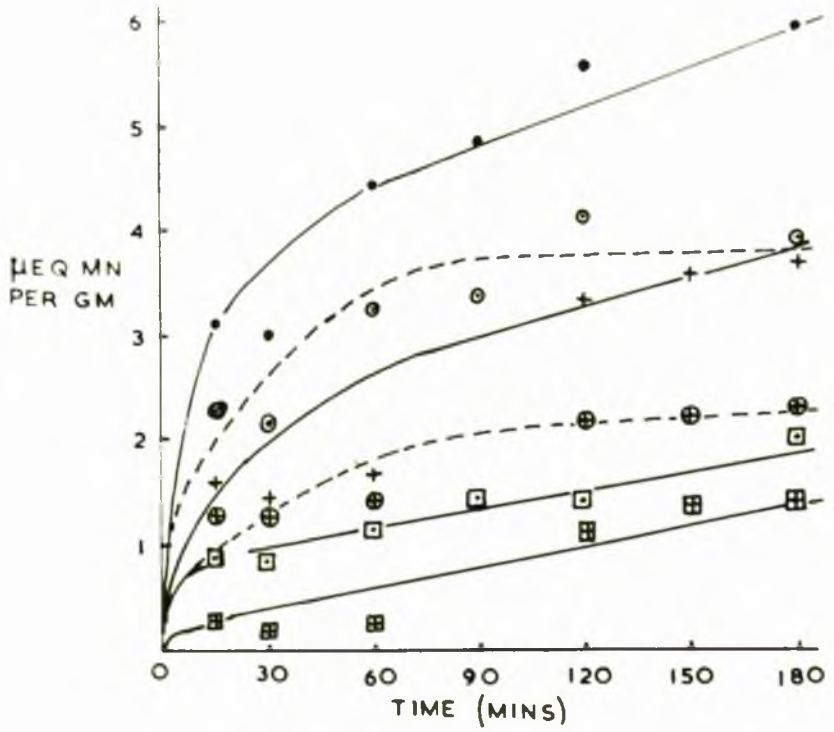


Fig. 41. Effect of temperature on manganese uptake from 10^{-4} M manganese chloride solution.

Uptake at 20° :- Total uptake •

Exchangeable manganese ⊙

Residual manganese ⊡

Uptake at 0° :- Total uptake of manganese +

Exchangeable manganese ⊕

Residual manganese ⊞

0°C. An uptake experiment was therefore carried out in which 0.5 g portions of roots were placed in 50 ml of 10^{-4}M manganese chloride, one series being at room temperature (20° ± 1°) and another in beakers placed in melting ice. The temperature of the solution in these beakers was 4°C ± 0.5°, except that for a short time the contents of the beakers in which uptake was for 30 minutes and 60 minutes the temperature fell to 0°C.

The results of this experiment are shown in Figure 40. It is quite evident that the slow phase of uptake has not been abolished by conducting the uptake at low temperature, indeed it has been affected less in proportion than has total uptake. There are some signs that equilibrium in the first phase of uptake has been reached rather more slowly at 4° than is usual at higher temperatures, as might be expected in an ion exchange process limited by the rate of diffusion of the ions, which would of course be slower at lower temperatures. The absorption shoulder for the slower phase of accumulation is rather large at room temperature, but appears to have been nearly abolished at 4°. It is interesting that the two batches of roots whose temperature fell for a short time to 0° did not seem to recover their power to absorb manganese above the residual level of the absorption shoulder.

Whatever may be the position for other ions, these experiments show that for manganese the slow phase of absorption is not dependent on metabolic energy. This might indicate that a physical process is

involved, but if so, Figure 40 shows that its rate is little affected by the temperature change from 20° to 4°C, so that any process rate controlled by diffusion would be excluded.

(5) TIME COURSE OF RELEASE OF MANGANESE FROM CAT ROOTS

The lgn of excised roots used were allowed to take up manganese from 100 ml of 10^{-4}M radioactive manganese chloride. At the end of two hours the roots were washed with distilled water and sucked dry on a sintered glass filter. They were then placed in 100 ml of 10^{-2}M calcium chloride solution.

At intervals 10 ml of solution were withdrawn and counted, and the 10 ml were replaced by fresh calcium chloride solution to maintain constant volume conditions. At the end of three hours the roots were washed with distilled water and transferred to a micro-Kjeldahl flask for wet ashing with nitric and sulphuric acids. The digest was made up to volume and counted. The total activity at zero time was calculated from the final activity plus the total activity removed into the calcium chloride solution, and from this successive deductions gave radioactivity remaining in the roots at each time interval. A semi-logarithmic plot of the radioactivity remaining in the roots against time is given in Figure 42A. Initially the radioactivity remaining in the roots falls very rapidly, but by the end of one and a half hours the slope of the line has become constant. By extrapolation backward of this straight line portion of the

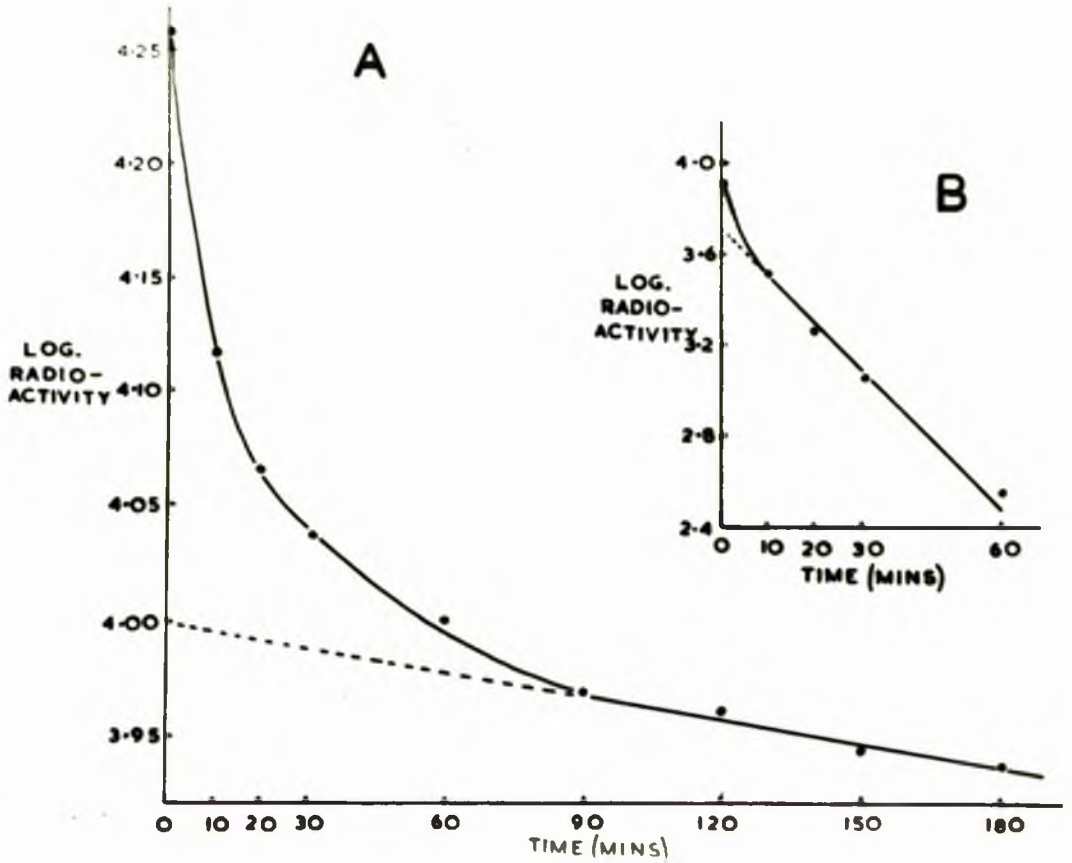


Fig. 42. Time course of release of manganese from oat roots.

A Total activity remaining in roots.

B Fast exchange, obtained by subtraction of straight line component of A from total activity at the respective times.

(1 gm of roots placed in 100 ml of 10^{-2} M calcium chloride solution)

curve and subtraction of the extrapolate from the total activity the curve may be separated into two fractions, an initial rapid release of manganese and a slower fraction. The figures obtained by subtraction of the extrapolate from the total activity are plotted semi-logarithmically in Figure 42B, giving the "fast" exchange. This is linear with time, indicating that only one exchange rate is involved, the half time of which, calculated from Figure 42 B, is 14 mins. The half-time, $t_{\frac{1}{2}}$, for the slow exchange is about 830 minutes. About 45% of the total activity is accounted for by the fast fraction, whereas 40% of the total activity is removed during the first 30 minutes. Since simple immersion of the roots in calcium chloride solution would be less efficient in the removal of exchangeable manganese than two washings with calcium chloride solution, it is considered that the two calcium chloride washings, each given for 15 minutes, in the routine treatment of the roots, gave reasonably complete removal of exchangeable manganese, which is identified with the fast fraction found in this experiment.

The slow fraction must be the fraction which has usually been regarded as metabolically accumulated, although it has been shown that for manganese metabolic energy is not involved in its uptake. Accumulation of ions against a concentration gradient would involve types of binding and possibly transport which would render the ions non-exchangeable; one would not therefore expect ions accumulated

metabolically to behave as exchangeable ions. The ions accumulated non-metabolically here are probably not held in an exchangeable form either. The rate of exchange of such ions would be limited by the speed at which calcium ions could diffuse to the exchange site, and manganese ions diffuse away. For a process rate limited by diffusion a half time of 830 minutes could only justified if the distance involved were much greater than could be possible in oat roots, or if diffusion were taking place through such narrow passages that a "queueing up" effect would be imposed.

It will be noted that in Figure 42 B, the point at zero time is not on the same straight line as the other points. This is because the equation for radial diffusion of ions is not linear with time at first, but becomes so after a short interval. If some irreversibly bound manganese were present, the point at zero time would be further removed from the extrapolate than would be accounted for by the diffusion equation. Although in this case the deviation appears to be large it is not possible to deduce the presence of irreversibly bound manganese because the information required to solve the diffusion equation is lacking.

(6) THE EFFECT OF TEMPERATURE ON THE UPTAKE OF MANGANESE BY OAT ROOTS

In a preliminary experiment 1 gm batches of roots were placed in 50 ml of 10^{-4}M manganese sulphate solution in beakers at 29°C in a thermostatically controlled water bath or in melting ice, and at the end of two hours the roots were filtered off, washed with distilled water, and analysed for manganese by the methane base method. After subtraction of the amount of manganese present in the fresh roots (0.145 μ eq. per gm) the uptake figures were found to be 2.29 μ eq per gm of roots at 29°C, and 1.80 μ eq per gm at 0°C.

A trial showed that in a similar experiment with 10^{-6}M manganese sulphate the determination of the small quantity of manganese (less than 0.1 μ eq) could not be made sufficiently precisely by this method, though the impression was gained that the variation with temperature was greater than with the more concentrated solution. This might, however, be due to the fact that the amount of manganese taken up under these conditions by the roots is almost equal to the total amount of manganese in solution, so that in the course of uptake the environmental conditions of the roots change very considerably. It would be necessary to use large quantities of solution in an experiment of this nature, and to use radio-active tracer techniques to obtain reliable figures representing genuine uptake.

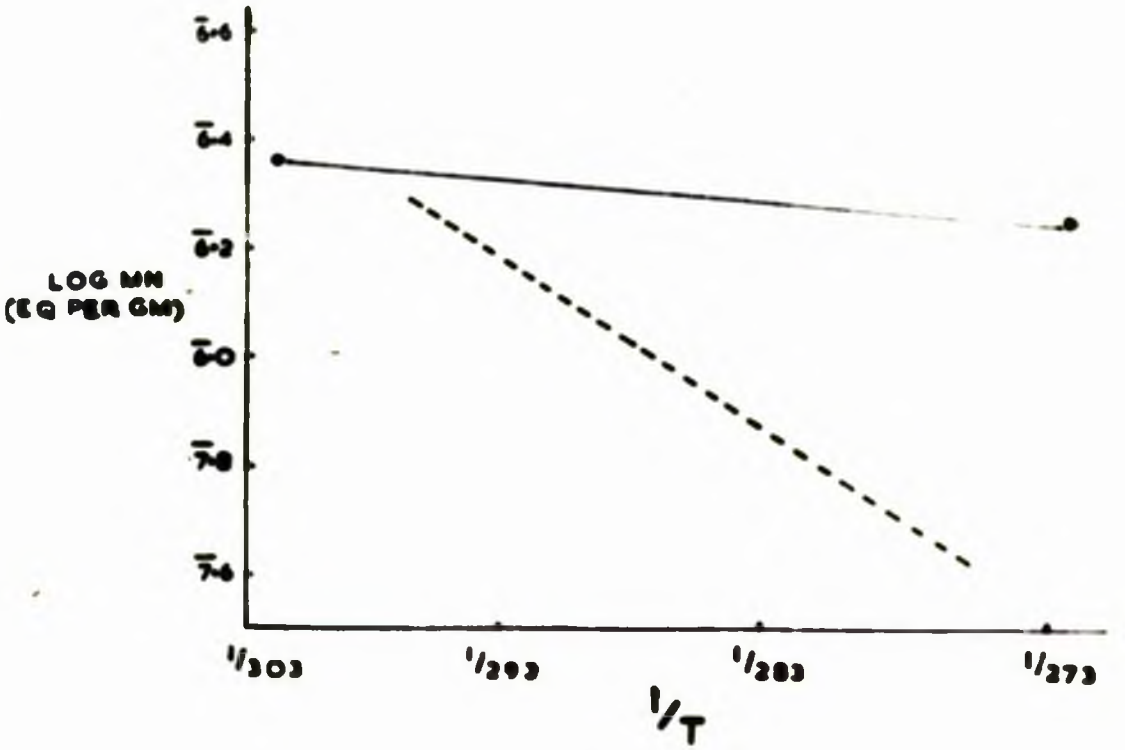


Fig. 43. Temperature dependence of manganese uptake.

Full line - Manganese taken up by 1 gm of oat roots from 50 ml of 10^{-4} M manganese sulphate solution.

Broken line - Temperature relationship of a reaction whose velocity doubles when temperature rises 10°C.

Most enzymic reactions show a marked temperature dependence, such that the rate of the reaction approximately doubles with every 10°C rise in temperature in the range 0° to 30°C. So long as the reaction continues steadily the amount of product is a measure of the velocity constant of the reaction. If this were the case with manganese uptake a plot of log Mn against 1/T, where T is the absolute temperature, would give a result something like that shown by the dotted line in Figure 43. The energy of activation of the reaction may be calculated from the slope of such a line, and in this case would be about 10,000 calories.

The figures obtained in the manganese uptake experiment described above, when plotted in Figure 43, give the full line, which is obviously different in slope from that expected for an enzymic reaction. The slope of this line cannot, however, be used to calculate a figure for energy of activation, since it has been shown in the previous sections that manganese uptake has two main phases, the faster being complete in much less than two hours, and the slower one only proceeding in a manner linear with time.

For the slower phase of uptake the energy of activation could be obtained from a plot similar to that shown in Figure 43, but for a reaction which has reached equilibrium this treatment gives the enthalpy, or heat content of the reaction.

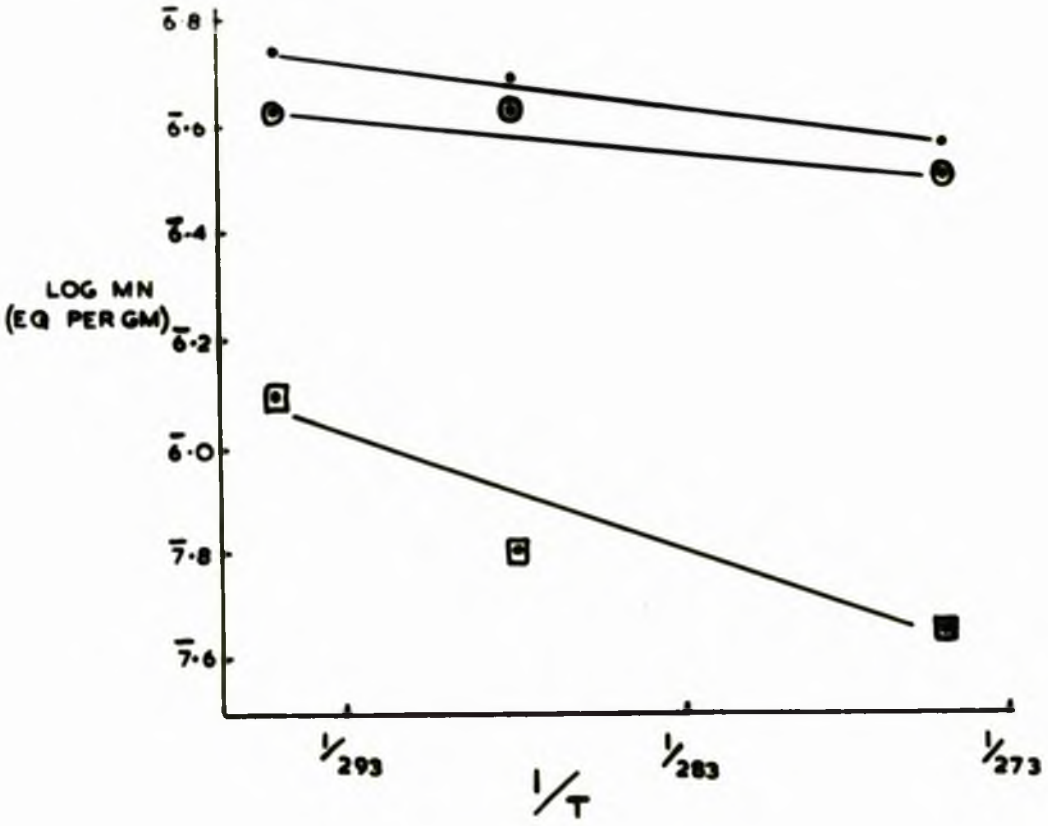


Fig. 44. Temperature dependence of manganese uptake.

Uptake by 0.5 gm roots immersed for 2 hrs. in 50 ml of 10^{-4} M radio-active manganese chloride solution.

- • Total uptake of manganese.
- ◎ Exchangeable manganese.
- ◘ Residual manganese left in root.

Fig. 44. Temperature dependence of manganese uptake.

Uptake by 6.5 gm roots immersed for 2 hrs. in 50 ml of 10^{-4} M radio-active manganese chloride solution.

Total uptake of manganese.

Exchangeable manganese.

Residual manganese left in root.

The experiment was therefore repeated, using radio-active manganese, and applying the standard procedure for removing exchangeable manganese by washing twice for 15 minutes each time with 10^{-2}M calcium chloride solution, and determining the manganese so removed, as well as the residual manganese remaining in the roots. 0.5 gm batches of roots were used, immersed in 10^{-4}M manganese chloride solution for two hours in beakers at room temperature (22°C), in continuously running tap water (14.5°C) and in melting ice (2°C). Each determination was in duplicate; the means of the duplicate values are used in the graph shown in Figure 44.

The temperature relationship of the total uptake is similar to that shown in Figure 43, and is almost the same as the temperature relationship of the exchangeable manganese. This fraction of the total manganese reaches its equilibrium value in much less than two hours, so that from the slope of the line the change of enthalpy, ΔH, of the reaction can be calculated. This is + 1,965 calories per equivalent of manganese, hence the reaction involved in the replacement of manganese for (presumably) calcium in the exchange complex is an endothermic one.

The much smaller fraction of manganese remaining in the root has a quite different temperature relationship. The uptake here has not reached equilibrium, so that the quantity of manganese

taken up is a measure of K, the velocity constant, and may be used to calculate the energy of activation of the reaction. This is 7,040 calories per equivalent of manganese.

(7) BINDING SITES INVOLVED IN MANGANESE UPTAKE

If the uptake of ions is selective in the sense that specific sites are utilised at which pairs of ions may, or may not, cause mutual interference, it seems surprising that two sites of uptake, neither more nor less, have been found in every example that has been investigated. Manganese uptake by yeast cells was shown by Rothstein and Hayes (1956) to utilise two binding sites; it was decided to use their technique to find out whether oat roots behaved similarly.

2 gm portions of roots, blotted firmly to remove surface moisture in the usual manner were placed in manganese sulphate solutions of various strengths for 2 hours at room temperature (21.5°C) 100 ml of solution was used in each case; the concentrations employed were 10^{-3} x 0.5M, 10^{-4} x 0.75M, 10^{-4} x 0.5M, 10^{-4} x 0.25 M, 10^{-6}M, 10^{-6} x 0.5M and 10^{-7}M, and a distilled water control.

At the end of the uptake period the roots were thoroughly washed with several changes of distilled water and sucked dry on a Buchner funnel; they were then ashed and manganese determined by means of the methane base method. Manganese taken up, or "bound" as Rothstein and Hayes called it, was obtained by

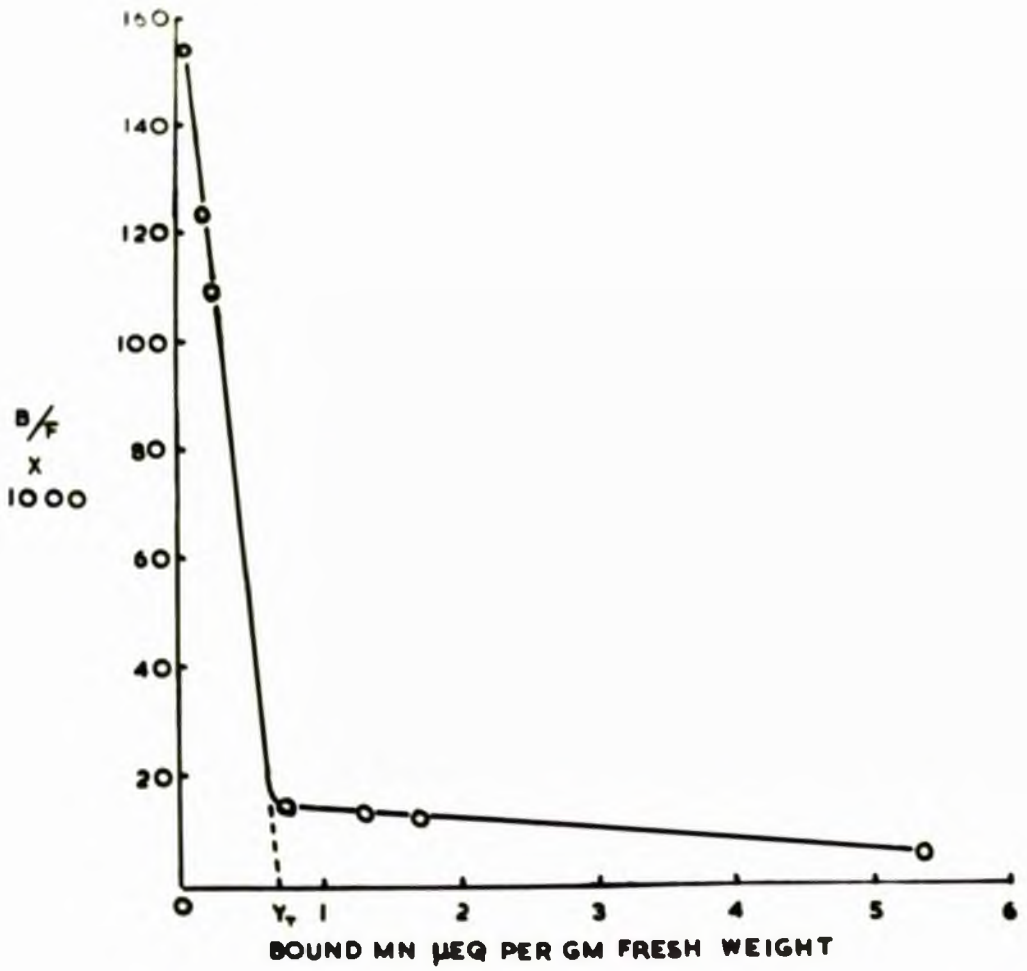


Fig. 45. Results of uptake experiment; 2 gm of roots were placed in 100 ml of manganese sulphate solutions of various concentrations for 2 hrs. at room temperature.

B = "Bound manganese" = Total uptake of manganese.

F = concentration of manganese in solution in µeq per litre.

subtracting the manganese content of the water control.

The results were plotted with "bound" manganese as abscissa, and "bound"/"free" manganese as ordinate, "free" manganese being the initial concentration of the manganese solution used. The graph obtained is shown in Figure 45, the form being similar to the plot obtained by Rothstein and Hayes (1956) for yeast cells, so that it would appear that the mechanisms of uptake of manganese by oat roots are similar.

This method of plotting the results was based on the application of enzyme kinetics, although Fried, Noggle and Hagen (1958) took care to point out that the equations are only formally identical when ion uptake is considered.

The Michaelis-Menten theory of enzyme action may be represented by the equation

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_3} E + P$$

when E is the free enzyme, S is the substrate, C is an intermediate enzyme - substrate complex, and P is the product of the reaction.

The reaction velocity at any instant is $v = k_3 C$. When all the enzyme is combined with substrate, that is when substrate is in great excess, v reaches its maximum value V_m.

It was shown by Michaelis and Menten (1913) that

$$\frac{v}{V_m} = \frac{[S]}{[S] + K_m}$$

where K_m is the Michaelis constant $= \frac{k_2 + k_3}{k_1}$

This was rearranged by Hofstee (1952) to give

$$v = V_m - \frac{v}{[S]} K_m$$

If the reaction continues at a steady rate, v may be measured by the quantity of product. Assuming that U = vt then U is the uptake in a time t, the uptake of manganese plotted against uptake/ concentration of manganese in solution would give a graph as shown in Figure 46A when one mechanism or enzyme is involved, or as in Figure 46B when two are involved.

Rothstein and Hayes based their reasoning on that of Scatchard (1949) and used the equation

$$\frac{(MY)}{(M)} = \frac{f_m Y_T}{K_1} - \frac{f_m (MY)}{K_1}$$

where M = free cation concentration in solution

Y_T = total concentration of free cell ion binding groups

MY = concentration of cations occupying binding sites

f_m = the activity coefficient of the cation in solution

K_1 = the velocity constant.

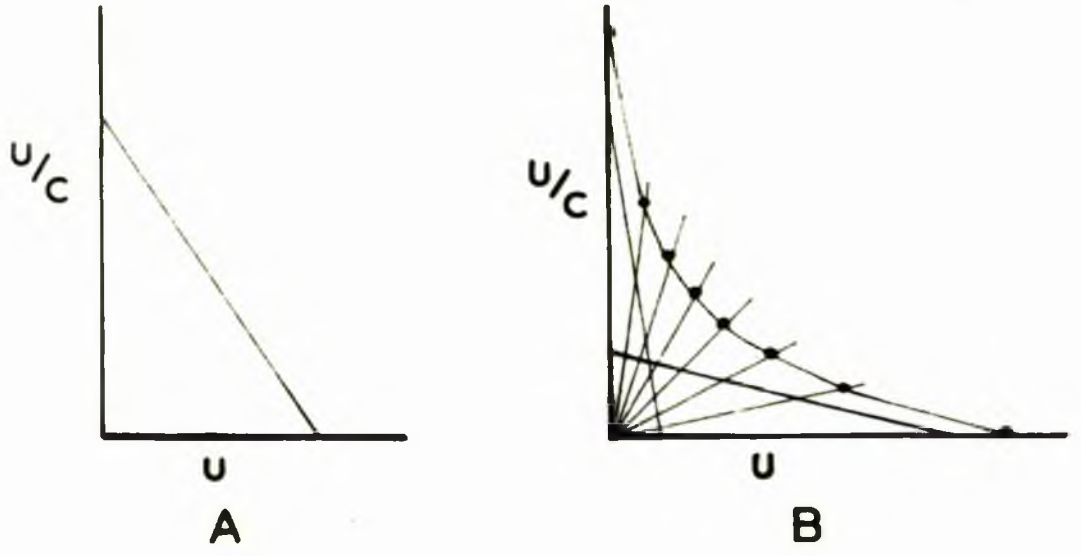


Fig. 46. Hofstee plots.

A - one uptake mechanism.

B - two uptake mechanisms. (Summation of the effects is by addition of the components for each triangle along the lines radiating from the origin of the graph.)

U = uptake.

C = concentration of substrate.

This is essentially the same as the treatment used by Hofstee. But since K_1 is a velocity constant, this form of equation can be used only when the process of uptake proceeds steadily throughout the period investigated. Consideration of the time uptake curve shown in Figure 34 makes it clear that this condition does not apply, so that Rothstein and Hayes interpretation of their results would appear to be at fault. (Incidentally, their method of obtaining Y_T by extending the steeper slope to cut the abscissa is also obviously incorrect; a figure obtained by the summation of two triangles each given by an equation similar to theirs, cannot be dissected into its components merely by extending one of its boundaries. Their erroneous method is illustrated in Figure 45; (compare Figure 46B).

A similar figure may be obtained, however, by plotting "bound" manganese against " bound"/"free" manganese when other considerations apply. If the case is considered where uptake of manganese takes place by ion exchange with calcium ions at exchange sites governed by the laws of Donnan equilibrium a graph of the same form is obtained.

Let the D.F.S. of the roots be v cm^3. and the volume of the external solution be V $cm.^3$ Let A $eq/cm.^3$ be the concentration of indiffusible anions in the D.F.S.

Then <u>at equilibrium</u> the following equations apply

$$\frac{[Ca^{++}]_i}{[Ca^{++}]_o} = \frac{[Mn^{++}]_i}{[Mn^{++}]_o} = r \qquad (1)$$

$$[Ca^{++}]_i + [Mn^{++}]_i = A \qquad (2)$$

$$v\,[Ca^{++}]_i + V\,[Ca^{++}]_o = v\,A \qquad (3)$$

$$v\,[Mn^{++}]_i + V\,[Mn^{++}]_o = v\,[Mn^{++}] \qquad (4)$$

when $[Mn^{++}]$ is the initial concentration of manganese in the external solution.

From (1) and (3) $$[Ca^{++}]_i = \frac{v\,A}{v + V/r} \qquad (5)$$

and from (1) and (4) $$[Mn^{++}]_i = \frac{V\,[Mn^{++}]}{v + V/r} \qquad (6)$$

Substituting (5) and (6) in (2)

$$v\,A + V\,[Mn^{++}] = A(v + V/r)$$

which gives $$r = A/[Mn^{++}] \qquad (7)$$

Substituting in (6)

$$[Mn^{++}]_i = \frac{V\,[Mn^{++}]}{v + \frac{V}{A}[Mn^{++}]}$$

$$= \frac{A\,[Mn^{++}]}{\frac{v\,A}{V} + [Mn^{++}]} \qquad (8)$$

Hence uptake, U, of manganese per gm of tissue, at equilibrium is given by $v[Mn^{++}]_1$

$$\therefore U = \frac{v A [Mn^{++}]}{\frac{v}{A} + [Mn^{++}]} \quad (9)$$ *

An alternative form of this equation is

$$U = vA - \frac{v}{A} \frac{U}{[Mn]}$$

if U is plotted against $U/[Mn]$ the same type of graph is given as is shown in Figure 46A.

There would seem to be an alternative explanation for the results of the manganese uptake experiment with oat roots, which would also apply to the findings of Rothstein and Hayes (1956) using yeast cells. Instead of two enzymic processes, or mechanisms having kinetics similar to enzymic processes, it seemed possible that only one such mechanism existed, and was accompanied by an ion exchange process which had reached equilibrium. This would appear to be consistent with the time curve of uptake which shows a rapid phase of uptake, believed to be due to ion exchange, and essentially complete in 15 minutes, followed by a slower continuous uptake, presumed to be enzymic in character. If this hypothesis should prove true, it would provide an explanation for the invariable finding that two mechanisms of uptake are involved whatever ion has

* I am indebted to Dr. J. Dainty for this proof.

been investigated.

To test this theory an experiment was carried out using radioactive manganese, on the same lines as before. 0.5 gm portions of roots were immersed for two hours in solutions of manganese chloride of the following strengths:- 10^{-3} x 0.5M, 10^{-4}M, 10^{-5}M, 10^{-5} x 0.5M, 10^{-6}M, 10^{-6} x 0.5M and 10^{-7}M. Except with the 10^{-3} x 0.5M solution two lots of solution were used, one of 50 ml, and one of 100 ml.

At the end of the absorption period of two hours the roots were washed with distilled water (15 ml) then placed in 50 ml 10^{-2}M calcium chloride for 15 minutes, then filtered off and washed with 30 ml of distilled water; this treatment was then repeated. Each total of 80 ml filtrate and washings was counted separately; the combined count gave the exchangeable manganese. The roots were transferred to micro-Kjeldahl flasks and ashed with nitric and sulphuric acids, and the digest counted to give adsorbed manganese. A count was also performed on the solution from which manganese had been taken up.

The actual figure for the counts obtained are given in Table XXIV of the Appendix. From these figures the actual quantities of manganese were calculated.

Figure 47 is obtained by plotting the total uptake of manganese against uptake divided by initial concentration of manganese in solution, and shows the same features as Figure 45.

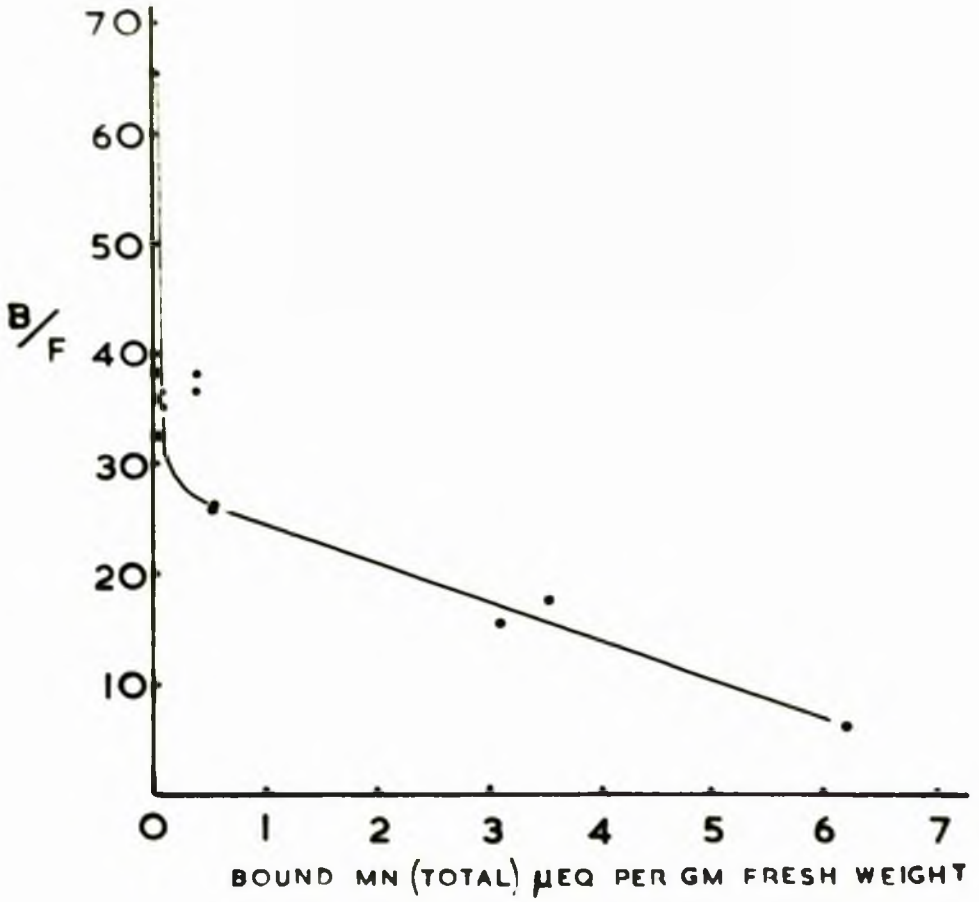


Fig. 47. Hofstee plot of manganese uptake by oat roots immersed in manganese chloride solutions of various concentrations.

B = "Bound manganese" (Total uptake)

F = "Free manganese" = Original concentration of manganese in µeq per ml.

(In these circumstances B/F represents a ratio of manganese per gm of root to manganese per ml of solution.)

The figures for exchangeable manganese, that is, the manganese removed from the roots by two consecutive washings, each of 15 minutes duration, with 10^{-2}M calcium chloride solution, are plotted in a similar manner in Figure 48. This is of the same form as Figure 46, within the fairly wide limits of experimental error; only the result obtained when uptake was from 100 mls of 10^{-7}M manganese chloride appears to deviate seriously, and here, of course, any error is likely to be large. The results are thus consistent with an equation of a type similar to equation (9) expressed in the form

$$U = vA - \frac{vA}{v} \frac{U}{[Mn^{++}]}$$

When $U/[Mn^{++}]$ tends to zero, that is when external manganese concentration is high, $U = vA$, so that an estimate of vA, the concentration of immobile anions of the D.F.S. can be obtained from Figure 48 by extrapolation. This is about 6.0 meq per gm (fresh weight) of roots.

If this value is inserted in equation (9) rearranged into the form

$$U = \frac{vA\,[Mn^{++}]}{\frac{vA}{v} + [Mn^{++}]}$$

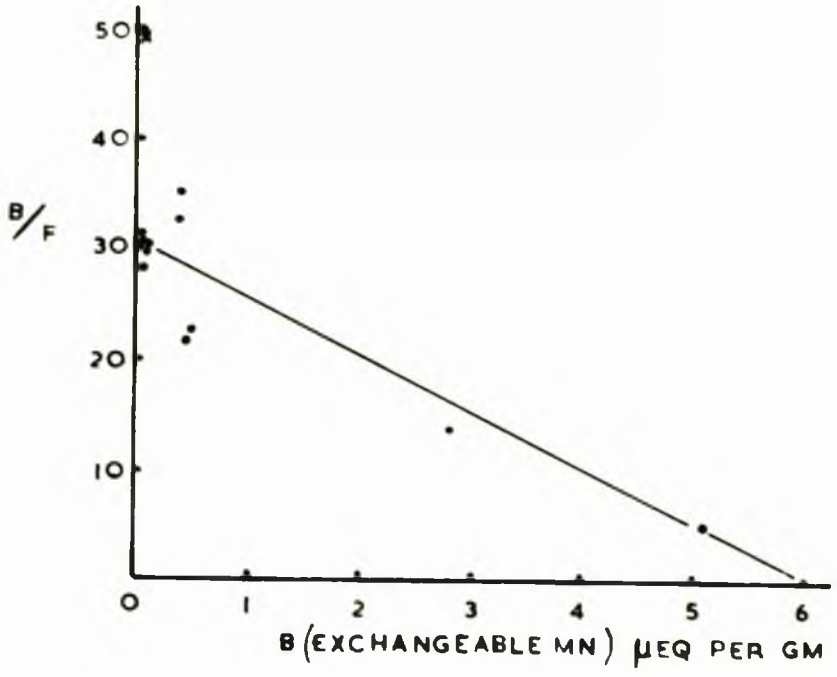


Fig. 48. Hofstee plot of exchangeable manganese (B) taken up per gm of roots from solutions of manganese chloride of various concentrations (F)

B = exchangeable manganese, µeq per gm.

F = initial manganese concentration, µeq per ml.

and using the appropriate initial values of $[Mn^{++}]$ theoretical values of uptake may be calculated, and compared with those actually found. The results obtained are shown in Table 26; column (1) shows the values obtained by inserting into the equation the values of V actually used in the experiment. It may be seen that the results deviate considerably from the measured uptake of exchangeable manganese. If however the calculation is repeated, inserting a value for V of 33⅓ ml to 1 gm of roots the figures are obtained which are shown in column (2); these are quite close to the amounts of exchangeable manganese actually taken up by the roots.

The explanation of this apparent deviation from the theoretical equation lies in the conditions of the experiment. The solutions were unstirred; evidently mixing by diffusion is inadequate during an uptake period of two hours, so that the effective volume V was less than that actually employed, and was something like 17 ml of solution for each 0.5 gm of roots. This explains why uptake was similar in solutions of the same strength whether 50 ml or 100 ml of solution were used, although considerable differences would be expected from equation (9,) as is shown in Table 26.

With adequate arrangements for stirring, for example by blowing a stream of moist air through the solutions to give thorough agitation, there is every reason to believe that equation (9) would be found to apply to the uptake of exchangeable manganese.

Table 26

Initial Mn conc. (eq/litre)	V ml	U = Mn uptake, μ eq/gm calculated (1)	(2)	observed
2×10^{-7}	50	0.0199	0.00665	0.00628
2×10^{-7}	100	0.0397	"	0.0099
10^{-6}	50	0.0984	0.0332	0.0282
10^{-6}	100	0.1937	"	0.0307
2×10^{-6}	50	0.1935	0.0658	0.0608
2×10^{-6}	100	0.375	"	0.0594
10^{-5}	50	0.858	0.326	0.328
10^{-5}	100	1.500	"	0.351
2×10^{-5}	50	1.900	0.600	0.434
2×10^{-5}	100	2.400	"	0.455
2×10^{-4}	50	4.61	3.16	2.77
2×10^{-4}	100	5.21	"	2.77
10^{-3}	50	5.66	5.08	5.07

N.B. The volume V corresponds to 0.5 gm of root material.

(1) U calculated from equation (9) using V = 50 or 100 ml per 0·5 gm roots.

(2) U calculated from equation (9) using V = 33⅓ ml per gm roots.

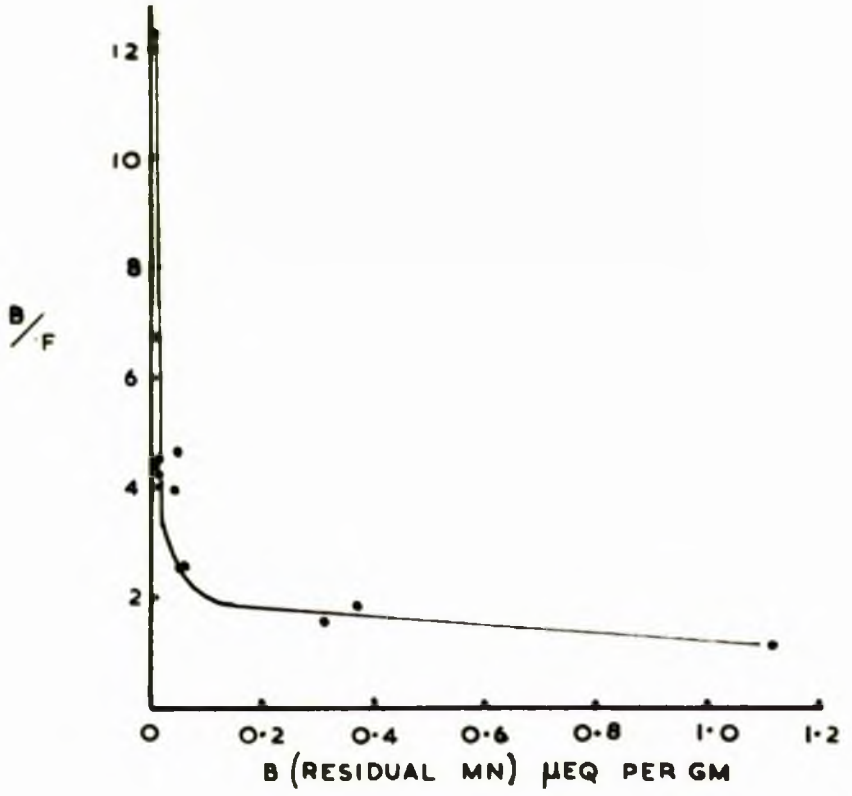


Fig. 49. Hofstee plot of residual manganese (B) taken up per gm of roots from solutions of manganese chloride of various concentrations (F)

B = residual manganese, µeq per gm (i.e. manganese not removed by washing with 10^{-2}M calcium chloride solution)

F = initial manganese concentration, µeq per ml.

If the figures for the residual manganese which remains in the root after washing with calcium chloride are plotted in the same way Figure 49 is obtained. It is quite clear that the removal of the exchangeable manganese has not left a simple situation in which only one uptake mechanism remains in operation as had been thought possible. It is still quite evident that two mechanisms of uptake, or two sites of uptake, remain; the two sites found by Fried and Noggle (1958) and Fried, Noggle and Hagen (1958) for K, Rb, Na and Sr, are not entirely explicable by the failure of their procedure to remove all the exchangeable ions.

Discussion

These experiments indicate that at least three mechanisms operate in the uptake of manganese by oat roots. These are the ion-exchange mechanism involving the D.F.S. of the tissue, and two mechanisms associated with the slower phase of uptake which has often been assumed to be metabolic, but which has been shown in this case at least to be non-metabolic. There may be a fourth mechanism connected with the "shoulder" noted in the slow uptake in Figures 39, 40 and 41, which may also account for the small fraction of irreversibly bound manganese indicated in Figure 42B.

Of these mechanisms ion exchange has been studied most. The immobile anions of the D.F.S. are now identified as unmethylated uronic acids, mainly galacturonic acid units in the pectin fraction and glucuronic acids in the hemicellulose fraction of the cell wall. The concentration of immobile anions in the manganese uptake experiment represented in Figure 48 was 6.0 μeq/gm fresh weight of roots. This appears to be lower than the recorded values of cation exchange capacity of oat roots found in the literature; Drake, Vengris and Colby (1951) give 13.68 μeq/gm and McLean, Adams and Franklin (1956) found 11.28 and 13.98 μeq/gm in two samples investigated. (Values have been recalculated from meq/100 gm dry matter, on the basis of 6% dry matter.) It is possible that their method of determination of cation exchange capacity

(electrodialysis followed by titration with potassium hydroxide solution) might result in some de-methylation of uronic acid carboxyl groups and consequent apparent increase in cation exchange capacity.

If values of cation exchange capacity are calculated from the figures for manganese uptake given in Table 25, the values are found to be 14.5 peq/g and 9.45 peq/g for tips and 3.63 peq/g and 4.72 peq/g for the remainder of the roots in the two experiments, values which do not conflict with the 6.0 peq/g found in the uptake experiment shown in Figure 48. Dr. N. King of Edinburgh University kindly determined the uronic acid content of a sample of these oat roots by a decarboxylation method (Anderson 1959) which would give a total for methylated plus non-methylated carboxyl groups, and found it to be 43 ueq/gm fresh weight. It would therefore seem likely that a high proportion of the carboxyl groups are methylated and do not contribute to the cation exchange capacity. The high cation exchange capacity of the tips of the roots is so far unexplained; there is no evidence that it is connected with any of the fractions obtained in the extraction experiment, so that pectins, protopectins, pectates and hemicelluloses are apparently not responsible. Crooke, Knight and MacDonald (1960b) found similarly that in leek roots the cation exchange capacity of the root tips far exceed the "pectin" content, although further up the root the "pectin" content was much

greater than the cation exchange capacity. This they ascribed to increasing methylation of the carboxyl groups of the pectin, but were unable to account for the high exchange capacity of the root tips. It is evident that there is a great deal of scope for enquiry into the nature and location of the substances responsible for the cation exchange capacity of roots.

The exchangeable manganese in oat roots behaves approximately according to the laws governing Donnan equilibria, although the interference effects shown by calcium ions deviate a little, probably because factors other than valency, such as ionic radius and degree of hydration, are involved in homovalent exchange. The exchange has a half-time in oat roots when the external calcium ion concentration is 10^{-2}M, of about 14 minutes, longer, as might be expected, since the tissue is more complex, than that found by Dainty and Hope (1959) for calcium, when the external concentration was 0.5×10^{-2}M using Chara australis walls ($t_{\frac{1}{2}}$ about 20 secs).

The distribution of the anions giving rise to the Donnan effect is not uniform throughout the oat root tissues; the concentrations of these anions in the cell walls is far greater in the meristematic tissue in the tips of the roots in the area immediately behind the root cap.

In short term experiments most of the manganese uptake is accounted for by this exchangeable fraction, which reaches its maximum value after about an hour in most cases, although in this

respect, as in others, different batches of roots were found to be very variable.

The significance of this rapid phase of uptake is not yet known. It may be a purely trivial property of plant cells, not playing any vital part for the nutrient economy of the plant as a whole. If this is so, exchange uptake by the cell walls must be unconnected with uptake by the cytoplasm and the rest of the plant, that is to say, it must be in parallel, not in series, with the uptake into cytoplasm and vacuole. Diamond and Solomon (1959) believe that uptake goes in series from medium to cell-wall to cytoplasm and then to vacuole in isolated cells, but their evidence does not apply to the step from cell wall to cytoplasm. If the cell wall holds ions as a result of its properties as an ion exchanger, and the cytoplasm can accumulate ions only from these, the rate of accumulation by the cytoplasm must be dependent on the concentration in the cell wall, and experiments such as those in which the interference effect of calcium sulphate was investigated should provide the required evidence. There appears to be a hint in Figure 37 that the rate of the slow phase of accumulation is depressed in the presence of calcium sulphate, but the material used here is too variable to make the matter certain. It is tempting to speculate that the complex character of the pattern of interference of other ions with manganese uptake in field or pot experiments may have some such simple explanation. In the introduction, pp 22-29,

many examples of interfering anions have been mentioned; in some cases, as, for example, with iron, more complicated explanations certainly must be invoked, but the interference of say, calcium and magnesium might be explicable by the depression of the level of manganese held on the exchange surface of the roots, if this were the only source for subsequent uptake by the root and thence by the rest of the plant. The variety of type and concentration of other ions which would exist in the various field soils of the experimental centres might then explain the different uptakes of manganese shown in Figures 2 to 10 even when the level of water soluble manganese in the soils was the same.

The slow phase of uptake of manganese ought, if uptake is in series from cell wall to cytoplasm, to begin with a zero rate of uptake, and rise only to its final steady value when the cell wall concentration reaches its maximum. There is no evidence for this in the experiments reported here, indeed, on the contrary, there is some evidence for an absorption shoulder, which would obscure the effect.

The slow phase of manganese accumulation is more complex than the rapid phase of exchange adsorption. It shows no signs of reaching completion in any of the experiments reported here, the duration of which did not exceed three hours. Stiles

and Skelding's experiments (1940) showed that the slow phase of accumulation continued for at least 100 hours. This phase has usually been regarded as metabolic, and with some ions, for example potassium, it is abolished by metabolic poisons, (Robertson 1957); yet in the experiments reported here it continued after the roots had been treated with potassium cyanide and chloroform, and at low temperature. Under these circumstances most metabolic processes would cease, so that manganese accumulation must be independent of current metabolic activity.

The Hofstee plot of the results obtained for accumulated manganese from solutions of different concentrations (Figure 49) shows that two types of site are involved in manganese accumulation as for phosphate, (Hagen and Hopkins 1955); Ca, Rb, and Sr, (Fried, Noggle and Hagen 1958); and K and Na (Fried and Noggle 1958). These sites presumably are situated in the cytoplasm, and probably represent two cytoplasmic constituents capable of binding manganese ions. Figure 49 shows that the two sites must differ very greatly in their firmness of binding with the manganese ion. The site represented by the steep slope takes up relatively little manganese, but does so very firmly, so that the second site scarcely begins its uptake until a concentration of external ions is reached at which the first site is saturated. The amount of manganese taken up at the first site must be less than 0.025 peq manganese per gram

(fresh weight) of root; approximately 1% of that taken up in two hours by the second site. It is possible that the first binding site is identical with that responsible for the "shoulders" in Figures 39, 40 and 41. Rothstein and Hayes (1956) tentatively suggested that in yeast cells phosphoryl and carboxyl groups might be involved, but there are many other possibilities, including the possibility that it is not a chemical difference between sites which is involved, but a spatial separation, so that one site might be represented by a species of binding group in the cytoplasm, and another by accumulation in the vacuole.

The release of accumulated manganese represented by the slow fraction in Figure 42A shows that its binding is reversible, but it should be noted that merely because release of a slow fraction occurs in calcium chloride solution the manganese is not necessarily held in an exchangeable form. The rate of uptake and release of this fraction of manganese gives a plot linear with time and this would be consistent with the formation or disruption of a reversible chemical combination in the presence or absence respectively of manganese in the external solution.

The rate of accumulation of manganese in this slow phase of uptake was shown to be scarcely affected by the difference in temperature between 4° and 20°. This means that the slow

ocurse of uptake cannot be the result of the time necessarily taken by manganese ions to diffuse through the cytoplasm to specialised sites distributed discontinuously in the cytoplasm, but is rather due to a slow reaction being the limiting factor.

There is no proof in this work that the processes involved in the slow phase of uptake of manganese are identical with those that proceed in the reverse direction during release of manganese. A slow phase exists in both processes; but whereas the slow phase of uptake has been shown to involve two anatomical structures or chemical processes there is no evidence that manganese is released by each of them.

VI. THESIS SUMMARY

The work described in this thesis was intended as a contribution to the understanding of the problem of the availability of manganese to plants. To unravel the problem requires attention to two principal topics; firstly the forms and behaviour of manganese in the soil, and secondly the means whereby the plant obtains its manganese from the soil. The topic thus embraces the whole field of plant-soil relationships in all their complexity, and its completion will require contributions from many scientific disciplines, ranging from mathematics to ecology.

The complexity of the problem is indicated by the necessarily incomplete review of the literature given in SECTION I. All the main aspects of the fields of work covered by the numerous investigators have been mentioned, and all the major contributors have been cited. There is no complete and up to date review of the world literature extant; it is hoped that this section meets the need for an interim assessment of the present position.

SECTION II gives an account of the routine methods used in this investigation. Further details of special techniques used in particular stages of the work are inserted in appropriate places in other sections.

The results of field experiments are presented in SECTION III. The long established field work of the Chemistry Department of the West of Scotland Agricultural College forms the background of the work. It is shown that both lime and phosphate applications affect manganese uptake by the oat crop, the former much more than the latter. It is shown further that the effect of phosphate is exerted in its own right, and not through an indirect effect of phosphate on pH.

The connection between both water soluble and total manganese of soil, and the uptake of manganese by the oat plant is described and discussed. The evidence suggests that total manganese in soil is a factor to be taken into account, and this must imply that oxides of manganese, which constitute the greater part of the total manganese of the soil, are available to the plant to some degree. This finding is in opposition to the old established theory that non-availability of manganese in soil is the result of oxide formation.

The relation of soil pH to water-soluble manganese is described and shown also to be incompatible with this theory of non-availability.

Experiments to investigate the extractable manganese of soil are described in SECTION IV. It is demonstrated that water soluble manganese is that fraction of extractable manganese which is

extracted by the dilute solution of ions provided by soil suspended in water. Certain misleading evidence available in the literature is examined critically, and fresh evidence is provided to show that extractable manganese in soil behaves according to simple ion exchange theory.

Experiments in which the pH of the soil was adjusted by addition of lime water rule out the possibility that bacterial or fungal activities control the changes in availability of manganese and provide further evidence against the theory that oxide formation account for non-availability of manganese. A theory is put forward to account for the pH relationship of manganese availability which suggests that complexes are formed between soil organic matter and manganous ions from the soil solution, and that the extent of complex formation is controlled by the soil pH.

A series of experiments is described in SECTION V.A in which oats were grown in agar cultures under sterile and non-sterile conditions with six oxides of manganese as the sole source of the element. It is shown that in both conditions all the oxides are available to oats, although to varying degrees. It is considered that this provides conclusive evidence against the "oxide" theory of manganese non-availability.

In SECTION V B. current theories of ion-uptake by plants are

considered, and experiments described which relate to the uptake of manganese from solution by excised oat roots. It is shown that the area of greatest uptake is located in the meristematic zone immediately behind the root cap, and that the manganese involved is exchangeable manganese. Two phases of uptake are demonstrated for manganese, superficially similar to those which have been found for other ions. The first rapid phase of uptake is shown to be of exchangeable manganese, involving a physical process of ion exchange consequent on the properties of the cell wall, as has been demonstrated for other ions by other workers. But the second slower phase of uptake, which for other ions has been described as metabolic uptake since it was abolished by metabolic poisons, has been found in these experiments to continue after the roots had been treated with chloroform, or potassium cyanide, or when the roots were subjected to a low temperature. The second phase of manganese uptake therefore is considered to be non-metabolic.

The mechanisms of uptake are considered to be at least three in number. There is the purely physical process of ion exchange uptake; its possible significance in the nutrient economy of the plant is discussed. Then there are two further mechanisms of uptake which involve specific binding sites, the nature of which are at present unknown, but it is shown that there is a great

difference in the firmness with which manganese is bound by these two sites.

SECTION VII lists the literature cited with author's names arranged alphabetically. The abbreviations of titles are those given in the "World List of Scientific Periodicals."

SECTION VIII is an Appendix, containing Tables I to XXXV, which present the results of experiments described in the text. There is no Table XVIII. These tables provide the basic information on which many of the tables and calculations in the text are based. Tables in the text are numbered with arabic numerals.

VII LITERATURE CITED

Adams, F., and Weir, J.I., 1957 Proc. Soil Sci. Soc. Amer. 21 305-308.

Adler, E., von Euler, H., Günther, G., and Plass, M., 1939 Biochem. J. 33 1028-1045.

Aldrich, D. J., and Martin, J. P., 1952 Soil Sci. 73 149-159.

Amdur, B.H., Rilling, H., and Bloch, K., 1957 J. Amer. chem. Soc. 79 2646-2647.

Anderson, I., and Evans, H.J., 1956 Plant Physiol. 31 22-28.

Arnon, D.I., 1939 Soil Sci. 48 295-307.

Arrhenius, O., 1923 Medd. CentAnst. Försökav. Jordbr. Stockh. No. 244 1-19 (quoted by Jacks and Scherbatoff 1940).

Arrhenius, O., 1924 Medd. CentAnst. Försökav. Jordbr. Stockh. No. 256 1 - 23 (quoted by Jacks and Scherbatoff 1940).

Association of Official Agricultural Chemists 1955 Official Methods of Analysis (8th Ed.) Ed. W. Horwitz.

Anderson, D.M.W., 1959 Talanta 2 73-78.

Barker, H.A., and Broyer, T.C., 1942 Soil Sci. 53 467-477.

Batalin, A. Kh., 1957 Pochvovedenie 9 124-126 (quoted in Soils and Fert. (1958) 21 (63).

Beckwith, R.S., 1955(a) Aust. J. agric. Res. 6 299-307.

" " " (b) " " " " 6 685-698.

Beeson, K.C., 1954 Southern Co-op. Bull. No. 36 174-198.

Beeson, K.C., Gray, L., and Adams, M.B., 1947 J. Amer. Soc. Agron. 39 356-362.

Beeson, K.C., Gray, L., Hamner, K.C., 1948 J. Amer. Soc. Agron. 40 533-562.

Bennet, J.P., 1945 Soil Sci. 60 91-105.

Berger, K.C., and Gerloff, G.C., 1947 Proc. Soil Sci. Soc. Amer. 12 310-314.

Bingham, F.T., Martin, J.P., and Chastain, J.A., 1958 Soil Sci. 86 24-30.

Bingham, F.T., and Garber, M.J., 1960 Proc. Soil Sci. Soc. Amer. 24 209-213.

Boken, K., 1952 Plant & Soil 4 154-163.

" " 1955 " " " 6 97-112.

" " 1956(a)" " " 7 237-251.

" " " (b)" " " 8 160-169.

" " 1958 " " " 9 269-285.

Bolle-Jones, E.W., 1955 Plant & Soil 6 45-60.

Bortner, C.E., 1935 Soil Sci. 39 15-33.

Bose, J.L., Foster, A.B., Stacey, M., Webber, J.M., 1959 Nature Lond. 184 1301-1303.

Bower, C.A., 1959 Soil Sci. 88 32-35.

Bradfield, E.G., 1957 Analyst 82 254-257.

Brenner, J.M., Heintze, S.G., Mann, P.J.G., and Lees, H., 1946 Nature Lond. 158 790-791.

Brenner, C., 1919 Thesis Zurich (quoted Kahane and Brard 1934).

Briggs, G.E., and Robertson, R.N., 1957 Annu. Rev. Pl. Physiol. 8 11-30.

Briggs, G.E., Hope, A.B., and Pitman, M.G., 1958 J. exp. Bot. 9 128-141.

Britton, H.T.S., 1955 "Hydrogen Ions" (2 vols.) 4th Ed.

Broomfield, S.M., 1956 Aust. J. bio. Sci. 9 238-252.

" " 1957 Plant & Soil 8 389-394.

" " 1958(a) " " 9 325-337.

" " 1958(b) " " 10 147-160.

Broomfield, S.M., and Skerman, V.B.D., 1950 Soil Sci. 69 337-348.

Broyer, T.C., Carlton, A.B., Johnson, C.M., and Stout, P.R., 1954 Plant Physiol. 29 526-532.

Burger, O.J., and Hauge, S.M., 1951 Soil Sci. 72 303-313.

Burriel, F., and Suares y Suares, A., 1951 An. edafol. fisiol. veg. 10 247-285 (quoted by Beeson 1954).

Burstrom, H., 1939 Planta 29 292-305 (quoted by Mulder and Gerretsen 1952 and by Beeson 1954).

Burstrom, H., 1939-40 Planta 30 129-150 (quoted by Mulder and Gerretsen 1952 and by Beeson 1954).

Calder, A.B., and Voss, R.C., 1957 Dept. of Agric. for Scotland. Consultative Ctte. for development of Spectrographic Work. Bull. 1.

Carlson, C.W., and Olsen, R.V., 1950 Proc. Soil Sci. Soc. Amer. 15 251-254.

Chenery, E.M., 1955 Plant & Soil 6 174-200.

Clinch, J., and Guy, M.J., 1958 Analyst 83 429-431.

Cole, W.F., Wadsley, A.D., and Walkley, A., 1947 Trans. electrochem. Soc. 92 133-158.

Collander, R., 1939 Protoplasma 23 215-257 (quoted by Stiles and Skelding 1940).

Comber, N.M., 1922 J. agric. Sci. 12 363-369.

Cook, J.W., 1941 Industr. Engng. Chem. (Anal.) 13 48-50.

Cornfield, A.H., and Pollard, A.G., 1950 J. Sc. Fd. Agric. 1 107-109.

Coulson, C.B., Davies, R.I., and Lewis, D.A., 1960 J. Soil Sci. 11 20-29, and 30-44.

Crooke, W.M., Knight, A.H., and MacDonald, I.R., 1960 (a) Plant & Soil 13 55-67.

Crooke, W.M., Knight, A.H., and MacDonald, I.R., 1960 (b) Plant & Soil 13 123-127.

Crum, W., 1845 Liebigs Ann. 55 219-220.

Dainty, J., and Hope, A.B., 1959 Aust. J. biol. Sci. 12 395-411.

Dainty, J., Hope, A.B., and Denby, C., 1960 Aust. J. biol. Sci. 13 267-276.

Davies, J.N., 1957 Rep. Glasshouse Crops Res. Inst. 1954/55 70-74.

Davis, D.G., 1959 Analyt. Chem. 31 1460-1463.

Denigès, G., 1932 Compt. rend. 194 895-897.

Dessureaux, L., and Ouellette, G.J., 1958 Canad. J. Soil Sci. 38 8-13.

Diamond, J.M., and Solomon, A.K., 1959 J. gen. Physiol. 42 1105-1121.

Dion, H.G., and Mann, P.J.G., 1946 J. agric. Sci. 36 239-245.

Dion, H.G., Mann, P.J.G., and Heintze, S.G., 1947 J. agric. Sci. 37 17-22.

Dische, Z., 1947 J. biol. Chem. 167 189-198.

Drake, M., Vengris, J., and Colby, W.G., 1951 Soil Sci. 72 139-147.

Dubois, P., 1936 Ann. chimie 5 411-482.

Elgabaly, M.M., and Wiklander, L., 1949 Soil Sci. 67 419-424.

Epstein, E., 1953 Nature Lond. 171 83-84.

Epstein, E., 1960 Amer. J. Bot. 47 393-399.

Epstein, E., and Hagen, C.E. 1952 Plant Physiol. 27 457-474.

Epstein, E., and Leggett, J.E., 1954 Amer. J. Bot. 41 785-791.

Erdey, L., and Szabadvary, F., 1957 Z anal. Chem. 155 90-96.

Eriksson, E., 1952 J. Soil Sci. 3 238-250.

Feitknecht, W., and Marti, W., 1945 Helv. chim. acta. 28 129-148 and 149-155.

Fiskel, J.G.A., and Mourikides, G.A., 1955 Plant & Soil 6 313-331.

Forsee, W.T., Jr. 1954 Proc. Soil Sci. Soc. Amer. 18 475-478.

Fried, M., and Noggle, J.C., 1958 Plant Physiol. 33 139-144.

Fried, M., Noggle, J.C., and Hagen, C.E., 1958 Proc. Soil Sci. Soc. Amer. 22 495-499.

Fujimoto, C.K., and Sherman, G.D., 1945 Proc. Soil Sci. Soc. Amer. 10 107-112.

Fujimoto, C.K., and Sherman, G.D., 1948 Soil Sci. 66 131-145.

Gallagher, P.H., and Walsh, T., 1943 J. agric. Sci. 33 197-203.

Gapon, E.N., 1933 Zhur. Obshen. Khim. 3 144-152 (quoted by Bower 1959).

Gauch, H.G., 1957 Annu. Rev. Pl. Physiol. 8 31-64.

Gavey, C.L., and Barber, S.A., 1952 Proc. Soil Sci. Soc. Amer. 16 173-175.

Gerretsen, F.C., 1936 Versl. RijkslandbProefst., Groningen. 42a 1-67 (quoted by Mulder and Gerretsen 1952).

Gerretsen, F.C., 1937 Ann. Bot. NS 1 207-230.

Gerretsen, F.C., 1950(a) Plant & Soil 2 159-193.

" " 1950(b) " " 2 323-343.

Gilbert, B.E., 1934 R.I. Agric. Expt. Sta. Bull. 246
(quoted by Jacks and Scherbatoff 1960).

Gisiger, L., and Hasler, A., 1948 Plant & Soil 1 18-30.

Gortner, R.A., and Rost, C.O., 1912 Ind. Eng. Chem. 4 522-524.

Gosselin, L., and Popjak, G., 1958 Biochem. J. 70 19P.

Greenberg, D.M., 1951 "The Enzymes" Vol. 1 893-921 Ed.
Sumner, J.B., and Myrback, C.K., Academic Press N.Y.

Gum, O.B., Brown, H.D., and Burrell, R.C., 1945 Pl. Physiol.
20 267-275.

Hagan, C.E., and Hopkins, H.T., 1955 Pl. Physiol. 30 193-199.

Hannay, J.N., Fletcher, B.L., and Street, H.E., New Phytol.
58 142-154.

Hardy, E., and Lewis, A.H., 1929 J. Agric. Sci. 19 17-25.

Harry, R.G., 1931 Chem. and Ind. 50 796.

Hasler, A., 1951 Schweiz. landw. Mh. 29 300-305.

Heintze, S.G., 1938 J. agric. Sci. 28 175-186.

" " 1946 " " 36 227-237.

" " 1956 Plant & Soil 8 218-236.

" " 1957 J. Soil Sci. 8 287-300.

Heintze, S.G., and Mann, P.J.G., 1946 Nature Lond. 158 791-792.

Heintze, S.G., and Mann, P.J.G., 1947 J. agric. Sci. 37 23-26.

" " " " " 1949 " " " 39 80-95.

" " " " " 1951 J. Soil Sci. 2 234-242.

Heisig, G.B., and Pollard, F.H., 1957 Anal. chim. acta. 16 234-237.

Hemstock, G.A., and Low, P.F., 1953 Soil Sci. 76 331-343.

Hester, J.B., 1941 Science 93 401.

Hewitt, E.J., 1946 Ann. Report Agr. Hort. Res. Sta. Long Ashton 1945 51-60.

Hewitt, E.J., 1947 Ann. Report Agr. Hort. Res. Sta. Long Ashton 1946 50-61.

Hewitt, E.J., 1948 Ann. Report Agr. Hort. Res. Sta. Long Ashton 1947 82-96.

Hewitt, E.J., 1949 Ann. Report Agr. Hort. Res. Sta. Long Ashton 1948 58-65.

Hewitt, E.J., 1954 J. exptl. Bot. 5 110-118.

" " 1957 Nature Lond. 180 1020-1022.

Hillman, W.H., and Galston, A.W., 1956 Physiol. Plant. 9 230-235.

Hiltner, E., 1924 Landw. Jb. 60 689-769 (quoted by Mulder and Gerretsen 1952).

Hiron, K.J., Doty, D.M., and Quackenbush, F.W., 1951 Soil Sci. 71 353-359.

Hoagland, D.R., and Arnon, D.I., 1950 Cal. Agr. Exp. Sta. Circ. No. 347 (revised).

Hoff, D.J., and Nederski, H.J., 1958 Proc. Soil Sci. Soc. Amer. 22 129-132.

Hofstee, B.H.J., 1952 Science 116 329-331.

Hopkins, E.F., Pagan, U., and Ramires-Silva, F.J., 1944 J. Agric. Univ. P.R. 28 43-101 (quoted by Mulder and Gerretsen 1952 and by Beeson 1954).

Hudig, J., 1911 Landw. Jb. 40 613-644 (quoted by Mulder and Gerretsen 1952).

Hunter, J.G., and McGregor, A.J., 1946 Scot. Agric. 26 30-33.

Hurwitz, C., 1948 Soil Sci. 66 267-272.

Iljin, W.S., 1951 Plant & Soil 3 339-351.

Iyer, C.R.N., and Rajagopalan, R., 1936 J. Indian Inst. Science A19 Pt. 7 57-66.

Jacks, G.V., and Scherbatoff, H., 1940 "The Minor Elements of the Soil" Imp. Bureau of Soil Sci. Tech. Comm. No. 39.

Jacobson, H.G.M., and Swanback, T.R., 1932 J. Amer. Soc. Agron. 24 237-245.

Jenny, H., and Overstreet, R., 1939 Soil Sci. 47 257-272.

Johnson, M.O., 1924 Hawaii Agric. Expt. Sta. Bull. 52.

Jones, L.H.P., 1957 Plant & Soil 8 328-336.

Jones, L.H.P., and Leeper, G.W., 1951(a) Plant & Soil 3 141-153.

Jones, L.H.P., and Leeper, G.W., 1951(b) Plant & Soil 3 154-159.

Kahane, E., and Brard, D., 1934 Bull. Soc. Chim. biol. Paris 16 710-719.

Kahane, E., 1935 Ann. Chim. anal. (Paris) 17 175-178.

Karanovich, G.C., 1956 Tr. Vses. Nauch. Inst. Khim. Reaktivov 21 14-17 quoted Anal. Abstract 1957 8 2605.

Keller, P., and Deuel, H., 1957 Z. Pflanzenernähr Düng . und Bodenk. 79 119-131.

Kelley, K.K., and Moore, G.E., 1943 J. Amer. chem. Soc. 65 782-785.

Kenten, R.H., 1955 Biochem. J. 61 353-359.

Kenten, R.H., and Mann, P.J.G., 1950 Biochem. J. 46 67-73.

" " " " " 1955 " " 61 279-286.

" " " " " 1957 " " 65 179-185.

Kirsch, R.K., Harward, M.E., and Peterson, R.C., 1960 Plant & Soil 12 259-275.

Kitchener, J.A., 1957 "Ion Exchange Resins" (Methuen Monograph).

Kolthoff, I.M., and Watters, J.I., 1943 Industr. Engng. Chem. (Anal.) 15 8-13.

Koroleff, F., 1947 Acta. chim. Scand. 1 503-506.

Krepelka, H., and Rejha, B., 1931 Coll. Czech. Chem. Comm. 1931 pp. 517-535 (quoted in Nature 129 284 1932).

Laine, T., 1934 Acta. Bot. Fenn. 16 1-64 (quoted by Stiles and Skelding 1940).

Laties, G.G., 1959(a) Annu. Rev. Plant and Physiol. 10 87-112.

" " 1959(b) Proc. nat. Acad. Sci. Wash. 45 163-172.

Latimer, W.M., 1952 "Oxidation Potentials" Constable (2nd Ed.)

Lee, H.A., and McHargue, J.S., 1928 Phytopath. 18 775-786 (quoted by Jacks and Scherbatoff 1940).

Leeper, G.W., 1935 Proc. roy. Soc. Viet. 47 (N.S.) 225-261.

" " 1939 " " " " 52 " 138-152.

" " 1947 Soil Sci. 63 79-94.

" " 1952 Annu. Rev. Pl. Physiol. 3 1-16.

Leeper, G.W., and Swaby, R.J., 1940 Soil Sci. 49 163-169.

Lineweaver, H., and Burk, D., 1934 J. Amer. chem. Soc. 56 658-666.

Lingane, J.J., and Davis, D.G., 1956 Analyt. chim. acta. 15 201-206.

Löhnis, M.P., 1936 Tijdschr. Plziekt. 42 159-167 (quoted by Jacks and Scherbatoff 1940).

Löhnis, M.P., 1950 "Trace Elements in Plant Physiology" Lotsya 3 63-76.

Löhnis, M.P., 1951 Plant & Soil 3 193-222.

" " 1954 VIII^{e} Cong. Intern. Bot. Paris Sect 11 33-35.

Löhnis, M.P., 1960 Plant & Soil 12 339-376.

Lowenstein, J.M., 1957 Biochem. J. 65 40P.

Lukin, A.M., and Cestrova, E.D., 1956 Tr. Vses. Nauch. Inst. Khim. Reaktivov 21 3-9 (quoted in Anal. Abstracts 1957 8 2605).

Lundegårdh, H., and Stenlid, G., 1944 Ark. Bot. 31 No. 10 1-27.

Lundegårdh, H., 1955 Annu. Rev. Pl. Physiol. 6 1-24.

Lyon, C.B., Beeson, K.C., and Ellis, G.H., 1943 Bot. Gaz. 104 495-514.

Lyon, C.B., and Beeson, K.C., 1948 Bot. Gaz. 109 506-520. (quoted by Richardson 1954).

Mann, P.J.G., and Quastel, J.H., 1946 Nature Lond. 158 154-156.

Marshall, H., 1901 Chem. News 83 76 (abstract in J. Chem. Soc. 80.2 350).

Masterman, A.T., 1939 Analyst 64 492-499.

Maton, J., 1947 Biologisch. Jaarb. Koninklijk Natuurw. Genootsch Dodonaia. Gent. 14 109-115.

Mattson, S., Eriksson, E., and Vahtras, K., 1948 Ann. Roy. Agr. Col. Sweden. 15 291-307.

Mederski, H.J., and Wilson, J.H., 1955 Proc. Soil Sci. Soc. Amer. 19 461-464.

Mehlich, A., 1957 Proc. Soil Sci. Soc. Amer. 21 625-628.

Mehlig, J.P., 1939 Indust. Engng. Chem. (Anal.) 11 274-277.

Michaelis, L., and Menten, M.L., 1913 Biochem. Z. 49 333-369.

Milliken, C.R., 1948 J. Dept. Agr. Victoria 46 511-517, 566-576.

" " 1949 " " " 47 37-41.

" " 1950 Aust. J. Sci. Res. B3 450-473.

Mitchell, R.L., 1948 Comm. Bureau Soil Sci. Tech. Comm. No. 44.

Mitra, S.P., and Prakash, D., 1957 Anal. Chim. acta. 16 431-435.

Morley Davies, W., 1939 Agric. Prog. 16 45-54.

Morris, H.D., and Pierre, W.H., 1947 Proc. Soil Sci. Soc. Amer. 12 382-386.

Morris, H.D., and Pierre, W.H., 1949 Agron. J. 107-112.

Mulder, E.G., and Gerretsen, F.C., 1952 Adv. Agron. 4 221-277.

McCool, M.M., 1935 Contr. Boyce Thompson Inst. 7 427-437 (quoted by Mulder and Gerretsen 1952).

McElroy, W.D., and Nason, A., 1954 Annu. Rev. Pl. Physiol 5 1-30.

McGregor, A.J., Schofield-Palmer, E.K., and Wilson, G.C.S., 1960 Fertil. Feed. St. J. 53 313-319.

McHargue, J.S., 1922 J. Amer. chem. Soc. 44 1592-1598.

" " 1923 J. agric. Res. 24 781-794.

McIlrath, W.J., deBruyn, J.A., and Skok, J., 1960 Soil Sci. 89 117-121.

McKenzie, R.M., 1955 Aust. J. agric. Res. 6 699-706.

MacLachlan, G.A., and Waygood, E.R., 1956(a) Physiol. Plant 9 321-330.

MacLachlan, G.A., and Waygood, E.R., 1956(b) Canad. J. Biochem. & Physiol. 34 1233-1250.

McLean, E.C., Adams, D., and Franklin, R.E., 1956 Proc. Soil Sci. Soc. Amer. 20 345-347.

McRobbie, E.A.C., and Dainty, J., 1958 J. gen. Physiol. 42 335-353.

Neal, D.C., and Lovett, H.C., 1938 Phytopathology 28 582-587 (quoted by Jacks and Scherbatoff 1940).

Nicol, H., 1954(a) West Scot. Agric. Coll. Ext. Staff Circ. No. 19.

Nicol, H., 1954(b) Trans. 5th Int. Soc. Soil Sci. Comm. III (Leopoldville) 166-170.

Nicol, H., 1958 Wallerstein Lab. Commun. 21 281-292.

Nicol, H., and Schofield-Palmer, E.K., 1952 Chem. & Ind. (Rev.) 1952 1080-1081.

Nicholas, D.J.D., 1946 Nature Lond. 157 696.

" " 1957(a) " " 179 800-804.

" " 1957(b) J. Sci. Fd. Agric. 8 Suppl. S15-25.

" " 1959 13th Symp. Soc. exp. Biol.

Nightingale, E.R., 1959 Analyt. Chem. 31 146-148.

Nydahl, F., 1949 Analyt. Chim. acta. 3 144-157.

" " 1951 Proc. Int. Ass. of Theor. and Appl. Limnology II 277-290.

Olsen, C., 1934 Biochem. Z. 269 329-348.

Olsen, R.V., Carlson, C.W., 1949 Proc. Soil Sci. Soc. Amer. 14 109-112.

Ordin, L., Cleland, R., and Bonner, J., 1955 Proc. nat. Acad. Sci. Wash. 41 1023-1029.

Page, E.R., 1961 Nature Lond. 189 597.

Pethybridge, G.H., 1936 J. Minist. Agric. 55-58.

Piper, C.S., 1931 J. agric. Sci. 21 762-779.

" " 1944 "Soil and Plant Analysis" University of Adelaide.

Pirson, A., 1955 Annu. Rev. Pl. Physiol. 6 71-114.

Possingham, J.V., 1956 Aust. J. Biol. Sci. 9 539-551.

Quastel, J.H., 1954 (Leeuwenhoek Lecture) Proc. roy. Soc. Ser. B. 143 159-578.

Reuther, W., Gardner, F.E., Smith, P.F., and Roy, W.R., 1949 Proc. Amer. Soc. hort. Sci. 53 71-84.

Reynolds, J.D., 1955 J. Sci. Fd. Agric. 6 725-734.

Richards, M.B., 1930 Analyst 55 554-560.

Richardson, L.R., 1954 South Co-op. Series Bull. No. 36 6-23.

Riches, J.P.R., 1947 Chem. & Ind. (Rev.) 656-658.

Robertson, R.N., 1951 Annu. Rev. Pl. Physiol. 2 1-25.

" " 1957 Endeavour 16 193-198.

Rothstein, A., and Hayes, A.D., 1956 Arch. Biochem. & Biophys. 63 87-99.

Ruiru, M.N., 1939 Biochem. Z. 301 238-244.

Russell, R.S., and Ayland, M.J., 1955 Nature Lond. 175 204-205.

Samuel, G., and Piper, C.S., 1929 Ann. appl. Biol. 16 493-524.

Seatchard, G., 1949 Ann. N.Y. Acad. Sci. 51 660-672.

Schachtschabel, P., 1956 Trans. 6th Congress Int. Soc. Soil Sci. (Paris) Vol. D 113-118.

Schofield, R.K., 1946 Soils & Fert. 9 265-266.

" " 1949 Rothamsted Expt. Sta. Rep. 1949 p 29.

Schofield, R.K., and Taylor, A.W., 1953 Proc. Soil Sci. Soc. Amer. 17 214-218.

Schofield, R.K., and Taylor, A.W., 1955 Proc. Soil Sci. Soc. Amer. 19 164-167.

Schofield-Palmer, E.K., and McGregor, A.J., 1955 Mimeographed Memo. West Scot. Agric. Coll.

Schofield-Palmer, E.K., 1956 Trans. int. Soc. Soil Sci. Comm. II (Paris) 32 641-649.

Schollenberger, C.J., 1927 Science 65 552-553.

Schutte, K.H., and Schendel, H.E., 1958 Nature Lond. 182 958-959.

Sherman, G.D., and Harmer, P.M., 1941 J. Amer. Soc. Agron. 33 1080-1092.

Sherman, G.D., and Harmer, P.M., 1942 Proc. Soil Sci. Soc. Amer. 7 398-405.

Sherman, G.D., McHargue, J.S., and Hodgkiss, W.S., 1942(a) Soil Sci. 54 253-257.

Sherman, G.D., McHargue, J.S., and Hodgkiss, W.S., 1942(b) J. Amer. Soc. Agron. 34 1076-1083.

Sherman, G.D., McHargue, J.S., and Hageman, R.H., 1943 Soil Sci. 56 127-134.

Sideris, C.P., 1937 Industr. Engng. Chem. (Anal.) 9 445-446.

" " 1940 " " " " 12 307.

Single, W.V., 1957 Nature Lond. 180 250-251.

Sjollema, B., and Hudig, J., 1909 Versl. Rijkslandb. Proefst. Groningen No. 5 29-157 (quoted by Mulder and Gerretsen 1952, and Jacks and Scherbatoff 1940).

Smith, A.M., and McCallum, E.S.R., 1956 Analyst 81 160-163.

Smith, A.M., 1959 Fertiliser Soc. Proc. No. 57 p. 20.

Smith, E.L., and Bergman, M., 1944 J. biol. Chem. 153 627-651.

Smith, E.L., 1951 "Enzymes and Enzyme Systems" edited by Edsall, J.T., Harvard Univ. Press Camb., Mass.

Smith, R.L., and Wallace, A., 1956 Soil Sci. 81 97-109.

Snider, H.J., 1943 Soil Sci. 56 187-195.

Sohngen, N.L., 1914 Zbl. Bakt. II 40 545-554 (quoted by Mulder and Gerretsen 1952).

Somers, I.I., Gilbert, S.G., and Shive, J.W., 1942 Plant Physiol. 17 317-320.

Somers, I.I., and Shive, J.W., 1942 Plant Physiol. 17 582-602.

Spencer, D., Takahashi, M., and Mason, A., 1957 J. Bact. 73 553-562.

Stark, S.M., 1950 Analyt. Chem. 22 1158-1160.

Starkey, R.L., 1955 Soil Sci. 79 1-14.

Steenbjerg, F., 1933 Tidsskr. Planteavl. 39 401-434.

Stoenbjerg, P., 1934 Tidsskr. Planteavl 40 337-367.

" " 1935 " " 40 797-824.

Steinberger, R., and Westheimer, F.H., 1949 J. Amer. chem. Soc. 71 4158.

Stemuit, D., Piot, R., and Boon, R., 1956 Trans. int. Soc. Soil Sci. Comm. IV (Paris) A 234.

Stevens, H.M., 1957 Anal. chim. acta. 16 435-438.

Stiles, W., and Skelding, A.D., 1940 Ann. Bot. N.S. 4 674-700.

Stumpf, P.K., and Loomis, W.D., 1950 Arch. Biochem. 25 451-453.

Sutton, C.D., and Hallsworth, E.G., 1958 Plant & Soil 9 305-317.

Swanback, T.R., 1939 Plant Physiol. 14 423-446.

Tanner, H.A., Brown, T.E., Hyster, C., and Treharne, R.W., 1960 Ohio J. Sci. 60 231-234.

Taper, C.D., and Leach, W., 1957 Canad. J. Bot. 35 773-777.

Tohen, T.T., and Vennesland, B., 1955 J. biol. chem. 213 535-547.

Timonin, M.I., and Giles, G.R., 1952 J. Soil Sci. 3 145-155.

Tisdale, S.L., and Bertramson, B.R., 1949 Proc. Soil Sci. Soc. Amer. 131-137.

Tobia, S.K., and Pollard, A.G., 1958 J. Sc. Fd. Agric. 9 705-713.

Tourky, A.R., Issa, I.M., and Mawaldy, I.F., 1957 Analyt. chim. acta. 16 151-154.

Utter, M.F., and Kurahashi, K., 1954 J. biol. Chem. 207 821-841.

Van der Merwe, A.J., and Anderson, F.G., Farm. S. Africa 12 439-440 (quoted by Jacks and Scherbatoff 1940).

Vanino, L., 1925 Hanbuch der preparative Chemie I. Anorg. Teil, 3 Auflage p. 695, F. Enke Verlag, Stuttgart. (quoted by Cole, Wadsley, and Walkley 1947).

Vavra, J.P., and Frederick, L.R., 1952 Proc. Soil Sci. Soc. Amer. 16 141-144.

Venturello, G., and Ghe, A.M., 1957 Analyst 82 343-352.

Verona, O., 1953 "Malattie nutrisionali delle piante coltivate," Bologna.

Wain, R.L., 1938 J.S.E. Agric. Coll. Wye 42 146-153.

Wain, R.L., Silk, B.J., and Wills, B.C., 1943 J. agric. Sci. 33 18-22.

Walker, D.A., 1957 Biochem. J. 67 73-79.

Walker, T.W., 1960 Soil Sci. 89 328-332.

Wallace, T., 1940 Ann. Rep. Agr. Hort. Res. Sta. Long Ashton 1940 19-23.

Wallace, T., 1951 "The Diagnosis of Mineral Deficiences in Plants" (2nd Ed.) London H.M.S.O.

Warington, K., 1951 Ann. appl. Biol. 38 624-641.

Watters, J.I., and Kolthoff, I.M., 1944 Ind. Engng. Chem. (Anal.) 16 187-189.

Weiss, A., and Fallab, S., 1954 Helv. chim acta. 37 1253-1256.

Whittles, C.L., 1952 Trans. int. Soc. Soil Sci. Comm. II & IV (Dublin) 2 379-390.

Whittles, C.L., and Schofield-Palmer, E.K., 1951 J. Soil Sci. 2 243-245.

Wieringa, K.T., and Bakhuis, J.A., 1957 Plant & Soil 8 254-262.

Wiklander, L., 1955 "Chemistry of the Soil" Ed. F.E. Bear Reinhold, N.Y. 1955.

Wilcox, H.V., and Kelley, W.P., 1912 Hawaii Agric. Expt. Sta. Bull. 28 (quoted by Fujimoto and Sherman 1948).

Willard, H.H., and Greathouse, L.H., 1917 J. Amer. chem. Soc. 39 2366-2377.

Yuen, S.H., 1958 Analyst 83 350-356.

Zende, G.K., 1954 J. Ind. Soc. Soil Sci. 2 55-61.

VIII APPENDIX

Tables I to XVII

and XIX to XXIV.

TABLE I

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XA 500609	E5	6.19	3.6341	8.75	0.66	67.3
XA 500610	D1	6.08	3.7367	8.42	0.85	64.1
XA 500611	C2	5.85	3.7979	7.90	1.35	69.7
XA 500612	B3	5.36	3.8356	6.88	2.32	116.1
XA 500613	A4	5.04	3.8443	6.24	3.53	155.4
XA 500614	A4	5.04	3.8840	6.19	3.71	161.0
XA 500615	B2	5.64	3.7582	7.52	2.31	99.3
XA 500616	A3	5.18	3.7474	6.61	4.27	193.8
XA 500617	E4	6.44	3.6698	9.21	0.65	107.3
XA 500618	D5	6.18	3.7479	8.61	0.99	107.3
XA 500619	C1	5.88	3.7292	8.03	1.44	93.7
XA 500620	C1	6.02	3.7531	8.29	1.53	135.3
XA 500621	D4	6.19	3.7081	8.67	0.54	143.4
XA 500622	C5	5.68	3.5620	7.80	2.35	135.3
XA 500623	B1	5.44	3.7773	7.10	2.11	124.1

Continued overleaf ....

TABLE I (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XA 500624	A2	5.08	3.7777	6.38	4.90	278.7
XA 500625	E3	6.74	3.6117	9.87	0.54	96.9
XA 500626	E3	6.56	3.6899	9.43	0.47	74.5
XA 500627	A1	5.06	3.8744	6.25	2.38	155.4
XA 500628	E2	6.29	3.6751	8.90	0.76	64.1
XA 500629	D3	6.00	3.6262	8.37	0.65	124.1
XA 500630	C4	5.70	3.8131	7.59	1.26	148.2
XA 500631	B5	5.58	3.7808	7.38	1.86	112.9
XA 500632	B5	5.37	3.8338	6.91	3.62	139.3
XA 500633	C3	5.82	3.8560	7.78	2.90	110.5
XA 500634	B4	5.21	3.7466	6.67	1.55	138.5
XA 500635	A5	5.05	3.7495	6.35	4.09	240.1
XA 500636	E2	6.62	3.7596	9.48	0.55	129.7
XA 500637	D2	5.83	3.8135	7.85	1.08	102.5
XA 500638	D2	5.85	3.7790	7.92	1.15	77.7

TABLE II

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XB 500346	D1	6.51	3.7076	9.31	0.325	68.7
XB 500347	C2	6.25	3.7899	8.71	0.288	76.6
XB 500348	B3	5.53	3.7637	7.30	1.105	90.0
XB 500349	A4	5.44	3.6970	7.18	1.885	130.2
XB 500350	E5	6.82	3.4130	10.23	0.375	68.7
XB 500351	E5	6.90	3.3309	10.47	0.475	68.7
XB 500352	A3	5.23	3.7993	6.66	2.930	135.0
XB 500353	E4	6.80	3.5399	10.06	0.233	60.8
XB 500354	D5	6.56	3.4802	9.64	0.415	71.0
XB 500355	C1	6.00	3.7238	8.28	0.555	95.5
XB 500356	B2	5.55	3.6939	7.41	1.475	95.5
XB 500357	B2	5.68	3.7530	7.61	0.980	76.6
XB 500358	C5	6.18	3.5312	8.83	0.840	68.7
XB 500359	B1	5.66	3.7833	7.54	0.780	95.5
XB 500360	A2	5.60	3.6679	7.53	1.680	115.3

Continued overleaf ....

TABLE II (continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XB 500361	E3	6.82	3.5157	10.12	0.290	58.4
XB 500362	D4	6.65	3.4961	9.80	0.250	68.7
XB 500363	D4	6.56	3.4887	9.63	0.315	63.2
XB 500364	E2	7.28	3.4993	11.06	0.180	52.9
XB 500365	D3	7.09	3.6013	10.58	0.180	58.4
XB 500366	C4	6.62	3.5996	9.64	0.420	65.5
XB 500367	B5	6.00	3.5901	8.41	1.095	105.8
XB 500368	A1	5.56	3.8510	7.27	1.320	130.2
XB 500369	A1	5.40	3.7674	7.05	3.050	137.4
XB 500370	B4	5.56	3.7374	7.38	1.590	103.4
XB 500371	A5	5.43	3.6295	7.23	3.785	150.8
XB 500372	E1	6.76	3.5817	9.94	0.410	58.4
XB 500373	D2	6.81	3.6073	10.01	0.395	65.5
XB 500374	C3	6.47	3.6234	9.32	0.525	68.7
XB 500375	C3	6.32	3.6484	8.99	0.675	76.6

TABLE III

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XC 500436	C2	5.88	3.8907	7.87	0.565	101.7
XC 500437	B3	5.49	3.8658	7.11	0.560	123.1
XC 500438	A4	5.65	3.9106	7.39	0.605	166.0
XC 500439	E5	6.39	3.6463	9.13	0.315	58.8
XC 500440	D1	5.91	3.8688	7.95	0.265	45.3
XC 500441	D1	6.33	3.9177	8.74	0.200	45.3
XC 500442	E4	6.51	3.6579	9.36	0.210	65.9
XC 500443	D5	6.30	3.6407	8.96	0.300	40.5
XC 500444	C1	6.18	3.7856	8.57	0.250	63.5
XC 500445	B2	5.61	3.9402	7.28	0.390	92.9
XC 500446	A3	5.35	3.9650	6.73	0.510	220.0
XC 500447	A3	5.42	3.9825	6.86	0.425	195.4
XC 500448	B1	5.88	3.9789	7.78	0.350	128.7
XC 500449	A2	5.37	3.9276	6.81	0.524	201.8
XC 500450	E3	6.90	3.7626	10.04	0.525	35.7

Continued overleaf ....

TABLE III (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XC 500451	D4	6.07	3.6964	8.44	0.335	61.2
XC 500452	C5	5.73	3.7051	7.55	0.340	92.9
XC 500453	C5	5.71	3.7377	7.68	0.365	96.1
XC 500454	D3	6.36	3.9527	8.77	0.335	58.8
XC 500455	C4	6.08	3.8036	8.36	0.295	65.9
XC 500456	B5	5.71	3.7010	7.72	0.493	104.1
XC 500457	A1	5.39	3.9932	6.79	0.400	166.0
XC 500458	E2	6.63	3.7270	9.53	0.053	40.5
XC 500459	E2	6.29	3.7806	8.80	0.058	40.5
XC 500460	A5	5.31	3.8282	6.79	0.873	242.3
XC 500461	E1	7.00	3.7375	10.26	0.053	40.5
XC 500462	D2	6.63	3.8077	9.45	0.155	45.3
XC 500463	C3	5.91	3.7995	8.02	0.175	55.6
XC 500464	B4	5.67	3.8811	7.46	0.200	101.7
XC 500465	B4	5.60	3.7543	7.45	0.400	117.6

TABLE IV

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XD 500713	B3	5.51	3.8414	7.18	1.29	187.7
XD 500714	A4	5.25	3.8801	6.62	1.69	277.1
XD 500715	E5	6.66	3.5915	9.73	0.27	131.8
XD 500716	D1	6.54	3.5702	9.51	0.725	107.8
XD 500717	C2	6.17	3.6826	8.66	0.66	129.4
XD 500718	C2	6.26	3.6623	8.86	0.51	102.2
XD 500719	D5	6.40	3.5746	9.23	0.785	129.4
XD 500720	C1	6.03	3.7794	8.28	0.86	123.8
XD 500721	B2	5.90	3.8406	7.96	0.85	170.1
XD 500722	A3	5.44	3.8999	6.98	0.445	224.2
XD 500723	E4	6.50	3.6004	9.40	0.81	115.8
XD 500724	E4	7.00	3.3950	10.00	0.50	118.2
XD 500725	A2	5.64	3.9427	7.34	1.32	194.1
XD 500726	E3	6.84	3.5308	10.15	0.325	123.8
XD 500727	D4	6.30	3.6156	9.38	0.555	110.2

Continued overleaf ....

TABLE IV (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XD 500728	C5	6.16	3.6047	8.72	0.635	131.8
XD 500729	B1	5.78	3.8575	7.70	1.025	158.1
XD 500730	B1	5.69	3.7960	7.58	1.155	152.5
XD 500731	C4	6.39	3.6948	9.09	0.40	120.6
XD 500732	B5	5.63	3.7132	7.55	1.075	181.3
XD 500733	A1	5.28	3.8990	6.66	1.36	196.5
XD 500734	E2	6.89	3.5601	10.22	1.29	91.0
XD 500735	D3	6.26	3.7529	8.77	0.655	91.0
XD 500736	D3	6.58	3.6266	9.53	0.32	93.4
XD 500737	E1	6.90	3.5892	10.21	0.22	87.9
XD 500738	D2	6.04	3.7435	8.34	0.335	120.6
XD 500739	C3	5.98	3.8139	8.15	0.25	115.8
XD 500740	B4	5.60	3.8018	7.40	0.68	143.8
XD 500741	A5	5.27	3.7598	6.78	1.20	258.8
XD 500742	A5	5.11	3.6858	6.53	1.905	266.7

TABLE V

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XP 500835	A4	5.24	3.7403	6.74	2.18	
XP 500836	E5	6.45	3.5528	9.35	0.59	
XP 500837	D1	6.70	3.6700	9.73	0.53	
XP 500838	C2	6.09	3.6036	8.58	0.74	
XP 500839	B5	5.76	3.5236	8.00	1.51	
XP 500840	B3	5.59	3.7328	7.45	1.57	
XP 500841	C1	6.07	3.7775	8.37	0.53	
XP 500842	B2	5.58	3.8147	7.35	0.97	
XP 500843	A3	5.32	3.7171	6.93	1.57	
XP 500844	E4	6.52	3.5487	9.49	0.52	
XP 500845	D5	6.64	3.5923	9.69	0.61	
XP 500846	D5	6.28	3.6343	8.93	0.77	
XP 500847	E3	6.70	3.6518	9.75	0.44	
XP 500848	D4	6.14	3.6604	8.62	0.61	
XP 500849	C5	5.72	3.5027	7.94	1.72	

No samples were available for analysis, due to crop failure.

Continued overleaf ....

TABLE V (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XF 500850	B1	5.66	3.8203	7.50	1.11	
XF 500851	A2	5.30	3.6063	7.00	2.29	
XF 500852	A2	5.32	3.8067	6.84	1.79	
XF 500853	B5	5.57	3.6485	7.49	1.28	
XF 500854	A1	5.39	3.6465	7.14	1.82	
XF 500855	E2	6.37	3.6188	9.12	0.45	
XF 500856	D3	6.30	3.7316	8.87	0.51	
XF 500857	C4	5.90	3.5205	8.28	1.03	
XF 500858	C4	5.81	3.6574	7.97	1.05	
XF 500859	D2	6.26	3.5501	8.97	0.62	
XF 500860	C3	6.26	3.5153	9.01	0.85	
XF 500861	B4	5.56	3.6154	7.51	1.78	
XF 500862	A5	5.34	3.6297	7.05	2.31	
XF 500863	E1	6.65	3.6249	9.68	0.35	
XF 500864	E1	6.49	3.6926	9.29	0.40	

No samples were available for analysis.

TABLE VI

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XG 500939	A	5.70	3.7024	7.70	0.425	100.3
XG 500940	B	5.78	3.7303	7.83	0.25	108.2
XG 500941	C	5.96	3.7204	8.20	0.285	116.0
XG 500942	D	5.74	3.1539	8.33	0.755	100.3
XG 500943	E	5.66	3.2385	8.08	0.755	121.5
XG 500944	F	5.60	3.1651	8.04	1.10	118.4
XG 500945	C	5.44	3.7505	7.13	0.62	102.7
XG 500946	D	5.32	3.3447	7.30	1.09	115.2
XG 500947	E	5.52	3.2290	7.81	1.50	110.5
XG 500948	F	5.34	3.2724	7.41	1.285	163.8
XG 500949	B	5.76	3.7466	7.77	0.38	108.2
XG 500950	A	5.68	3.7831	7.58	0.42	105.0

Continued overleaf ....

TABLE VI (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XG 500951	E	5.54	3.1503	7.93	0.495	129.3
XG 500952	F	5.61	3.2057	8.01	2.20	145.0
XG 500953	A	5.61	3.7141	7.51	1.31	100.3
XG 500954	B	5.74	3.7337	7.75	0.455	89.4
XG 500955	C	5.71	3.7678	7.66	0.375	100.3
XG 500956	D	5.40	3.2979	7.50	1.695	145.0
XG 500957	F	5.32	3.1175	7.52	3.125	135.6
XG 500958	C	5.79	3.7502	7.83	1.165	91.7
XG 500959	D	5.48	3.2811	7.68	1.765	140.3
XG 500960	E	5.61	3.2878	7.93	1.545	110.5
XG 500961	A	5.87	3.7470	7.99	0.45	116.0
XG 500962	B	5.93	3.8079	8.05	0.46	91.7

Continued overleaf ....

TABLE VI (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XG 500963	B	5.61	3.8211	7.40	0.49	102.7
XG 500964	A	5.61	3.8052	7.41	0.455	116.0
XG 500965	F	5.45	3.0970	7.80	1.22	155.0
XG 500966	C	5.72	3.7567	7.68	0.54	105.0
XG 500967	D	5.52	2.9921	8.05	4.84	157.5
XG 500968	E	5.38	3.1664	7.59	4.58	127.0
XG 500969	D	5.38	3.2917	7.47	1.47	145.0
XG 500970	E	5.38	3.2074	7.55	2.155	160.7
XG 500971	B	5.84	3.7560	7.92	0.37	97.2
XG 500972	A	5.76	3.7783	7.73	0.31	62.7
XG 500973	F	5.45	3.2251	7.67	3.625	177.9
XG 500974	C	5.72	3.7916	7.65	1.15	141.1

TABLE VII

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XJ 500793	B2	5.76	3.8465	7.67	0.74	127.8
XJ 500794	C3	6.00	3.7259	8.27	0.40	101.5
XJ 500795	D4	6.46	3.6007	9.32	0.38	97.6
XJ 500796	E5	6.92	3.4386	10.40	0.25	90.6
XJ 500797	A1	5.61	3.8615	7.36	0.825	147.9
XJ 500798	E4	6.65	3.5440	9.76	0.255	72.8
XJ 500799	A5	5.75	3.5916	7.91	0.94	141.7
XJ 500800	B1	6.32	3.6684	8.97	0.385	118.5
XJ 500801	C2	6.15	3.7006	8.60	0.40	93.7
XJ 500802	D3	6.52	3.4960	9.54	0.525	106.9
XJ 500803	C1	6.13	3.7466	8.51	0.385	85.2
XJ 500804	D2	6.46	3.5833	9.34	0.465	106.9

Continued overleaf ....

TABLE VII (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XJ 500805	E3	6.98	3.4821	10.48	0.24	84.4
XJ 500806	A4	5.76	3.6070	7.91	0.835	147.9
XJ 500807	B5	5.66	3.5178	7.80	1.00	158.8
XJ 500808	A3	5.46	3.7241	7.20	1.095	175.8
XJ 500809	B4	5.63	3.6579	7.60	0.985	144.8
XJ 500810	C5	6.29	3.3767	9.20	0.63	150.3
XJ 500811	D1	6.45	3.5398	9.36	0.375	93.7
XJ 500812	E2	6.72	3.4752	9.96	0.36	88.3
XJ 500813	D5	6.37	3.4788	9.26	0.44	147.9
XJ 500814	E1	6.85	3.5361	10.16	0.20	82.1
XJ 500815	A2	5.54	3.5866	7.49	1.03	130.9
XJ 500816	B3	5.71	3.6404	7.78	0.77	120.1
XJ 500817	C4	6.17	3.5707	8.77	0.465	96.0

TABLE VIII

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XK 500522	C3	5.97	3.6865	8.25	2.465	148.9
XK 500523	D4	6.29	3.6063	8.97	1.60	148.9
XK 500524	E5	6.80	3.4213	10.18	1.095	170.7
XK 500525	A1	5.15	3.8732	6.43	4.86	321.1
XK 500526	B2	5.23	3.8326	6.63	4.485	232.2
XK 500527	A5	5.03	3.6830	6.38	5.54	370.2
XK 500528	B1	5.53	3.7983	7.26	3.285	179.3
XK 500529	C2	5.71	3.7833	7.64	2.12	153.5
XK 500530	D3	6.52	3.6158	9.42	1.075	141.8
XK 500531	E4	6.58	3.5533	9.61	1.175	139.5
XK 500532	D2	6.47	3.5743	9.37	1.335	131.7
XK 500533	E3	6.66	3.5079	9.81	1.195	147.3

Continued overleaf ....

TABLE VIII (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XK 500534	A4	5.12	3.7478	6.49	3.75	236.9
XK 500535	B5	5.34	3.6716	7.01	4.475	184.7
XK 500536	C1	5.64	3.7622	7.52	2.505	161.3
XK 500537	B4	5.39	3.6535	7.13	3.995	173.0
XK 500538	C5	5.82	3.6014	8.04	2.415	159.0
XK 500539	D1	6.52	3.6536	9.39	0.855	126.3
XK 500540	E2	6.64	3.5795	9.70	1.035	113.0
XK 500541	A3	5.06	3.8484	6.27	4.21	308.6
XK 500542	E1	6.49	3.5820	9.40	1.17	170.7
XK 500543	A2	5.03	3.8712	6.19	4.735	404.5
XK 500544	B3	5.35	3.8611	6.84	2.69	243.2
XK 500545	C4	5.69	3.7529	7.63	2.21	128.6
XK 500546	D5	6.03	3.5755	8.48	2.34	161.3

TABLE IX

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XL 501110	D4	6.71	3.4625	9.96	0.595	80.6
XL 501111	E5	6.91	3.4016	10.42	0.45	86.1
XL 501112	A1	5.11	3.8167	6.40	2.11	190.9
XL 501113	B2	5.54	3.7285	7.35	1.77	149.5
XL 501114	C3	5.96	3.7675	8.15	0.89	124.4
XL 501115	B1	5.59	3.8021	7.38	1.12	121.3
XL 501116	C2	6.26	3.6226	8.90	0.64	70.4
XL 501117	D3	6.45	3.5885	9.31	0.535	75.9
XL 501118	E4	6.79	3.5029	10.08	0.46	94.7
XL 501119	A5	5.05	3.6936	6.41	2.33	177.6
XL 501120	E3	6.78	3.5379	10.02	0.515	75.9
XL 501121	A4	5.10	3.6972	6.50	1.735	170.6

Continued overleaf ....

TABLE IX (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn. content of plant p.p.m. dry matter
XL 501122	B5	5.33	3.6858	6.97	1.695	157.3
XL 501123	C1	6.12	3.7616	8.48	0.455	75.9
XL 501124	D2	6.78	3.5482	10.01	0.41	75.9
XL 501125	C5	6.15	3.6211	8.68	0.42	94.7
XL 501126	D1	6.53	3.6450	9.41	0.31	75.9
XL 501127	E2	6.92	3.4005	10.44	0.35	64.9
XL 501128	A3	5.47	3.7459	7.19	1.23	137.7
XL 501129	B4	5.62	3.7548	7.49	0.98	134.6
XL 501130	A2	5.44	3.7987	7.08	0.56	166.7
XL 501131	B3	5.55	3.7420	7.36	0.62	97.0
XL 501132	C4	6.00	3.6488	8.35	0.59	72.8
XL 501133	D5	6.17	3.6018	8.74	0.85	70.4
XL 501134	E1	6.82	3.5498	10.09	0.39	50.1

TABLE X

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XN 501034	E5	6.64	3.4071	9.87	0.11	114.6
XN 501035	A1	5.38	3.7485	7.01	0.84	204.6
XN 501036	B2	5.75	3.7010	7.80	0.485	170.6
XN 501037	C3	6.20	3.6125	8.79	0.395	160.2
XN 501038	D4	6.47	3.5529	9.39	0.34	152.8
XN 501039	C2	5.89	3.6951	8.08	0.235	116.2
XN 501040	D3	6.23	3.5814	8.88	0.14	98.9
XN 501041	E4	6.70	3.4888	9.91	0.12	103.6
XN 501042	A5	5.43	3.6822	7.18	0.845	253.8
XN 501043	B1	5.72	3.7530	7.69	0.385	229.2
XN 501044	A4	5.37	3.6504	7.09	0.94	181.6
XN 501045	B5	5.52	3.5064	7.53	0.54	106.8

Continued overleaf ....

TABLE X (Continued)

Plot sample number	Treatment	pH	pC	pL	W.S. Mn p.p.m. soil	Mn content of plant p.p.m. dry matter
XN 501046	C1	5.99	3.6554	8.33	0.36	98.9
XN 501047	D2	6.39	3.5111	9.27	0.155	88.5
XN 501048	E3	6.78	3.4453	10.11	0.255	106.8
XN 501049	D1	6.41	3.5451	9.27	0.21	77.5
XN 501050	E2	6.65	3.4535	9.75	0.155	64.9
XN 501051	A3	5.42	3.7139	7.13	0.93	170.6
XN 501052	B4	5.61	3.6134	7.61	0.96	147.6
XN 501053	C5	5.84	3.4527	8.23	0.59	147.6
XN 501054	B3	5.55	3.6527	7.45	0.60	168.5
XN 501055	C4	5.80	3.4681	8.13	0.645	134.0
XN 501056	D5	6.13	3.3844	8.08	0.53	124.0
XN 501057	E1	6.59	3.5483	9.63	0.195	98.9
XN 501058	A2	5.26	3.7406	6.18	0.92	262.2

Table XIa

Analysis of variance of manganese uptake due to treatments at centre XA.

Adjustment for mean		404,089.0624	
Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	1,842.0776	460.5194
Lime	4	39,275.5736	9,818.8934
Rows	4	11,285.9496	
Columns	4	6,477.4376	
Residual Error	8	3,449.9792	431.2474
Total	24	62,331.0176	

F (phosphate) = 1.068 F (lime) = 22.769

Table XIb

Analysis of variance of manganese uptake due to treatments at centre XB

Adjustment for mean		186,313.0896	
Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	502.8584	125.7146
Lime	4	17,376.3544	4,344.0886
Rows	4	380.5344	
Columns	4	334.2184	
Residual Error	8	416.9448	52.1181
Total	24	19,010.9104	

F (phosphate) = 2.412 F (lime) = 83.351

Table XIc

Analysis of variance of manganese uptake due to treatments at centre XC.

Adjustment for mean 234,004.3876

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	1,042.3184	260,5796
Lime	4	78,132.9704	19,533.2426
Rows	4	762.2504	
Columns	4	4,108.7264	
Residual Error	8	2,163.5568	270.4446
Total	24	86,209.8224	

F (phosphate) = 0.964 F (lime) = 72.226

Table XId

Analysis of variance of manganese uptake due to treatments at centre XD.

Adjustment for mean 554,250.4704

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	2,976.7216	769,1804
Lime	4	52,340.6516	13,085.1629
Rows	4	2,689.7496	
Columns	4	1,988.0456	
Residual Error	8	1,623.3812	202.9152
Total	24	61,618.4896	

F (phosphate) = 3.791 F (lime) = 64.486

Table XIe

Analysis of variance of manganese uptake due to treatments at centre XJ.

Adjustment for mean 339,213.0564

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	3,249.2456	812.3114
Lime	4	12,956.2976	3,239.0744
Rows	4	1,534.0976	
Columns	4	465.8536	
Residual Error	8	1,784.1992	223.249
Total	24	19,989.6936	

F (phosphate) = 3.639 F (lime) = 14.509

Table XIf

Analysis of variance of manganese uptake due to treatments at centre XK.

Adjustment for mean 943,307.1376

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	6,192.4944	1,548.1236
Lime	4	124,042.5784	31,010.6446
Rows	4	8,362.1104	
Columns	4	2,903.3944	
Residual Error	8	6,942.3648	867.7956
Total	24	148,442.9424	

F (phosphate) = 1.784 F (lime) = 35.730

Table XIg

Analysis of variance of manganese uptake due to treatments at centre XL.

Adjustment for mean		289,831.489	
Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	793.007	198.2517
Lime	4	34,226.543	8,556.636
Rows	4	3,305.603	
Columns	4	542.487	
Residual Error	8	2,532.711	316.5888
Total	24	41,400.351	

F (phosphate) = 0.626 F (lime) = 27.028

Table XIh

Analysis of variance of manganese uptake due to treatments at centre XN.

Adjustment for mean		513,486.8964	
Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	262.6976	65.6744
Lime	4	44,836.5176	11,209.1294
Rows	4	9,884.0016	
Columns	4	10,297.3936	
Residual Error	8	1,859.6832	232.4604
Total	24	67,140.2936	

F (phosphate) = 0.283 F (lime) = 48.220

Table XIIa

Analysis of variance of pH due to treatments at centre XA.

Adjustment for mean 830.938276

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.047984	0.011996
Lime	4	5.654264	
Rows	4	0.111224	
Columns	4	0.045824	
Residual Error	8	0.253528	0.031691
Total	24	6.112824	

F (phosphate) = 0.379

Table XIIb

Analysis of variance of pH due to treatments at centre XB.

Adjustment for mean 963.233296

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.103664	0.025916
Lime	4	8.103264	
Rows	4	0.669424	
Columns	4	0.023264	
Residual error	8	0.086088	0.010761
Total	24	8.985704	

F (phosphate) = 2.408

Table XIIc

Analysis of variance of pH due to treatments at centre XC.

Adjustment for mean 898.920324

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.091296	0.022824
Lime	4	4.939816	
Rows	4	0.153016	
Columns	4	0.421096	
Residual error	8	0.186952	0.023369
Total	24	5.792176	

F (phosphate) = 0.977

Table XIId

Analysis of variance of pH due to treatments at centre XD.

Adjustment for mean 918.817344

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.055336	0.013834
Lime	4	5.933736	
Rows	4	0.139216	
Columns	4	0.152536	
Residual error	8	0.246232	0.030779
Total	24	6.527056	

F (phosphate) = 0.449

Table XIIe

Analysis of variance of pH due to treatments at centre LJ.

Adjustment for mean 952.586496

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.078384	0.019596
Lime	4	4.655584	
Rows	4	0.090864	
Columns	4	0.170224	
Residual error	8	0.186044	0.0232555
Total	24	5.181100	

F (phosphate) = 0.843

Table XIIf

Analysis of variance of pH due to treatments at centre KK.

Adjustment for mean 853.340944

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.041896	0.010474
Lime	4	8.579936	
Rows	4	0.102336	
Columns	4	0.116776	
Residual error	8	0.095912	0.011989
Total	24	8.936856	

F (phosphate) = 0.874

Table XIIg

Analysis of variance of pH due to treatments at centre XL.

Adjustment for mean 913.8529

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.17852	0.04463
Lime	4	9.00788	
Rows	4	0.05932	
Columns	4	0.08080	
Residual error	8	0.20548	0.025685
Total	24	9.53200	

F (Phosphate) = 1.738

Table XIIh

Analysis of variance of pH due to treatments at centre XN.

Adjustment for mean 896.643136

Source of variation	d.f.	Sums of squares	Mean square
Phosphate	4	0.044904	0.011226
Lime	4	5.458264	
Rows	4	0.127024	
Columns	4	0.046744	
Residual error	8	0.043324	0.0054155
Total	24	5.720260	

F (Phosphate) = 2.073

TABLE XIII

Total manganese content in p.p.m. of soils of individual plots of centre XA (Abington). Soil samples taken in 1953. Figures are average of duplicate determinations.

645	580	643	655	655
623	618	650	645	635
638	638	678	685	635
613	628	625	665	635
693	655	643	635	570

Average manganese content 639 p.p.m.

TABLE XIV

Total manganese content in p.p.m. of soils of individual plots of centre XJ (Dalbeattie). Soil samples taken in 1953. Figures are averages of duplicate determinations.

535	568	560	543	523
575	548	535	568	563
553	528	563	543	548
593	570	585	470	460
605	555	528	465	505

Average manganese content 544 p.p.m.

TABLE XV

Total manganese content in p.p.m. of soil of individual plots of centre XL (Lugton). Soil samples taken in 1953. Figures are single determinations.

675	705	675	715	715
580	720	620	690	730
560	605	650	650	560
560	560	575	590	560
715	560	560	540	565

Average manganese content 625 p.p.m.

TABLE XVI

Centre	Reference	Year of Sample	Total Manganese p.p.m. soil
Abington	XA	1953	639 *
Cartmore	XB	1953	630
Cowie	XC	1953	350
Methven	XD	1953	817
Mauchline	XF	1953	642
Kilmacolm	XG	1954	752
Dalbeattie	XJ	1953	544 *
Bearsden	XK	1954	800
Lugton	XL	1953	625 *
Cleland	XN	1953	340

* Average figure obtained from determinations of total manganese for each of the individual plots in the centre.

Table XVIIa

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XA.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	2.303119917	
Regression of pMn on pH	1	2.299696556	
Additional variation due to inclusion of pC	1	0.003423361	0.00342336
Residual error	27	0.508971083	0.01885078
Total	29	2.812091000	

F = 0.18

Table XVIIb

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XB.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	3.631384690	
Regression of pMn on pH	1	3.435415849	
Additional variation due to inclusion of pC	1	0.195968841	0.195968841
Residual error	27	0.42199031	0.0156292707
Total	29	4.053375000	

F = 12.54

Table XVIIc

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XC.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	1.123892629	
Regression of pMn on pH	1	1.122652198	
Additional variation due to inclusion of pC	1	0.00124043	0.00124043
Residual error	27	1.509604781	0.05591128818
Total	29	2.633497410	

F = 0.022

Table XVIId

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XD.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	0.8405109861	
Regression of pMn on pH	1	0.7609867460	
Additional variation due to inclusion of pC	1	0.0795242401	0.0795242401
Residual error	27	1.0832489076	0.04012032991
Total	29	1.9237598937	

F = 1.98

Table XVIIe

Analysis of variance of regression equation of pMn on pH and on pH and pC combined for centre XF.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	1.78368144	
Regression of pMn on pH	1	1.61782215	
Additional variation due to inclusion of pC	1	0.16585928	0.165859282
Residual error	27	0.08239313	0.003051597
Total	29	1.86607457	

F = 54.35

Table XVIIf

Analysis of variance of regression equation of pMn on pH and on pH and pC combined for centre XG.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	2.860059693	
Regression of pMn on pH	1	2.238014889	
Additional variation due to inclusion of pC	1	0.622044804	0.622044804
Residual error	33	1.540137687	0.04968186087
Total	35	4.400197380	

F = 12.52

Table XVIIg

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XJ.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	1.077701941	
Regression of pMn on pH	1	0.998681591	
Additional variation due to inclusion of pC	1	0.079020350	0.079020350
Residual error	22	0.094933799	0.004315172688
Total	24	1.172635740	

F = 18.31

Table XVIIh

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XK.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	1.5024752449	
Regression of pMn on pH	1	1.4615021790	
Additional variation due to inclusion of pC	1	0.0409730659	0.0409730659
Residual error	22	0.0694584751	0.0031572034
Total	24	1.5719337200	

F = 12.98

Table XVIIi

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XL.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	1.296865723	
Regression of pMn on pH	1	1.189337151	
Additional variation due to inclusion of pC	1	0.107528572	0.107528572
Residual error	22	0.347360010	0.01578909136
Total	24	1.644225733	

F = 6.81

Table XVIIj

Analysis of variance of regression equations of pMn on pH and on pH and pC combined for centre XN.

Source of variation	d.f.	Sums of squares	Mean square
Bivariate regression	2	1.819038611	
Regression of pMn on pH	1	1.769281441	
Additional variation due to inclusion of pC	1	0.049757170	0.049757170
Residual error	22	0.478439709	0.0217472595
Total	24	2.297478320	

F = 2.29

TABLE XIX

Effect of time of shaking on extraction of manganese by a solution of M calcium nitrate. 20 gms. of soil R 30647 extracted with 200 mls. of solution. pH of extractant 3.60; pC = 1.0476.

Experiment 1.

Time (hours)	½	1	1½	2	2½	3
Manganese extracted as p.p.m. of soil	30	30	31	31.5	32	31.5
pH of extract	3.97	4.00	4.00	4.00	4.00	3.98
pC of extract	1.0476	1.0476	1.0476	1.0476	1.0476	1.0477

Experiment 2.

Time (hours)	1	2	3	4	5	6
Manganese extracted as p.p.m. of soil	28.5	29	31.5	30	32	32

(Manganese determined by periodate method in the calcium nitrate solution)

TABLE XX

Manganese extracted by N calcium nitrate solution from soil R 30647. Effect of variation of volume ratio. Shaking time, 2 hours.

Ratio of weight soil/ volume of extract	1:1	1:2.5	1:5	1:10	1:20	1:40	1:80	1:160
Mn Extracted as p.p.m. of soil	22.0	25.6+	24.9	25.4	24.1	25.6	23.8	26.1
pH of Extract	-	4.15*	4.20	4.25	4.41	4.56	4.73	4.96
pC of Extract	-	1.2761*	1.2754	1.2746	1.2708	1.2738	1.2727	1.2728
Characteristics of Extracting Solution	pH = 6.41; pC = 1.2329					pH = 6.60; pC = 1.2608		

Manganese extracted values are means of four determinations;
pH and pC values are average of duplicate samples.
* Single determinations only.
+ Mean of two determinations only.

TABLE XXI

Manganese extracted by 2 N calcium nitrate solution from soil R 30647. Effect of variation of volume ratio. Shaking time, 2 hours.

Ratio of weight of soil/ volume of extract	1:1	1:2.5	1:5	1:10	1:20	1:40	1:80	1:160
Mn extracted as p.p.m. of soil	25.1	29.1	27.7	32.5	33.5	26.5*	25.9	29.9
pH of extract	-	-	3.95	4.07	4.16	4.40	4.63	5.20
pC of extract	-	-	1.1006	1.1008	1.0953	1.1060	1.1072	1.1040
Characteristics of extracting solution	pH = 6.96; pC = 1.0941				pH = 7.02; pC = 1.0907			

Manganese extracted values are means of four determinations; pH and pC values are average of duplicate samples.

* mean of three determinations only.

TABLE XXII

Manganese extracted by 4 N calcium nitrate solutions from soil R 30647. Effect of variation of volume ratio. Shaking time 2 hours.

Ratio of weight of soil/ volume of extract	1:1	1:2.5	1:5	1:10	1:20	1:40	1:80	1:160
Mn extracted as p.p.m. of soil	25.4	27.6	27.4	26.2	27.7	25.6	29.5	30.7
pH of Extract	-	-	3.69	3.80	3.90	4.16	4.40	5.16
pC of Extract	-	1.0274	1.0257	1.0227	1.0168	1.0203	1.0212	1.0186
Characteristics of Extracting Solution	pH = 5.88; pC = 0.9917					pH = 7.16; pC = 1.0037		

Manganese extracted values are means of four determinations.

pH and pC values are average of duplicate samples.

TABLE XXIII

Effect of concentration of extracting solution

10 gms. of soil R 30647 shaken for 2 hours with 200 ml. of calcium nitrate solutions of varying concentrations and with water.

Extractant			Extract			Manganese extracted as p.p.m. of soil
Normality	pH	pC	pH	pC	pL	
4 N	5.88	0.9917	3.90	1.0168	6.78	27.7
3 N	6.60	1.0220	4.05	1.0280	7.07	28.4
2 N	6.76	1.0912	4.17	1.0980	7.24	25.6
N	6.71	1.2712	4.33	1.2709	7.39	24.2
N/2	6.30	1.4923	4.38	1.5027	7.26	26.6
N/4	5.76	1.7376	4.43	1.7525	7.11	25.4
N/8	5.66	1.9962	4.56	2.0091	7.11	25.8
N/16	5.66	2.2575	4.67*	2.2603*	7.08	25.0
N/64	6.28	2.8765	4.92*	2.7784*	7.06	24.8

Continued Overleaf ...

TABLE XXIII

(Continued from previous page)

Extractant			Extract			Manganese extracted as p.p.m. of soil
Normality	pH	pC	pH	pC	pL	
N/256	5.53	3.3902	4.67	3.2804	6.08	22.2
N/512	5.27	3.6746	5.16*	3.4804*	6.84	18.4
N/1024	5.16	3.8487	5.63*	3.5501*	7.71	14.7
N/2048	3.93	4.0432	5.44*	3.7102*	7.17	12.3
Water	5.37	5.0426	5.70*	3.8774*	7.52	6.4

All figures represent average of duplicate determinations, except those marked * which are single results.

Table XXIV

Extraction of Soil No. 801560 with N $CaCl_2$ pH adjusted by addition of N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0385.

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
6.25	1.1525	4.18	1.1698	4.04	1.1518	24.30	3.6558
11.06	1.1502	4.45	1.1862	4.34	1.1533	22.20	3.6950
11.40	1.1467	4.74	1.1863	4.68	1.1516	19.80	3.7447
11.56	1.1449	5.14	1.1924	5.07	1.1516	16.40	3.8266
11.68	1.1429	5.58	1.1941	5.40	1.1521	14.40	3.8830
11.78	1.1406	5.86	1.1853	5.70	1.1525	10.00	4.0414
11.84	1.1369	6.09	1.1794	6.03	1.1523	8.20	4.1276
11.92	1.1358	6.54	1.1811	6.22	1.1524	5.40	4.3090
12.00	1.1325	7.00	1.1805	6.31	1.1523	3.00	4.5643

Table XXV

Extraction of soil No. 801560 with 0.4 N $CaCl_2$.pH adjusted by addition of 0.4 N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0373

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
6.28	1.4893	4.22	1.5102	4.22	1.4895	19.72	3.7465
11.40	1.4839	4.56	1.5099	4.56	1.4904	17.84	3.7900
11.73	1.4770	4.92	1.5081	4.92	1.4900	17.00	3.8110
11.93	1.4692	5.40	1.5065	5.38	1.4897	13.80	3.9015
12.06	1.4626	5.85	1.5111	5.79	1.4833	10.62	4.0153
12.15	1.4569	6.28	1.5112	6.05	1.4887	7.28	4.1793
12.24	1.4489	6.71	1.5087	6.22	1.4891	4.44	4.3940
12.32	1.4416	7.05	1.5110	6.32	1.4893	2.24	4.6912
12.38	1.4350	7.34	1.5075	6.43	1.4881	0.98	5.0502

Table XXVI

Extraction of soil No. 801560 with 0.1 N $CaCl_2$.pH adjusted by addition of 0.1 N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0387

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
6.19	2.0185	4.26	2.0491	4.26	2.0223	16.72	3.8182
11.71	1.9908	4.70	2.0532	4.67	2.0200	13.96	3.8965
12.00	1.9595	5.18	2.0450	5.15	2.0198	11.32	3.9876
12.18	1.9326	5.69	2.0474	5.70	2.0189	8.08	4.1340
12.31	1.9038	6.15	2.0507	6.09	2.0182	5.28	4.3188
12.41	1.8797	6.70	2.0441	6.49	2.0176	2.88	4.5820
12.49	1.8574	7.19	2.0443	6.66	2.0186	1.74	4.8009
12.55	1.8347	7.54	2.0429	6.80	2.0177	1.00	5.0414
12.63	1.8098	7.91	2.0443	6.93	2.0163	1.00	5.0414

Table XXVII

Extraction of soil No. 801560 with 0.05 N $CaCl_2$.pH adjusted by addition of 0.05 N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0402.

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.80	2.2934	4.30	2.3182	4.30	2.2934	16.68	3.8192
1.82	2.2294	4.84	2.3065	4.84	2.2918	13.28	3.9183
2.12	2.1658	5.43	2.3030	5.42	2.2930	9.32	4.0720
2.31	2.1113	5.92	2.3184	5.88	2.2909	5.88	4.2720
2.44	2.0659	6.60	2.3069	6.26	2.2893	2.80	4.5942
2.53	2.0242	7.07	2.3122	6.45	2.2879	1.32	4.9208
2.60	1.9856	7.39	2.3130	6.56	2.2866	0.76	5.1606
2.66	1.9523	7.95	2.3101	6.90	2.2882	0.66	5.2219
2.70	1.9186	8.14	2.3129	6.98	2.2899	0.60	5.2632

Table XXVIII

Extraction of soil No. 801560 with 0.02 N $CaCl_2$.pH adjusted by addition of 0.02 N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0438.

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.82	2.6671	4.29	2.6865	4.32	2.6633	9.92	4.0449
11.85	2.5082	4.88	2.6865	4.96	2.6589	7.28	4.1793
12.15	2.3817	5.51	2.6827	5.59	2.6562	4.76	4.3638
12.31	2.2926	6.06	2.6835	6.18	2.6535	2.92	4.5760
12.42	2.2195	6.59	2.6827	6.66	2.6505	1.76	4.7959
12.52	2.1567	7.14	2.6772	7.00	2.6467	1.04	5.0244
12.60	2.1029	7.44	2.6783	7.11	2.6461	0.76	5.1606
12.66	2.0579	7.85	2.6757	7.22	2.6457	0.60	5.2632
12.72	2.0100	8.33	2.6714	7.64	2.6433	0.56	5.2932

Table XXIX

Extraction of soil No. 801560 with 0.01 N $CaCl_2$ pH adjusted by addition of 0.01 N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0443

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.53	2.9464	4.49	2.9571	4.42	2.9301	10.64	4.0145
11.90	2.6685	5.18	2.9461	5.10	2.9169	7.28	4.1393
12.20	2.4860	5.81	2.9461	5.68	2.9191	3.72	4.4709
12.38	2.3639	6.40	2.9392	6.13	2.9344	1.56	4.8483
12.58	2.2626	7.12	2.9315	6.66	2.9074	.70	5.1963
12.58	2.1915	7.54	2.9248	6.67	2.9066	0.44	5.3979
12.67	2.1299	7.94	2.9216	6.83	2.9005	0.36	5.4851
12.73	2.0747	8.32	2.9211	7.00	2.8965	0.36	5.4851
12.78	2.0298	8.53	2.9026	7.35	2.8953	0.42	5.4182

Table XXX

Extraction of soil No. 801560 with 0.005 N $CaCl_2$ pH adjusted by addition of 0.005 N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0455

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.48	3.2261	4.63	3.2156	4.59	3.1858	9.56	4.0609
12.00	2.7574	5.37	3.1987	5.31	3.1720	5.24	4.3221
12.31	2.5275	6.01	3.1864	5.86	3.1588	2.94	4.5731
12.48	2.3795	6.59	3.1796	6.27	3.1507	1.28	4.9342
12.59	2.2857	7.11	3.1630	6.60	3.1372	0.76	5.1606
12.68	2.2020	7.80	3.1592	6.76	3.1345	0.30	5.5643
12.76	2.1351	8.25	3.1440	6.92	3.1270	0.16	5.8373
12.81	2.0803	8.51	3.1455	7.02	3.1187	0.08	6.1383
12.87	2.0252	8.85	3.1429	7.30	3.1135	0.08	6.1383

Table XXXI

Extraction of soil No. 801560 with 0.0025 N $CaCl_2$.pH adjusted by addition of 0.0025 N $CaCl_2$ saturated with calcium hydroxide. Hydroxyl ion normality = 0.0455.

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.33	3.5073	4.70	3.4550	4.64	3.4360	6.38	4.2366
11.94	2.8167	5.46	3.4363	5.42	3.4173	2.96	4.5701
12.30	2.5677	6.14	3.4065	6.07	3.3926	1.64	4.8266
12.53	2.4076	6.76	3.3654	6.47	3.3422	0.80	5.1383
12.62	2.3006	7.40	3.3612	6.78	3.3528	0.32	5.5363
12.72	2.2202	7.72	3.3559	6.93	3.3356	0.16	5.8373
12.80	2.1408	8.20	3.3512	7.16	3.3275	0.08	6.1383
12.86	2.0849	8.60	3.3381	7.36	3.3183	0.06	6.2632
12.92	2.0185	8.95	3.3340	7.60	3.3028	0.04	6.4393

Table XXXII

Extraction of soil No. 801560 with water. pH adjusted by addition of a saturated solution of calcium hidroxide. Hydroxyl ion normality = 0.0473 .

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.40	5.1308	5.06	4.0477	5.10	4.0204	1.33	4.9175
12.00	2.8938	5.64	3.8194	5.66	3.7987	1.295	4.9291
12.30	2.6068	6.28	3.8860	6.30	3.8673	0.62	5.2490
12.46	2.4384	6.85	3.8078	6.80	3.7918	0.335	5.5164
12.58	2.3196	7.41	3.7669	7.06	3.7476	0.16	5.8373
12.66	2.2343	7.91	3.7339	7.21	3.7113	0.115	5.9807
12.73	2.1623	8.19	3.7014	7.35	3.6708	0.056	6.2932
12.79	2.0989	8.76	3.6827	7.43	3.6455	0.016	6.8373
12.85	2.0425	9.04	3.6637	7.85	3.6181	0.016	6.8373

Table XXXIII

Extraction of soil No. 801560 with a saturated solution of $CaSO_4$. pH adjusted by addition of a saturated solution of $CaSO_4$ saturated with calcium hydroxide.
Hydroxyl ion normality = 0.0414

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.70	2.7087	4.48	2.7147	4.45	2.6861	16.96	3.8120
11.88	2.5577	5.01	2.7258	4.94	2.7029	12.36	3.9495
12.14	2.4372	5.52	2.7139	5.45	2.6863	9.84	4.0484
12.29	2.3519	6.04	2.7331	5.97	2.7033	6.16	4.2518
12.42	2.2743	6.90	2.7283	6.64	2.7042	1.68	4.8161
12.51	2.2117	7.22	2.7306	6.80	2.7092	0.56	5.2932
12.60	2.1516	7.28	2.7575	6.83	2.7333	0.56	5.2932
12.67	2.1061	7.71	2.7804	7.02	2.7460	0.36	5.4851
12.72	2.0556	8.09	2.7721	7.18	2.7464	0.14	5.8953

Table XXXIV

Extraction of soil No. 801560 with 0.02 N KCl. pH adjusted by addition of 0.02 N KCl saturated with calcium hydroxide. Hydroxyl ion normality = 0.0473

Extractant		Suspension		Extract			
pH	pC	pH	pC	pH	pC	Mn p.p.m. soil	pMn
5.60	2.5988	4.302	2.6355	4.314	2.6102	18.04	3.7852
11.84	2.4459	4.875	2.6429	4.900	2.6139	13.52	3.9104
12.14	2.3272	5.457	2.6427	5.500	2.6114	7.96	4.1405
12.30	2.2356	6.130	2.6364	6.220	2.6109	3.76	4.4662
12.42	2.1644	6.652	2.6317	6.679	2.6117	1.97	4.7469
12.50	2.1027	7.160	2.6298	7.038	2.6040	1.20	4.9629
12.57	2.0519	7.551	2.6295	7.157	2.6028	0.48	5.3602
12.63	2.0059	8.049	2.6259	7.404	2.6017	0.08	6.1383
12.67	1.9730	8.244	2.6293	7.597	2.6010	0.04	6.4393

Table XXXV

0.5 g oat roots immersed in 50 or 100 mls of radio-active manganese chloride solution for 2 hours.

Counts below obtained as follows:-

Original Solution - 10 ml counted, count multiplied by 5 or 10 as case might be.

Final Solution " " " " " " " " " " " "

$CaCl_2$ washings (a) and (b) - 10 ml counted, count multiplied by 8

Residual manganese left in roots - roots wet ashed, and made up to 25 ml. 10 ml counted, count multiplied by 2.5.

Original solution			Final solution	$CaCl_2$ washings		Residual
Conc.	Vol.	c.p.m.	c.p.m.	c.p.m. (a)	(b)	c.p.m.
10^{-7} M	50	3055	1780	896	64	205
10^{-7} M	100	3055 x 2	3830	1448	64	350
10^{-6} x 0.5 M	50	15,535	9080	4152	232	683
10^{-6} x 0.5M	100	15,535 x 2	24470	4528	232	593

Continued overleaf ...

Table XXXV continued

Original solution			Final solution	$CaCl_2$ washings		Residual
Conc.	Vol.	c.p.m.	c.p.m.	c.p.m. (a)	(b)	c.p.m.
10^{-6} M	50	16,955	10,265	4968	184	683
10^{-6} M	100	16,955 x 2	27,200	4800	240	743
10^{-5} x 0.5 M	50	15,780	10,200	4960	208	743
10^{-5} x 0.5 M	100	15,780 x 2	25,770	5328	208	633
10^{-5} M	50	17,455	11,940	3664	120	403
10^{-5} M	100	17,455 x 2	29,210	3816	160	428
10^{-4} M	50	16,825	14,280	2192	136	260
10^{-4} M	100	16,825 x 2	30,230	2400	208	305
10^{-3} x 0.5 M	50	-	15,990	808	56	190