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Micronutrient studies on cotton growth on Syrian calcareous soils.

Mohammad, S Y.

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MICRONUTRIENT STUDIES ON COTTON GROWTH

ON SYRIAN CALCAREOUS SOILS

A Thesis for the Degree of Ph.D.

by

Shaher Youssef Mohammad B.Sc. (Damascus)
Dip.Agric.Sci.(Wales)

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SUMMARY

A field experiment was conducted on a recently reclaimed calcareous soil in the Euphrates' basin, Syria, to study the effect of soil applications and foliar sprays of Fe, Mn, Zn and Cu sulphates on cotton (var. Alepo 40) yield and fibre characteristics.

The soils are highly calcareous (15-25% $CaCO_3$), very low in organic matter (<1%), and rich in total iron and manganese oxides, but DTPA extracted very little "available" iron. There appeared to be adequate Mn and Cu but DTPA extractability of Zn declined to near critical levels after 90 days of cotton growth.

Submergence (anaerobism) alone was ineffective in increasing iron solubility when no organic source was applied to the soil. Easily decomposed organic material (starch or freshly collected green manure) could bring much Fe^{2+} into solution. This solubility increased greatly when fresh hydroxides were also included.

Green manure (clover) enhanced Fe, Mn and Zn availability in the field up to 75 days after incorporation. In pot experiments, green manure was effective in improving Mn, Zn and particularly Fe nutrition of cotton. Iron supplied by the organic complexing activity of green manure or its products was clearly quite different from inorganic iron from ferrous sulphate additions. The green manure prevented iron precipitation within the root even in the presence of phosphate, and it increased iron translocation to the upper plant portions. Natural organic chelate sources of iron are thus much more mobile and active within the plant.

Iron and zinc sulphate application to soil caused 5 and 10% increases in the cotton yield, while their application to plants as sprays resulted in 12 and 23% yield increases respectively. Iron also improved significantly lint smoothness.

The yield of cotton in the field was increased by ferrous sulphate sprays and soil applications largely because of the predominance of Mn solubility over Fe in the root medium rather than because iron "availability" was low. There was clear Mn/Fe and also P/Fe antagonism in the plants.

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LITERATURE REVIEW

Introduction

Investigation of the role of trace elements in soils, plants, animals and also in human nutrition has increased in the past few decades. These elements are not regularly applied to soil by usual fertilization programmes in most countries. The removal of these nutrients by leaching and plant uptake contributes towards the accelerated exhaustion of their available resources in soil, particularly as less farmyard manure and other natural fertilizers are being used. This leads to the appearance of deficiency symptoms on the plant, first reported at the end of the 19th Century, and today it is known that the extensive areas have insufficient micro-nutrients in available forms to meet plant demands for maximum growth and high yields (e.g. on calcareous soils).

Modern cultivation methods, improved varieties, better control of plant diseases, pests and weeds, increasing use of mineral fertilizers and increases in irrigated areas are all factors responsible for the general increase in crop yields in recent years. These increases have been necessary to support the rapidly increasing population of the world.

Trace element problems, now of local importance, may well become more serious in the near future with intensive agricultural practices and much continuous research is needed to avoid the development of trace element deficiencies. An understanding of features causing their fixation in unavailable forms in both soils and plants under varied physical, chemical, biological and environmental conditions

is needed and also the introduction of species and varieties tolerant or capable of responding to micronutrient stress in the soil medium.

This chapter is a literature review of the behaviour of Fe, Mn, Zn and Cu in both soils and plants.

1.1 Micronutrients in the Soil

1.1.1 Iron in the soil

In the field of geochemistry the element iron can be considered as a major one since it ranks third in abundance (about 5%) among the mineral elements in the earth's crust after silicon and aluminium, and soils rarely contain less than 1% of iron (Sillanpaa, 1972). Despite this abundance of iron in the soil, it is a trace element in the plant nutrition field and this is due to the extremely low solubility which does not exceed, for iron oxides, 10^{-11} moles Fe/l at pH 7-8 and this value is totally inadequate compared with the minimum requirements of a crop like maize which is about 10^{-6} moles Fe/l by mass flow (O'Connor et al., 1971).

In rocks iron occurs in both the ferrous and ferric state. During weathering, iron is released from minerals to form its free oxides and also to substitute for Al in clay minerals. The forms and nature of the oxides depend on the conditions under which the weathering occurs.

The total amount of iron in the soil is generally a very poor indicator of its availability to plants due to its low solubility and despite the low demands for Fe by plants for normal growth, approximately 1kg Fe/ha per year for a crop of corn or wheat (Marschner, H., 1978); its deficiency seems a common phenomena on many soils and for many species especially under calcareous soil conditions.

The principal factors affecting the solubility and availability of hydrous oxides are numerous such as Eh, pH, concentration of the metal of interest, concentration of

competing metals, concentration of other ions capable of forming inorganic complexes, and organic chelates (Jenne, 1968). pH and Eh are probably the most significant.

Amorphous iron oxides can be present in the soil in both active and inactive forms with very different solubilities. The greatest solubility recorded by Schindler et al. (1963) is 10^{-7} moles Fe/l and this value is higher than for goethite or inactive oxides.

These active oxides of iron under moist and aerobic conditions will be subjected to recrystallisation giving other inactive compounds such as lepidocrocite and goethite (Hsu and Ragone, 1972).

Fe-oxides of soils adsorb considerable amounts of trace elements depending on the soil chemical conditions e.g. pH. Grime (1968) has shown in his study on a grey brown podzolic soil that 300-600 p.p.M of Cu were adsorbed by Fe-oxides in B-horizons, and the adsorption was pH dependent and began in the order Cu, Zn, Co, Mn at pH values of about 3.0, 4.0, 4.6 and 5.0 respectively. The adsorption began with concentration of ions at the surface, the ions being occluded and irreversibly fixed. Hematite and amorphous Fe-oxide behaved in the same way as goethite.

The plant's ability to acquire iron from the soil depends on the soil conditions in which there exist a demand for oxygen, restricted diffusion and soluble organic materials. For these reasons the estimation of potential iron uptake by plants from the soil by the chemical extractants has been frequently unsuccessful (Metwally and Abdellah, 1978).

Iron plays an important role in soil chemistry being

involved in numerous reactions affecting the chemical and physical properties of soils. Due to its interaction with other elements, it may considerably affect the availability of micro- as well as macronutrients.

In neutral and alkaline soils Fe^{2+} is oxidized to Fe^{3+} which is so insoluble that plants have difficulty in absorbing it. Granich (1958) reported that at pH 7, the solubility of Fe^{3+} is only 10^{-7} mole per litre, while that of Fe^{2+} is 10^{-4}, and so increasing oxidation potential decreases Fe availability to plants.

Under alkaline and calcareous conditions, soils which undergo alternate cycles of reversible oxidation-reduction processes were found to maintain a higher solubility of Fe and Mn (Sinha et al., 1978). pH and organic matter content were found to affect significantly the solubility.

The solubility of Fe^{3+} in equilibrium with its hydroxide increases 1.000-fold for each unit of pH decrease (Oertli and Opoku, 1974), and evidence has been presented by Jacobson et al. (1956) that K^+ uptake by plants stimulates Fe nutrition from inorganic forms by inducing pH reduction and this is in agreement with the observations of Hoffer and Krantz (1949) in calcareous soil.

Chelates are better than Fe salts due to their ability to maintain the iron in the soluble form, but both hydrolysis and the substitution of iron and manganese by calcium in chelates in calcareous soil decreases their effectiveness as Fe sources.

The stability of Fe-EDTA in calcareous soil is too low to supply the plants with enough Fe + Mn (Boxma et al., 1971). Similar results were obtained by Hill-Cottingham (1957) and Wallace et al. (1957). Wallace and Lunt (1956) attributed the cause of inactivation of Fe-chelates in calcareous soils also to the hydrolysis of Fe chelates and fixation of Fe on the clay portion. While Lindsay, Hodgson and Norvell (1967) reported that the reason was the displacement of Fe by Ca and Zn at pH values above 7.5. Wallace's (1962b) results agreed with Lindsay. Lunt, Hemaidan et al. (1956) added to Lindsay's investigation by showing that the entire iron chelate molecule is fixed on clay colloids, and that the iron fixed by this procedure was found to be slightly available to plants. Wallace and Lunt (1956) reported that fixation of Fe-EDTA occurs not on the basal plane surfaces of the clay but rather on the edges of the clay and is greater for kaolinite than for montmorillonite.

Although in most cases Fe-DTPA is very successful in correcting iron chlorosis it may have some unfavourable effects. This chelate depresses the manganese uptake (Boxma, et al., 1971) and the high and rapid fixation of Fe-DTPA reduce its value as a source of iron. Fe-EDDHA overcomes the problems of fixation in the soil and in addition Fe-EDDHA is the least toxic of all iron chelates.

Reduction of iron and manganese as a result of anaerobic conditions on flooding is a consequence of the anaerobic metabolism of bacteria and appears to be chiefly a

chemical reduction by bacterial metabolites (Bloomfield, 1951; Motomura, 1961).

It is known that lower initial pH, higher total iron content, higher organic carbon with higher soil temperatures favour greater increases in the availability of iron under waterlogged conditions (Ponnamperuma, 1965; Mandal, 1961; Ponnamperuma, 1972).

Reduction of iron and manganese may occur in micro-sites even in unflooded soil conditions and a maximum accumulation of exchangeable iron and manganese was found when soil was kept at field capacity with high temperatures (up to 32^{o}C) (Cheng et al., 1972).

Sinha et al. (1978) indicated that fluctuating periods of anaerobism and aerobism will also increase the extractable iron and manganese fractions. Ottow et al. (1971) isolated 71 facultative anaerobic bacteria capable of reducing iron oxide in pure culture, and among these 71 iron-reducing bacteria, all except 3 were capable of reducing nitrate to nitrite.

Under extremely anaerobic conditions with a high organic matter content an accumulation of organic acids and other phytotoxic substances induce some detrimental effects on the rice (Okajima et al., 1970).

In the acid soils most of the inorganic phosphorus is as a ferric phosphate and the use of the lime and then of organic matter will result in an increase in the availability of soil phosphorus under waterlogged conditions. In the first stage, most of the inorganic soil phosphorus occurs as ferric phosphate and lime would convert part of the ferric phosphate

to a calcium phosphate, which would subsequently be made soluble by the CO_2 formed by the decomposition of the added organic matter as a second stage (Mandal, 1961). Marschner et al. (1975) reported that micro-organisms play no significant role, since under both sterile and non-sterile conditions, the sunflower plants were able under iron stress to decrease the pH, release reducing substances and riboflavin. There appears to be no direct function for riboflavin in the root media. However there are many simple organics such as homoserine, aspartate, glutamate, lactate, glucose and fructose which can stimulate microbial activity and increase microbial populations (Egeraat et al., 1975 and Matsumoto et al., 1979). In oxygen limited conditions the iron reducing bacteria may become dominant (Trolldenier, 1973, 1977).

In swamp soils the presence of soluble iron removes the harmful soluble sulphides present in the reduced zone. Green manure in a simulation of this gave increased soluble Fe and Mn in the soil solution which could react with sulphides giving Fes and Mns besides providing the plant with adequate iron and manganese (Sturgis, 1936; Mitsui et al., 1951).

1.1.2 Manganese in the soil

Most of the rocks of the earth's crust contain manganese, the average content being about 900-1000 p.p.m. (Vinogradov, 1959; Kovda et al., 1964). The average in lime-stone is 400-600 p.p.m.

Manganese occurs in soil in several oxidation states in water soluble, exchangeable and easily reducible forms and in primary minerals or higher oxides of manganese (Viets, 1962;

Lopez and Graham, 1972). A dynamic equilibrium is believed to exist between the manganese forms so that the tetravalent form is most likely to occur in alkaline soils, the trivalent form is presumably favoured by soil pH values near neutrality and the divalent form is found in both water soluble and exchangable form under acidic conditions (Leeper, 1947; Tisdale et al., 1966).

The last two forms of soil manganese (water soluble plus exchangable) are probably in equilibrium with the higher oxides and hydroxides of manganese in soil, but Metwally et al. (1973) in a study of manganese forms that are in equilibrium with soil solution for both alluvial and calcareous soils added that both chelated and easily reducible forms of manganese also participate in that equilibrium.

The problems of how to determine the plant available manganese pool have not been overcome, despite an extensive literature on the subject, and so far there is no satisfactory method for estimating available soil manganese and the classical methods for estimating readily available manganese include that water soluble, exchangeable, and easily reducible. Several investigators were unable to differentiate between manganese deficient and non-deficient soils on the basis of estimating the aforementioned forms (Heintze, 1938; Leeper, 1947; Jones and Leeper, 1950; Fick, 1954). Metwally et al. (1973) also obtained the same results showing great variability with poor correlation between soil extractable manganese and plant uptake of manganese under calcareous soil conditions.

There are indications that manganese oxidation in soils near neutrality is largely microbiological, but a high

concentration of Mn^{2+} in the soil solution can inhibit the microbial oxidation of manganese. The optimum pH for microbial oxidation is 6-8 (Leeper and Swaby, 1940). Bromfield (1974) added that only small changes of 0.1-0.2 of a pH unit outside the range 5.8-7.8 inhibited manganese oxidation by Arthro-bacter Sp.

Bromfield's (1974) results suggest that CO_2 is considered as a stimulatory factor in manganese microbial oxidation as a result of its acidifying effect, while Uren et al. (1977) indicated that where manganese oxidizing micro-organisms are involved the supply of CO_2 is important and that the microbial oxidation of Mn^{2+} can occur at low oxygen pressure provided CO_2 is adequate. Leeper (1947) was of the opinion that the reoxidation was bacterially mediated and those reoxidation products could be identified by hydroquinone extraction. Bromfield and David (1978) have agreed with Leeper's findings.

Manganese oxides have a great capacity for absorbing cobalt and they also occur as nodules of iron and manganese segregated in separate phases within the crystalline mass (Brewer, 1973).

Higher available manganese was correlated positively with organic matter increasing, and the reverse was true with respect to pH and $CaCO_3$ content. Khan and Ryan (1978) in their investigations on Lebanese calcareous soil found that manganese availability to the crops increased with organic matter increase, while decreased ~~with every higher decline~~ to an even greater extent with increase in pH and $CaCO_3$ content in the soil. Tisdale et al. (1966) reported that the presence of a high organic matter

content in soils frequently results in the appearance of manganese deficiency symptoms at a lower pH than in soils with a lower humus content. This has led to the assumption that certain types of organic matter will form insoluble complexes with divalent Mn^{2+} (Boischot et al., 1950). On the other hand, higher oxides of manganese do not predominate in organic soils and addition of organic matter to soils with a low manganese content has been found to increase the availability of manganese (Christensen et al., 1950).

The chemistry of manganese is very important in the nutrition of lowland rice. The chemical changes which occur when a soil is submerged are complex and were reviewed recently by Ponnamperuma (1972). The changes include Eh, pH and the breakdown of organic matter with the production of CO_2 and new organic compounds. At pH 5.0 almost all of the reducible soil manganese was converted to the water soluble plus exchangeable fraction even at a redox potential as high as +500 (Gotoh and Patrik, 1972). At pH between 6.0-8.0 most of the conversion took place at relatively lower redox potentials of +200 to +300 mV.

Soils which were subjected to periodic changes of flooding and drying were found to contain higher available manganese and the reoxidation of the reduced manganese produces an enhanced plant available form (Leeper, 1947).

1.1.3 Zinc and copper in soil

Zinc and copper have been recognized as micro constituents of soils for many years. The total contents of zinc and copper in soils have often been found to be

unreliable indices because several factors affect their presence in plant available forms.

A knowledge of the mechanism controlling soil zinc availability would make it possible to predict zinc availability under a wide variety of conditions, and thereby facilitate the development of more efficient agronomic practices for minimizing zinc fixation. Clay minerals (Bingham, Page and Sims, 1964), sesquioxides (Chu, 1968; Stanton and Burger, 1970), carbonates (Udo, Bohn and Tucker, 1970) and also soil organic matter (Tan, King and Morris, 1971), have all been suggested as constituents involved in the fixation and/or precipitation of added zinc in soils.

Jenne (1968) reported that hydrous oxides of iron and manganese were the major matrices accommodating zinc and some of the other micronutrients in soil, and the capacity of adsorption of iron and manganese oxides was a function of both pH and the amount of phosphate ions adsorbed by the oxides (Stanton and Burger, 1970), so we can conclude that iron oxides are very closely associated with the adsorption and retention of zinc (Grime, 1968; Shuman, 1979), especially by the more crystalline fractions in the presence of phosphate (Stanton and Burger, 1970).

In calcareous soils variations in calcium carbonate content have a major effect on zinc availability (Navrot, et al., 1969) but the mineralogical composition of these soils also plays an important role in influencing both the total and available zinc (Navrot et al., 1971), and it is probable that soil rich in montomorillonite can contain more

zinc, but due to stronger zinc bonding the content of available zinc may be lower.

Copper can be readily adsorbed by goethite but it has been found more closely associated with the soil organic matter in alkaline soils (Shuman, 1979), and it was reported by Hodgson et al. (1966) that more than 98% of the copper in solution was in an organic complexed form, suggesting that in neutral soils very small quantities of free Cu^{2+} are available for adsorption reactions, and Cu fixation by organic matter has often been considered the main cause of Cu deficiency in organic soils (Szalay et al., 1968). It is adsorbed as $CuOH^{+}$ by carboxyl groups and as Cu^{2+} usually by phenolic groups (Lewis and Broadbent, 1961a).

Although Cu may occur in soils in both mono- and divalent forms, the effect of oxidation-reduction on its availability is generally considered smaller and less clear than that of Fe and Mn.

Randhawa and Broadbent (1965a) found that the species of zinc complexed by the humic acid varied with pH, at pH 3.6 they concluded that 70% of the zinc retained by humic acid was present as Zn^{++} whereas at pH 7.0 75% was $ZnOH^{+}$, and the stability constants for the zinc humic acid complex were calculated to be 4.42 at pH 3.5, 6.1 at pH 5.6 and 6.8 at pH 7.0 (Randhawa et al., 1965b).

The high solubility of some organic complexes of zinc and copper recorded by McBride and Blasiak (1979) have been confirmed by the improvement in prediction of plant available zinc from chemical extractions, if the soil organic content was

determined (Iyengar and Deb, 1977; Shukla and Prasad, 1976).

In some attapulgite rich soils, zinc fixation by clay minerals is reduced and consequently they can supply sufficient zinc e.g. for maize (Navrot and Gal, 1971).

Under submerged soil conditions zinc and copper deficiency in rice plants is reported to be fairly widespread. This phenomenon is attributed to the formation of insoluble compounds like carbonates, sulphides, hydroxides and organic chelates and to microbiological immobilisation (Yoshida and Tanaka, 1969).

It is known that in submerged soil, especially if organic matter content is high, an increase in the intensity of soil reduction accompanied with an increase in Mn^{2+}, Fe^{2+} and phosphates in solution may influence the available zinc and copper content (Mandal, 1964; Misra, 1975).

Haldar and Mandal (1979) suggested that microbiological immobilisation and the antagonistic effect of increased concentrations of soluble iron, manganese and phosphorus have been the possible reasons for the observed decrease in the availability of zinc and copper.

1.2 Mechanism of Micronutrient Movement in Soil Toward Plant Roots

Movement of micronutrients from the soil and the soil solution to the root is the most important stage for uptake by the plant. This movement is governed by three different mechanisms: massflow, diffusion and the direct contact between the roots and the solid phase of the soil, and no doubt all three mechanisms occur depending upon the nature of the nutrient within the soil.

Barber (1962) defined mass flow as the movement of dissolved nutrients carried by water flow through the soil to the plant roots, and this movement occurs as the result of a water deficiency at the plant root induced by transpiration.

If mass flow cannot supply as much nutrient to the root surface as is adsorbed by the plant, the concentration at the root surface is lowered, and the ions in solution being subject to thermal movement move in the medium from points of higher concentration to points of lower concentration by the process known as diffusion.

In practice it is very difficult to distinguish between nutrient movement by mass flow and by diffusion. If no water flow occurs, diffusion can be measured alone (micro-nutrients, especially zinc and copper, move if there are differences in availability in different regions of the soil) but if there is water flow, an interaction between the mass flow and diffusion process occurs. Thus the plant first stimulates nutrient movement by mass flow and secondly,

stimulates the transfer of ions by diffusion if its rate of nutrient absorption is greater than the rate of movement of ion to the roots by mass flow.

Factors that effect the rate of micronutrient movement to plant roots are:

1) Plant species: plants vary in their ability to absorb micronutrients from dilute solutions (Loneragan, 1968). Plants that have a high rate of transpiration will develop a higher H_2O suction at the root surface accompanied by a faster rate of water movement and thus of ions to the root by mass flow.

2) The affinity between ions and the solid phase of soil affects movement in the soil, and the diffusion coefficient of Cu^{2+}, Mn^{2+}, Zn^{2+} in clays of increasing cation exchange capacity decreases in the order kaolinite > illite > montmorillonite > vermiculite (Ellis et al., 1970a). The adsorption strength also depends on the ion size and charge and polarisability.

3) Insoluble compounds and solubility coefficient of these compounds at high pH values (Fried and Broeshart, 1967; Lindsay and Norvell, 1969) limit the release and movement of ions in the soil solution. Even at low pH values, Fe and Mn occur as oxides and hydroxides, thus the level of ion mobility in the soil depends on the soil pH and the nature of precipitation.

4) Forming a chelated ion which decreases the tendency of the free ion to react with the soil (O'Connor et al., 1971).

5) Soil water content, a decrease can cause: a) Reduction of cross-sectional area available for flow. b) Increased tortuosity of the flow path. c) Decreasing mobility of the water near the clay surface.

The plant itself plays a very important role in altering the availability of these ions in the soil solution by respiration, exudation and microbial stimulation. These factors will be discussed in more detail later.

1.3 Mechanism of Micronutrients Uptake and Translocation

The nature of the mechanisms that control or govern uptake and translocation of micronutrients have not been fully clarified and there is considerable disagreement in the literature concerning the role of metabolic activity in the uptake of micronutrients by plant tissues especially that of zinc.

Micronutrient absorption is affected by a number of physiological and chemical factors in soils, viz:

1. Their presence in an extremely low concentration.
2. The positive correlation between concentration and uptake.
3. Being sensitive to temperature and especially aeration.
4. Their interaction with other ions in soil.
5. Variations between species in ability to absorb them from soil.
6. Their reductive reactivity at the root surface.

1.3.1 Iron

Seeds usually contain sufficient iron to meet the seedling's requirements (Brown, 1961), and the factors that interfere with iron uptake by plant roots from the growth medium, do not interfere with the use of iron from the cotyledons (Ambler and Brown, 1974).

Green plants require a continuous supply of iron as they grow because iron does not move from the old to the new leaves (Brown, 1961), and iron deficiency often is attributed not to a lack of iron in the plant, but rather the plant

itself fails to translocate iron from the root to the shoots, and for some reasons (e.g. nutrient imbalance) iron inactivation in the leaf occurs despite the presence of iron in quantities over the usual required amount.

Both the growth medium and the plant species affect the uptake and use of iron by plants (Brown et al., 1972), and above pH 4.0, the solubility of Fe^{3+} decreases 1000-fold for each unit increase in pH (Latimer, 1952).

In an early study by Kliman (1937), it was concluded that plant roots do not take up the iron until released as cationic Fe^{2+}. Some time later, several studies were published (e.g. Tiffin and Brown, 1959; Brown et al., 1961), concerning the mode of iron uptake also suggesting its initial reduction.

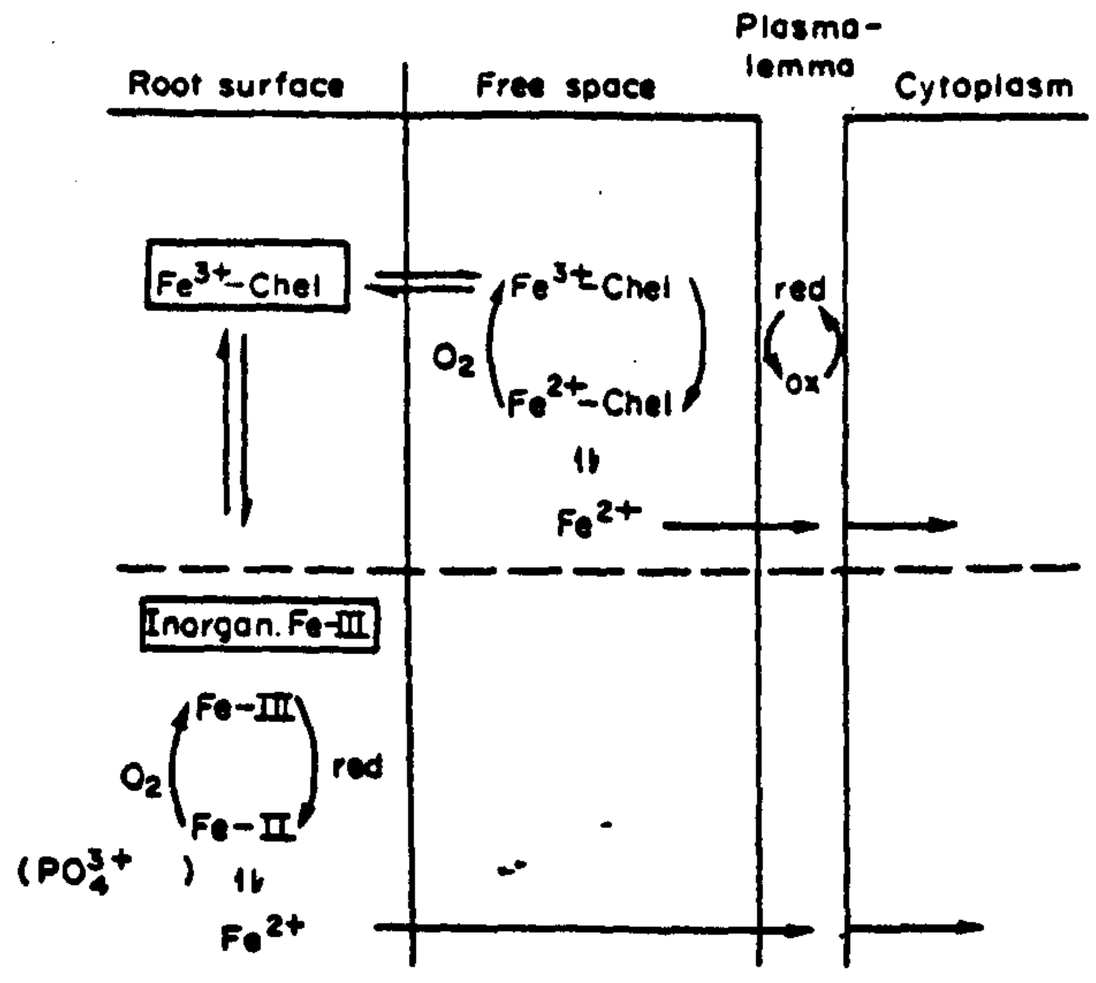
Ambler et al. (1971) in their study to identify areas of iron reduction at the soybean root reported that reduction was most pronounced between the regions of root elongation and root maturation at both the epidermis and the endodermis, and the reducing capacity was greatest in the young lateral roots indicating that these roots contribute significantly to the ability of plants to take up iron. Genotype characteristics of plants which have the ability to reduce iron partially explain why they can obtain iron from synthetic chelates and can utilize iron in calcareous soils more effectively than other plants.

Kashirad et al. (1973), in their investigation of the form of iron absorbed by plant roots, found that at least in the corn variety "velox" with normal iron nutritional status the source of iron (organic or inorganic) and presence

or absence of phosphate in the absorption solution influence the form in which iron is absorbed and translocated.

In the case of Fe-EDTA supply, uptake and translocation seems to take place mainly in the form of the entire chelate molecule, when $FeCl_3$ is supplied in the absence of phosphate, presumably iron is mainly accumulated and translocated as Fe^{2+}, whereas in the presence of phosphate due to impaired reduction, iron probably remains mainly in the form of Fe^{3+} during accumulation and translocation (Kashirad et al., 1973).

The iron uptake model proposed by Chaney et al. (1972) is presented below in the modified form of Marschner (1978). It demonstrates that plants take up iron from the soil either in chelated form or in inorganic form, and that with organic iron, the chelates can enter the free space of the roots. Therefore, all cells of the cortex can take up iron from the chelates. With inorganic Fe^{3+}, however, the iron is practically exclusively present in insoluble form as particles which can be in close contact with the root surface, but cannot enter the free space; with this in mind, the importance of organic substances excreted by the roots into the soil bulk should be the subject of concentrated research in a study of inorganic iron uptake to identify the conditions under which different plant species can utilize this source of iron for normal growth.



Model for iron uptake by plant roots.

It is evident from this model that both root surface and the rhizosphere are of primary importance for the utilization of inorganic iron, whereas with iron chelates the diffusion of the chelate into the free space allows its uptake into the root cell or its reduction prior to uptake at the plasmalemma, as assumed by Chaney et al. (1972).

Iron is translocated in the root as either Fe^{2+} or Fe^{3+} in phosphate deficient and sufficient conditions respectively (Kashirad et al., 1973) and the oxidation of Fe^{2+} occurs only when it is translocated from the lateral root xylem into the main root xylem in soybean plants (Ambler et al., 1971).

Plants vary widely in their ability to change iron into a useful form and the plants are classed as Fe-efficient if they respond to iron deficiency stress by inducing bio-

chemical reactions that change iron into a useful form, and Fe-inefficient if they do not.

Brown et al. (1974) have concluded that monocots were less effective at iron uptake, i.e. making it available from its organic and inorganic sources, than dicots which have a great ability to obtain adequate iron even when it is in a very low concentration in solution by influencing its insoluble forms in soil, releasing organic substances in the plant root medium and reducing the soil pH, all of which release iron to plants. However, when the iron is supplied in the divalent form, the differences between monocots and dicots are completely suppressed (Christ, 1974).

The supply of iron to the plant leaves needs to be continuous (Oserkowsky, 1933), and this is also true with respect to other micronutrients. Very little redistribution of zinc and manganese from the older leaves to the new leaves occurs. Copper is much more freely redistributed (Kannon, 1977; Loneragan, 1975). In the case of iron there is a mobile phase in the old leaves but this does not supply biochemically active iron to new tissues.

Present information indicates that citrate is the major compound involved in keeping Fe mobile in plants, and ferric citrate has been identified in exudates of sunflower (Tiffin, 1966a), and in soybean (Tiffin, 1970), and the adequacy of sap citrate concentration to provide the necessary complexing capacity for Fe^{3+} translocation has also been demonstrated (Tiffin, 1970).

1.3.2 Manganese

Manganese as was reported by Morre (1972) shows a two phase process, a rapid initial uptake thought to be passive and a slower phase thought to be metabolic.

Munns et al. (1963) defined three fractions of manganese in oat roots, replaceable, labile and nonlabile. The replaceable manganese was rapidly removed by electrolyte solutions. The labile manganese equilibrated rapidly with the ambient solution and moved readily to the shoot. The non-labile fraction was only slowly transported to the shoot, and was concentrated in the older root tissue, while the labile fraction was localized in the younger tissue. The non-labile form had no metabolic involvement.

In contrast, Bowen (1968) showed that manganese absorption by sugarcane leaf tissue was strongly inhibited by amytal and succinate and was also strongly temperature dependent, concluding that the process was under metabolic control.

The manganese in extracts of ryegrass exists as a single cationic and probably non complexed form, and its translocation within the plant occurs as a divalent ion (Tiffin, 1972).

1.3.3 Zinc

Gutknecht (1961, 1963) concluded that Zn uptake by algae was non-metabolic and essentially a process of cation exchange. Rathore et al. (1970) in their study on bean plants agreed with Gutknecht's conclusion, but Bowen (1969)

investigating zinc and copper absorption by sugarcane leaf tissue found that both had characteristics of an active process and uptake of zinc and copper was reduced by low temperature, DNP, N_3^-, CN^- and arsenate and was completely inhibited by amytal and nembutal, indicating that even at relatively high concentrations metabolic processes were involved.

1.3.4 Copper

Reilly (1969) in his study using extractants of plant tissues showed that much of the accumulated copper was not in ionic form and an increase in total nitrogen occurred with an increase in copper content, and it is suggested that copper may be bound as a protein complex.

Copper is much more strongly bound in organic complexes than zinc with only one carrier between roots and shoots, and because of the affinity between copper and the N atom of amino carboxylic acids, the expected carriers are probably amino acids (Tiffin, 1972).

1.4 Biochemical functions of micronutrients in plants

The biochemical function of micronutrients and their metabolic role within plant tissues has been studied through the metabolic changes occurring under their stress.

1.4.1 Iron

Plants suffering from iron deficiency, genetic chlorosis or certain virus infections, and also young leaves, contain more potassium relative to calcium (Dekock and Hall, 1955), and the potassium concentration in the exudate sap from chlorotic plants can be four times more than with non-chlorotic plants (Iljin, 1952).

It has been suggested by Hewitt (1963) that high concentrations of potassium in the plant leaves as a result of iron deficiency is associated with an increase of iron translocation, promoting phosphorylation, and decreasing phosphate uptake by plants.

The present state of our knowledge of the role of iron in the maintenance of greenness of plants is not clear, but iron is suggested to take a key position in growth and respiration, by regulating chlorophyll synthesis and the light depending process of photosynthesis.

Jacobson et al. (1956) concluded that if iron is supplied at a uniform rate, a good correlation is obtained between iron and chlorophyll contents in sunflower leaves, but when the plant undergoes a preliminary period of iron deficiency, then no correlation is found when the iron supply subsequently becomes adequate and it is suggested that iron is involved in chloroplast formation via protein synthesis

directly or indirectly.

It was recently shown that iron is an essential component of many heme and non-heme Fe enzymes and carriers, and it is generally accepted that iron does not play any role in the enzymatic synthesis of prophyrins either in plants (Carell and Price, 1965) or in porphyrin-secreting bacteria (Kortstee, 1970).

An alternative hypothesis is emerging from the work of Price et al. (1972) that when the alga Euglena gracilis was grown under iron deficient conditions, the iron deficient chloroplast ribosomes had less than half as much chloroplast RNA as the control. This result has agreed with the view of Fuwa et al. (1960) suggesting that iron may be playing an essential role in nucleic acid metabolism.

Chlorotic leaves contained enhanced amounts of free amino acids depending on their iron status, as reflected by the phosphorus : iron ratio (Dekock and Morrison, 1958) and their content of citric acid was markedly more than that of malic acid, and the citric acid : (malic + oxalic acid) ratio varies as the phosphorus : iron and potassium : calcium ratios. Green leaves contain large quantities of either malic or oxalic acid. Sugarcane green leaves can synthesize malate more efficiently and also utilize it for sucrose synthesis more rapidly than chlorotic ones. In contrast, more amino acids, reducing sugars and sugar phosphates are synthesized in the chlorotic leaves (Naik et al., 1979). An accumulation of citrate, glutamate and tartrate in the chlorotic leaves also occurred. These results indicate that sucrose synthesis is disturbed in the chlorotic leaves and can be corrected by

foliar sprays of ferrous sulphate.

When the plant itself fails to show iron deficiency symptoms, the peroxidase activity in plant leaf tissue is a very good indicator (Perur et al., 1965). Perur (1965) also concluded in his study on soybean plants that when the plant was given inadequate iron, peroxidase activity was reduced by 71%, showing that latent Fe deficiency can be detected by measurement of peroxidase activity.

In contrast to Perur's results, Dekock et al. (1960) have reported that in mustard leaves peroxidase activity showed only small variation between chlorotic and green leaves, whereas catalase activity was several-fold greater in green than in chlorotic leaves and a linear relationship of 60:1 between chlorophyll and hematine was found. In pea plants catalase and chlorophyll were closely related to iron supply. An inverse relationship was observed between peroxidase and catalase activity (Del Rio et al., 1978) since peroxidase was increased in both deficient and excess iron leaves. Peroxidase/catalase ratio varied with iron supply. Thus it appears that under iron stress peroxidase and catalase activities in the leaves are affected by plant genotype and there is no constant relationship by which iron deficiency level can be assessed.

1.4.2 Manganese

Manganese appears to participate in the O_2-evolving reactions of photosynthesis, and is required for glycollate formation in green algae (Tanner et al., 1960), and manganese substitution for magnesium in many of the ATP-dependent enzymes of glycolysis.

Manganese also acts as a catalytic agent in nitrate

reduction where it may be replaced by iron. It constitutes a part of some respiratory enzymes and of some enzymes responsible for protein synthesis.

1.4.3 Zinc

In zinc deficient leaves the content of RNA and the rate of protein synthesis is very low. Zinc ions take part in amino acid formation.

Numerous dehydrogenases contain zinc (Vallee, 1955, 1960), and thus alcohol dehydrogenase from yeast with a molecular weight of 15.000 contains 0.18% zinc. Four atoms of zinc bind nicotinamide adenine dinucleotide (NAD) to the enzyme, and zinc is firmly bound to the ADH protein and NAD is reversibly bound to zinc.

Zinc deficiency is another cause of the inhibition of chlorophyll synthesis. Ohki (1976) in his study on the cotton plant reported that when zinc content in the blade of the third leaf of cotton was less than the critical level (14 ug/g), the chlorophyll content was inhibited, while it remained constant when the zinc content was above the critical level. He attributed the growth reduction under zinc deficient conditions to the inhibition of chlorophyll synthesis.

Zinc is also an essential constituent of carbonic anhydrase (Evans et al., 1966) by which the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions is catalysed. Graham and Reed (1971) suggested that zinc may be very important to photosynthesis.

1.4.4 Copper

Copper is a constituent of important enzymes such as phenol oxidases, ascorbic acid oxidase and the "key enzyme" phenylalanime ammoniumlydase, which controls the lignification process. It is also important in protein metabolism, and may be associated with chlorophyll formation.

1.5 Causes of Chlorosis in the Field

The factors that induce plant chlorosis in the field are numerous and complicated, the majority of these factors are outside the terms of this thesis, but are listed for the sake of completeness.

- Environmental factors such as: light, temperature, aeration, moisture and frost.
- Plant genotype.
- Disease or toxaemia from microorganisms.
- Soil properties (chemical + physical).
- Interaction with the major elements.
- Interaction between the micronutrients themselves.

The major emphasis in this discussion will concentrate on P, Fe, Zn interactions due to their agricultural importance, particularly under calcareous soil conditions, drawing into the discussion other nutrient interactions where it is thought appropriate.

Wallace and Lunt (1960) concluded their review with respect to iron chlorosis by giving a list of problems to be understood and also to be solved before iron chlorosis can be corrected. Some of these are:

- How do plants ordinarily obtain iron which is very insoluble in soil?
- How is iron translocated in plants?
- Why are some plants susceptible to lime-induced chlorosis while others are not?
- What happens to iron in plants when it becomes "inactive"?

- Why is the micronutrient balance so intimately related to the development of iron chlorosis?
- How are bicarbonates and CO_2 related to iron chlorosis?
- How does phosphorus metabolism in roots affect iron chlorosis?
- Why is iron chlorosis so nearly irreversible?

Iron deficiency in plants is believed to arise both from insufficient supply of this element to plants under certain soil conditions, and also through its inactivation within plant tissue, especially in roots after it had been taken in. Its accumulation occurs on or in plant roots (McGeorge, 1949) and this is attributed chiefly to iron fixation by phosphate ion (Biddulph, 1951; Somers et al., 1942), and partially due to excessive absorption of calcium by plants (Wildon, 1957), or through the combined effect of calcium and phosphate present in growth media (Brown et al., 1950).

In intact tomato roots , suitable supplies of chelating agents were effective in mobilization and translocation of iron to the aerial tissue. An increase of leaf phosphate concentration accompanied iron uptake enhancement. Thus it is believed that both absorbed iron and phosphate are a part of iron fixed in or on the roots (Ayed, 1970).

The primary interference between iron and phosphate occurs by either the precipitation of insoluble iron phosphates in the soil diminishing the area of iron free oxide surfaces (Brady, 1974), by forming phosphate bridges between the oxides and other micronutrients e.g. zinc (Stanton and Burger, 1967), or acting internally within the root (Cumbus

et al., 1977), and stem (Esters and Bruetsch, 1973) inhibiting micronutrient transport to the plant shoots (Marschner, 1978).

To elucidate this, Kashirad et al. (1973) supplied phosphorus and inorganic Fe^{3+} to different zones of the primary root of corn plants and demonstrated that the interactions within the plant root and also within the shoot were very small compared to that in the substrate and at the root surface.

Extensive investigations have linked high P/Fe ratios in the leaf with the intensity of chlorosis (Dekock et al., 1960b; Elgala et al., 1971) reflecting the reduction of active iron in the plant, and also preventing the dilution of leaf phosphate and thus resulting in a uniformity of P/Fe ratios independent of the actual cause of chlorosis e.g. heavy metal toxicity (Dekock, 1956).

It has long been known that iron chlorosis is associated with high potassium levels in leaves (Jacobson et al., 1956). Since potassium is taken up faster than most other ions, it will often tend to produce lower solution pH at least in the vicinity of the roots. At this lower pH, Fe dissolves and because of the close proximity of the root, some iron ions are absorbed before they diffuse into the bulk solution and become precipitated as the hydroxide (Oertli and Opoku, 1974).

In calcareous soil, owing to rapid precipitation of insoluble calcium phosphate, gradual additions of phosphatic fertilizer in areas of intensive cropping will be necessary for maximum production, and continuous supply like this after some time creates an iron deficiency problem (Matter, 1976).

Iron uptake is also reduced by high contents of heavy metals such as manganese, copper, molybdenum. There is an evident antagonism between iron and manganese, in that an increase in manganese brings about a decrease in soluble iron and an increase in the percentage of insoluble iron in the plants. Kelley (1914) found crystals of manganese dioxide in the tissues of plants that showed manganese toxicity. Furthermore, since the oxidation potential of manganese is higher than that of iron it may exert a preventive action against the reduction of iron by the reducing systems of the plant (Hopkins, 1930).

The classical case of the importance of micronutrient balance was investigated by Somers and Shive (1942), in which they reported that Fe/Mn ratio affects the growth and the condition of the plant more than the absolute concentrations of these nutrients, and that with soybean, the growth and condition of plants were normal when this ratio was approximately within the range of 1.5 to 2.6, but when the ratio of soluble iron to soluble Mn in the plant leaves was outside this range, pathological symptoms tended to develop.

Twyman (1951) did not consider the Fe/Mn ratio very significant in determining manganese deficiency or toxicity, but thought that the ratio might be important in the metabolism of healthy plants as a factor in determining growth and yield.

It has long been recognised (Brown et al., 1959) that a low oxygen content in soils in some cases is related to iron deficiency under widely differing conditions (Boxma, 1972; Kovanci et al., 1978). The reason for this might be the

increased carbon dioxide content of the soil which, with soil water, may form bicarbonate ions in a calcareous medium. The bicarbonate ions may indirectly decrease the availability of iron by increasing the solubility of calcium phosphates which both interfere with iron availability in the root medium, or cause inactivation of iron in the plant itself (Olsen et al., 1960). Miller and Thorne (1956) found that the respiration rate of root tips of plants susceptible to bicarbonate was much reduced in the presence of bicarbonate, and was affected in plants not susceptible to lime-induced chlorosis. It was also observed that cytochrome oxidase activity of root preparations was consistently less in the presence of HCO_3^- than in the presence of Cl^-, $SO_4^=$, NO_3^-, $HPO_4^=$ or $H_2PO_4^-$ ions (Miller and Evans, 1956b).

Interaction between zinc and phosphate has been widely studied (e.g. Rudgers et al., 1970; Warnock, 1970; Brown and Tiffin, 1962; Jackson et al., 1967), and all have shown that zinc deficiency may be related to high levels of phosphate in the soil: The so-called "phosphate induced zinc deficiency". On the other hand, several investigators either failed to show any effect of P on Zn nutrition (Boawn et al., 1954; Bingham, 1963) or revealed an increase in uptake of micronutrients caused by the presence of phosphate.

Malavolta and Lopez (1972), in a series of experiments with excised barley roots, studying the causes of the phosphate induced zinc deficiency concluded that the deficiency occurs by several processes: non competitive inhibition of zinc uptake, precipitation of zinc by phosphate at the root surface, reduction in translocation to the tops, and dilution effects

resulting from higher growth rate caused by phosphate.

An antagonism is well known between zinc and copper in wheat (Brar and Sekhon, 1976; Chaudhry and Loneragan, 1970) , zinc and iron in maize (Clark and Brown, 1974), and copper and iron in wheat (Brown et al., 1977).

Problems of plant susceptibilities to micronutrient interactions not only depend on the species but also on the individual genotype, and plants may be classified as lime hating and lime loving (Hutchinson, 1967). The lime induction of chlorosis in a plant reflects the adaptation of the plant to non-calcareous soil conditions.

There is a very strong manganese and zinc uptake suppression by iron, which itself is suppressed by Mo (Olsen and Watanabe, 1979). In tomatoes, Berry and Reisenauer (1967) reported Fe/Mo interactions, and Mo also depressed the ability of plants to reduce Fe^{3+} to Fe^{2+}.

1.6 Role of Rhizosphere in Micronutrient Nutrition and Plant Response to Iron Stress

The concentration of Fe^{3+} in the soil solution is far too low (= 10^{-10}M) to meet the demands of fast-growing crop plants (Lindsay, 1974) due to high pH and redox potential. Despite this low value of iron availability, most plants seldom develop iron chlorosis in soils, and this may be attributed to several factors: A "2-phase-effect" at the soil/root interface (Jenny, 1961, 1965), an increase in iron solubility by complex-forming organic compounds released by the plant roots such as organic acids and amino acids (Scheffer et al., 1965, 1967), or humic acids (Badurova et al., 1967), phenolic substances (Scheffer et al., 1968), or a lower pH and redox potential within the rhizosphere.

Root exudates could be involved directly in iron availability via complexation, or indirectly via enhanced microbial activity leading to a lowering of the redox potential and an increase in the Fe^{2+} concentration (Trolldenier, 1971). The mechanism by which rhizosphere micro-organisms can accelerate iron uptake is not yet fully understood. There are, however, good indications that the redox potential is one of the main factors involved. High microbial activity in the rhizosphere means a high demand for O_2. This high demand for oxygen makes it limiting leading to a drop in the redox potential in the rhizosphere. However this contribution of microorganisms should not be overestimated because, at least in the case of "iron-efficient" plant

species such as alfalfa, utilization of Fe^{3+}-oxide in sand culture is not dependent upon the presence of microorganisms in the rhizosphere (Anton et al., 1965).

Marschner and Barber (1975), in their study on sunflower plants under sterile and non-sterile conditions, concluded that microorganisms play no significant role since the ability of plants under iron stress, to decrease the pH of the nutrient solution and to release reducing substances and riboflavin and to make Fe^{3+} available for uptake and chlorophyll formation was similar under both sterile and non-sterile conditions.

A wide experimental study has been carried out by Trolldenier (1973) on rice and wheat, who concluded that an insufficient supply of other nutrients can increase the root exudation rate.

Potassium deficiency induces an increase in exudation rate and number of bacteria, leading to the decrease in oxygen concentration and redox potential. This drop in redox potential shifts the equilibrium from Fe^{3+} to Fe^{2+} increasing the iron uptake of the plants as Fe^{2+} is the main form of iron taken in and translocated up the shoot. All these changes in pH, Eh and the formation of microbial products with Fe chelating properties can take place in the rhizosphere, improving the iron nutrition even in soils with a high pH.

Influence of potassium supply to rice on number of bacteria, O_2 and Fe^{2+} in the solution (After Trolldenier, 1973).

K treatment	No. of bacteria x 10^{-6}	Concentration in the nutrient solution	
		mg/O2/l	mg/Fe^{2+}/l
K_2	1244	17.5	1.0
K_1	1686	8.6	2.4
K_2/K_0*	2036	0.5	10.6

* 55 days K_2 and then 21 days K_0

The nitrogen source also affects the solubility of other plant nutrients, iron in particular. It has been demonstrated by Farrahi and Aschtiani (1972), that in calcareous soil, $Ca(NO_3)_2$ as a N source depressed shoot growth and induced iron chlorosis, while $(NH_4)_2\ SO_4$ increased both growth and chlorophyll content. This type of effect is due to pH changes within the rhizosphere induced by different cation/ anion uptake.

Effect of 2g N per pot as NH_4^+ or NO_3^- on chlorophyll content and growth of Vinca minor on calcareous soil of pH 8.3 (After Farrahi, 1972).

Treatment	Chlorophyll content in the dry matter/ relative value	Dry matter production per 100 plants/ relative value
Control	100	100
$Ca(NO_3)_2$	69	39
$(NH_4)_2SO_4$	177	260

Plants under iron deficiency stress take up cations rather than anions from solutions (e.g. K^+, Ca^{++}, Mg^{++}) which cause a rise in carboxylic acids in the plants in addition to high levels of hydrogen ion release from the roots in the soil (Hofner and Grieb, 1979).

Plant species or varieties differ widely in their iron stress response. Brown and Jones (1974) in a study of two varieties of tomato plants showed the ability of the iron efficient plants to drop the nutrient solution pH even when nitrogen was supplied as nitrate. This drop of pH was associated with more release and uptake of iron by plant roots, while the inefficient tomato variety failed to show any change in solution pH or response to iron stress.

In maize, iron efficient and iron inefficient varieties have been studied by Brown and Bell (1969) showing that only under high iron stress, the efficient variety was able to induce a slight reduction in the nutrient solution pH, in contrast to the tomato varieties which have the ability to cause a pH drop of 2.0 units in a few hours (Brown and Ambler, 1974).

Monocotyledonous species required a substantially higher iron concentration in the nutrient solution in order to attain optimum growth than did the dicotyledonous species. These differences disappeared when Fe^{2+} was used (Christ, 1974) confirming the postulation that Fe^{3+} is reduced before uptake as chelated iron by the root, and the reduction also takes place when iron is used in ionic forms. The efficiency of iron uptake seems to depend on the efficiency of the root system of the particular plant species in reducing Fe^{3+}. The removal of iron from the chelate complex after reduction to Fe^{2+} seems also to present no difficulty to many plant species.

Much attention has been given to the ionic balance in investigating the efficiency by which iron stressed plants take up iron.

Venkat Raju (1972) demonstrated that the pH lowering effect in the initial stages of iron deficiency takes place as a result of excess cation over anion uptake with the assumption that the accumulation of organic anions or carboxylates play an important role in this response of the plant to iron deficiency.

Considering ionic balance, cereals and grasses grown with nitrate as the nitrogen source excrete considerable amounts of HCO_3^- or OH^- ions (Dijkshoorn et al., 1968) whereas in young tomato (Kirkby, 1974) and sugarbeet (Egmond et al., 1977) plant root excretion of OH^- ions is considerably lower.

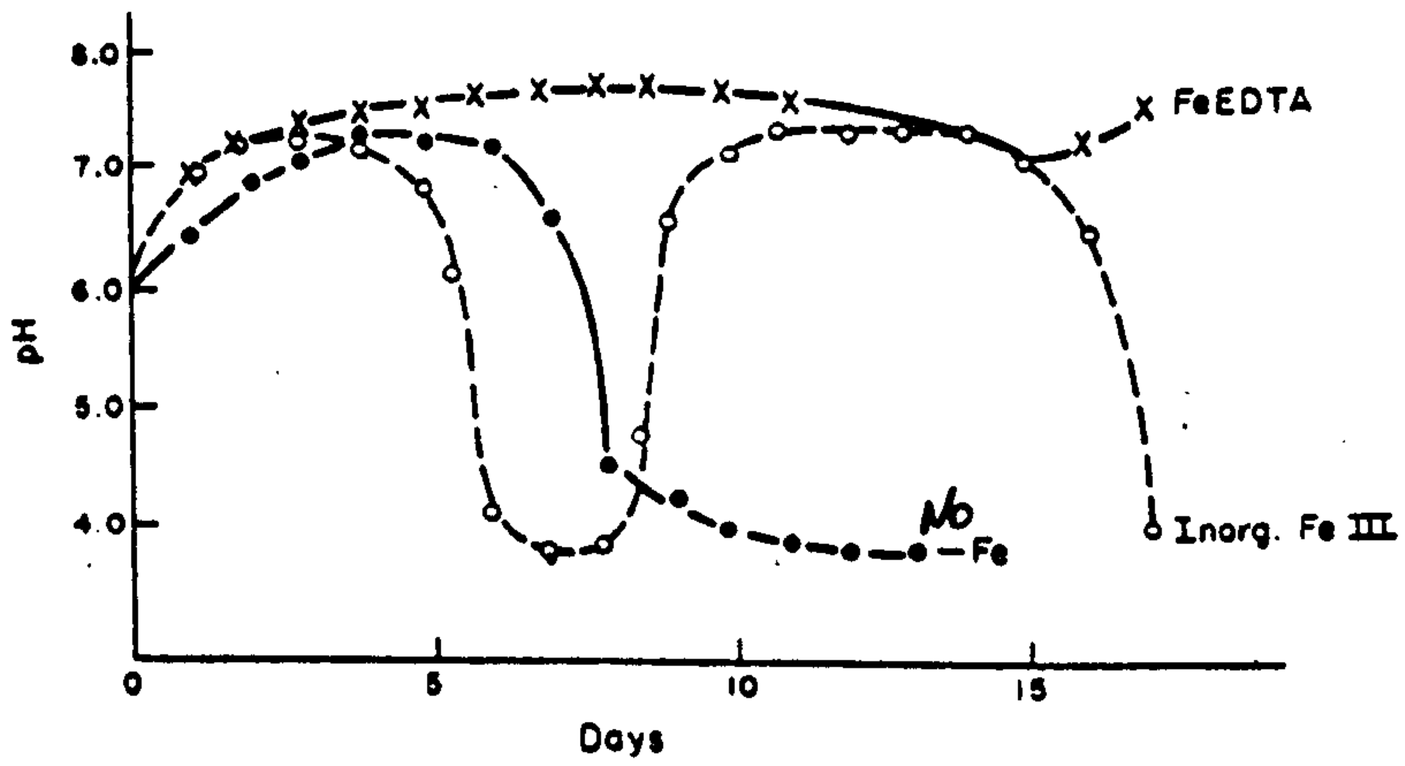
It can be concluded that those plant species which normally excrete relatively low amounts of OH^- ions when grown on nitrate N and respond to iron stress by lowering the pH of the nutrient medium and decreasing anion uptake, may be considered as being Fe efficient. Those which normally excrete relatively high amounts of OH^- ions when grown on nitrate N and which continue to increase the pH of a nutrient medium when under Fe stress may be considered as Fe inefficient (Egmond and Aktas, 1977). They proposed four classes of response to iron stress:

Normal full nutrient solution N as NO_3^-	Iron deficiency stress
1) High OH^- release	Fe stress lowers this but still with a net OH^- release, e.g. cereals.
2) Low OH^- release	Fe stress OH^- goes up e.g. soybeans, inefficient varieties.
3) Intermediate OH^- release	Fe stress reduced anion over cation uptake a net H^+ release to solution, e.g. soybeans, efficient varieties (Hawkeye).
4) Intermediate OH^- release	Fe stress reduced anion uptake, increased cation uptake - much H^+ release, e.g. sunflowers.

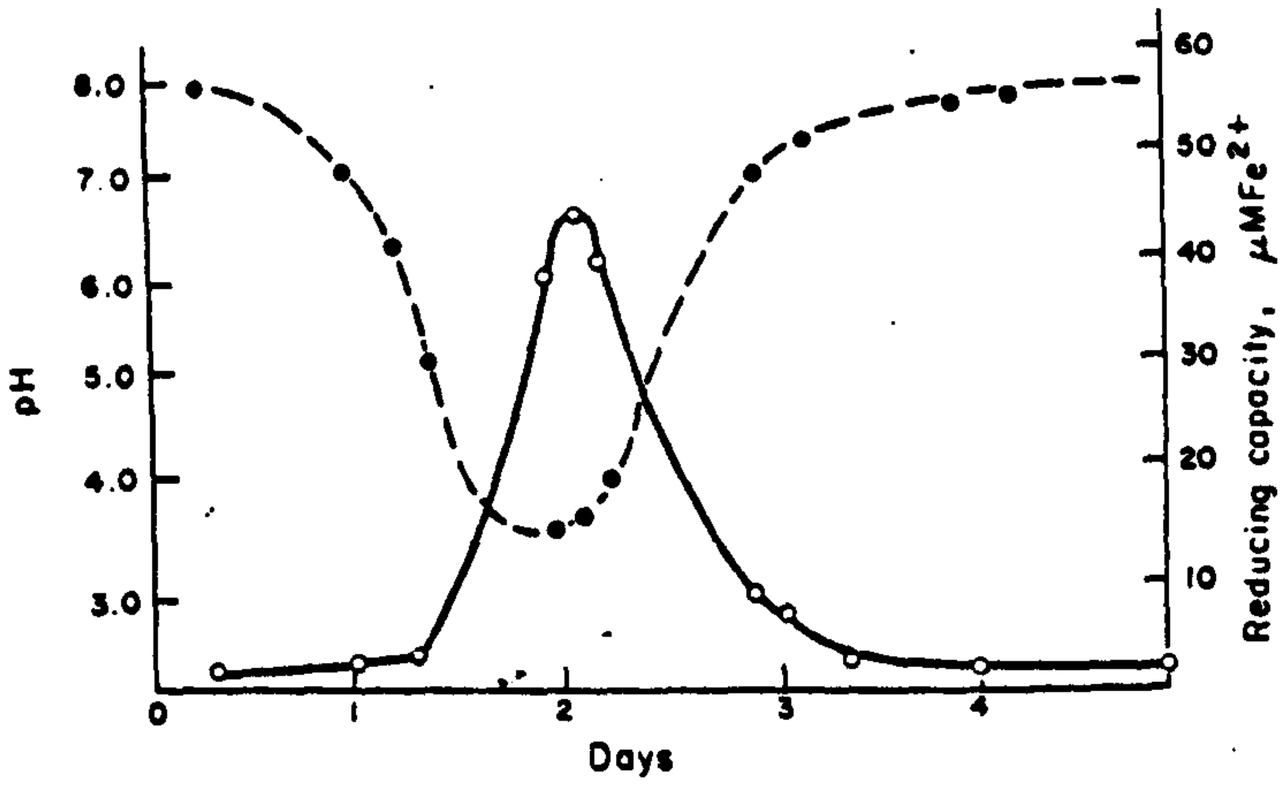
It is evident from this classification that species belonging to class 1 and 2 are Fe-inefficient, and the species belonging to class 3 and 4 are Fe-efficient. This classification also is very useful in deducing the causes of the widely differing results obtained by investigation of the effects of mixing iron efficient and inefficient plants in the same nutrient solution.

The iron efficiency reaction control was considered to be dependent on the root genotype (Brown et al., 1971), and was closely governed by the conditions in the rooting media. pH lowering is not only caused by increased release of organic acids from the roots, but by a shift in cation/anion uptake. Under iron stress the cation uptake remains almost constant with a sharp decrease in anion uptake leading to excessive H^+

release. If there is no iron present, then the pH of the substrate remains low and the plant also becomes severely chlorotic. In the presence of Fe^{3+} in the substrate, this lowering of the pH causes mobilization of Fe^{3+} via reduction to Fe^{2+} and an increasing Fe uptake. The iron stress is overcome rapidly and the cation/anion uptake is normalized, leading to an increase in pH. This sequence of events repeats depending on the iron nutritional status of the plant, and are represented below by the data as demonstrated by Marschner et al. (1974).



Effect of different iron supplies to sunflower plants on the pH changes in the nutrient solution.



Time course of pH value and reducing capacity of the nutrient solution with sunflower supplied with inorganic Fe-III.

In a comparison between sunflower and maize roots subjected to both iron sufficient and deficient conditions, looking particularly at root morphology, Romheld and Marschner (1979) have shown that when iron deficient, the main root growth of sunflower was inhibited and extensive lateral roots developed behind the root tips with a high accumulation of riboflavin causing the tissues to become yellow. This phenomenon was associated with a twelve-fold increase in the number of long root hairs from the enlarged rhizodermis. In contrast, the root hairs are very short under iron rich conditions, while on the other hand with maize, roots subjected

to the same conditions did not show any morphological changes detectable by the authors.

Rayle (1979) has concluded that enhanced cell division, expansion and hydrogen ion efflux is caused under the influence of auxin, whereas it has been suggested by Romheld and Marshner (1979) that auxin accumulation in sunflower roots is caused by the suppression of IAA oxidase activity. This suggestion is interesting as accumulation of phenolic materials has been long associated with iron stress responses.

Chlorogenic acid has been recently isolated from sunflowers and acts as an auxin protection, accumulating at the site of cell division, poising the Eh of the cell in a reduced state and bringing about a total halt to IAA breakdown until it is wholly oxidized (Stonier et al., 1979). Micronutrient availability in the soil medium under the rhizospheric influence can be varied. Manganese can be rendered more soluble by reduction and complexing (Bromfield, 1958). Bromfield's results have shown that both oats and vetch plants released substances which dissolved MnO_2, and these substances were readily decomposed by micro-organisms. They became more effective as the pH dropped below 7.0 and also as their concentration increased. The substances released were different for each plant although the process of solution appeared similar.

Both zinc and copper can have enhanced solubility and plant uptake in the presence of micro-organisms (Tiller et al., 1972; Barber and Lee, 1974).

Under alkaline soil conditions, zinc is not in equilibrium with soil solution in the presence of plants and

must be brought into solution by agents associated with the root environment (Rovira, 1969). This supports the view expressed by Wilkinson, Loneragan and Quirk (1968) when they found depletion of zinc around wheat roots by autoradiographic techniques and is the possible basis for species differences in their feeding power for soil zinc (Gladstones et al., 1967).

1.7 Micronutrient deficiency correction in plants

A knowledge of the causes in the field of micronutrient deficiencies is necessary if the best method of correction is to be selected. There are three different techniques for controlling micronutrient deficiency, and the technique selected depends on both soil and plant genotype characteristics:

1) By supplying micronutrients to soil or direct to plant.
2) By altering the availability of nutrients native within the soil.
3) By altering the activity of the micronutrients within the plant.

1.7.1 Micronutrient soil treatments

1.7.1.1 Iron

Most inorganic iron fertilizers, including $FeSO_4.7H_2O$ are rapidly fixed on soils and provide little correction of Fe deficiency (Lindsay et al., 1967) and so a continuous supply is necessary throughout the season to maintain healthy plants, especially when the iron availability in the soil is inadequate to cover plant requirements.

Follett and Lindsay (1971) examined the decrease in extractable Zn, Cu, Mn and Fe following fertilization, and concluded that during a 14 week experimental period the copper declined to 61%, Zn to 44%, Mn to 14% and only 20% of the Fe added as $FeSO_4.7H_2O$ remained extractable after one week. On the other hand, 70% of Fe-EDDHA was extractable after seven weeks and 26% after fourteen weeks.

Soil application of $FeSO_4.7H_2O$ did not alleviate iron

chlorosis of the rice seedlings, while the foliar spray greened up the leaf tissue slowly. The best method of correcting was acidulation of soil prior to sowing of paddy seeds which made seedlings become very vigorous, green and healthy and free from chlorosis (Patel et al., 1977).

Withee and Carlson (1959) concluded that soil application of ferrous sulphate for correcting iron deficiency chlorosis of grain sorghum was somewhat effective but economically impractical. Using a 4.0% ferrous sulphate solution spraying on the leaves was an effective method of improving the yield of grain.

Murphy et al. (1970) found that applying the range of 200-600 kg/ha of ferrous sulphate was inadequate for correcting iron chlorosis in rice or sorghum (even if banded). An increase in sorghum growth and yield occurred when Mathers et.al. (1970) used higher rates of iron sulphate (1100-2780 kg/ha) banded below the seeds.

In contrast to Murphy's results, Stewart-Jones (1980) found that in Hofuf, Saudi Arabia, treatment of soil (calcareous soil low in iron) with some 200 kg/ha $FeSO_4 \cdot 7H_2O$ was adequate to provide iron for summer crops of sorghum increasing yields by some 200% or more. On the other hand, 200 kg/ha of "sequestrene 138" was less effective in correcting iron chlorosis on sorghum due to leaching losses in irrigation water.

1.7.1.2 Manganese

Manganese fertilizers behave differently, depending on soil pH. Under acidic soil conditions, manganese remains

available for several years, whereas in neutral and alkaline soils the residual value is less (Cook and Davis, 1957) due to the rapid oxidation and precipitation as insoluble manganic oxides (Leeper, 1947).

On cotton, Puche (1963) obtained the highest yield of cotton by applying a comprehensive mixture of minor elements including Mn. Joham and Amin (1963) did not obtain any deleterious effect on cotton growth from weekly sprayings of 4.0 ppm manganese, but an increase of manganese in the root zone from 1 to 27 ppm advanced the date of flowering and decreased lint yield.

1.7.1.3 Zinc

A residual response is longer with respect to zinc fertilizers in the soil depending on the soil characteristics and rate of application. Brown, Krantz and Martin (1964) have shown that addition of 3.2 ppm of zinc as $ZnSO_4.7H_2O$ corrected zinc deficiency for six to seven successive croppings in the greenhouse. They also concluded that when dithizone-extractable zinc fell below 0.55 ppm, the plants responded to further additions of zinc.

The cotton crop showed a high response to both soil and foliar applications of zinc (Singh et al., 1970) and that a yield increase of 19% followed application of 20 kg/ha of zinc sulphate ($ZnSO_4.7H_2O$), whereas an increase of 22% with foliar spray occurred using one spray 60 days after sowing and a total of 2.0 kg/ha of $ZnSO_4.7H_2O$.

In Australian calcareous soil, correction of zinc

deficiency on cotton plants induced iron chlorosis (80 kg $ZnSO_4.7H_2O$/ha), but applying 200 kg $FeSO_4.7H_2O$ + 40 kg $ZnSO_4.7H_2O$/ha corrected both iron and zinc deficiency (Chapman and Boundy, 1977).

Soaking seeds of cotton variety (216 F) in 0.01 M to 0.001 M solutions of zinc sulphate ($ZnSO_4.7H_2O$) before germination, showed zinc absorption particularly by the testa without any improvement in the yield (Singh, 1961).

1.7.1.4 Copper

Copper deficiency is frequently associated with soils high in organic matter and highly weathered sandy mineral soils (Reuther, 1957; Berger, 1965). Copper deficiency can be corrected by applying both inorganic and organic forms. Copper sulphate ($CuSO_4.5H_2O$) is most commonly used for both soil and foliar application, but other products such as copper ammonium phosphate and copper oxide can be used and also chelated copper compounds are commercially available.

The normal rates of copper sulphate ($CuSO_4.5H_2O$) application to the soil range between 5.0 to 20 kg/ha depending on soil properties and the crop used.

Copper fertilizers generally remain available for several years. Harris (1947) found that 34 kg/ha of $CuSO_4.5H_2O$ was effective for five years. Cook and Davis (1957) also reported that applying 6.5 kg/ha of Cu^{++} in 1953 still increased grass production in 1956 from 6.5 to 33.0 metric tons/ha.

1.7.1.5 Organic complexing agents

In calcareous soil conditions, owing to the low solubility of micronutrients applied to the soil, as a result of rapid fixation or precipitation, utilization of organic complexing agents by which the micronutrients remain in an available form is considered under some conditions a better method of micronutrient deficiency correction.

Wallace (1956) listed the characteristics that an acceptable chelate must have:

- The metal (Fe, Mn, Zn, Cu) in the chelate ring must not be easily replaced by other metals.
- The metal chelate must be stable against hydrolysis.
- The chelating agent must not be decomposed by soil micro-organisms.
- The metal chelate must be water soluble.
- Must not be easily fixed in soil.
- Must be available to the plant either at root surface or somewhere in the plant.
- Must be nontoxic to plants in the amounts needed.
- Must be in a form easily applied to soil or plants.
- Must be inexpensive.

Formation of natural chelates between micronutrient elements and various ligands derived from soil organic matter, plant or microbial production seems to be the primary explanation for plant uptake of micronutrient at soil pH higher than neutrality in arid and semi-arid regions (Parsa et al., 1979). They tested 30 plant materials incorporated in

a highly calcareous soil growing sorghum; all the organic materials increased both dry matter yield and uptake of iron significantly as compared with $FeSO_4.7H_2O$ and even Fe-EDDHA. Sorghum yield increases from $FeSO_4.7H_2O$ were small as compared with thöse from organic manure, whereas effect of manure plus iron on yield was significant in the third crop indicating that organic compounds in manures were effective in keeping iron available (Thomas and Mathers, 1979). They also found that $FeSO_4.7H_2O$ helped cure chlorosis but its effectiveness was much inferior to the manure.

Iron deficiency is more difficult to correct than zinc. Inorganic forms of iron do not often correct iron chlorosis unless enormous quantities of iron are applied. Iron chelates supply adequate available iron to plants but the high cost of these forms preclude their extensive use.

Chelated micronutrient fertilizers vary in their effectiveness for correcting deficiency depending on the stability constants of the metal chelate (Lindsay et al., 1967). Fe-EDDHA chelating agent has repeatedly proved the best for correcting iron deficiency in calcareous soils (Aso and Dantur, 1972; Hodgson et al., 1972; Mortvedt and Giordano, 1971; Abo-Eldahab, 1977). Boxma and DeGroot (1971) corrected both manganese and iron deficiency by using Mn-DTPA in Dutch flowers, fruit and green crops. The higher stability of Mn-DTPA confirms that it is better to use manganese dressing on calcareous soil than Mn-EDTA. Its effectiveness in treating Fe also is due to the partial replacement of Mn by Fe from the soil. In this respect in their soils application of Mn-

DTPA offered the advantage of a well-balanced iron and manganese supply to the plant and it was preferable to Fe-EDDHA for the iron chlorosis correction in plants which were susceptible to manganese deficiency.

Brown (1965) used 11 sources of zinc (including zinc chelate) that were equally effective in correcting zinc deficiency, but when the zinc is not thoroughly mixed in to the soil, adding zinc as the metal chelate gives a better distribution of the metal into the soil because of its greater solubility.

1.7.2 Foliar spray treatments

Foliar application of micronutrients is usually one of the most efficient ways of correcting their deficiency. Relatively low rates of application are often as effective as much higher rates of soil application. Adding micronutrients directly to plant leaves with wetting agents to ensure intimate contact, has been repeated by many investigators.

It has been suggested by Wallace et al. (1957) that the following advantages of foliar application in general over soil application of iron:

- Elimination of complicated soil reactions.
- Irrigation not required to move the compounds in the root zone.
- A considerable economy of materials is effected by foliar applications.
- A more rapid response to the applied iron.

On the other hand, they pointed out that disadvantages of

foliar application of iron also exist, namely:

- Greater chance of toxicity.
- Incomplete coverage and subsequent uneven response.
- Need for repeated applications.

1.7.2.1 Iron

Patel et al. (1977) have reported that 4% $FeSO_4.7H_2O$ sprays were not adequate in correcting bicarbonate affected rice, but with the careful selection of surfactants were effective for citrus (Newman and Prinz, 1974). Increasing the rate to 5% was adequate to cure chlorosis in sugarcane over a period of a year (Naik and Joshi, 1979). Higher concentrations were needed for curing iron chlorosis on sorghum ranging from 1.33-6% $FeSO_4.7H_2O$. Krantz and Brown (1967) have concluded that applying a 3% $FeSO_4.7H_2O$ solution as a foliar spray is sufficient for treating grain sorghum and related crops. They also noted that $(NH_4)_2SO_4$-$FeSO_4.6H_2O$ has been equally effective in sprays at the same concentrations as $FeSO_4.7H_2O$.

In Arizona, Richardson (1967) pointed out that foliar applications of Fe-polyflavonoid material was effective in overcoming chlorosis of grain sorghum.

Aso and Dantur (1972) found that citrus responded to Fe-EDDHA as much as $FeSO_4.7H_2O$. However, although 0.3 and 0.2% 'sequestrene 138' sprays were moderately successful in stimulating growth in peas and maize grown on calcareous soil and allowed high iron uptake by the plants, the iron was of very low physiological activity (Dungerwal et al., 1974; Mathur et al., 1976).

1.7.2.2 Manganese

$MnSO_4.4H_2O$ is considered to be the most effective inorganic form of manganese for foliar spray (Ozaki, 1955) but as an organic form Mn-EDTA is considered the most effective. 2-5 kg/ha of $MnSO_4.4H_2O$ is sufficient to correct Mn deficiency on most crops, and it has been found that spray application of 2.2 kg/ha of $MnSO_4.4H_2O$ optimized soybean yield (Cox, 1968) and increasing the rate to 4.4 kg/ha did not give any further increase.

In a comparison between foliar application of $MnSO_4.4H_2O$ and Mn-EDTA on soybean, Randall and Schulte (1971) found that 0.56 kg/ha of Mn as $MnSO_4.4H_2O$ was approximately equal in effectiveness to 0.17 kg/ha of Mn as Mn-EDTA.

1.7.2.3 Zinc

Like other micronutrients, foliar application of zinc is considered as a temporary treatment to correct zinc deficiency in most crops and such applications are usually made after zinc deficiency symptoms occur on the plants. In Australia, Duncan (1967) observed in corn that solutions of 0.5, 1.0,1.5% $ZnSO_4.7H_2O$, corrected zinc deficiency when sprayed twice. Equally successful results were obtained by Naik and Bas (1968) for wheat in India, especially when sprayed at the tillering stage. It has been shown that foliar application of zinc is more effective, with a higher rate of absorption, when it is applied in ionic form rather than complexed with EDTA (Thorne, 1957).

Cotton is an important crop in the economy of many countries and micronutrient soil and foliar application have recently been given more attention, especially on calcareous soils where the crop suffers from decreased supplies of micronutrients, especially that of iron and zinc. Dargan et al. (1965) in their study on the effect of micronutrients on the yield and economic returns for American cotton reported that application of Cu, Mn, Zn increased cotton yields by 7.0, 6.9 and 6.2% respectively. $ZnSO_4$ and $FeSO_4$ increased lint index by 4.2 and 2.9% respectively, and seed index by 6.9 and 7.6%. A 22% increase in the cotton yield followed spraying 2 kg/ha of $ZnSO_4.7H_2O$ (Singh, S.Singh, 1970). Tagi-Zade (1956) looked at environmental effects of the response of cotton to zinc, and found that in warm weather the cotton yield increased by 34% but in cold weather by only 20%.

1.7.2.4 Copper

Copper sprays have been used for years on trees and field crops to correct copper deficiency, and $CuSO_4.5H_2O$ is considered the best inorganic source of copper for this objective. Bridger et al. (1962) concluded that $CuNH_4PO_4.H_2O$ is an acceptable source of sopper for foliar application when suspended in H_2O.

A comparison between soil and foliar application of $CuSO_4.5H_2O$ for mature oats and barley has been conducted by Reith (1968) who concluded that in both crops, leaf copper concentrations were unaffected by 11.0 to 22.0 kg/ha as soil application, but foliar application caused great increases of copper in leaf tissues.

Leonard's (1967) recommendations for a foliar treatment with $CuSO_4.5H_2O$ consider that 360g Cu/400 litres of H_2O/ha are adequate for controlling fungus diseases in citrus and for supplying nutritional Cu.

1.7.3 The modification of micronutrient availability within the soil

It is possible in calcareous soil to make the iron available for plants without any addition to either soil or plants by releasing the nutrients in a soluble form from its native sources in the soil.

Attacking the soil natural sources of iron with acids was proposed by many authors as soil amendments to transfer some of the native iron in soil to a soluble form preventing iron chlorosis (Ryan et al., 1974; Saxena et al., 1971). Sulphur and H_2SO_4 have been used to correct Fe chlorosis (Olson, 1951; Ryan et al., 1973; Miyamoto et al., 1975). Wallace and Mueller (1978) concluded that for a 10% $CaCO_3$ soil, 0.37% of H_2SO_4 was adequate for neutralization of all the $CaCO_3$ in a pot experiment. They also reported that the mixing of H_2SO_4 with all soil was not necessary and the lowest rate of H_2SO_4 in spots improved leaf yield and iron content as much as did the highest rates as a mixture. Plants treated with iron chelates were slightly smaller than H_2SO_4-spot treated and the chelates resulted in reduced Zn, Mn and Cu concentrations in leaves (Wallace and Alexander, 1973). The acid spots which also increased Fe uptake gave the same results as the chelate regarding Mn, Zn and Cu although not greatly pronounced at the 0.37g H_2SO_4 rate (Wallace et al., 1978) and these results are

of interest since it can be expected that soil acidification would increase them (Wallace et al., 1976). With sorghum a similar result has been obtained by Mathers et al. (1970) treating with banded sulphuric acid in a soil with 2.5% free lime.

Singh (1970) found that the application of sulphur, which oxidises in soil, was effective in preventing the occurrence of chlorosis on peas, and that 250 kg/ha of sulphur increased the grain yield of peas 100% with a 40% increase in the iron leaf content. An increase of 10, 17, 10, 11 and 25% in N, K, S, Mg and B respectively occurred, showing that the application of sulphur often creates balanced nutritional conditions in the plant.

Sorensen (1968) reported that nitrogen concentration of fodder increased with the application of sulphur to soil. Reding (1956) indicated that application of sulphur induced an increase in both nitrogen and sulphur content of plants. Patel et al. (1977) and Okajima et al. (1970) obtained similar results by using sulphuric acid and it was the only satisfactory way for bicarbonate affected rice.

Ibrahim et al. (1979) summarized the procedures which may help in correcting iron deficiency in alkaline soils with an ample content of iron as follows:

1) Inducing soil acidity by applying acid-forming material.
2) Inducing soil reduction by means of lowering the redox potential.
3) Raising the level of natural chelating materials in the soil.

In their study on some effective soil amendments, they

concluded that the acid treatment affected the levels of mobile iron significantly at about pH 4.0 and maximum values of mobile iron were obtained by a further increase in soil acidity to pH 3.1 and 3.25, but further acidification caused a reduction in the quantities of mobile iron.

1.7.4 The modification of iron activity within the plant

Growth substances are known to increase, retard or modify plant growth by affecting diverse metabolic processes (Trewavas, 1968; Van, 1966) and it is likely that these substances also affect the mechanism of iron uptake (Muller et al., 1966). However, very little information relating to the action of growth substances on the absorption and transfer of Fe is available in the literature.

Mathur et al. (1976) developed the practical findings of Singh (1970) on the sulphur stimulation of chlorotic peas suggesting that the sulphur reactivated native plant iron deposits. Dungerwal et al. (1974) used a 0.1% sulphuric acid leaf spray on maize plants suggesting that it was the best treatment for restoring enzymic activity.

With bean plants, Kannan and Mathew (1970) examined the effects of a number of growth substances, e.g. GA3 and CCC, on the absorption and translocation of iron reporting that absorption of iron by the primary leaf was increased by treatment of the trifoliate leaf with CCC or GA3. The increase was significantly more with CCC than GA3 and iron transport to trifoliate leaves was enhanced by GA3. Kinetin showed a tendency to draw the iron towards the site of application, as did GA3. However they found no relationship between absorption

of leaf and root applied iron and its translocation within the plant and that none of the growth substances used governed iron absorption/translocation in either beans or maize.

The growth substances differ in their effects on the absorption and transport of root and foliar applied iron. The treatment of roots with TIBA significantly reduced the transport of Fe from the primary leaf to upper shoot, and also to the stem and root, and foliar-applied TIBA reduced the transport to the upper shoot, and although Kessler and Moscicki (1958) reported that TIBA increase the translocation and utilization of foliar applied iron, the studies of Bar-Akiva and Hewitt (1959) did not support their findings.

CHAPTER 2

METHODS AND MATERIALS

2.1 Field Work

2.1.1 Soil profiles

In the summer of 1978 after a sugarbeet crop had been harvested, three soil profiles at three locations on the experiment site were sampled to study some of the relevant mechanical and chemical properties of the various horizons of soils. The soil samples were collected from the depths: 0-5, 5-20, 20-40, 40-60 and 60-80 cm, air dried and crushed to pass a 2mm sieve.

2.1.2 Clover sowing for "green manure"

White clover (Trifolium repens Var. unknown) is used as a leguminous winter crop in Syrian rotations. The objective is the improvement of the physical, chemical and nutritional properties of especially new reclaimed saline soil. The crop was sown in autumn 1978 (40 kg/ha) by hand, irrigation water was supplied when appropriate. 220 kg P_2O_5/ha were broadcast when seeding.

2.1.3 Land preparation, fertilizer and cotton seeding

In mid-March 1979 (one month before the cotton crop was seeded) and in the late flowering stage of clover growth, the land was ploughed incorporating the whole clover crop within the soil, rotovated twice in two opposite directions breaking down the larger clods of soil to produce both a suitable tilth and also suitable seed bed.

Land leveling is of importance under Deir Zor soil

conditions due to the variation in salt accumulation on the soil surface as a result of capillary water movement when the ground water is near the surface 2-3.3 meters). In addition it is needed to ensure that irrigation water is uniformly distributed within the plots. The experiment site was mechanically levelled twice and in opposite directions. The site was divided into 100 plots (5 x 4 m^2 each). Each plot contained six furrows (70 cm apart).

The recommended applications of the major fertilizers, under the Euphrates basin conditions for cotton crops, have been used as a basal dressing in this experiment. The treatments were as follows:

Nitrogen as ammonium nitrate (33% N) 150 kg N/ha

Phosphate as Triplesuperphosphate (46% P_2O_5) 220 kg P_2O_5/ha

Potassium as potassium sulphate (50% K_2O) 100 kg K_2O/ha

The phosphatic fertilizer was applied to the soil once, after the plots were built and before furrowing.

The potassium fertilizer was applied twice together with the nitrogen fertilizer, the first half was supplied one month after emergence while the remainder was supplied to the plant two months after emergence. With the nitrogen fertilizer, the decided amount was divided equally in three applications being at monthly intervals.

All fertilizers were broadcast by hand from pre-weighed bags containing the correct amount for each plot.

The local variety of cotton Allepo 40 (the only

improved variety in Syria) was seeded on the sunny side of the furrows, 20 cm apart (4-6 seeds in each hole), in mid April when the average temperature was 20°C, the favourable temperature for cotton seed germination.

2.1.4 Soil and plant micronutrient applications

2.1.4.1 Soil application of micronutrients

The trace elements were applied at the following rates:

$FeSO_4.7H_2O$	50 kg/ha (100g plot)
$MnSO_4.4H_2O$	40 kg/ha (80 g plot)
$ZnSO_4.7H_2O$	20 kg/ha (40 g plot)
$CuSO_4.5H_2O$	10 kg/ha (20 g plot)

These fertilizers were applied broadcast on rows under the cotton seeds at seeding time.

2.1.4.2 Plant spray of micronutrients

The following solution concentrations of micronutrients were sprayed on the leaves as follows:

1200 ppm Fe as $FeSO_4.7H_2O$ (6.0 g litre)

750 ppm Mn as $MnSO_4.4H_2O$ (3.0 g litre)

340 ppm Zn as $ZnSO_4.7H_2O$ (1.5 g litre)

110 ppm Cu as $CuSO_4.5H_2O$ (0.5 g litre)

A 10 litre sprayer was used. Sprays were acidified with hydrochloric acid to drop the pH to 6.5 to prevent rapid precipitation of trace elements, since that irrigation water reaction is pH 7.8. 0.045% wetting agent Agral 90 (90% alkyl phenolethylene oxide) was added to ensure complete wetting of

plant leaf blades. The spray solution was applied at the rate of 1000 litre/ha. The plants were sprayed three times during the growth stages of plants, at monthly intervals, the first spray occurred 1 month after planting when the plants were 25 cm long, the second was associated with the early blooming stage 60 days after planting, and the last one when the plants were at the early bolling stage 90 days after planting.

The spray involved all the plants for each plot, avoiding any contamination between plots.

All spraying was conducted at dawn to obtain maximum benefit from the slightly higher humidity, low wind speeds and air temperatures at that time of day.

2.1.5 Crop protection and irrigation

The field and irrigation canals were kept free of weeds, with cultivation to break down the soil surface cracks once between irrigations up to the early flowering stage of plants, to minimize soil moisture evaporation. The plants were thinned to two in each hole, 120 plants in each plot (5x4 m^2) on average.

Irrigation water was given to the plots by practiced irrigation labourers at the rate of approximately 10 cm of water/irrigation. The frequency of irrigation to the plants varied in accordance with the plant growth stage. During germination the soil surface was maintained moist to prevent hard crust formation and raise the seed germination and establishment rate to the maximum. The water volume given to plants during this stage was less than average due to the short periods between irrigations, and because the seedlings

were still too young to withstand heavy irrigation. At later stages of plant growth the water given in each irrigation was increased to normal (12 days between irrigation). The irrigation factor, especially under salt affected soils, is important to maintain a low ground water table and in addition prevent salt formation on the soil surface by capillary movement of ground water especially when the drainage system is inadequate to remove excess irrigation water.

2.1.6 Soil sampling

Surface soil samples (one from each plot) were collected on 4 occasions at monthly intervals. The samples (0-10 cm) generally were collected from the centre of each plot and from the furrow side treated with the relevant trace element. The soil samples were collected a few hours before each spray.

2.1.7 Leaf sampling

Thirty days after each spray, a leaf sample was collected from each plot at random. The first group of samples were collected a few hours before the first spray (thinned plants) and were considered as representative samples for all plots and treatments.

2.1.8 Harvest

In mid September and when 90% of the plant bolls were matured, each plot was harvested separately by hand. Each plot yield was directly weighed in the field. A 500g sample of seed cotton (one from each plot) was collected and

sent to the cotton research laboratories in Alepo for the determination of cotton fibre characteristics. Twenty days later the residual yield was also harvested and added to the first harvest to obtain the final total yield.

2.2 Laboratory Work

2.2.1 In Syria

2.2.1.1 Determination of the seed cotton fibre characteristics including lint strength, lint length, lint smoothness, fibre percentage and maturity factor in addition to oil content of cotton seeds.

The object of these analyses was to find out the effect of the various micronutrient treatments on these important economic characteristics (work done by the Cotton Research Center Laboratories, Alepo, Syria).

2.2.1.2 Preparation of soil samples for elemental analysis

Surface soil samples were air dried, crushed to pass a 2mm sieve and stored in polythene bags.

2.2.1.3 Preparation of plant samples for elemental analysis

Fresh plant samples were submerged in distilled water containing 0.05% Agral 90, rinsed three times in distilled water, dried in a forced air 70^{o}C oven for 24 hours. The samples were ground in a Glen Creston stainless steel hummer mill and stored in polythene bags.

2.2.2 In Bangor

2.2.2.1 Soil organic carbon

Soil organic carbon was determined in three profile samples by the Tinsley method (Bremner and Jenkinson, 1960) modified with the addition of 1.0 gram red mercuric oxide to

remove free chloride ions and by using 0.2% N phenylanthranilic acid as the redox indicator (Kalembasa and Jenkinson, 1973).

2.2.2.2 Calcium carbonate % of soil profile samples

The soil total calcium carbonate in samples from three profiles was determined by the calcimeter method (Bascomb, 1961) using apparatus modified to permit samples with a wider range of carbonate. Despite the slowness of equilibration, this method is considered more accurate than the speedier acid back titration.

2.2.2.3 Mechanical analysis of the soil profile samples

The mechanical analysis of the soil profile samples was carried out using the Bouyoucos hydrometer (Bouyoucos, 1962) and sieves.

2.2.2.4 Active calcium carbonate % of the soil profile samples

The active calcium carbonate % of the soil profile samples was determined using ammonium oxalate method (Drouineau, 1942).

2.2.2.5 Soil profile ~~analysis~~determination of plant available Fe, Mn, Zn and Cu by the DTPA extraction method

The DTPA (diethyltriaminepentaacetic acid) soil extraction procedure was first proposed by Lindsay and Norvell (1969). The procedure: 1/2 soil/extractant ratio, extractant being:

0.005 M DTPA

0.01 M $CaCl_2$

0.1 M triethanolamine (TEA).

The extractant solution was adjusted to exactly pH 7.3 with HCl. To 10g of air dried soil, 20ml of DTPA extractant were added, shaken for two hours and filtered through Whatman No.1 filter paper. Fe, Mn, Zn and Cu concentrations in the filtrate were determined by atomic absorption spectrophotometry.

2.2.2.6 Determination of the DTPA-extractable Fe, Mn, Zn and Cu in surface soil

Four hundred surface soil samples (0-10 cm) collected from the cotton experiment plots were analysed for the DTPA extractability of Fe, Mn, Zn and Cu by the above procedure.

2.2.2.7 Preparation of leaf samples for elemental analysis

Each crushed leaf sample was mixed and subsampled to give a 1 gram sample in a high sided glazed porcelain crucible. These were then heated in a silica lined furnace to 200°C for exactly two hours, raising the temperature to 450°C ± 10°C for a further four hours when they were removed from the hot furnace. This procedure was essential to reduce copper fixation by plant silica bodies while oxidizing sufficient organic matter to obtain complete extraction of metal ions (Stewart-Jones, 1980).

The cooled ash was wetted in the crucible with 7.5 mls of 1 N HNO_3 and transferred to a glass vial, a further 7.5 mls

of acid being used to rinse out the crucible and ensure complete transfer.

2.2.2.8 Determination of Fe, Mn, Zn and Cu in both soil and leaf solutions by the atomic absorption spectrophotometer

These elements were analysed by aspirating the solution directly into a Pye Unicam SP 2900 atomic spectrophotometer. Curve correction was used where appropriate to facilitate analysis, the air/acetylene flame being used in all cases. Blank samples of extractant were checked to see if this contained or interfered with the determination of each element.

2.2.2.9 Determination of phosphorus in solution

Phosphorus was determined spectrophotometrically by measuring the optical density of the stannous chloride reduced phosphomolybdate blue complex at 660mm on a Cecil 202 spectrophotometer (Jackson, 1962).

2.3 Outline of Laboratory Experiments

2.3.1 Laboratory pot experiments

A pot experiment in the greenhouse was designed to answer some of the questions arising from field work. The treatments were as follows:

Pot No.	Treatment	
1	Control	
2	1% dried green manure	) mixed with whole soil volume.
3	2% dried green manure	)
4	4% dried green manure	)
5	5 ppm Fe as $FeSO_4.7H_2O$	) mixed with the top 2.0cm of the pot soil prior to seeding.
6	10 ppm Fe as $FeSO_4.7H_2O$	)
7	20 ppm Fe as $FeSO_4.7H_2O$	)
8	2 ppm Zn as $ZnSO_4.7H_2O$	) mixed with the top 2.0cm of the pot soil prior to seeding.
9	5 ppm Zn as $ZnSO_4.7H_2O$	)
10	10 ppm Zn as $ZnSO_4.7H_2O$	)

These 10 treatments were repeated 3 times in Groups I II and III.

Group I received no phosphate.

Group II received 100 ppm of phosphorus as KH_2PO_4 added on the pot soil surface before watering. This was repeated 2 weeks later, also on the surface before watering.

Group III received 200 ppm of phosphorus as KH_2PO_4 added on the pot soil surface before seeding and again 2 weeks later.

The dried green manure was mixed with whole pot contents, while iron and zinc sulphates were mixed with the top 2.0 cm of the pot soil before seeding.

300 ppm of nitrogen as ammonium nitrate were supplied to all pots at seeding and again 2 weeks later in association with phosphate fertilization where appropriate.

No potassium fertilization took place. Five weeks after planting the plants were harvested, divided into leaf, stem and root and were treated in the same way as the field experiment plant samples (page 68).

Three soil samples were collected from each pot after harvest at depths of 0-2, 2-5 and 5-10 cm. These samples were analysed for DTPA extractable micronutrients in the same way as field soil samples (page67). Green manure was 80% white clover and 20% ryegrass field herbage collected early in May, 1980.

2.3.2 Anaerobic incubation studies

Stationary soil samples (70g) were incubated at 30°C under anaerobic conditions while an irrigation mixture was drawn off the bottom and recycled.

Experiment I used starch as an organic source, while green manure was the organic source in experiment II. Green manure was 80% white clover and 20% ryegrass.

The objective of these two procedures was to investigate the influence of both starch and "green manure" substrates in addition to temperature on iron and manganese release from Deir Zor soil into solution under anaerobic conditions and the effect of flooding on the soil DTPA

Fig. 2.3.1 Schematic diagram of the anaerobic culture circulatory system

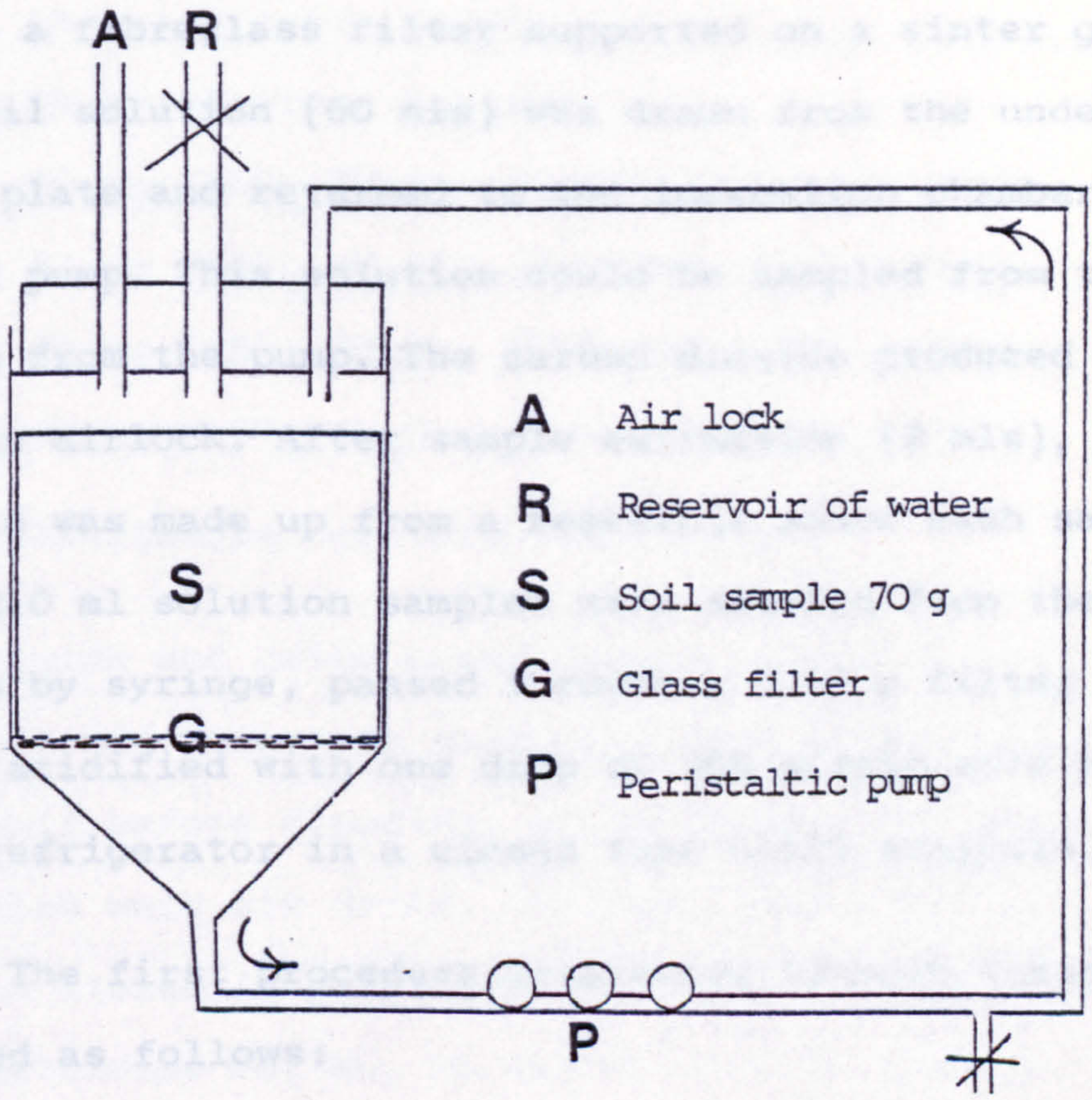


Photo 2.3.1 shows the anaerobic culture equipment

extractability of Fe, Mn, Zn and Cu.

The soil sample (70g) was retained in the incubation chamber above a fibreglass filter supported on a sinter glass plate. The soil solution (60 mls) was drawn from the underside of the glass plate and returned to the incubation chamber by a peristallic pump. This solution could be sampled from the return tubing from the pump. The carbon dioxide produced was released by an airlock. After sample extraction (2 mls), the loss of liquid was made up from a reservoir above each sample.

The 2.0 ml solution samples were sampled from the cycling tubes by syringe, passed through a 0.22 μ filter (millipore), acidified with one drop of 50% nitric acid and stored in a refrigerator in a closed tube until analysis.

2.3.2.1 The first procedure treatments (starch treatments) are summarized as follows:

Tube No.	Treatment
1	Control
2	50 mg $FeSO_4.7H_2O$ + 2% starch
3	50 mg $MnSO_4.4H_2O$ + 2% starch
4	50 mg $FeSO_4.7H_2O$
5	50 mg $MnSO_4.4H_2O$

These treatments were duplicated.

2.3.2.2 The green manure procedure treatments are summarized overleaf:

Tube No.	Treatment
1	Control
2	1% dried green manure
3	2% dried green manure
4	50 mg $FeSO_4.7H_2O$ + 1% dried green manure
5	50 mg $MnSO_4.4H_2O$ + 1% dried green manure
6	50 mg $FeSO_4.7H_2O$ + 2% dried green manure
7	50 mg $MnSO_4.4H_2O$ + 2% dried green manure

These treatments were also duplicated.

The starch and dried green manure were mixed with the soil while iron and manganese sulphates were mixed with the top 2 cm of dry soil before flooding. After 16 days of incubation, the soil samples were air dried, crushed, passed through a 2mm sieve and then stored in polythene bags. The DTPA extractant was used to determine Fe, Mn, Zn and Cu availability as in page

2.3.3 Water and sand culture experiments

Cotton (Alepo 40) was grown in a water and sand culture system in the greenhouse. The seeds were first soaked in distilled water for 24 hours and germinated between filter paper for a further 48 hours. The seedlings were then transferred for 2 days to a complete nutrient solution with the exception of both iron and phosphate. After this treatment, the seedlings were placed in holes in plastic rings which were supported on 500ml beakers containing complete nutrient solutions or sand + solution with varied levels of both iron and phosphate. The solution was circulated from the sand to

the corresponding water culture container and back again by a peristaltic pump during the experiment period.

The nutrient solutions had the following composition in milliequivalent per litre: 5 K^+ (as K_2SO_4), 8 Ca^{++} (as $Ca(NO_3)_2.4H_2O$), 4 Mg^{++} (as $MgSO_4.7H_2O$) and 2 Mg^{++} (as $MgCl_2.6H_2O$). Minor elements, namely, boron, manganese, zinc, copper and molybdenum were added at the concentrations of 1.0, 2.0, 0.5, 0.5, 0.2 ppm respectively. Iron and phosphate were applied at the following concentrations:

Fe : 1.0, 2.0, 5.0, 10.0 ppm (as $FeSO_4.7H_2O$)

P : 0.5, 1.0, 5.0, 10.0, 20.0, 40.0 ppm (as KH_2PO_4)

The solutions were adjusted to pH 6.5 before addition and were replaced weekly. The plants were grown at a temperature of 25-30°C day and 20-25°C night, with a 16h day length.

2.3.3.1 Experiments in water culture

500ml beakers were used with two replicates, each with 4 plants. Nutrient solutions were aerated constantly. The solution levels in the beakers were maintained by topping up twice daily with the same nutrient solution freshly prepared. The plants were harvested after 28 days of growth (4-6 leaf stage).

2.3.3.2 Experiments in sand culture

For a comparative study, a very simple "solid substrate" of quartz sand (1.0mm grain size) was used. The sand was washed thoroughly with 15% hydrochloric acid and then with distilled water to neutrality (pH 7). 800g of dried and

washed sand were put in each beaker. This procedure was also replicated twice.

2.3.3.3 Harvest and chemical analysis

The plants were separated into 3 fractions after harvest (leaf, stem, root), washed thoroughly with distilled water containing 0.05% Agral 90, rinsed 3 times in distilled water, dried in a forced air 70°C oven for 24 hours, crushed in a Glen Creston stainless steel hummer mill. Iron, manganese, zinc and copper concentration was determined by atomic absorption spectrophotometer. The quantities of plant matter obtained did not allow the simultaneous determination of phosphate.

CHAPTER 3

THE FIELD CONDITIONS AND SOIL CHARACTERISTICS

3.1 A Physical and Chemical Review of Soil Properties

The Arab Center for the studies of arid zones and dry lands (ACSAD), started in summer, 1973, a programme of reclamation on salt-affected soils at Bani-Tagleb experiment station, 10 km east of Deir Zor. The main objective of this study was to establish criteria for drainage and reclamation for the area.

The soils studied are alluvial in origin on the lower terrace of the Euphrates river, deposited mainly by flood water and to a lesser extent, by the runoff from the hills south of the study area. The soils are characterized by weak to medium structure, with clear stratification due to the mode of the deposition. The infiltration rate and permeability of the soils were classified as medium.

The area consists of recent river terrace deposits of quaternary age. The surface material of the recent terrace (approximately 1m) shows a textural variation from sandy loam to silt clay; the underlying layers locally contain sand or even gravel within a depth of two metres. This is an indication of the existence of former stream beds where this coarser material had been deposited. With regard to the bulk density values, the surface layer is strongly compacted and the underlying horizons are compacted. Total porosity is uniform within the profile and is slightly lower than normal (43%). The field capacity ranges from 25 to 30% H_2O, the wilting point from 10 to 15% H_2O and the available moisture content is about 20% by volume (Dougramaji, 1977).

Table 3.1.1 Chemical characteristics of soil before leaching

		Crust 0-2 cm	W. average 2-360 cm	Ground water		Crust 0-2 cm	Top soil 2-8 cm	Top soil 8-25 cm	W. average 25-360 cm
	pH	7.2	7.9	7.3	$CaCO_3$%	18.0	17.0	19.5	24.3
**	E.C.e	400.0	31.4	40.5	Active	8.0	5.6	4.4	10.7
	C.E.C	8.4	19.3		Gypsum %	1.2	2.2	1.2	5.0
**	ESP%	50.0	34.1		O.M %	1.8	0.8	0.4	0.23
**	SAR	69.8	37.6		C. org %	1.05	0.46	0.232	0.135
Cations m.e./ 100 ml	Ca	1203.0	52.7	60.0	Nt %	0.12	0.08	0.05	0.04
	Mg	1365.0	74.7	60.0	C/N	8.7	5.8	4.6	3.2
	Na	2500.0	274.5	300.0	Available p.p.m. P	2.5	3.0	4.0	2.7
	K	0.4	1.4	0.2	K	200.0	170.0	240.0	111.9
	Total	5068.0	406.9	420.2	NO_3	tr	1.0	0.28	0.18
Anions m.e./ 100 ml	CL	4809.0	249.7	240.0	B	1.11	0.48	0.32	0.68
	SO_4	198.0	143.1	182.0					
	HCO_3^-	1.5	2.9	0.9					
	Total	5008.5	395.7	422.9					

** See Richards CA (1954) Salin o Alkali Soils USDA No 60

The results of X-ray diffraction analysis of the clay fractions have shown that the dominant constituent is montmorillonite. It is accompanied by attapulgite, chlorite-montmorillonite "intergrade" mineral and vermiculite with interlayer hydroxy alluminum and some halosite (Dougramaji and Pavel, 1978).

Before reclamation, the exchangable sodium percentage (ESP) was high in an average 34.2%. The lime and gypsum were high in soil. In the top soil they were 19.5% and 1.2% while in the subsoil they constituted 24% and 5% respectively. The soil salinity of the saturated extract, expressed in terms of specific electric conductivity was 31 mmhos/cm in the soil surface, while the ground water was 40.5 mmhos/cm. The dominant ions in the soil were sodium, calcium, chloride and sulphate. The average cation exchange capacity (CEC) was 19.3 me/100gm soil reaching its highest values at the depth from 220 to 300cm, being 25.6, this was obviously attributed to the clay content and varying due to differences in the composition of clay minerals.

It is evident from the figures mentioned above that the soil was highly saline, the ground water was also saline and characterized by the same ionic distribution. Initial work on the soil included:

a) Research on drain spacing in relation to a predetermined drain depth which is governed by the existing outfalls. The depth of the draining system was 1.50 m at the upper end with 0.1% slope.

b) Research on hydrological conditions through the installation of a network of observation wells, piezo-meters and drain discharge measurements.

c) Research on soil reclamation practices: a comparison of summer fallow with non-fallow, surface ploughing and mulching techniques on the fallow land, studies of leaching methods, especially comparing continuous ponding with intermittent leaching.

The area used in this experiment was effectively reclaimed and salinity levels were from 3.0 to 6.5 mmhos/cm (Table 4.1.1) in surface soil samples. This is low enough to be ignored for the cotton crop, and no correlation between yields within treatment blocks and salinity were found in the experiment.

3.2 The Climate

The climate of Deir Zor region is semi-arid. The mean annual temperature ranges from 7.4°C in January to 32.6°C in July while the mean annual precipitation ranges from 33 to 165mm, with a maximum in December to March. The relative humidity ranges from 27% in June, July and August rising to 76% in January. The annual total evaporation is about 1900mm with minimum loss in January (50mm) and maximum in July (500mm). The wind speeds tend to be strong, 2-5.6 m/s.

The evaporation determined by an open water pan in the experimental station site indicated that of the total water loss, 72% was between April and September with the wind speed being the most important factor.

Most of the solar radiation is consumed by evapo-transpiration or reflected, but there is an estimated 16% heating the soil. This can lead to such extreme temperatures at the soil surface (grass cover crop) and at 2.0cm depth as 49°C, 45°C respectively.

The climatic data is summarized in Table 3.2.1for the period 1965-1977.

Table 3.2.1* Summary of the mean monthly climatic conditions in accordance with the Deir Zor Climatic Station records for the 1965 to 1977 period

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Daily temperature C^{O}	7.4	9.5	13.2	18.6	24.4	29.7	32.6	32.2	27.7	21.2	13.8	8.4	19.9
Maximum daily temperature C^{O}	12.9	15.7	19.7	25.2	31.6	37.3	40.3	40.1	35.7	29.5	21.2	14.4	27.0
Minimum " "	2.4	3.8	6.8	11.7	16.8	21.9	25.0	24.7	20.2	13.9	7.9	3.3	13.2
Precipitation/mm	33.3	25.2	25.1	24.2	8.8	.6	-	-	.40	6.8	11.2	29.3	164.9
Relative humidity %	76.0	66.0	57.0	49.0	38.0	27.0	27.0	27.0	32.0	43.0	60.0	75.0	48.0
Wind speed m/sec.	2.6	2.9	3.5	3.7	3.9	5.6	4.3	4.3	3.3	2.3	2.0	2.4	3.5
Sunshine hours/day	5.1	6.6	8.0	8.6	10.1	12.4	11.9	11.9	10.7	8.6	7.1	5.3	8.9
Daily evaporation mm	1.5	2.2	5.1	6.3	9.8	13.9	15.7	13.2	6.9	5.1	2.7	1.7	7.0
Dusty days	.2	.3	.5	.6	2.1	1.2	.5	.5	.4	.9	.1	.2	7.6

Longtitude 40.09 east
Latitude 35.20 north
Altitude 203.0 meters

*Climatic Station records in Deir Zor.

3.3 Properties of both Euphrates River in Deir Zor and the Feeder Irrigation Canal Water at the Agricultural Research Station.

Agriculture in the Euphrates basin entirely depends on irrigation water because of the limited rainfall and lack of uniformity of rainfall distribution. The Euphrates river is the only source of irrigation water which is pumped and carried by concrete earth canals

There are no problems in using the Euphrates river water for irrigation especially when both drainage and irrigation systems are carefully maintained, but the continuous supply of heavy irrigation to the soil under both hot and dry climatic conditions in the absence of good drainage systems causes the ground water table to rise and additional deposits of salts on the surface to form.

The high evaporation causes deposition of $CaCO_3$, $MgCO_3$ and $CaSO_4$ deposits. Table 3.3.1 summarizes properties of both the Euphrates river water and the main irrigation canal water at the experimental research station site.

Table 3.3.1 Euphrates river water and the main irrigation canal water properties. From Dougramaji, J.S.(1977)

	Euphrates River water properties						Canal water properties at the field
	1973 Aug.	1973 Nov.	1974 Feb.	1974 April	1974 Sept.	Average	Av. of Aug. & Sept. 1973
Salinity m mhos/cm	0.75	0.49	0.57	0.67	0.55	0.61	0.85
pH	7.5	7.9	7.9	7.8	7.7	7.7	7.4
Ca^{2+} meq/litre	2.8	2.7	3.9	3.0	3.6	3.2	3.15
Mg^{2+} "	2.4	1.7	3.3	3.1	1.2	2.34	4.10
Na^{+} "	4.1	1.9	2.1	2.5	1.2	2.36	4.05
K^{+} "	.12	0.05	0.16	traces	0.11	0.11	0.15
Cl^{-} "	4.2	3.0	2.2	3.3	2.6	3.02	5.00
$SO_4^{=}$ "	2.6	1.8	1.7	2.5	1.9	2.10	3.00
HCO_3^{-} "	1.6	3.2	3.2	3.0	2.2	2.64	2.90
$CO_3^{=}$ "	-	-	-	-	-	-	-
B p.p.m.	-	-	0.18	0.50	0.12	0.23	0.31

3.4 Background of the Field Work at Bani-Tagleb Experimental Research Station on Reclaimed Saline Calcareous Soil.

The Middle East region is mostly characterized by vast dry areas with limited rainfall and/or underground water. Such areas produce many important food crops where supplemental irrigation is practised. Nevertheless, rates of agricultural production are often rather low with great possibilities for improvement.

Recently more research has been directed towards increasing productivity of dry lands with the majority of such efforts concentrated on breeding crop varieties adaptable to the environmental conditions of such areas.

The use of fertilizers, one of the major factors affecting agricultural productivity often does not receive much attention under the pretext that crops do not respond sufficiently to fertilizer applications under the conditions of dry lands. Fertilizer studies are confined to limited studies on N P K with very little attention given to micro-nutrient studies.

With respect to the role of micronutrients, research investigations in the last decade have proven beyond doubt the importance of micronutrients for higher crop production (Huguet, 1970; Mortvedt and Giordano, 1970; Sandoin, 1971; Lindsay, 1972). Therefore, the integrated use of micro- and macronutrient fertilizers are being practiced in some areas of the world.

Among the major factors contributing to micronutrient deficiencies in some areas are: alkalinity of soils (high pH), variable soil contents of calcium carbonate and/or gypsum with calcium being the dominant cation in the soil, very low soil contents of organic matter and low total contents of micronutrients in soil parent materials.

These factors combined lead to low micronutrient supplies in most soil types dominating the dry regions of the world.

Applications of micronutrients are not important by themselves but they may be very beneficial for better utilization of macronutrients (Polikarpochkina and Kharkin, 1972; Olsen, 1972).

The importance of micronutrients for plant growth is enhanced also under conditions of drought and high salt concentrations in root media (Bagouri, 1977).

Investigations in several countries have demonstrated the importance of micronutrient fertilizers on calcareous soils (Wassief, 1973; Bagouri et al., 1974; El-Kadi et al., 1975).

Applications of micronutrients have led to large and highly significant increases in the yields of several crops, even when no deficiency symptoms were apparent. This phenomenon is known as "hidden hunger". The dominance of calcium ions in the soil might be one of the contributing factors to such conditions.

Despite these factors, application of micronutrients is not generally practiced in Syria on irrigated and non-irrigated areas.

After initial experiments on rotation, NPK and irrigation requirements for wheat, cotton, legumes and sugar beet, a preliminary experiment on sugarbeet micronutrients was carried out in summer, 1977, by Bagouri and Mohammad. The application of iron, manganese, zinc and copper sulphate individually sprayed twice on the leaves at concentrations of 5.0, 10, 5.0 and 0.5 g/litre respectively (on the basis of 800 litre solutions/ha) increased the sugar beet crop yield compared with control by 232, 286, 234 and 196% respectively; the yields being: control 13.8, manganese sulphate 39.5, iron sulphate 32.3, zinc sulphate 32.3 and finally copper sulphate 27.0 ton roots/ha (unpublished data).

It was evident from the results with micronutrient leaf applications to sugar beet and from other experimental results carried out studying the interaction between macro- and micronutrients on sugar beet, that attention must be given to the role of micronutrients in increasing the crop yields.

Due to the importance of cotton as a major crop in the Syrian economy, especially in the Euphrates basin region, and due also to the low productivity of cotton under Euphrates basin conditions (1850 kg seed-cotton/ha), any improvement of productivity is considered valuable whether achieved by soil management methods or by fertilization. .

A field experiment was designed to study the single and combined effects of both iron and manganese sulphates, applied to soil or to plants on cotton yield and cotton fibre characteristics. This was carried out in summer, 1978. Initially, yield observations indicated a positive response of cotton plants to micronutrient fertilization, but undesirable

climatic conditions towards the end of the season with night temperatures dropping far below normal limits caused damage to 40-60% of the later crop bolls. Variable damage occurred between treatments, so any estimation of micronutrient effects on yield were unsatisfactory. Any treatment which delayed "ripening", although it might normally increase yields, would severely depress yields when such "frosts" occur.

In summer, 1979, another cotton field experiment, (the main topic of this thesis) was carried out, the objective was to study the response of the Syrian cotton variety "Alepo 40" under Euphrates basin conditions to soil and plant applications of Fe, Mn, Zn and Cu.

CHAPTER 4

THE FIELD WORK

This chapter is divided into three sections. The first two sections are concerned particularly with field experiment results, while the final section summarises the results, assessing them in relation to the published literature.

Section 4.1: Effect of soil micronutrient applications on a cotton crop under calcareous soil conditions.

Section 4.2: Effect of foliar sprays of micronutrient on a cotton crop under calcareous soil conditions.

Section 4.3: General discussion, assessment, problems.

The objective of the field work is outlined at the beginning of this chapter. The results are presented and the discussion is confined to their significance within the section. Each section ends with a brief summary.

Introduction and Aims

It is widely recognized that under calcareous soil conditions in general and newly reclaimed saline calcareous soils in particular, that soils often suffer from an unbalanced nutrition mostly associated with insufficient micronutrient availability for plants. The degree of this deficiency depends on a number of factors related to the physical and chemical properties of soil such as: percentage calcium carbonate, pH, organic matter content, irrigation and drainage systems as well as environmental conditions and management practices.

Efforts have been made to increase cotton yield and to improve the fibre quality. To this end, a new variety "Alepo 40" has been introduced. Better agricultural practices in which nutrition plays an essential and important role has been developed.

Although some work has been carried out investigating the effects of the major nutrients (N P K) on both cotton production and fibre properties, there has been little research on the influence of micronutrients on cotton production and fibre properties. The fibre strength, length, smoothness and maturity are the main properties that determine the quality of cotton. Fibre length is the most obvious feature of cotton fibre and Lord (1961) reported that of all the characteristics of raw cotton, staple length is the property most valuable to the spinner. Other things being equal, finer yarns can generally be spun from longer cotton. Fibre maturity is the degree of cellulose deposition in the

secondary wall of the spinners (Pierce and Lord, 1939).

Soil applied micronutrients are rapidly fixed and precipitated under the soil conditions studied particularly with too low a biodegradable organic matter content to keep these cations in an easily soluble complex form for absorption by plant roots.

The data in Table 4.1.1, a preliminary analysis of micronutrient availability in Deir Zor experimental research station, show that soil DTPA extractability of both iron and zinc are close to the critical levels of availability for most crops. The critical level of DTPA extractable iron below which Fe deficiency might occur has been reported as 2.5 ppm (Whitney et al., 1973). Follett and Lindsay (1970) reported that values for DTPA extractable Fe adequacy are grouped as: Low 0-2.0 ppm; Marginal 2.0-4.5 ppm; Adequate 4.5 ppm.

It can be seen later when discussing soil iron availability at various stages of cotton growth that 90 days after planting, iron availability decreased to the level of 2.1-2.7 ppm.

For zinc 0.5 ppm DTPA extractable is considered the critical level below which zinc deficiency is likely to occur (Whitney et al., 1973; Brown et al., 1971). The data in Table 4.1.5 (soil zinc analysis) show that DTPA extractable zinc in soil is close to this level (0.8-0.5) both before and 90 days after planting. Copper deficiency may be expected when its DTPA extractability declines below 0.1-0.2 ppm (King and Alston, 1974).

These preliminary results indicated that a field research programme on a cotton crop should give fresh

Table 4.1.1 The physical and chemical properties of the soil profiles samples

(Samples were collected prior to clover crop sowing)

	Depth/cm	Clay %	Salt %	Sand %	pH	E.C (s.e.)	Organic matter %	$CaCO_3$ %	Active Lime %	D.T.P.A. extractable ppm Fe	Mn	Zn	Cu
Profile I	0-5	38	32	30	7.8	6.3	0.83	19.0	10.2	4.7	6.8	0.80	2.4
	5-20	44	32	24	8.0	4.7	0.71	19.5	9.0	5.5	6.6	0.66	2.1
	20-40	44	30	26	7.7	3.3	0.53	20.3	11.2	4.0	5.4	0.62	2.1
	40-60	34	30	36	7.5	4.2	0.42	20.5	9.2	4.0	3.6	0.60	1.8
	60-80	38	32	30	7.5	4.5	0.24	19.5	12.7	3.7	1.7	0.60	1.20
Profile II	0-5	32	34	34	7.5	6.5	0.77	20.0	9.0	4.4	8.0	0.70	2.3
	5-20	38	36	26	7.7	4.4	0.68	21.0	8.8	4.8	6.4	0.55	2.0
	20-40	36	34	30	7.6	3.9	0.51	21.4	8.9	3.4	4.2	0.55	1.80
	40-60	26	46	28	7.6	4.7	0.39	23.0	12.0	3.0	3.5	0.50	1.50
	60-80	38	30	32	7.4	4.4	0.21	24.1	9.7	3.0	2.5	0.60	1.30
Profile III	0-5	35	36	29	7.9	6.0	0.80	18.9	8.2	4.2	7.8	0.65	2.0
	5-20	46	30	24	8.2	4.5	0.70	20.3	7.3	4.8	5.8	0.56	1.8
	20-40	40	32	28	7.6	4.3	0.61	20.7	9.3	4.5	4.6	0.54	1.6
	40-60	31	40	29	7.5	5.6	0.48	21.3	10.9	3.3	2.5	0.52	1.7
	60-80	35	31	34	7.2	6.0	0.27	22.9	11.3	3.0	2.5	0.50	1.5

information as to what extent applications of iron and zinc compounds to the soil are necessary, especially as no information or previous studies were available for the local cotton variety.

The same Table shows that Mn and Cu availability is adequate and that no response from their application to soil can be expected. The soil organic matter content is low (0.8-0.2%) while calcium carbonate concentrations are high (19-24%), half of this concentration being "active lime". The electrical conductivity ranged between 3.3-6.3 in accordance with sample depth, and it is acceptable for cotton growth where a good drainage system is maintained.

All soil profile samples were collected from the experimental field site before sowing clover for green manure.

Due to some advantages of foliar application in general over soil application, since foliar application avoids interactions in the soil and offers more rapid responses and a considerable economy of material, and also due to the lack of any experience in the field with regard to foliar micro-nutrient applications to cotton (Alepo 40), a comparative study between soil applied and plant leaf applied micro-nutrients was necessary.

In the future an advanced field programme can be designed with respect to a fertilizer regime for cotton.

The main objective of this work is to study the effect of both soil and plant application of Fe, Mn, Zn and Cu sulphates on both cotton production and fibre properties.

SECTION 4.1

Effect of soil micronutrient applications on cotton crop under the newly reclaimed saline calcareous soil conditions.

The experiment

A field experiment was conducted to examine the effectiveness of the following soil applied treatments on cotton grown on the experimental site at Bani Tagleb Research Station, Deir Zor (Table 4.1.2).

Table 4.1.2

Treatment	kg/ha	Addition time	Frequency
Control	-	-	-
$FeSO_4.7H_2O$	50	Sowing time	Once
$MnSO_4.4H_2O$	40	Sowing time	Once
$ZnSO_4.7H_2O$	20	Sowing time	Once
$CuSO_4.5H_2O$	10	Sowing time	Once

The procedure

There were four replicates of each treatment. Cotton was seeded on the 20th April on furrows 70 cm apart, with 20 cm between positions, for a total of 120 plants in each plot (5 x 4 m^2), thinning each position to two plants. The trace elements were banded by hand on furrows in the seed bed under the seeds. The major nutrients (N, P, K) were applied at the rates of 150, 220 and 100 kg/ha as N, P_2O_5 and K_2O respectively. The germination was good (85-100%); cultivation and irrigation operations were carried out when appropriate. Leaf and soil

Photo 4.1.3 The experimental field showing the canals for basin irrigation

Photo 4.1.4 A neighbouring field showing the influence of saline crust formation at the soil surface on crop germination and final yield

Photo 4.1.1 The irrigation water carried 3 Km by an earth canal to the experimental research station

Photo 4.1.2

An open field drain showing the necessity of periodical maintenance

(0-10 cm) samples from the whole experiment were taken four times at monthly intervals.

Results and Discussion

Soil micronutrient availability

Soil micronutrient availability was determined at four different times of crop growth by DTPA analysis, indicating the changes brought about by micronutrient treatments within the soil, but not necessarily their impact on plant micronutrient uptake equilibria (Tables 4.1.3, 4.1.4, 4.1.5, 4.1.6).

Iron

Table 4.1.3 The soil DTPA extractability of iron (ppm) at four plant growth stages

Treatment	Days after planting			
	0*	30	60	90
Control	4.7[f]	5.2	3.3	2.6
Soil applied $FeSO_4.7H_2O$	4.6	5.6	3.6	2.7
Soil applied $MnSO_4.4H_2O$	4.3	4.8	3.0	2.4
Soil applied $ZnSO_4.7H_2O$	4.4	4.7	2.3	2.3
Soil applied $CuSO_4.5H_2O$	4.5	5.2	2.4	2.3
LSD 5%	N.S.	0.25	0.24	0.21

*Soil samples were collected at planting time.

fEach figure represents a mean of four replicates.

Regardless of the applied micronutrient, iron DTPA extractability was high until the 30th day of plant growth. This

can be attributed to the effectiveness of green manure incorporated in the soil, decomposition by microorganisms occurring over this period.

This high iron availability for all treatments masked any plant response to iron applied. A considerable decrease occurred at the 60th and 90th days, this decrease was caused either by the fixation and precipitation of mobilized iron or its rapid absorption by cotton plants or by both. Table 4.1.3 shows that the application of 50 kg iron sulphate, despite the rapid fixation by various soil components, as well as the overall influence of green manure, had a small influence on its DTPA extractability in the top 0-10 cm soil over the 30-90 day period. Mn, Zn and Cu sulphate are effective in depressing significantly iron availability as compared with both control and Fe treatment (Table 4.1.3).

Manganese

Table 4.1.4 The soil DTPA extractability of manganese (ppm) at four plant growth stages

Treatment	Days after planting			
	0*	30	60	90
Control	23.0[f]	9.7	9.0	6.6
Soil applied $FeSO_4.7H_2O$	22.7	10.0	9.2	5.9
Soil applied $MnSO_4.4H_2O$	22.0	10.2	10.0	9.7
Soil applied $ZnSO_4.7H_2O$	23.1	9.7	8.2	6.6
Soil applied $CuSO_4.5H_2O$	22.3	9.9	7.9	7.5
LSD 5%	N.S.	N.S.	.40	.52

*Soil samples were collected at planting time.

[f]Each figure represents a mean of four replicates.

High available manganese at planting time reflects the early release of manganese in the soil prior to iron. However, 30 days after green manure incorporation, both aerobic and anaerobic microbial activity in the moist conditions of soil, and the organic compounds released from the organic material and attacking manganese mineral surfaces in soil, declined. Therefore a rapid fall in manganese availability occurred after 30 days, decreasing to about half and a further slight decrease occurred between 30 and 90 days except that when manganese sulphate was added it maintained a similar manganese concentration during the 30-90 day period.

As with iron, 40 kg of manganese sulphate had little effect on soil manganese due to the initial soil content of various manganese compounds and also to the rapid oxidation of added manganese.

Zinc

Table 4.1.5 The soil DTPA extractability of zinc (ppm) at four plant growth stages

Treatment	Days after planting			
	0*	30	60	90
Control	.79[f]	1.1	.84	.55
Soil applied $FeSO_4.7H_2O$	.71	.92	.84	.50
Soil applied $MnSO_4.4H_2O$	.61	1.2	.91	.57
Soil applied $ZnSO_4.7H_2O$	.71	2.5	2.4	1.7
Soil applied $CuSO_4.5H_2O$	.79	1.1	.73	.61
LSD 5%	N.S.	.21	.18	.14

*Soil samples were collected at planting time

[f]Each figure represents a mean of four replicates.

It is evident from the data in Table 4.1.5 that zinc availability increases in all treatments including control treatment after 30 days due to the influence of green manure and declines again to a critical level after 90 days. With soil zinc application a remarkable increase occurred raising its availability 2-3 times that of the control even after 90 days. Generally, using 20 kg/ha of $ZnSO_4.7H_2O$ on the soil, banded in the seed bed, enhanced zinc availability to a high level, and it is likely that an increase like this provides adequate zinc for the plants especially after 90 days of plant growth and at the early bolling stage during which any imbalance in the nutritional conditions may be reflected in plant growth and final cotton production including fibre characteristics.

The differences between zinc treatment and that of Fe, Mn and Cu are significant and zinc availability is close to the critical level (0.5 ppm). It is also likely from the data that this soil zinc fertilization provides the soil with adequate available zinc for further successive crops (six to seven crops: Brown, Krantz and Martin, 1964), since three months after using 20 kg/ha of $ZnSO_4.7H_2O$ the available zinc detected was 3 times the control and Fe treatment (critical level).

Copper

Table 4.1.6 The soil DTPA extractability of copper (ppm) at four plant growth stages

Treatment	Days after planting			
	0*	30	60	90
Control	2.4[f]	2.3	2.3	1.9
Soil applied $FeSO_4.7H_2O$	2.5	2.1	2.1	1.8
Soil applied $MnSO_4.4H_2O$	2.5	2.1	2.0	1.7
Soil applied $ZnSO_4.7H_2O$	2.6	2.2	1.9	2.0
Soil applied $CuSO_4.5H_2O$	2.5	6.2	3.3	3.4
LSD 5%	N.S.	.24	.30	.24

*Soil samples were collected at planting time.

[f]Each figure represents a mean of four replicates.

Green manure incorporation in the soil induced no increase in copper availability unlike the other micronutrients, probably due to the formation of insoluble complexes by organic compounds from decomposed organic material.

Copper availability also slightly decreased with time, but despite this decrease, it remained adequate to provide for plant requirements. 10 kg/ha $CuSO_4.5H_2O$ significantly increased copper availability in soil and this influence may continue for years as copper fertilizers in the soil generally remain 'available' for several years (Harris, 1947; Cook and Davis, 1957).

The differences in copper extractability using DTPA resulting from the application of other micronutrients to the soil are very slight and insignificant compared with control,

since under the conditions of rapid fixation and precipitation by soil constituents these have no opportunity to inhibit each other.

Micronutrient analysis of soils and plants

Iron

The soil DTPA extractable and plant leaf iron concentrations are shown for the first three sampling periods in Table 4.1.7.

Table 4.1.7 The iron concentration (ppm)

Treatment	Soil extractable (Days after planting)			Plant uptake (Days after planting)		
	30	60	90	30	60	90
Control	5.2*	3.3	2.6	210	190	179
Soil applied $FeSO_4.7H_2O$	5.6	3.6	2.7	229	195	181
Soil applied $MnSO_4.4H_2O$	4.8	3.0	2.4	202	184	174
Soil applied $ZnSO_4.7H_2O$	4.7	2.3	2.3	218	175	174
Soil applied $CuSO_4.5H_2O$	5.2	2.4	2.3	229	183	171
LSD 5%	.25	.24	.21	N.S.	N.S.	N.S.

*Each figure represents a mean of four replicates.

Although the soil iron treatment significantly enhanced iron "availability" in soil, the plant leaf iron concentration was not increased and there is no significant difference between treatments, and no interaction between treatments reflected in the leaf iron concentration. There is a decrease in iron availability in soil with time, and a decline in plant iron concentration also due to dilution as a result of plant growth.

Soil iron application did not give any significant increase in iron in plants, and soil application of other micronutrients did not reduce iron uptake by plants.

Manganese

The results are presented in Table 4.1.8 showing the soil DTPA extractable and plant manganese for the first three samples.

Table 4.1.8 The manganese concentration (ppm)

Treatment	Soil extractable (Days after planting)			Plant uptake (Days after planting)		
	30	60	90	30	60	90
Control	9.7	9.0	6.6	240	282	248
Soil applied $FeSO_4.7H_2O$	10.0	9.2	5.9	258	262	249
Soil applied $MnSO_4.4H_2O$	10.2	10.0	9.7	260	274	262
Soil applied $ZnSO_4.7H_2O$	9.7	8.2	6.6	243	261	242
Soil applied $CuSO_4.5H_2O$	9.9	7.9	7.5	258	266	244
LSD 5%	.46	.40	.52	N.S.	N.S.	N.S.

It appears from the data in Table 4.1.8 that plant manganese concentration remained constant through the season. The presence of a high and constant concentration of manganese in cotton leaf samples indicates rich sources of plant available manganese providing more than the plants required. A very important conclusion can be made that there is no response to and no need to supply manganese to the soil under Deir Zor soil conditions for cotton crops.

Zinc

The results are presented in Table 4.1.9 showing soil DTPA extractable and plant zinc for the first three sampling periods.

Table 4.1.9 The zinc concentration (ppm)

Treatment	Soil extractable (Days after planting)			Plant uptake (Days after planting)		
	30	60	90	30	60	90
Control	1.1*	.84	.55	21.8	17.0	15.3
Soil applied $FeSO_4.7H_2O$	.92	.84	.50	20.5	18.1	15.0
Soil applied $MnSO_4.4H_2O$	1.2	.91	.57	21.7	18.0	14.0
Soil applied $ZnSO_4.7H_2O$	2.5	2.5	1.7	24.9	20.3	17.7
Soil applied $CuSO_4.5H_2O$	1.1	.73	.61	20.3	16.2	15.5
LSD 5%	.21	.18	.14	4.4	2.8	2.6

*Each figure represents a mean of four replicates.

The soil and plant zinc concentration show significant differences at different sampling times. The enhancement of soil zinc availability resulting from application of 20 kg/ha zinc sulphate increased zinc plant uptake significantly. Leaf contents decreased with time. It can also be seen from plant leaf zinc that there is no inhibition of zinc uptake by addition of Fe, Mn or Cu, probably due to the low amounts supplied and to rapid fixation and precipitation in thr soil. Soil zinc application created better conditions of nutrition providing adequate plant zinc for various biochemical functions and gave better yields. Zinc concentration in plant leaves after 90 days of growth decreased to near the critical level

under which zinc deficiency symptoms are expected (11.0 ppm - Ohki, 1975).

Copper

The results are presented in Table 4.1.10 showing the soil DTPA extractable and plant copper concentration for the first three sampling periods.

Table 4.1.10 The copper concentration (ppm)

Treatment	Soil extractable (Days after planting)			Plant uptake (Days after planting)		
	30	60	90	30	60	90
Control	2.3*	2.3	1.9	12.7	12.9	13.3
Soil applied $FeSO_4.7H_2O$	2.1	2.1	1.8	13.1	12.4	13.1
Soil applied $MnSO_4.4H_2O$	2.1	2.0	1.7	12.2	11.8	14.1
Soil applied $ZnSO_4.7H_2O$	2.2	1.9	2.0	11.5	11.9	13.8
Soil applied $CuSO_4.5H_2O$	6.2	3.3	3.4	12.4	12.1	13.3
LSD 5%	.27	.30	.24	N.S.	N.S.	N.S.

* Each figure represents a mean of four replicates.

Despite the significant increase of copper availability in soil resulting from application of 10 kg/ha copper sulphate to soil, there is no significant difference in copper concentrations in plant leaves. The data in Table 4.1.10 also show that the decline in copper availability was not accompanied by a depression in its concentration in the plant, and soil iron, manganese and zinc applications did not further decrease copper concentration in plants, and this is probably due to adequate soil sources of copper with no need for copper fertilizer under these soil conditions.

Effect of soil micronutrient application on the phosphorus concentration in both soil and plants

a) Effects of soil micronutrient application on phosphorus availability in soil at four varied times of plant growth (Table 4.1.11).

Table 4.1.11 Phosphorus availability (ppm)

Treatment	Days after planting			
	0*	30	60	90
Control	2.85[f]	8.27	5.22	3.87
Soil applied $FeSO_4.7H_2O$	2.79	7.35	4.37	2.70
Soil applied $MnSO_4.4H_2O$	2.93	8.18	5.97	3.40
Soil applied $ZnSO_4.7H_2O$	2.48	6.63	4.60	3.30
Soil applied $CuSO_4.5H_2O$	2.56	6.76	3.41	3.40
LSD 5%	N.S.	.80	.61	.41

*Sampling occurred at planting time

[f]Each figure represents a mean of four replicates.

Phosphorus availability in soil greatly increased after 30 days, which was attributed both to phosphate fertilization (220 kg/ha P_2O_5) and green manure (clover) incorporation in the soil; this green manure effect was also noted with micro-nutrients. The data in Table 4.1.11 also show that after 90 days of plant growth, phosphorus availability in soil remained significantly higher than before planting for all treatments.

It is evident from the data that all soil micronutrient treatments with the exception of Mn depressed significantly the

phosphate availability in soil compared with control. These differences would probably have been higher if green manure had not been used.

The manganese sulphate influence on soil phosphate availability remained slight and non significant, while iron, zinc and copper applications caused a clear and significant depression in the later stages of plant growth.

b) Effect of soil micronutrient application on the phosphorus concentration in plants at four plant growth stages (Table 4.1.12).

Table 4.1.12 Phosphorus concentration in plants (parts per thousand)

Treatment	(Days after planting)			
	30	60	90	120
Control	5.20*	3.20	2.24	1.64
Soil applied $FeSO_4.7H_2O$	4.32	2.45	2.09	1.62
Soil applied $MnSO_4.4H_2O$	4.31	2.70	2.12	1.63
Soil applied $ZnSO_4.7H_2O$	4.41	2.65	2.09	1.72
Soil applied $CuSO_4.5H_2O$	4.37	2.75	2.14	1.76
LSD 5%	.73	.28	.20	.18

*Each figure represents a mean of four replicates.

The data in Table 4.1.12 reveals a decrease in leaf phosphorus concentration with time due to both dilution as a result of plant growth and a decline in phosphate availability in the soil. The data also shows significant differences between treatments and all soil micronutrient applications

depressed phosphate concentration in plants compared with control up to 60 days after which their influence decreased.

Iron-manganese ratio in relation to the soil micronutrient treatments and plant growth stages

It is evident from the results of several investigators that iron and manganese are functionally interrelated in some way and that an increase in manganese solubility brings about a decrease in the iron uptake and an increase in the percentage of insoluble iron in the plant. It has been suggested that manganese IV, with its high oxidizing potential, is responsible for the decrease of soluble iron by oxidation of the ferrous iron ions to ferric ions, and Kelley (1914) found crystals of manganese dioxide in the tissues of plants that showed manganese toxicity.

Tottingham et al. (1916) reported that the best ratio of Fe/Mn in the substrate for growth of wheat was 1.0 to 2.5, and Somers and Shive (1942) and Lockman (1972) showed that a 2.0 ratio was best for good growth and development of plants free from pathological symptoms. If the Fe/Mn ratio of 2.0 was altered, chlorotic symptoms developed. However cotton is probably less susceptible and can tolerate a wider range of Fe/Mn ratio, the actual iron concentration being more important (Carlson and Olson, 1950), but any increase in manganese will mean that the plant requires more total iron in order to have sufficient physiologically active iron for biochemical processes.

The data in Table 4.1.13 show Fe/Mn concentration ratio in both soil and cotton leaves as influenced by soil micro-nutrient applications and as a reflection of their availability in the soil at varied plant growth stages.

Table 4.1.13 The Fe/Mn concentration ratio in both soil and plant

Treatment	Days after planting					
	30		60		90	
	Soil*	Leaf	Soil	Leaf	Soil	Leaf
Control	0.53	0.88	0.36	0.67	0.37	0.72
Soil applied $FeSO_4.7H_2O$	0.56	0.89	0.39	0.74	0.45	0.72
Soil applied $MnSO_4.4H_2O$	0.47	0.78	0.30	0.67	0.24	0.66
Soil applied $ZnSO_4.7H_2O$	0.48	0.90	0.28	0.67	0.34	0.72
Soil applied $CuSO_4.5H_2O$	0.53	0.89	0.30	0.69	0.30	0.70

*DTPA extractable

Several conclusions may be drawn from this table concerning Fe/Mn concentration ratio. It can be seen that their ratio in both soil and plants decreased with time and this depression was caused by the sharp decrease in iron availability in soil with time and the slight decrease of manganese. This decrease of iron in the soil was followed by a decrease in plant iron concentration while the slight decrease in manganese availability in soil was associated with constant manganese concentrations in plants as noted earlier.

In general, the wide variation between the generally

accepted optimum ratio and that actually present (Table 4.1.13) probably demonstrates unfavourable conditions for plants, since much of the iron will be transformed to inactive or insoluble forms within plant tissue.

A foliar spray of iron sulphate quickly applied could modify this ratio - increasing iron concentration in plants with improved yields following.

Effect of soil micronutrient applications of Fe, Mn, Zn and Cu on cotton crop yield and fibre quality grown on newly reclaimed calcareous soil

Table 4.1.14 shows the effect of soil micronutrient applications on seed-cotton yield and fibre characteristics.

Table 4.1.14

Treatment	Yield kg/ plot (5 x 4 m^2)	Yield kg/ha	Fibre %	Oil %	Lint strength per-sly	Lint length (inch)	Lint smoothness micronaire	Maturity factor
Control	6.32[f]	3160	37.8	20.5	8.37	1.15	5.08	82.3
$FeSO_4.7H_2O$	6.64	3320	38.6	20.9	8.24	1.13	5.28**	83.6
$MnSO_4.4H_2O$	6.57	3285	38.1	20.6	8.96**	1.12	5.14	83.8
$ZnSO_4.7H_2O$	6.95**	3475	38.2	21.0	8.57	1.11	5.20	83.7
$CuSO_4.5H_2O$	6.26	3130	37.4	20.5	8.75**	1.11	5.14	82.7
LSD 5%	.48		N.S.	N.S.	.40	N.S.	.15	N.S.
LSD 1%	.58		N.S.	N.S.	.52	N.S.	.19	N.S.

** Significant at the level of 1% compared with control.

[f]Each figure represents a mean of four replicates.

Effect of iron sulphate application to soil on cotton yield and fibre properties

The increase in yield resulting from using 50 kg/ha of iron sulphate (160 kg/ha) compared with control is not significant but may suggest the possibility of using higher amounts of iron on soil when designing new field experiments in these areas.

The fibre properties of the iron sulphate treated plants show that soil iron application had no significant result on: seed oil content, lint strength, lint length and maturity factor, but may improve (non-significantly) fibre percentage compared with control. Lint smoothness in comparison with control increased significantly at the 1% level.

The data in Table 4.1.14 indicate that iron sulphate application to soil enhanced both cotton yield and fibre properties although statistically insignificant as compared with control.

Effect of manganese sulphate application on cotton yield and fibre properties

The yield increased by 4% (non-significant) as a result of using 40 kg/ha $MnSO_4 \cdot 4H_2O$ compared with control, but generally there was no effect on: fibre percentage, oil content, lint smoothness, lint length and fibre maturity, while Mn had an effective influence significant at the 1% level on lint strength. The average of four replicates show lint strength for manganese treatment as 5.28 and for control as 5.08.

Effect of soil zinc sulphate application on cotton yield and fibre properties

With soil zinc sulphate application, the crop yield improved significantly at the 1% level by 315 kg/ha compared with control. A considerable improvement in lint strength, from 8.37 to 8.57, oil content from 20.5 to 21.0%, fibre maturity from 82.3 to 83.7, fibre percentage from 37.8 to 38.18 and finally lint smoothness from 5.08 to 5.20 was also noted. Better results might be expected with higher amounts of zinc sulphate, since the used amount - 20 kg/ha - compared with recommended figures - 40 kg/ha (Chapman et al., 1977) is low.

It is important to continue this research using a wider range of zinc applications.

Effect of soil copper sulphate application on cotton yield and fibre properties

In addition to the negative results of copper application on cotton yield by -0.9% compared with control, its application had undesirable effects on all fibre characteristics except lint strength which increased significantly and lint smoothness which improved slightly.

Summary of Section 4.1.

This field experiment on a newly reclaimed saline calcareous soil studied the effect of soil application of Fe, Mn, Zn and Cu sulphate at the levels of 50, 40, 20 and 10 kg/ha respectively, banded once at planting time in the seed bed, on both seed cotton yield and fibre characteristics.

The results indicate that application of Fe, Mn, Zn and Cu increased significantly their "availability" in soil but their concentration in the plant remained non-significant except zinc. Zinc application increased the concentration in plant tissues significantly. Zinc application also increased significantly the cotton yield and had desirable effects on the fibre quality since it substantially increased fibre percentage, seed-oil content, lint length, lint smoothness and fibre maturity. Iron application improved yield, fibre percentage and fibre maturity but non-significantly, while it increased significantly lint smoothness and decreased both lint length and lint strength.

A 4% increase in cotton yield was obtained by manganese application. It also showed a significant effect on the fibre strength, and a slight increase in both fibre maturity and fibre percentage as compared with control.

Copper application decreased the cotton yield, lint length and fibre percentage, but had no effect on seed-oil content and fibre maturity, while it increased substantially lint strength, the only advantage of copper application to soil. This increase in lint strength also followed foliar copper application (see later).

Section 4.2

Effect of foliar spray of certain micronutrients on a cotton crop under reclaimed saline calcareous soil conditions

The experiment

The experiment was conducted on the experimental site at Bani Tagleb Research Center Station. The plant sprayed micronutrient concentrations are shown in Table 4.2.1.

Table 4.2.1

Treatment	Concentration g/litre	kg/ha	Addition time
Control	-	-	-
$FeSO_4.7H_2O$	6.0	12.0	on the 30th, 60th and 90th days after planting.
$MnSO_4.4H_2O$	3.0	6.0	
$ZnSO_4.7H_2O$	1.5	3.0	
$CuSO_4.5H_2O$	0.5	1.0	

The procedure

As in the soil micronutrient experiment, there were four replications of each treatment. Cotton was seeded on the 20th April on furrows 70cm apart with 20cm between positions, and a total of 120 plants in each plot (4x5 m^2).

The major fertilizers (N, P, K) were supplied at the rates of 150, 220 and 100 kg/ha as N, P_2O_5 and K_2O respectively. The germination rate was good (88-100%). The plants were thinned to two in each position. Operations of cultivation, weeding and irrigation were done when appropriate.

The micronutrients, Fe, Mn, Zn and Cu sulphate were sprayed at monthly intervals on the 30, 60 and 90th days of plant growth. The Fe, Mn, Zn and Cu concentration in the spraying solution was 1200, 750, 340 and 110 ppm respectively. The spray involved all plot plants for each treatment. Plant leaf samples were taken at random from the middle four rows, avoiding outer rows.

Plant leaf samples were associated with the following stages of plant development:

1) when the plants were 25cm long (30 days after planting)
2) at the early flowering stage (60 days after planting)
3) at the early bolling stage (90 days after planting)
4) at the late bolling stage (120 days after planting)

Results and discussion

Table 4.2.2., 4.2.3., 4.2.4. and 4.2.5 show the results of foliar sprays of Fe, Mn, Zn and Cu sulphate on their concentrations in the plant tissue at four different times of plant growth.

Iron

Table 4.2.2 The iron concentrations (ppm) in cotton leaf at four different times of plant growth

Treatment	(Days after planting) 30f	60	90	120
Control	212*	170	153	144
$FeSO_4.7H_2O$	220	223	274	247
$MnSO_4.4H_2O$	210	181	155	130
$ZnSO_4.7H_2O$	228	176	143	128
$CuSO_4.5H_2O$	218	175	154	136
LSD 5%	N.S.	22.2	20.8	14.8

*Each figure represents a mean of four replicates

fSampling occurred immediately before the first spray.

Iron concentration in plant leaves for all treatments, and also at all sampling times, was higher than reported critical levels (85-112 ppm. Kouskoleka and Kallinis, 1968) for cotton. Treatments had no significant influence on iron concentration except that of zinc sulphate which caused a significant decrease after 120 days in iron concentrations.

As expected, iron sulphate sprays (1200 ppm) increased significantly iron concentration in leaf. The usual decrease in iron concentration with time was absent with iron sprays.

It can be seen that from the plant response to iron sprays, that iron spraying probably created a modification in the Fe/Mn ratios and increased soluble or active iron inside the plant cell.

Manganese

Table 4.2.3 The manganese concentration (ppm) in cotton leaf at four different times of plant growth

Treatment	(Days after planting)			
	30[f]	60	90	120
Control	221*	252	252	226
$FeSO_4.7H_2O$	220	239	239	215
$MnSO_4.4H_2O$	213	279	368	255
$ZnSO_4.7H_2O$	223	241	240	210
$CuSO_4.5H_2O$	232	253	244	219
LSD 5%	N.S.	27.2	29.6	26.8

*Each figure represents a mean of four replicates.

[f]Sampling occurred immediately before the first spray.

The manganese concentration in the plant for all treatments including control increased with time up to the 90th day, after which a slight reduction occurred. This is different from the usual behaviour with respect to iron.

As expected, manganese foliar sprays increased leaf manganese concentration. There is no influence from other treatments on manganese content in plants.

Zinc

Table 4.2.4 The zinc concentration (ppm) in cotton leaf at four different times of plant growth

Treatment	(Days after planting)			
	30[f]	60	90	120
Control	15.6*	16.6	14.3	16.5
$FeSO_4.7H_2O$	15.6	15.8	15.5	16.4
$MnSO_4.4H_2O$	15.0	18.4	14.5	15.2
$ZnSO_4.7H_2O$	14.4	40.4	32.8	21.0
$CuSO_4.5H_2O$	14.5	18.6	13.6	15.6
LSD 5%	N.S.	4.4	2.8	2.9

*Each figure represents a mean of four replicates

[f]Sampling occurred immediately before the first spray.

Similar conclusions to that of iron and manganese are drawn from Table 4.2.4. Significant differences are found only from zinc sulphate spray application, while its concentrations in plants for all other treatments are close to the critical level (11 ppm. Ohki, 1975). The increase following zinc spray

probably created better conditions of zinc metabolism in the plant and this is reflected in better plant growth and higher yield.

Copper

Table 4.2.5 The copper concentration (ppm) in cotton leaf at four different times of plant growth

Treatment	(Days after planting)			
	30[f]	60	90	120
Control	12.0*	11.4	10.5	8.0
$FeSO_4.7H_2O$	12.8	11.2	10.2	9.7
$MnSO_4.4H_2O$	12.9	11.2	10.5	10.2
$ZnSO_4.7H_2O$	11.9	11.5	10.5	10.0
$CuSO_4.5H_2O$	12.3	15.8	21.0	22.4
LSD 5%	N.S.	1.8	2.6	2.6

*Each figure represents a mean of four replicates.

[f]Sampling occurred immediately before the first spray.

For all treatments, except copper spray treatment, copper concentration in the plant decreased slightly with time. As expected, copper sulphate treatment enhanced the copper content significantly doubling its concentration. It is also evident from the data that there is no clear interaction between treatments.

Fe/Mn ratio in plant tissue and the influence of micronutrient foliar sprays on this ratio

The data in Table 4.2.6 show Fe/Mn concentration ratio in cotton leaf as influenced by the plant micronutrient applications at four stages of plant growth.

Table 4.2.6 Fe/Mn concentration ratio

Treatment	(Days after planting)			
	30	60	90	120
Control	0.96	0.67	0.61	0.64
$FeSO_4.7H_2O$	1.0	0.93	1.15	1.15
$MnSO_4.4H_2O$	0.99	0.65	0.42	0.51
$Zn\ SO_4.7H_2O$	1.02	0.73	0.59	0.61
$CuSO_4.5H_2O$	0.94	0.69	0.63	0.62

Iron sulphate sprays maintained a Fe/Mn ratio near to 1.0 in plant tissue at the four stages of plant growth, while on the other hand, plant manganese sulphate spray severely depressed this ratio especially at the 90th and 120th day period.

It can be seen, despite a lack of knowledge as to the quantity of iron and manganese in plants coming from both extracellular "contamination" and "inactive" material, that the iron sulphate spray modified Fe/Mn ratio. Favourable conditions for iron metabolism were indicated by the yield data. Fe/Mn ratios arising from soil applications are not subject to this criticism.

Effect of the foliar application of Fe, Mn, Zn and Cu on the cotton yield and fibre quality grown on reclaimed saline calcareous soil

Table 4.2.7 shows the influence of plant micronutrient sprays on the seed-cotton yield and fibre characteristics.

Table 4.2.7

Treatment	Yield kg/ plot (4x5 m^2)	Yield kg/ha	Fibre %	Oil %	Lint strengthpersly	Lint smoothness (micronaire	Lint length (inch)	Maturity factor
Control	6.04[f]	3020	38.2	21.1	8.56	5.16	1.13	83.7
$FeSO_4.7H_2O$	6.75**	3375	38.1	20.6	8.53	5.34**	1.11	83.3
$MnSO_4.4H_2O$	6.25	3125	37.3	20.9	8.61	5.08	1.13	82.6
$ZnSO_4.7H_2O$	7.43**	3715	38.7	21.0	8.43	5.17	1.13	83.4
$CuSO_4.5H_2O$	6.27	3135	37.4	20.3	8.96**	5.08	1.12	83.3
LSD 5%	.48		.80	N.S.	.40	.15	N.S.	N.S.
LSD 1%	.58		1.02	N.S.	.52	.19	N.S.	N.S.

** Significant at the 1% level compared with control.

[f]Each figure represents a mean of four replicates.

Effect of iron sulphate on cotton lint yield and fibre properties

It appears from the results that iron sprays to the plant improved the cotton yield at the 1% level, causing a 12% increase over control. This increase was probably caused by Fe/Mn ratio modification and is not attributed only to the provision of higher iron concentrations in plants, because iron concentrations in plant tissue for all treatments remained over

the accepted critical levels for cotton crops (85-112 ppm, Kouskoleka and Kallinis, 1968) and plants did not show any obvious iron deficiency symptoms in the field. The high manganese may have caused specific problems in iron metabolism. Iron sprays also improved significantly the lint smoothness, but seed oil, lint strength and lint length were unaffected by iron application. The average values for seed oil and fibre maturity for the four replicates were 20.6 and 83.3, and those for the control were 21.1 and 83.7 respectively.

Tables 4.1.14 and 4.2.7 also show that both soil and plant applied iron sulphate improved significantly lint smoothness.

Effect of manganese spray on cotton lint yield and fibre characteristics

From the results given in Table 4.2.7 it appears that manganese application caused only a 3% increase (not significant) in the cotton yield. It had no effect on lint length and fibre maturity, but had undesirable effects on fibre percentage, seed oil and lint smoothness.

A slight increase in lint strength was obtained by manganese applications and this agrees with that reported in Table 4.1.14 where manganese applications to the soil improved significantly lint strength.

Effect of zinc spray on cotton lint yield and fibre properties

The yield of plants treated with zinc were considerably increased, significant at the 1% level, with a 23% increase in comparison to 10% increase from soil zinc application.

With respect to fibre properties for the zinc treated plants, the data in Table 4.2.7 shows that zinc application had no effect on seed oil, lint smoothness and lint length compared with control. However, lint strength and fibre maturity showed a slight decrease compared with control, while fibre percentage showed a slight increase with zinc application.

In general it appears that zinc treatment greatly improved the yield (by 23%) and maintained cotton fibre characteristics at the values associated with control.

Effect of copper spray on cotton lint yield and fibre properties

The results given in Table 4.2.7 show that a slight non-significant increase occurred in cotton yield, 4% compared with control. Copper spray application resulted in a considerable decrease in seed oil content, lint smoothness, maturity and fibre percentage. Lint strength increased significantly at the 1% level as compared with control. This result is similar to that with soil copper sulphate applications. There is a clear relationship between copper concentration in plant and fibre strength.

Summary of Section 4.2.

A field experiment was carried out on a recently reclaimed saline calcareous soil, studying the influence of foliar sprays of micronutrients on cotton yield and fibre characteristics. The results obtained indicate that their concentrations in the leaf significantly increased as a

result of plant sprays without any interaction occurring between treatments.

The results also indicate that the micronutrients studied differ considerably in their effect on both cotton yield and fibre properties. Fe, Mn, Zn and Cu increased the seed cotton yield by 12, 3,23 and 4% respectively compared with control. Zinc increased fibre percentage while copper and manganese decreased it. Iron had no effect on fibre percentage. All the elements decreased seed-oil content slightly. Little variation in fibre maturity was obtained with Fe, Zn and Cu but remarkable decreases occurred with Mn application. All elements studied had no effect on lint length. Iron significantly increased lint smoothness while manganese and copper decreased it and zinc application had no effect. Only copper application clearly increased lint strength, but a slight increase was obtained by manganese application, while iron and zinc decreased it.

Section 4.3 General discussion of the field work

Results of the analysis of soil samples collected from the field experimental site show that the soil contents of iron and manganese oxides are high. The three major forms of these two elements were determined using DTPA (Diethylene-triamine penta acetic acid), HHQ (Hydroxyhydroquinine) and DIth (Dithionite) extractants for assessing the available, reactive and free oxides respectively.

Table 4.3.1 shows the average of 11 surface soil analyses for Deir Zor soils compared with data obtained on Hofuf soils, Saudi Arabia, which showed severe iron deficiency on many crops (Stewart-Jones, 1980).

Table 4.3.1 The iron and manganese extracted from 1 gram of soil by various methods

	Syrian soil		Saudi soil	
	Feμg/g	Mn μg/g	Fe μg/g	Mn μg/g
Available (DTPA)	4.76	4.80	2.60	6.80
Reactive (HHQ)	36.90	31.10	14.70	3.20
Free iron oxides (Dithionite)	4800.00	239.00	789.0	32.20

Despite the high soil content of free iron oxides as compared with Saudi Arabia, the availability of iron remained restricted within narrow limits near to levels considered critical and this is because iron is present in highly crystalline insoluble forms especially where the soil has a very low organic matter content. The plant's ability to absorb

adequate iron depends mainly on the distribution of iron oxides through the soil profile and the nature of these oxides, and also the plant's ability to modify rhizosphere conditions and so release more iron into solution.

Green manure (clover crop) incorporation into the soil enhanced Fe, Mn and Zn DTPA extracability in the soil during the limited period of its decomposition (75 days for the summer cotton crop).

The data in Table 4.3.2 show changes in micronutrient availability of the control treatments, resulting from green manure incorporation, through the period of cotton growth.

Table 4.3.2 DTPA extractable micronutrients (µg/g)

	Before clover Sowing	Days after clover incorporation				From Tables
		30	60	90	120	
Fe	4.1	4.7	5.2	3.3	2.6	4.1.3
Mn	8.2	23.0	9.7	9.0	6.6	4.1.4
Zn	0.71	0.79	1.1	0.84	0.55	4.1.5
Cu	2.20	2.40	2.30	2.30	1.90	4.1.6

The effects of "green manure" organic compounds released into the soil solution on the micronutrients in the soil seemed variable depending on the nature of these compounds and their reaction with exposed surfaces of oxides. The results indicated the ease with which the Mn IV oxides can be attacked, reduced and organically complexed in more available forms in the soil solution, as compared with iron oxides which revealed only a slow and low availability increase under the same conditions.

The organic material exerted a positive and significant role in enhancing zinc extractability in soil for up to 75 days following incorporation after which a rapid reduction occurred giving a return to critical levels of availability on the 120th day when plants were at the peak bolling stage and plant requirements for zinc are considered very urgent for determining boll size and fibre characteristics. This is discussed further in relation to zinc sulphate application.

The short benefit obtained from green manure, in the enhancement of micronutrient availability is attributed to the fact that with high soil temperatures, moisture and microbial activity, the decomposition of organic material occurred rapidly over about two months. The green manure material was incorporated at the late flowering stage of clover growth, before plant maturity, when it is rapidly biodegraded.

However, these results reveal that in Deir Zor area the use of a green manure can improve the soil nutritional status in general and for micronutrients in particular, desirable results can be obtained for one summer crop growth period.

Despite the advantages of green manure application to the soil, both from soil physical, chemical and nutritional views, a detailed micronutrient study under natural soil conditions without any amendments such as green manure needs to be carried out for the following reasons:

1) Green manure added to the soil throughout the entire experimental area created such better nutrient availability in control plots that a full assessment of the role of soil

micronutrient applications in improving cotton growth and its final yield could not be determined.

2) The plant response to micronutrient fertilizers during the 75 days after application seemed to be somewhat limited, for example, soil applied iron and, to a lesser extent, manganese applied before planting changed to unavailable forms as a result of fixation and precipitation within the soil.

3) Green manure is not used commercially by farmers as it is thought to waste land and water, so it is probable that micronutrient fertilizers supplied either to soil or sprayed on plants under natural soil conditions without the application of any green manure would be more attractive to commercial users. The comparison of these fertilizers and the plant responses in growth and yield, and the economic factors in using micronutrient fertilizers and/or green manure should be evaluated.

The level of iron sulphate used (50 kg/ha as $FeSO_4.7H_2O$) showed no spectacular indications of plant response due to rapid fixation and precipitation, and also the overall green manure application masked any responses which may have been evident on unamended soils. Iron sulphate caused a small but significant increase in the iron DTPA extractability for up to 60 days following application, after which the extractable iron returned to control levels (Follett and Lindsay, 1971; Lindsay et al., 1967). This enhancement in iron extractability remained restricted to the top 0-10 cm layer, so there is little leaching of this element down the profile.

The main factor preventing a cotton crop response to soil iron sulphate application is probably the natural iron

content in the soil, and there were no severe iron deficiency chlorosis symptoms on the crops in the area studied. Organic manure also contributed towards this phenomenon and a return to the data in Table 4.3.2 demonstrates how much the organic compounds enhanced manganese availability in the soil (some 300%), 30 days after incorporation compared with only an 11% increase in iron extractability. Due to the high antagonism between Mn and Fe, the plants were unable to absorb any further iron despite enhancement of its "available" concentration in the soil. It is also likely that the amount applied 50 kg/ha was inadequate and larger quantities were used by several investigators (e.g. 200 kg/ha Stewart-Jones, 1980; 560 kg/ha Mathers, 1970). Finally, the heavy phosphate fertilization of the soil practiced in the research station supplied twice yearly at the rate of 220 kg/ha P_2O_5 for summer and winter crops probably has an important influence on iron nutrition. Phosphate may form insoluble iron phosphates in the soil diminishing the area of iron oxide surfaces (Bray, 1974) and may form phosphate bridges between the oxides and other micronutrients such as zinc (Stanton and Burger, 1967) or it may act internally within the root (Cumbus et al., 1977) inhibiting iron translocation to the shoots (Marschner, 1978).

DTPA extractable manganese was significantly increased by 40 kg/ha of $MnSO_4.4H_2O$ to the soil as compared with control. This enhancement was apparent 60 days after crop planting, since during the first two months the high available manganese coming from the influence of green manure on native soil manganese meant that all treatments had manganese availability similar to the control. There appears to be no need for

manganese fertilization on this soil for cotton and its application probably causes undesirable interference with iron uptake and metabolism in the plant.

Based upon the work of Ohki (1975) the Fe/Mn ratio appears of little importance as such. However it can be used as an indicator of the iron status of the nutrient supply as shown by Lockman (1972). Lockman used hydroponic solutions to identify the effects of nutrient deficiencies and their balance in young sorghum and found that plants grown with a complete nutrient solution had a Fe/Mn ratio of about 2.0. Both macro- and micronutrient deficiency tended to lower this ratio except that of manganese when the ratio could rise to 11.0. Iron deficiency reduced the ratio to 0.30. In the water culture experiment which I carried out in the greenhouse, using the same cotton variety (Alepo 40), iron deficiency symptoms first appeared on the plants when Fe/Mn ratio in the shoots dropped to 0.20 (Table 6.3.3). Both iron and sulphur deficiency cause a 2-3 fold increase in plant manganese levels over that of any other nutrient and a 5-6 fold increase over balanced nutrition.

The following Table summarizes the leaf iron and manganese ratios of cotton at Deir Zor and also the influence of soil and plant micronutrient applications on this relation (Table 4.3.3).

It appears from the data in this Table that iron concentrations in the leaves remained adequate for developing healthy plants without approaching critical concentrations for cotton (85-112 ppm, Kouskoleka et al., 1968). Fe/Mn ratio under all soil and plant Fe, Mn, Zn and Cu treatments remained

Table 4.3.3 Fe/Mn ratio in cotton leaf and influence of the soil and foliar spray treatments on this ratio

		A Soil treatment				B Foliar spray treatment			
Days after planting		30	60	90	120	30	60	90	120
Control	Fe	210	190	179	153	212	170	153	144
	Mn	240	282	248	217	221	252	252	226
	Fe/Mn	0.88	0.67	0.72	0.71	0.96	0.67	0.61	0.64
$FeSO_4.7H_2O$	Fe	229	195	181	158	220	223	274	247
	Mn	258	262	249	211	220	239	239	215
	Fe/Mn	0.89	0.74	0.72	0.75	1.0	0.93	1.15	1.15
$MnSO_4.4H_2O$	Fe	202	184	174	162	210	181	155	130
	Mn	260	274	262	227	213	279	368	255
	Fe/Mn	0.78	0.67	0.66	0.71	0.99	0.65	0.42	0.51
$ZnSO_4.7H_2O$	Fe	218	175	174	159	228	176	143	128
	Mn	243	261	242	224	223	241	240	210
	Fe/Mn	0.90	0.67	0.72	0.71	1.02	0.73	0.59	0.61
$CuSO_4.5H_2O$	Fe	229	183	171	154	218	175	154	136
	Mn	258	266	244	230	232	253	244	219
	Fe/Mn	0.89	0.69	0.70	0.67	0.94	0.69	0.63	0.62

Fe from table 4.1.7

Mn from table 4.1.8

Fe from table 4.2.2

Mn from table 4.2.3

below 1.0 and higher than that for absolute deficiency (0.30) recorded by Lockman (1972). In the same Table (side B) when iron was supplied to the leaves, the ratio significantly increased to near 1.0 at the varied stages of plant development, while spraying Mn sulphate on the leaves severely reduced Fe/Mn ratio to a range close to 0.5. This is to be expected, and indeed all the Fe and Mn measured in these leaves may not be biochemically active as some may be precipitated extracellularly.

The cotton production and fibre characteristics were the only means by which we could evaluate the balance in nutrition through the plant life cycle, because neither applied Fe caused Mn deficiency nor applied Mn induced actual Fe deficiency chlorosis symptoms on the plants during the plant growth period.

Spraying plants with a 6% Fe^{2+} solution as $FeSO_4.7H_2O$ at three stages of plant growth (monthly intervals) significantly increased Fe/Mn ratio and increased cotton yield by 12% as compared with control. The manganese dominance over iron is considered a clear problem for plant metabolism of iron. Neither soil manganese application nor foliar spray resulted in an increase in cotton yield and fibre characteristics except lint strength (Anter et al., 1978) and this might support the Fe/Mn ratio influence in plant development.

It can be seen from the results of zinc and copper applications to the soil that their DTPA extractability significantly increased and they are effective as soil treatments over long periods unlike iron and manganese. They are

not leached from the soil and are expected to supply micro-nutrients for some years (6-7 years for zinc; Brown, Krantz and Martin, 1964 and 5 years for copper; Cook and Davis, 1957) and their persistence depends on the soil properties, rate of application and the crop used (Boawn, 1974).

While the cotton plants responded to soil zinc application and also foliar spray resulting in 12 (Table 4.1.14) and 23% (Table 4.2.7) increases in the cotton yield respectively, it was clear that there was no need for copper fertilization to enhance cotton yield.

The plant leaf zinc concentration shown in Tables 4.1.9 and 4.2.4 confirms the efficiency of both soil and plant applications on the enhancement of zinc in the leaf. Ohki (1975) in his study on cotton in the greenhouse using 14 levels of zinc from 0 to 10.000 µg/litre, reported that when Zn levels in the leaf were less than 20 µg/g dry matter, top weight, root dry weight, plant height, node number produced were reduced as compared to plants supplied with adequate zinc. He also concluded that the lower critical Zn levels were from 8 to 11 µg/g. The results obtained from my field experiment indicate, except for those plants supplied with zinc in general and foliar spray in particular, that zinc concentration in leaves is below the established value of Ohki (20 µg/g) and a further decrease in its concentration occurs approaching the critical level at the late bolling stage of plant growth (120 days after planting). On the other hand, while zinc application to soil increased its concentration in the leaf, this was not true in the case of copper and this was probably due to the rich reserves of copper in the soil since its DTPA

extractability even after 120 days of plant growth, in control treatment, remained 10 times the critical level (0.1-0.2 ppm; King and Alston, 1974).

CHAPTER 5

LABORATORY STUDIES

This chapter is divided into four sections dealing with problems arising from the field experiment.

Section 5.1 deals with a laboratory pot experiment on the effects of "green manure", iron and zinc sulphate, under different levels of phosphate on micronutrient concentration in both soil and the cotton plant.

Section 5.2 deals with anaerobic studies on the iron and manganese release from flooded calcareous soil.

Section 5.3. The role of the cotton plant rhizosphere in utilization of inorganic iron as affected by varied levels of phosphate and iron in both water and sand culture.

Section 5.4. General discussion and conclusions from the laboratory work.

Section 5.1 The Pot Experiment

5.1.1 Introduction and aims

It is known that several advantages are obtained when leguminous crops are grown and then incorporated in the soil especially when soil is deficient in some critical nutrients needed in very low concentrations such as micronutrients under calcareous soil conditions. These nutrients become more available to plants. In practice some of these green manures can be used instead of nitrogen fertilizers with less cost.

Incorporation of green manure within the soil under field experimental conditions was believed to have reduced to a large extent cotton responses to the soil micronutrient applications and this was clear from analysis of soil samples taken at monthly intervals during plant growth. Many published reports indicate that green manures can effectively supply necessary elements to plants during their decomposition. Cavaleri, P.A. et al. (1963) have reported that in 10 experiments on sandy soils in San Paulo, green manure without N increased yields by 355 kg/ha and with N by 280 kg/ha, and green manure + P gave an increase of 51%. The residual effect of green manure was 14% of its immediate effect. Green manure combined with deep ploughing of the lucerne practically doubled cotton yield in a nine-year experiment (Maksumov et al., 1975).

The content of iron and manganese oxides and hydroxides in the soil used are high, but they are present in the unavailable forms under calcareous conditions. The numerous

organic compounds released by decomposing plant material can modify soil reaction and attack these oxide surfaces and more iron and manganese can become organically complexed and in soluble forms which can be utilized by plants, in addition to those nutrients present in the original plant material.

The preliminary analysis of soil and plants in the field indicated that there was no plant response to manganese or copper following soil enrichment with these two elements. Although the free iron oxides content of the soil was very high, the iron availability determined by D.T.P.A. extractant was very low probably due to these oxides being crystalline, stable and of low reactivity under the local conditions. The zinc availability in the soil was close to established critical levels and its addition to the soil enhanced both availability and uptake. Phosphate was studied in this pot experiment as it interferes with micronutrient availability and uptake in soil, especially with iron and zinc.

The objective of this greenhouse experiment was to answer some of the following questions arising from field work:

a) To what extent the green manure incorporated in the cotton experimental site in Deir Zor contributed to the improvement in cotton yield.

b) The green manure influence on the availability of micronutrients in soil and their concentrations in plant leaves.

c) The effect of the soil application of one micronutrient on the D.T.P.A. extractability of the other micronutrients.

d) Redistribution of the soil applied micronutrients through the soil profile of the pots under the influence of irrigation water during the period of plant growth.

e) The effect of the phosphate fertilization on the D.T.P.A. extractable Fe, Mn, Zn and Cu and their concentration in the cotton plant fractions (leaf, stem, root).

f) A study of the micronutrient interactions in both soil and plant.

5.1.2 <u>The procedure</u>

To 1 kg of air dried calcareous soil (collected from the field experimental site in Syria) passing through 5 mm sieve, the levels (1, 2, 4%) of dried green manure, iron sulphate (5,10,20 p.p.m. Fe) zinc sulphate (2,5,1o p.p.m. Zn) and K H_2 PO_4 (0, 100, 200 p.p.m. P) were applied replicated twice giving a total of 60 pots. The green manure was uniformly mixed with the whole pot soil, iron and zinc sulphates were mixed only with the top 2 cm of the pot soil while phosphate was added on the soil surface immediately before watering.

Six seeds of cotton (Hellenic Cotton Board, "Variety 45") were seeded per pot on the 23rd May 1980, 2 cm below the soil surface. The crop germinated after 7 days. The germination rate was good for all treatments. Irrigation was applied daily by using 300 ml of tap water. The appearance of the crop was recorded regularly. The plants were perfectly healthy except for a slight reduction in growth associated with mild chlorosis symptoms on the pots receiving 10 p.p.m. zinc and

200 p.p.m. P. These two treatments combined caused the appearance of iron deficiency chlorosis symptoms on the plants in both replicates. After five weeks growth the plants were harvested. Leaf, stem and root samples were collected separately. The soil was air dried and subsampled to the depths of 0-2, 2-5 and 5-10 cm to show micronutrient distribution down the pot. Fe, Mn, Zn and Cu in soil were assessed by the D.T.P.A. extractant method while their concentrations in the plant samples were determined by atomic absorption spectrophotometry as described before.

5.1.3 Results and discussion

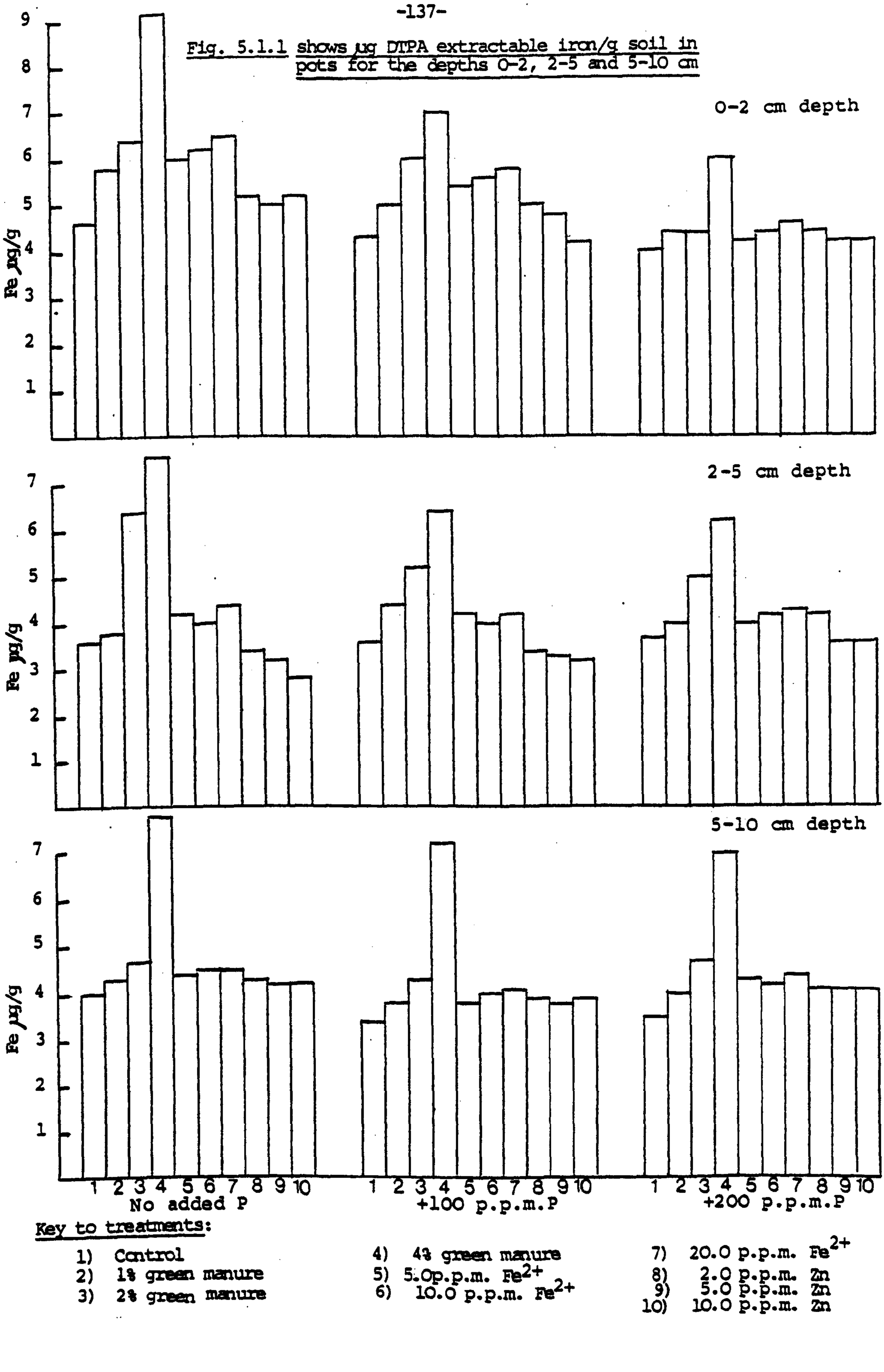
5.1.3.1 Effect of the varied soil treatments on the D.T.P.A. extractable Fe, Mn, Zn and Cu in the soil and their distribution down the pot soil profile

Iron

The distribution of D.T.P.A. extractable iron and the influence of the treatments of green manure, iron, zinc and phosphate on this distribution is shown in figure 5.1.1.

The uniformly distributed green manure caused considerable enhancement in D.T.P.A. extractable iron in all pot soil depths as compared with control. Using 4% green manure for example released 9.2, 7.6 and 7.8 p.p.m. Fe for the depths 0-2, 2-5 and 5-10 cm while for control these values were only 4.6, 3.6 and 4.1 p.p.m. respectively. Differences in soil near the top of the pots caused by green manure, were much lessened when phosphate was applied, although differences were still seen at depth. The slight decrease in iron availability with depth,

Fig. 5.1.1 shows µg DTPA extractable iron/g soil in pots for the depths 0-2, 2-5 and 5-10 cm



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. Fe^{2+}
8) 2.0 p.p.m. Zn
9) 5.0 p.p.m. Zn
10) 10.0 p.p.m. Zn

although the soil was uniformly mixed with green manure, may have been caused by plant root absorption in addition to leaching loss especially as the seeds were initially planted 2 cm below the soil surface and the top 2 cm was mostly outside plant root activity. This layer was subjected to dryness after each irrigation and so anaerobic conditions rarely occurred in this layer, thus little change can be expected in iron compounds in this region when the organic matter content in the soil is low.

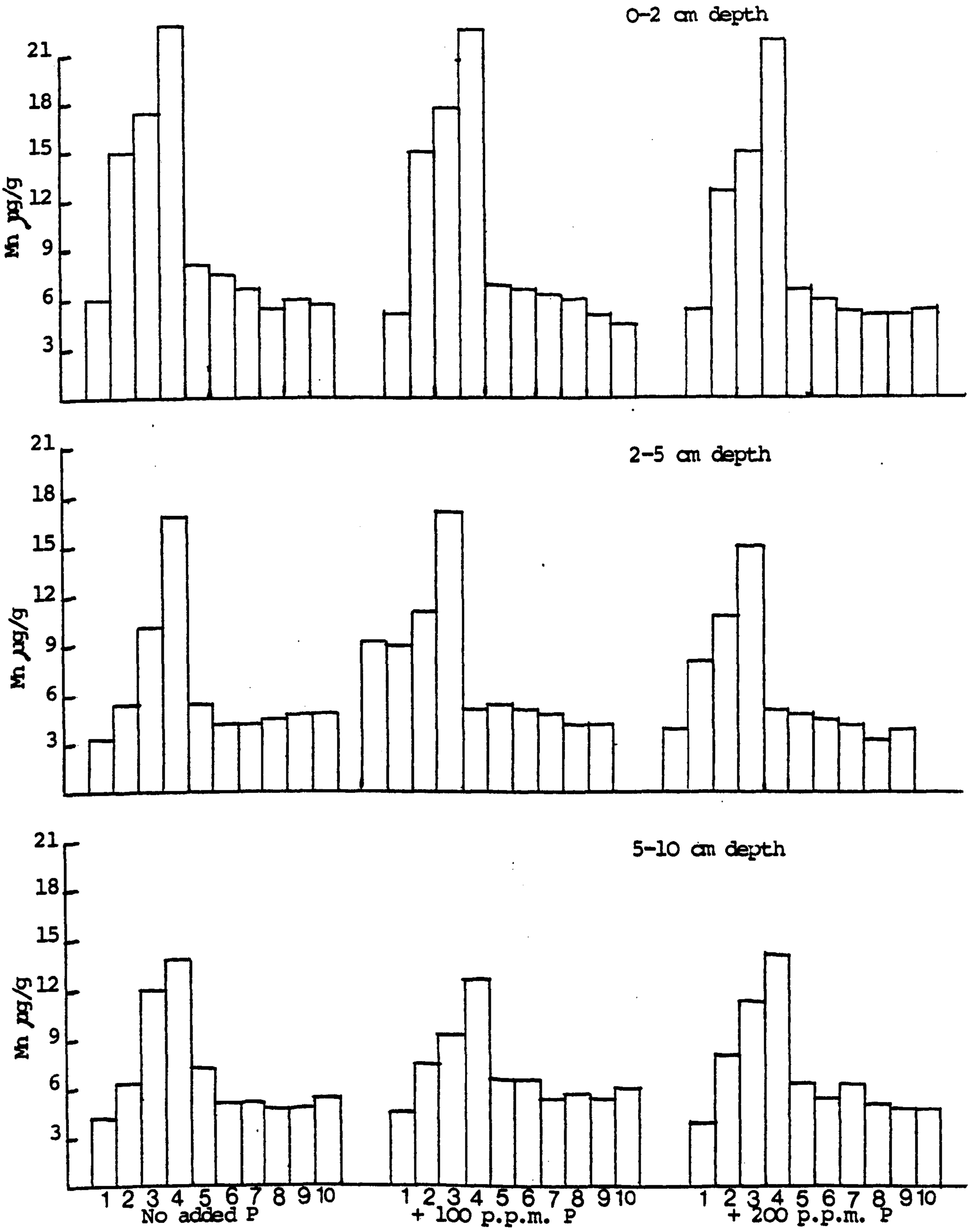
In the lower layers probably short periods of anaerobic conditions could have occurred, increasing the content of soluble compounds of iron and manganese, under the heavy irrigation (300 ml daily) which was used. The results in figure 5.1.1 also indicate that for all treatments iron concentration at the bottom (5-10 cm) was slightly more than in the upper (2-5 cm) zone. Some of this increase may have occurred as a result of unavoidable contamination by roots when sampling, since after five weeks of growth the majority of the root hairs appeared to be at the bottom of the pot.

Phosphate application to the soil caused a remarkable decrease in the D.T.P.A. extractable iron at the top of the pots while its effect was slight with depth, reflecting the slow movement of phosphates in these calcareous soils.

Due to the rapid fixation and precipitation of iron applied to the soil surface especially under very low organic matter, iron incorporation in the top 2 cm of soil caused no increase in its availability in the bottom layer as compared with control.

With zinc application to the soil a reduction in available iron occurred (in the absence of phosphate applications)

Fig. 5.1.2 shows µg DTPA extractable Mn/g soil in pots for the depths 0-2, 2-5 and 5-10 cm



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0 p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. F
8) 2.0 p.p.m F
9) 5.0 p.p F
10) 10.0 F

at both 0-2 and 2-5 cm depths, but not at the bottom 5-10 cm depth.

Manganese

Manganese was not applied in any of the treatments. However there is clear evidence of the differential distribution of D.T.P.A. extractable manganese within the pot soil profile as can be seen in figure 5.1.2.

The maximum values of manganese availability were associated with those of the organic matter treatments inducing approximately 154, 177 and 287% increases for 1, 2 and 4% green manure treatments respectively. These high differences in manganese availability resulting from green manure treatments at the top 0-2 cm, decreased with depth despite the original uniform distribution of green manure within the whole pot soil. In this respect iron and manganese are similar in this experiment.

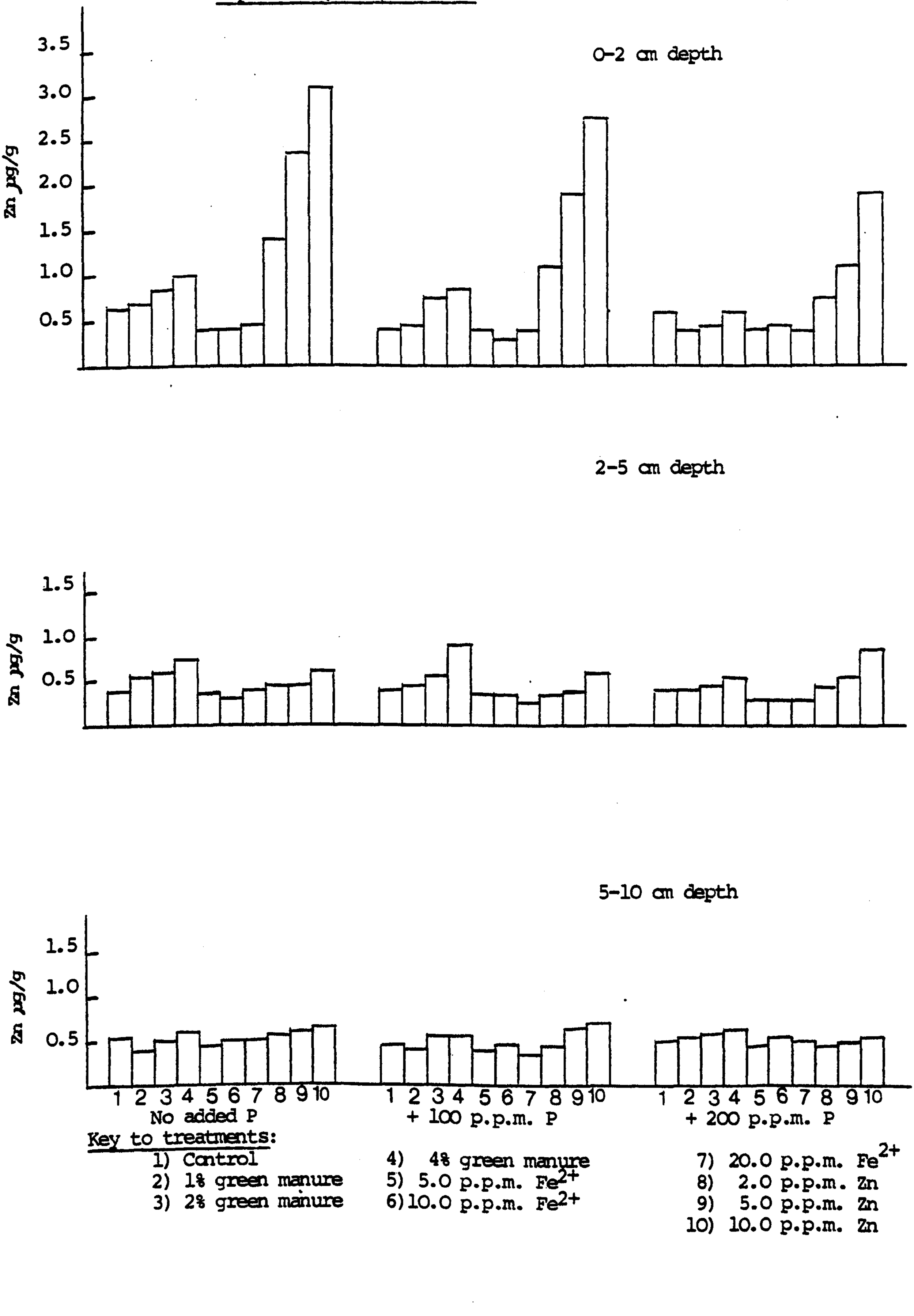
It can also be seen from the figure 5.1.2 that applications of phosphorus, iron and zinc to the soil at their varied levels did not appreciably influence manganese extractability, due to very high original soil content of native manganese compounds.

Zinc

Zinc was applied to the soil at three different levels (2,5, 10 p.p.m.) as $ZnSO_4.7H_2O$. The D.T.P.A. extractable zinc is presented in figure 5.1.3.

Green manure had a role in enhancing zinc availability in the soil when phosphate was absent and when it was applied at the 100 p.p.m. level, but application of 200 p.p.m.P as

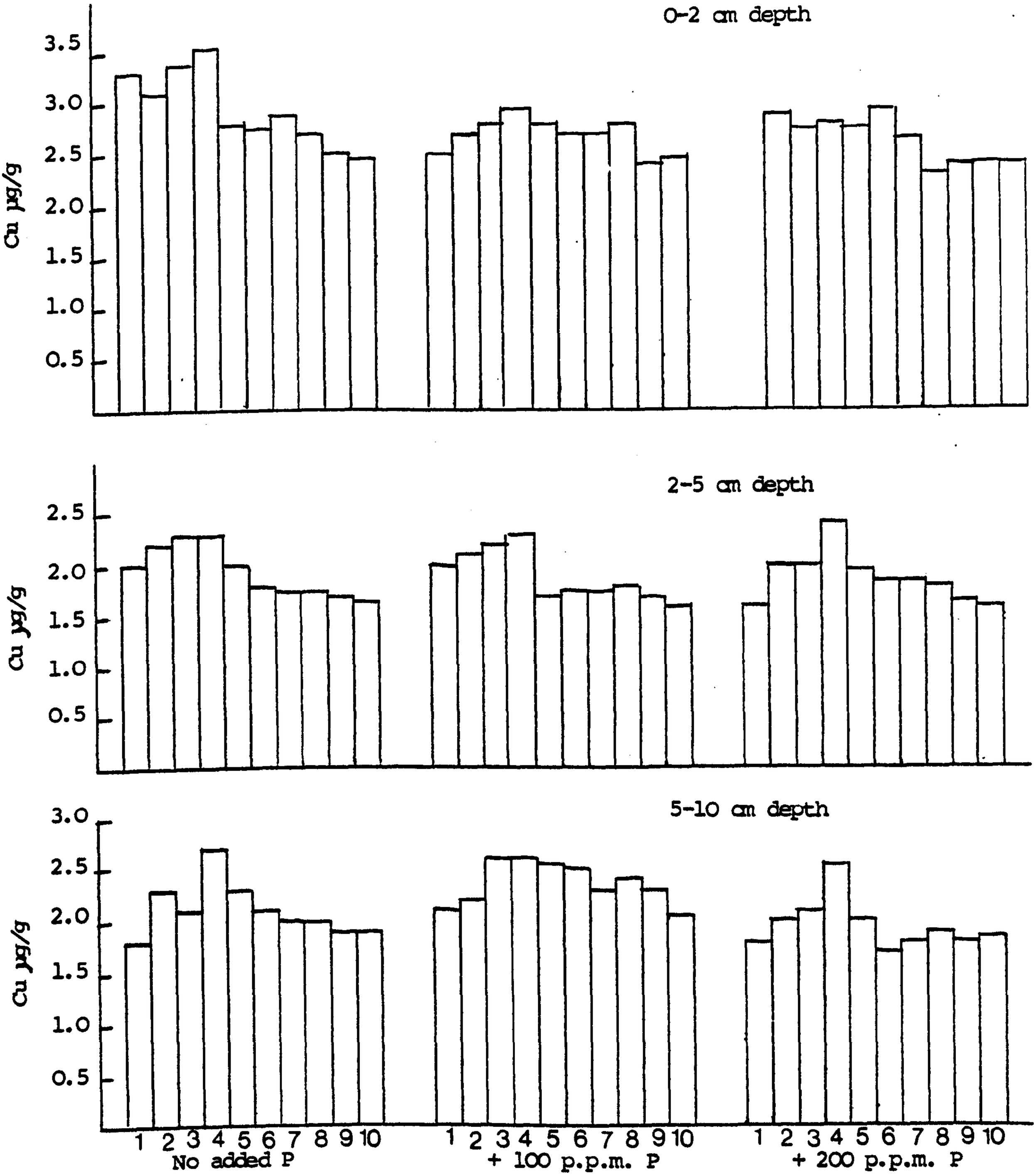
Fig. 5.1.3 shows µg DTPA extractable Zn/g soil in pots for depths 0-2, 2-5, 5-10 cm



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0 p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. Fe^{2+}
8) 2.0 p.p.m. Zn
9) 5.0 p.p.m. Zn
10) 10.0 p.p.m. Zn

Fig 5.1.4 shows ug DTPA extractable Cu/g soil in pots for depths 0-2, 2-5, 5-10 cm



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0 p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. Fe^{2+}
8) 2.0 p.p.m. Zn
9) 5.0 p.p.m. Zn
10) 10.0 p.p.m. Zn

phosphate to the soil caused a considerable reduction in zinc availability even in soil treated with zinc.

A six-fold increase in D.T.P.A. extractable zinc occurred in the top 0-2 cm layer when the zinc was applied at 10 p.p.m. level. At the 2-5 cm depth differences were less, while at the bottom of the pot the zinc availability for all treatments was comparable to control. These results reflect the rapid fixation of zinc applied to the soil and its slow movement down the profile.

Iron application caused a reduction in zinc availability in the top layer when there was no phosphorus supplied to the soil, but the role of iron decreased with increasing phosphate application to the soil. Iron/zinc interactions decreased with depth for all phosphate levels.

Copper

Copper like manganese was not applied in any of the treatments. It appears from copper D.T.P.A. extractability (fig. 5.1.4) that neither iron, zinc or phosphorus application caused any appreciable variation in D.T.P.A. extractable copper in the soil, with the exception of a slight increase as a result of green manure application.

The slight noticeable reduction of copper availability in the middle soil layer (2-5 cm) compared with the bottom may be due to similar reasons to those governing iron distribution in the soil (viz plant root absorption).

5.1.3.2 Effect of the various treatments of green manure, Fe, Zn and phosphate on cotton yield and Fe and Zn uptake

Altering the growth medium by using varied levels of

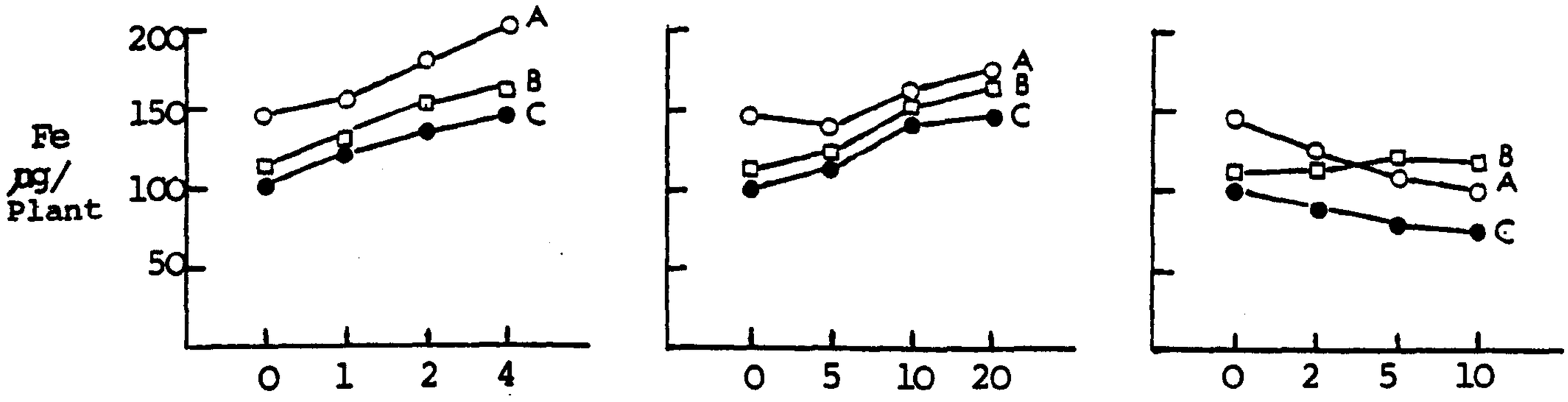
phosphate, iron sulphate and zinc sulphate, and green manure as an organic source in the pot soils, modified plant growth and the relations between Fe and Zn with respect to total uptake and distribution within the plant fractions (table 5.1.1).

Table 5.1.1 Influence of the various levels of green manure, Fe, Zn, and phosphate on the fresh and dry cotton yield g/pot

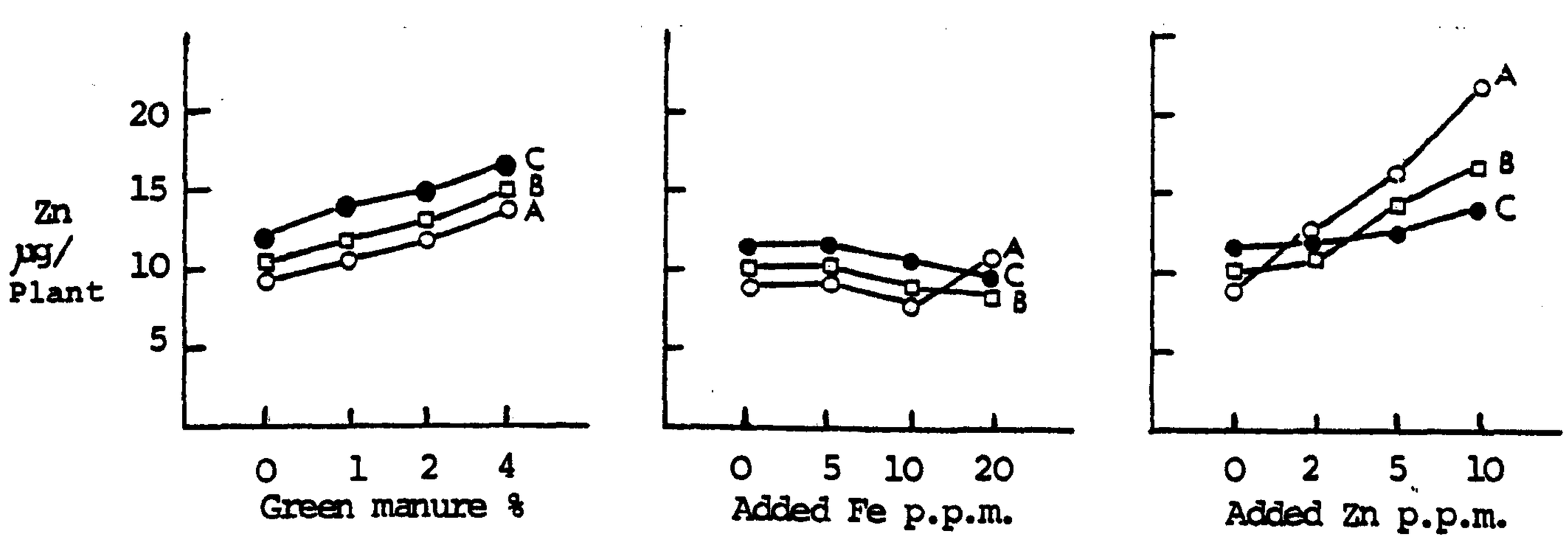
		Control	Green manure			Fe/p.p.m.			Zn/p.p.m.		
		0	1	2	4	5	10	20	2	5	10
No added P	wet	13.3	16.8	17.7	18.1	17.0	15.7	16.8	14.7	13.0	12.6
	dry	2.13	2.73	2.9	2.97	2.8	2.65	2.69	2.29	1.91	1.52
100 p.p.m.P	Wet	18.6	18.8	20.4	21.2	19.7	19.0	18.4	19.9	16.4	11.4
	dry	2.59	2.84	3.10	3.22	3.0	2.9	2.84	2.10	1.75	1.6
200 p.p.m.P	wet	11.9	11.6	15.9	17.9	17.3	16.4	18.6	11.9	10.8	9.5
	dry	1.67	1.62	2.23	2.93	2.7	2.27	2.62	1.73	1.84	2.00

It is clear from the data in table 5.1.1 that optimum yields were obtained when 100 p.p.m. P was supplied to soil. Green manure seemed very effective in increasing plant growth, the more green manure applied to the soil the higher the yield obtained. Ferrous sulphate treatments also increased yields. When phosphate was present at a high level, growth of both green manure and iron sulphate treated plants was reduced. Severe reduction in both wet and dry yields together with iron deficiency chlorosis symptoms on the plants occurred when plants received the highest two levels of zinc (5.0, 10.0 p.p.m) combined with 100 and 200 p.p.m. P levels.

Fig. 5.1.5



a) The average Fe(µg/g) in whole cotton plant grown in pots with green manure, Fe, Zn and various phosphate treatments. (Data for plant parts in fig. 5.1.7)



b) The average Zn (µg/g) in whole cotton plant grown in pots with green manure, Fe, Zn and various phosphate treatments. (Data for plant parts in fig. 5.1.9)

A = No added (P)
B = +100 p.p.m. (P)
C = +200 p.p.m. (P)

Plant growth looked normal under all the iron concentrations used, but a slight reduction in yield was caused by 200 p.p.m. P applications. There was close correlation between wet and dry yields except for zinc treated plants. Those which were very stunted had a high dry matter content compared with control and other treatments.

In general it might be concluded that the optimum levels for healthy plants were 100 p.p.m. P, 5.0 p.p.m. Fe and 2.0 p.p.m. zinc. Further application of P and Zn causes lower yields because of poor uptake/translocation of iron.

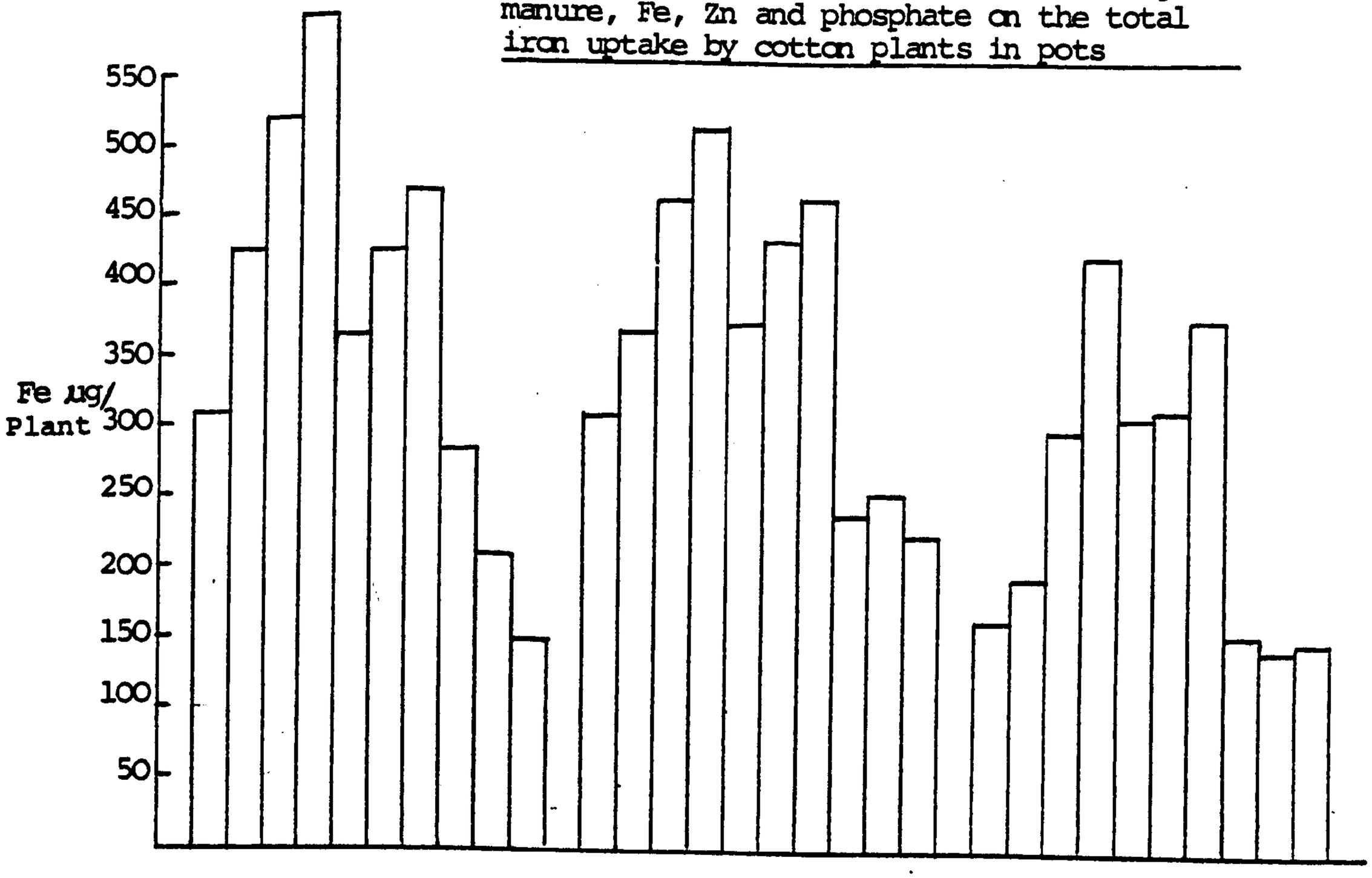
The average concentration (µg/g) of Fe and Zn in whole plant as affected by the various concentration of Fe, Zn, green manure and P is illustrated in fig. 5.1.5, and total Fe and Zn uptake (µg/plant) in fig. 5.1.6.

Plant growth and the final yield would be a reflection of the uptake and movement of P, Fe and Zn in the plant. It is clearly evident from figs. 5.1.1 and 5.1.3 that green manure applications enhanced Fe and Zn uptake by plants as a whole. Higher concentrations of phosphate decreased the average iron concentration in the plant. Similar trends occurred when iron was supplied as increasing levels of ferrous sulphate, but when zinc was added both growth and iron uptake were severely reduced. The inhibition of growth of cotton plants is attributed not to iron precipitation in the lower parts of plant but to high concentration of zinc in soil depressing plant uptake of iron. The situation was worse when extra phosphate was combined with zinc treatments. This result indicates Fe/Zn interference in the substrate before absorption and not within the plant.

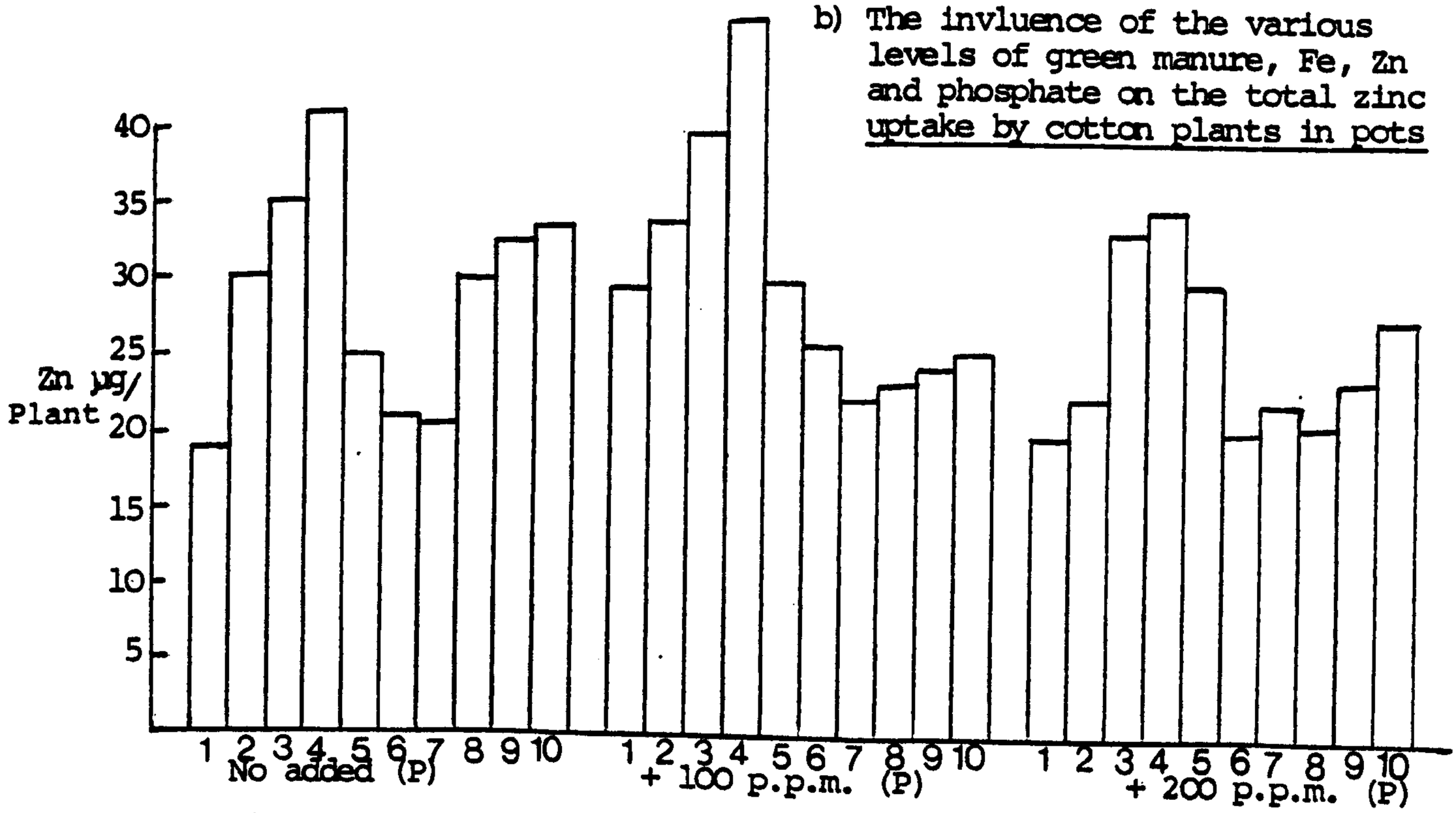
Green manure to soil created better conditions for zinc uptake by plants, but zinc uptake was little affected by phosphate

Fig. 5.1.6

a) The influence of the various levels of green manure, Fe, Zn and phosphate on the total iron uptake by cotton plants in pots



b) The invluence of the various levels of green manure, Fe, Zn and phosphate on the total zinc uptake by cotton plants in pots



Key to treatments:

1) Control
2) 1% dried green manure
3) 2% dried green manure
4) 4% dried green manure
5) 5 p.p.m. Fe
6) 10 p.p.m. Fe
7) 20 p.p.m. Fe
8) 2 p.p.m. Zn
9) 5 p.p.m. Zn
10) 10 p.p.m. Zn

levels. This shows that greater interference occurs between P and Fe than between P and Zn, thus plants tend to take up zinc much more easily than Fe. Iron sulphate addition induced only slight reduction in zinc uptake by plants.

The increased additions of zinc sulphate to soil resulted in higher zinc uptake, and only at high levels of added Zn was any inhibition by high levels of P noted.

The total uptake of Fe and Zn is illustrated in fig. 5.1.6. Consideration of this reduces the dilution and concentration effects of actual plant growth and yield. Increased Fe and Zn uptake accompanied green manure increases in soil at all phosphate levels. A slight reduction in uptake followed phosphate increase in soil with respect to Fe, but 100 p.p.m. P was the required level for optimum total Zn uptake. This again indicates stronger Fe/P interactions than Zn/P in the soil. At 100 p.p.m. added P, the plants probably acquired more Zn on account of less Fe uptake. The highest level of P (200 p.p.m.) inhibited both Fe and Zn uptake from the soil.

Total Fe uptake was severely decreased when Zn was applied in the absence of added P to the soil, but the role of Zn was very slight when 200 p.p.m. P were supplied. Fe/Zn interactions appear to be less important than Fe/P interactions.

An increase in total uptake resulted from increased iron sulphate supply and phosphate seemed to inhibit this only when it was supplied at its highest level (200 p.p.m.).

Higher Zn application levels had little effect on the total zinc uptake. Data on Zn concentration in fig. 5.1.6 are due to severe depression of plant growth under both 5.0 and 10.0 p.p.m. Zn.

The micronutrient contents (µg/seed) of the cotton seeds used are negligible and the seed contribution towards the total uptake of these micronutrients is considered very limited as compared with those of iron and zinc taken up by each plant (fig. 5.1.6).

The average of Fe, Mn, Zn and Cu content in each cotton seed (µg/seed)

Fe	Mn	Zn	Cu
1.37	0.40	1.07	0.27

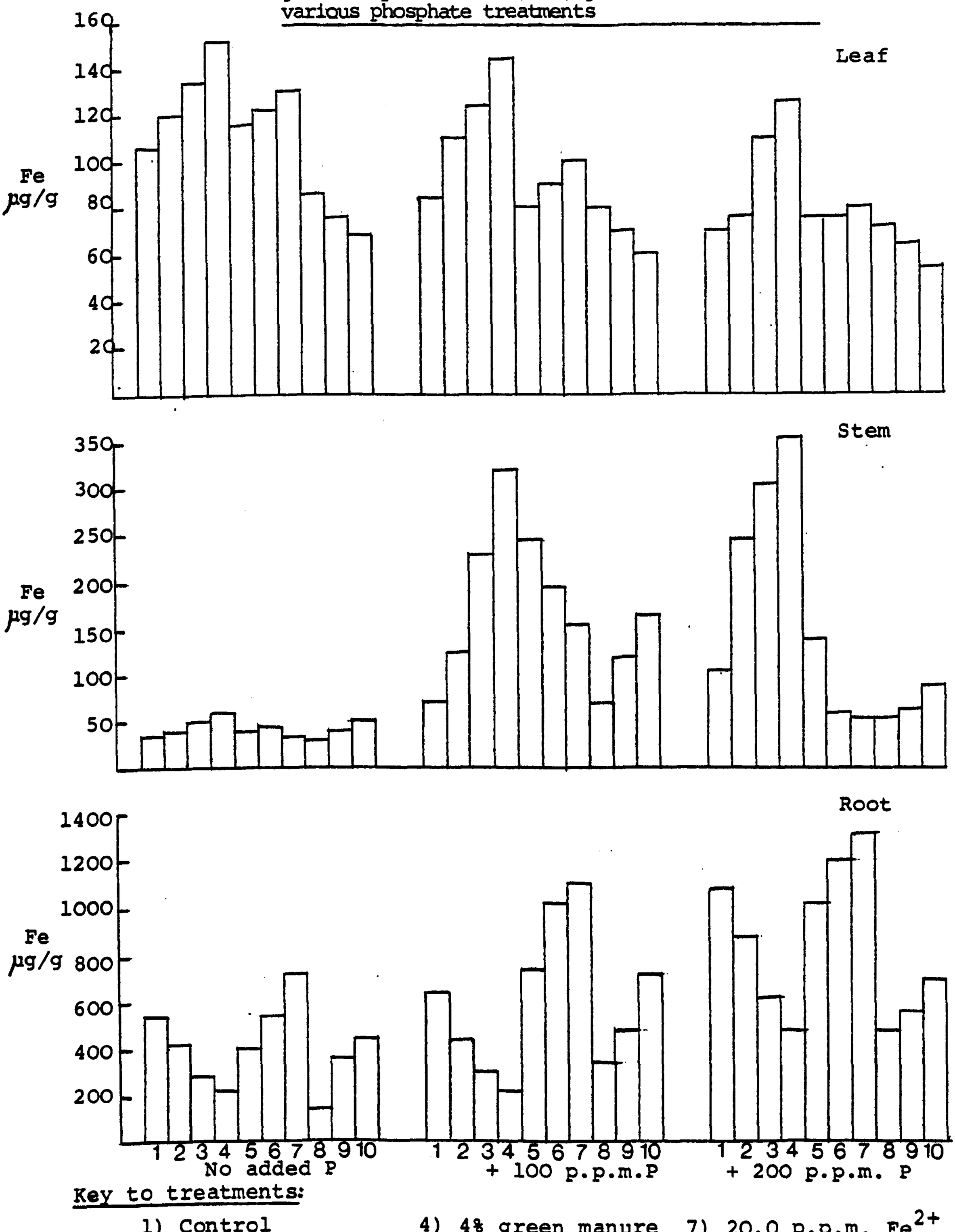
5.1.3.3 <u>Effect of the varied soil treatments on the Fe, Mn, Zn and Cu uptake and their concentration within plant fractions (leaf, stem, root)</u>

<u>Iron</u>

Green manure application considerably enhanced iron concentration in plant leaves (fig. 5.1.7). The decrease observed due to phosphorus application was found not only in the green manure treatments but also in all other treatments including control. It also appears that soil iron application increased its concentration in plant leaf when no phosphorus was added, but using 200 p.p.m. P inhibited any enhancement of both iron uptake and translocation to the upper portion of the plant as indicated by leaf analysis.

Soil zinc application caused a depression in plant leaf iron concentration. When the highest two levels of zinc (5 and 10 p.p.m.) were combined with either 100 or 200 p.p.m. P a further reduction in Fe concentration occurred in the plant shoots, and iron deficiency chlorosis symptoms appeared one

Fig. 5.1.7 shows Fe concentration (µg/g) in plant fractions grown in pots with Fe, Zn, green manure, and various phosphate treatments



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0 p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. Fe^{2+}
8) 2.0 p.p.m. Zn
9) 5.0 p.p.m. Zn
10) 10.0 p.p.m. Zn

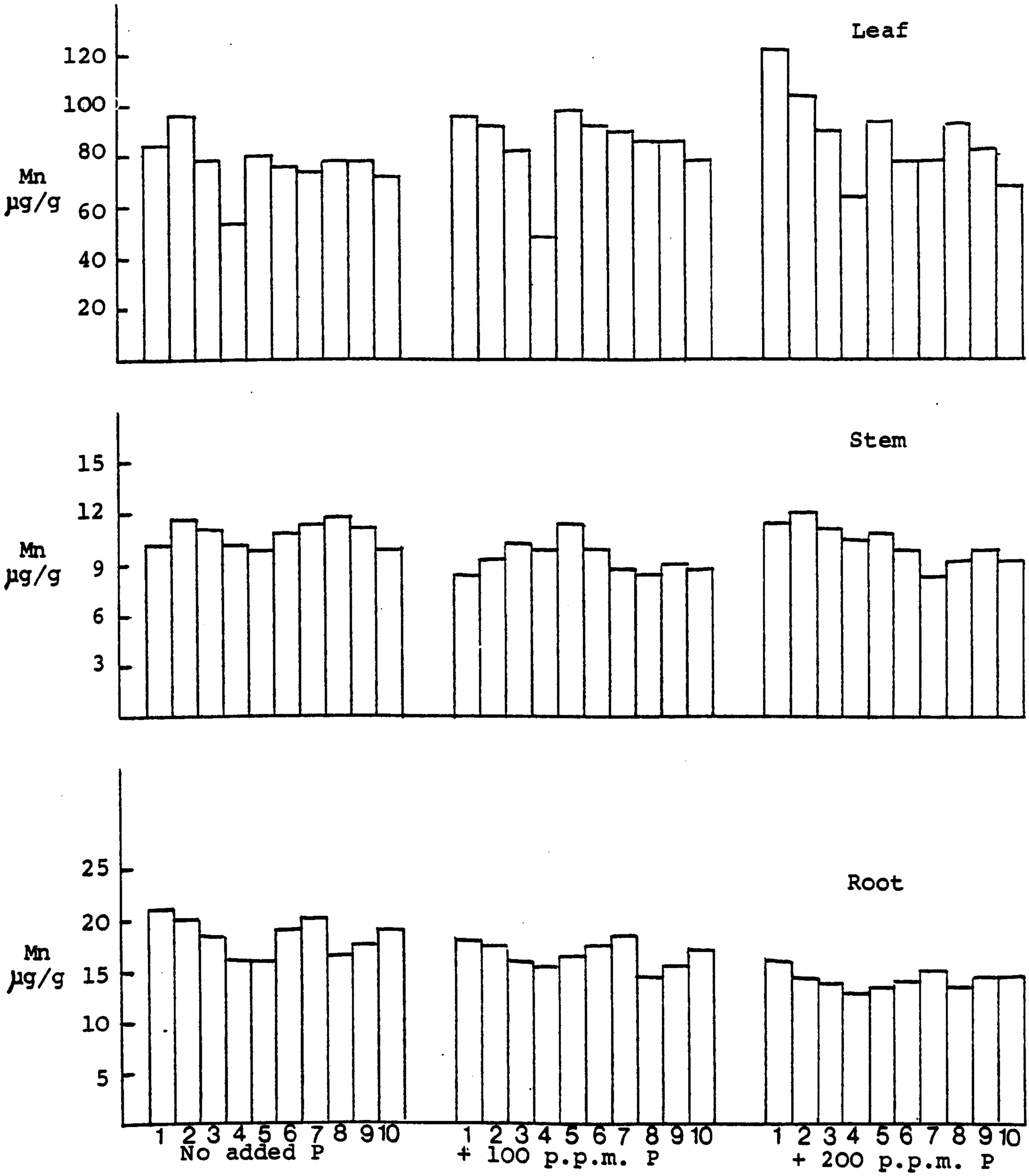
week before harvest.

The figure also shows that no iron accumulation occurred in the stem in the absence of phosphorus applications to the soil and similar values were obtained for all treatments including control. A sharp increase in the iron concentration in the stem was associated with P treatments especially those of the green manure treatments reflecting the role of P in inhibiting iron translocation as a result of its accumulation within the lower portions of plant (stem and root), as well as its precipitation in the soil media before absorption.

A combination of P and Fe treatments to the soil caused very great Fe accumulation in the root. Zinc treatments also prevented Fe movement to leaves, but the opposite was true regarding green manure treatments where high Fe concentrations in both leaf and stem were associated with low concentrations in the root when higher green manure levels were added to the soil.

In general from figure 5.1.7 it can be seen that iron uptake inhibition was largely caused by phosphorus treatments supplied to the soil. However interaction between Fe and P appears to occur mostly in soil solution and within the plant root after absorption. Similar results were obtained when zinc sulphate was associated with P treatments. The question arises as to why, with soil phosphorus treatments, iron distribution in plant fractions depends on the iron source used to the soil. Green manure treatments cause high Fe in both leaf and stem and low Fe in the root, while inorganic Fe treatments of the soil cause precipitation of Fe in the root with lower values in the stem, confirming that the iron source applied to the soil

Fig. 5.1.8 shows Mn concentration (µg/g) in plant fractions grown in pots with Fe, Zn, green manure and various phosphate treatments



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0 p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. Fe^{2+}
8) 2.0 p.p.m. Zn
9) 5.0 p.p.m. Zn
10) 10.0 p.p.m. Zn

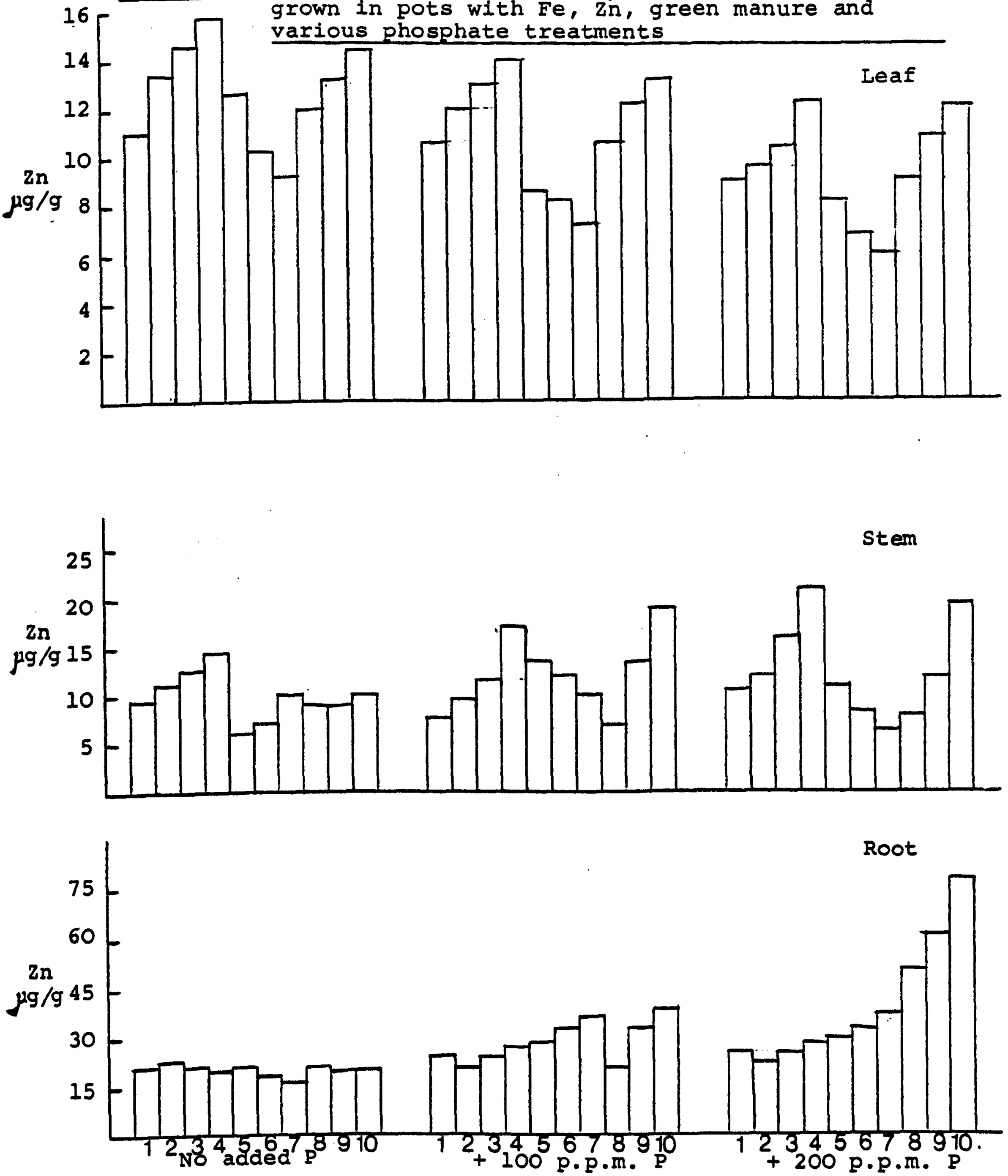
(organic or inorganic) define the form of iron absorbed by plant roots in the presence of phosphorus (Kashirad et al., 1973 and Marschner, 1978). Marschner (1978) suggested that organically complexed iron could pass directly cells, while inorganic iron could remain in cell-free space in the roots (see Chapter 1.3 page 20). This is also clearly shown in this experiment where higher green manure applications lead to lower Fe in roots and much higher Fe in leaves in the presence of phosphate. These results will be discussed with more detail later in this section.

Manganese

Manganese uptake and concentration in plant fractions proved particularly interesting. Manganese concentration in both soil and plant was sharply affected by iron concentrations (fig 5.1.8). Figures 5.1.1 and 5.1.2 show that green manure application significantly increased both D.T.P.A. extractable iron and manganese in soil, but the increased uptake of iron by plants influenced manganese uptake as a result of the antagonism of iron on manganese uptake, regardless of the high availability of manganese in the soil. For example 4% of green manure to the soil caused the highest concentration of iron in the leaves while it induced a minimum content of manganese in the plant especially in leaves.

Phosphate application slightly enhanced manganese concentration in both leaf and stem for most treatments and this is probably due to depression of iron availability in the soil and iron transport in the plant as a result of an increase in phosphate and little P-Mn interaction in either soil or plants.

Fig. 5.1.9 shows Zn concentration (µg/g) in plant fractions grown in pots with Fe, Zn, green manure and various phosphate treatments



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0 p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. Fe^{2+}
8) 2.0 p.p.m. Zn
9) 5.0 p.p.m. Zn
10) 10.0 p.p.m. Zn

Iron and zinc at the levels used had only a slight depressing influence on manganese uptake by plants. Thus iron made available by green manure was the main factor which evidently inhibited manganese uptake by plants, and it could inhibit uptake even when Mn concentration was double that of the Fe concentration extractable from the soil by D.T.P.A.

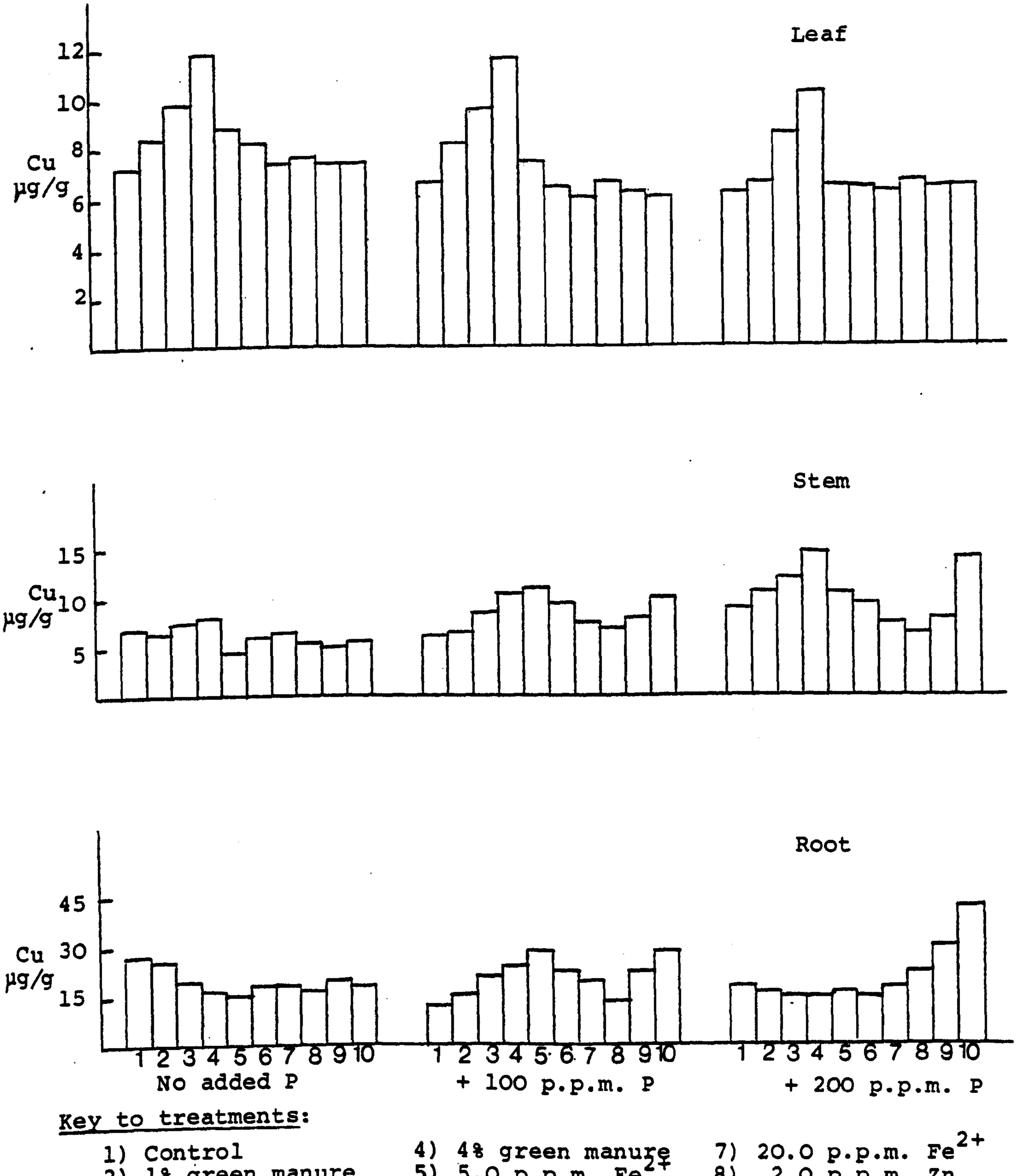
Zinc

Zinc uptake by cotton plants was considerably enhanced by green manure applications to the soil (fig.5.1.9). A slight decrease in zinc concentration was caused by phosphate application to the soil. The inhibitory effects of iron on zinc in both stem and leaf suggest some Fe/Zn interaction either in the root or more particularly in soil solution before absorption. Addition of zinc sulphate to the soil significantly enhanced zinc concentrations in the leaf providing similar results to those of the green manure treatments. Considerable accumulation of zinc occurred in the root following phosphate application, indicating that phosphate inhibited both absorption and translocation of zinc by the root.

Copper

A slight increase in copper concentration is seen in figure 5.1.10 resulting from green manure application to the soil. Phosphate treatments seemed to have no effect on copper in the plant, but a little accumulation occurred in the stem. The only factor inducing a slight increase in root copper concentration was the combination of phosphate and zinc to the soil (fig. 5.1.10).

Fig. 5.1.10 shows Cu concentrations (µg/g) in plant fractions grown in pots with Fe, Zn, green manure and various phosphate treatments



Key to treatments:

1) Control
2) 1% green manure
3) 2% green manure
4) 4% green manure
5) 5.0 p.p.m. Fe^{2+}
6) 10.0 p.p.m. Fe^{2+}
7) 20.0 p.p.m. Fe^{2+}
8) 2.0 p.p.m. Zn
9) 5.0 p.p.m. Zn
10) 10.0 p.p.m. Zn

5.1.3.4 Role of green manure in cotton plants

Green manure in the pot experiments is clearly effective in providing iron for the plants. Much of this iron is no doubt organically complexed. These Fe-chelates will have widely varying stabilities depending on the nature of organic compounds released by the decomposition of green manure. The influence of these compounds on the highly insoluble iron oxides is also affected by the nature of these oxides. The chelated iron in many cases probably remains in solution during its movement in the soil solution towards plant roots.

Two hypotheses have been advanced for the mechanism by which a root utilizes Fe from Fe-chelates.

a) Fe and chelating agent are separated before Fe uptake by root cells.

b) The iron chelate enters the plant intact and is separated as the iron is utilized, at various sites within the plant.

Tiffin et al. (1960) and Tiffin and Brown (1961) found only extremely slow entry of chelator into both green and chlorotic plants. Recent reports support the conclusion that Fe chelator can be separated at the root, especially in the case of Fe-deficient dicotyledonous plants (Hill-Cottingham et al., 1965; Jeffreys et al. 1968). Tiffin (1970) reported that there was considerable Fe but no chelate in the stem exudate from decapitated soybean plants. However Jeffreys et al. (1968) reported that when Fe was supplied to the roots in chelated form, Fe-chelate was found in leaves of plants.

It seems that so far there is no clear evidence and no agreement between plant physiologists with respect to the

mechanism by which the iron is taken up by plants.

Marschner (1978) makes similar suggestions but points out that the Fe can enter the root either as Fe-chelates (organic iron) or directly from its insoluble compounds in the soil by the intimate contact between the root and these particles (inorganic iron). Fe-chelates can easily enter the free space of the root and the root surface is not a diffusion barrier, so all cortex cells can take up Fe from the chelates. When Fe is supplied as inorganic ions then these can precipitate outside cells in the free space of the root. However Fe release from its highly insoluble oxides by root contact is evidently rhizosphere dependent. The root surface and the rhizosphere are of primary importance for the utilization of insoluble inorganic iron. Crops and even varieties differ in their ability to acquire iron. The iron reduced (Fe^{++}) in the rhizosphere cannot enter the free space (Marschner, 1978).

The results obtained (fig 5.1.11) illustrate the importance of green manure in Fe uptake by cotton plants under calcareous soil conditions. It is clearly evident that increased green manure applied to soil considerably increased iron in both leaves and stems. However when inorganic Fe was supplied to soil, much more Fe accumulated in the roots. This accumulation increased with increased phosphate applications to the soil.

It is well known that phosphate is a strong inhibitor of Fe uptake. Phosphate significantly reduces Fe solubility from its oxides, and causes the precipitation of Fe as Fe phosphate in both soil solution and plant roots - possibly in the free space outside cortical cells.

The chelated Fe probably entered the root up to the position where it is oxidized at the junction of the protoxylem

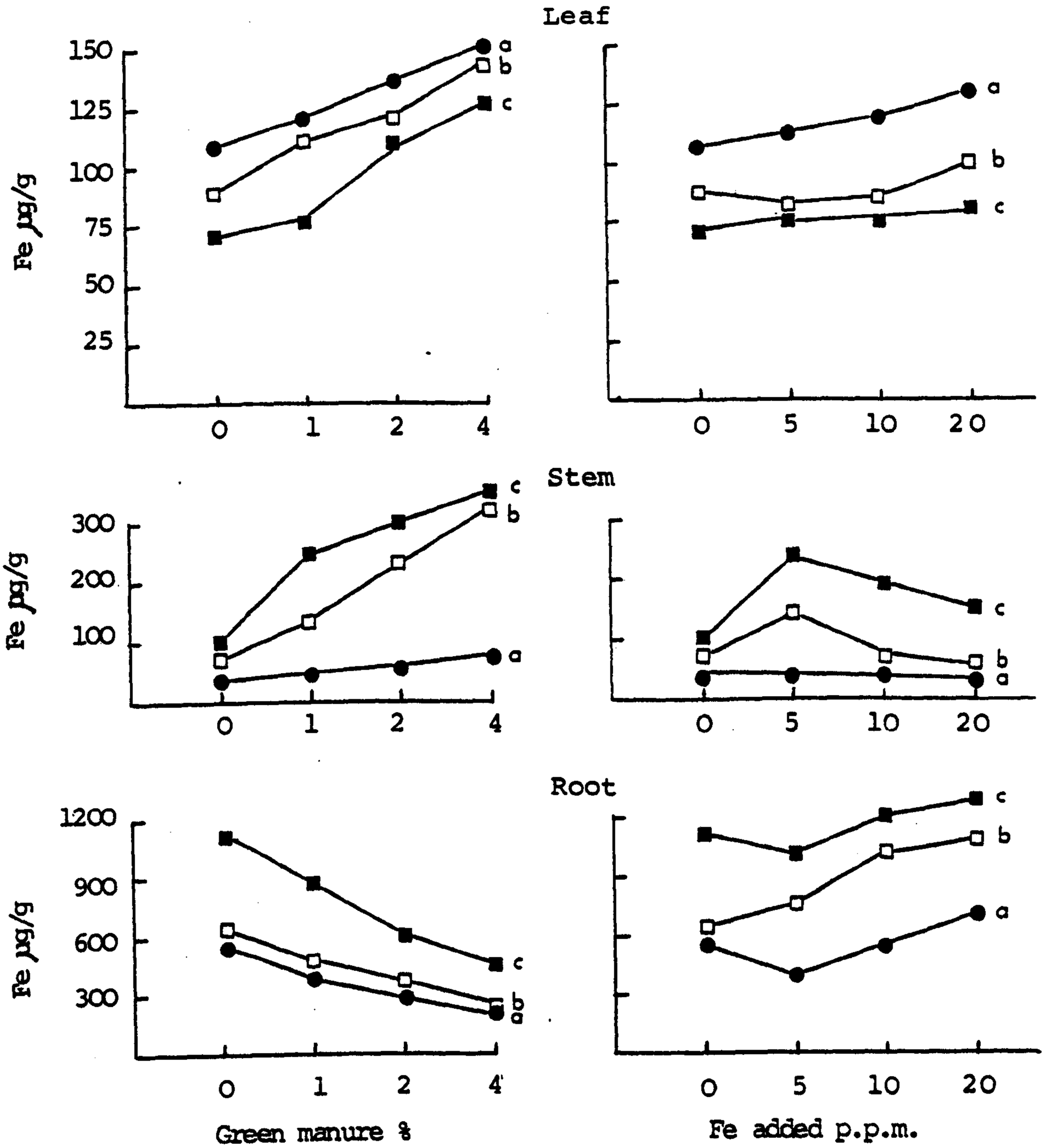


Fig. 5.1.11 The influence of organic and inorganic iron applied to soil on iron concentration in plant fractions grown in pots under various phosphate treatments

a = no added (P)
b = + 100 p.p.m. (P)
c = + 200 p.p.m. (P)

and the metaxylem, chelated by citrate and then transporated in the metaxylem to the plant top (John C. Brown, 1978). Formation of insoluble iron phosphate was thus prevented in the free space and/or in the plasmalemma.

The citrate is considered the main Fe carrier from the roots up to the shoots. A striking relationship existed between Fe and citrate transported in the xylem exudate (Brown and Tiffin, 1965; Brown and Chaney, 1971). When Fe increased, the citrate increased. Enough citrate was always present to chelate the metal, and citrate in excess of that needed for the chelation migrated as an Fe-free fraction behind the Fe-citrate band.

The high Fe levels accumulated in the stem under both 100 and 200 p.p.m. phosphate applied to the soil in comparison with control indicate that phosphate was the main Fe inhibitor in the stem. This is due probably either to inadequate citrate formation by cells to chelate Fe (in disagreement with Brown and Chaney, 1971 and also Brown and Tiffin 1965) and/or the weakness of citrate as a chelator, so much Fe could be precipitated in the stem when phosphate levels were high.

Despite the increased iron accumulation in the stem as a result of phosphate application to soil, its level in the leaf remained adequate. Its concentration significantly increased with green manure increase to soil.

The plant clearly 'strives' to maintain Fe in the leaf, and this becomes increasingly difficult with higher phosphate additions. A decline in leaf Fe with addition of P is accompanied by an increase in root Fe contents and also increased Fe in stems.

Increasing inorganic Fe leads to increased Fe in leaf and

root and little change in yield (fig. 5.1.11 and table 5.1.1).

Increasing green manure, and therefore organic complexes of Fe, increased Fe in leaf (fig. 5.1.11) and increased yields (table 5.1.1), but caused a dramatic fall in root retention of Fe particularly noticeable in high P treatments.

Thus it is apparent that under high P conditions where Fe is particularly limiting, the use of green manure as a source of soluble chelated iron leads to much more efficient distribution and utilization of iron within the plant.

These results reveal the importance of green manure in Fe nutrition. It not only provides a soluble form for plant uptake, but also prevents phosphate interaction in both the substrate (soil solution) and roots. Phosphate fertilization is necessary, especially under intensive irrigated cropping for most crops. This is especially true in the calcareous conditions of the Deir Zor area, and here for the maintenance of adequate Fe nutrition for optimum growth especially of Fe-inefficient crops, green manure provides advantages as compared with the inorganic iron salts.

In retrospect it is unfortunate that non green manure treated areas were not available for field experiments, although the risk of poor seedling establishment would have been very great.

Clearly much work is needed, both on a glasshouse and field scale, with green manures, farmyard manures, plant wastes, etc. as sources of soluble iron. Inorganic iron e.g. $FeSO_4.7H_2O$ could be mixed with these. Green manures could be sprayed with $FeSO_4$ before ploughing under during further field work. Various organic chelating agents could also be investigated. The behaviour of these with phosphate, and the distribution of iron (leaf, root) would be particularly interesting.

5.1.4 Summary of Section 5.1

Green manure application significantly enhanced Fe, Mn and Zn D.T.P.A. extractability in soil. Cu extractability slightly increased probably due to copper complex formation with large insoluble organic compounds/materials.

Iron application enhanced Fe extractability only in the absence of phosphate applications. Zinc applied to the soil was very effective in increasing Zn availability, but a clear reduction occurred as a result of phosphate supply. Mn extractability seemed unaffected by phosphate supply to the soil.

A remarkable increase in iron uptake by plants was associated with both green manure and Fe treatments in the absence of phosphate application, but with P applied, a slight depression caused by P was much more evident when iron was supplied as $FeSO_4$.

The chelated iron as a result of green manure application to soil probably entered the main root cells where it was oxidized before transfer to the other parts of the plant. Thus the phosphate role in Fe precipitation in the soil solution and the root was very slight, on the reverse of inorganic iron, since rapid iron precipitation in the roots accompanied its additions to soil.

All treatments causing an increase in Fe uptake (i.e. green manure and iron sulphate) reduced manganese uptake. High contents of zinc in leaf were brought about by both green manure and Zn treatments.

From the distribution of Fe, Mn, Zn and Cu in cotton plant fractions under varied concentrations of phosphate, it

appears that Fe/Zn antagonism mostly occurs in the soil solution and on the roots before absorption, whereas Mn/Fe antagonism probably takes place at the absorption sites before uptake, and an increase in the uptake of one causes a depression in the other. Zn/Cu inhibition occurs in both root and stem, but there was no indication of Fe/Cu interaction in soil or in plants.

Section 5.2 Anaerobic Studies on Iron and Manganese Release in Flooded Soils

5.2.1 Introduction and aims

Iron and manganese are found as their insoluble oxides under aerobic calcareous soil conditions (Oertli and Jacobson, 1960; Jones and Leeper, 1951). Their presence in and disappearance from soil solution under submerged conditions depends on processes involving:

1) microorganisms and their metabolic activity
2) organic complexes
3) precipitation of insoluble compounds

In the field when a soil is submerged or saturated by water, a sequence of chemical and biological reactions take place because oxygen diffusion is curtailed. In the absence of oxygen, anaerobic microorganisms become active and reduced organic and inorganic substances are produced. The level of oxygen supply and the type and amount of organic matter have direct effects on the oxidation-reduction status.

Oxygen is the first soil component to be reduced, the next oxidant to be attacked is nitrate when the oxygen concentration in the soil medium drops to a very low value (Greenwood, 1962; Turner and Patrick, 1968). Manganese dioxide is the next in the reduction sequence being used as an electron acceptor in anaerobic respiration. Ponnamperuma and Castro (1964); Pannamperuma et al. (1965), reported that native or added MnO_2 retards the decrease in Eh of flooded soils and prevents building up a high concentration of Fe^{2+}

and other reduction products. The next is the $Fe(OH)_3$-Fe^{2+} system and, finally, $SO_4^=$ reduction occurs.

Attempts made by Ponnamperuma (1972) to define the potentials at which each system comes into operation, have not been successful because of the wide range of critical potentials, but Patrick (1964); Connell and Patrick (1968); Turner and Patrick (1968) provided a rough guide to the progress of reduction as follows:

Observation	Eh (volt)
Oxygen (undetectable)	0.33
Nitrogen (undetectable)	0.22
Manganese (detectable)	0.20
Iron (detectable)	0.12
Sulphate (undetectable)	-0.15

The actual values of potentials given are of course suspect due to the irreversible nature of some reactions, and poisoning of the inert electrode by insoluble products.

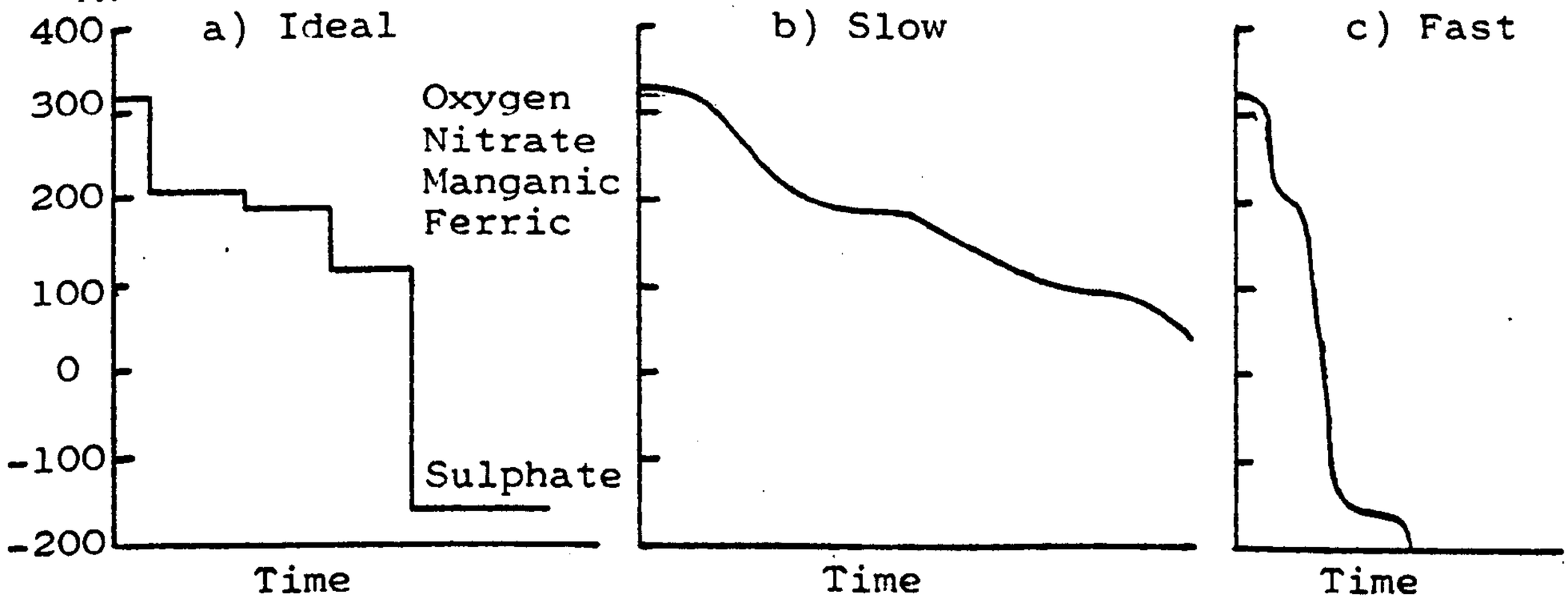
Ponnamperuma (1972); Munch et al. (1978) and Asami (1970) have reported that the influence of iron and manganese will depend on microbial metabolic rates, since the Eh drop depends on how fast the microbial metabolism proceeds.

a) If metabolism is slow then the Eh decrease will be gradual allowing the insoluble oxides the greatest chance to poise the system.

b) If, on the other hand, microbial metabolism is fast then periods of poise would be short.

Figure 5.2.1 illustrates the hypothetical case of an Eh poised system (Stewart-Jones, 1980).

Fig. 5.2.1 Poised Redox Potentials



As a result of soil submergence, a variety of electrochemical changes are brought about including:

a) A decrease in redox potential

b) A slight increase in pH of acid soils and a decrease in pH of alkaline soil (Nicol and Turner, 1957; Ponnamperuma et al., 1966a; Friedman and Gavish, 1970).

c) Changes in specific conductance and ionic strength

d) Drastic shifts in mineral equilibria

e) Cation and anion exchange reactions

f) Sorption and desorption of ions.

Only very low concentrations of reduced iron and manganese will exist under aerobic calcareous agricultural soil conditions. Lindsay (1972) reported that even Fe^{2+} and Mn^{2+} salts applied under these conditions are rapidly oxidized and precipitated within the soil forming hydrated Fe III and Mn IV oxides. Oxides rapidly formed in this way will be more hydrated and in smaller crystals than native oxides in the soil (Hsu and Ragone, 1972). Nye and Tinker (1977) have pointed out that the nature of crystals of iron oxides, their composition, orientation and exposed surfaces which undergo the most rapid changes, will play a very important role in plant mineral nutrition.

Iron and manganese reduction and the release of Fe^{2+} and Mn^{2+} into solution is greatly enhanced under anaerobic conditions and when a soil is heavily irrigated, such conditions could be established especially when subsoil is poorly drained. Conditions of reduced oxygen potential may persist in flooded soil pores or water films between surfaces where there is a demand for oxygen (Bowen and Rovira, 1969; Greenwood, 1969) for plant root and microbial respiration.

Microbial growth depends on moisture, warmth and a supply of substrates (Wilson and Griffith, 1975), and this substrate demand, and the synthesis of respiratory products, will generate concentration gradients and thus microsites.

Rovira (1969) has shown that soil/root interface (rhizosphere) is enriched in a wide range of organic compounds, exudated by plant roots, and their nature depends on circumstances such as the presence of many dead and ruptured cells. These organic compounds generally stimulate micro-

organisms which themselves will contribute to the characteristics of the rhizosphere (Rovira and Davey, 1974; Hall et al., 1971; Barber and Martin, 1976). Plant mineral nutrition is thus largely dependent on the rhizospheric soil solution.

In considering the composition of the rhizosphere environment, when the plant root is in close contact with various soil mineral surfaces, a rapid depletion of dissolved oxygen is expected from rapid microbial growth, and the generation of anaerobic microsites may well take place. The nature of the soil particles which are in contact with the plant root define to a large extent the associated changes to the rhizosphere environment especially in the case of reduction of iron compounds. When contact occurs between plant roots and recently precipitated iron oxide particles, reduced iron will appear in the rhizosphere at a much higher level than when the contact is with aged highly crystallised particles of iron. It has been known for a long time (Leeper, 1947) that iron and manganese availability to the plant is much greater under soil conditions subjected to alternative periods of flooding and drying, where most of the oxides are in this more reactive crystal form.

As well as the nature of iron oxides in the soil, their concentration and wider distribution through the soil can create maximum areas of contact with plant roots and so better provide the plant iron requirements.

In Deir Zor owing to the dry and hot climate, frequent irrigation is necessary to keep the soil occupied by plant roots near field capacity. Due to the basin method of

irrigation and to the moderate permeability of soil (fine texture and blocky structure), the soil surface remains flooded for 4-10 hours after water application, after which the plant root region (its depth depends on the crop and growth stage) requires other 4-8 hours to fall to field capacity. Under these conditions many pockets in the soil profile especially those occupied by the plant roots are subject to short periods with a partial lack of oxygen. Plant root exudates and other organic substrates of a biodegradable nature, and also the forms of iron and manganese oxides, govern release during these periods of sufficient iron and manganese for plant uptake.

An incubation study under anaerobic conditions using both starch and 'green manure' as organic sources for anaerobic bacterial metabolism was used to assess the iron and manganese oxides in Deir Zor soil. Their solubility under these conditions and their ability to provide reduced Fe^{2+} and Mn^{2+} in the presence and absence of organic substrate was examined.

Field studies with detailed redox and/or O_2 level measurements, following irrigations, would be necessary if this work is to be extended in the future.

5.2.2 The experiments

Two experiments were conducted to study the effects of anaerobism on iron and manganese release to soil solution as affected by varied levels of both starch and 'green manure' as well as iron and manganese salt additions.

5.2.3 The procedure

The procedure that was used for both experiments was illustrated schematically in Fig. 2.3.1 70g of air dried surface soil (0-10 cm < 2mm) from the field experimental site, was retained in an air free chamber under water. The soil solution was circulated at approximately 3 mls/minute from the bottom to the top of the soil. Gas release from the flooded soil was vented through a fermentation trap, preventing internal pressurization and increases in biocarbonate concentration. 2 ml samples were drawn off at regular intervals by syringe and expressed through 0.22 u millipore filters to filter out any soil and deposited particles and ensure that only soluble iron and manganese were analysed. The actual equipment used was shown in photograph 2.3.1. It was kept inside an incubator at 30 ± 1°C.

The following treatments were used in the first experiment:-

1) flooded control
2) 50 mg/$FeSO_4.7H_2O$/70g soil
3) 50 mg/$FeSO_4.7H_2O$ + 1% starch/70 g soil
4) 50 mg/$MnSO_4.4H_2O$/70 g soil
5) 50 mg/$MnSO_4.4H_2O$ + 1% starch/70 g soil

While the second experiment consists of the following treatments:-

1) flooded control
2) 1% dried green manure
3) 2% dried green manure
4) 50 mg/$FeSO_4.7H_2O$ + 1% dried green manure
5) 50 mg/$MnSO_4.4H_2O$ + 1% dried green manure

6) 50 mg/$FeSO_4.7H_2O$ + 2% dried green manure

7) 50 mg/$MnSO_4.4H_2O$ + 2% dried green manure

Samples in both procedures were kept incubated for 16 days. Any changes in appearance were recorded when appropriate.

5.2.4 Results and discussion

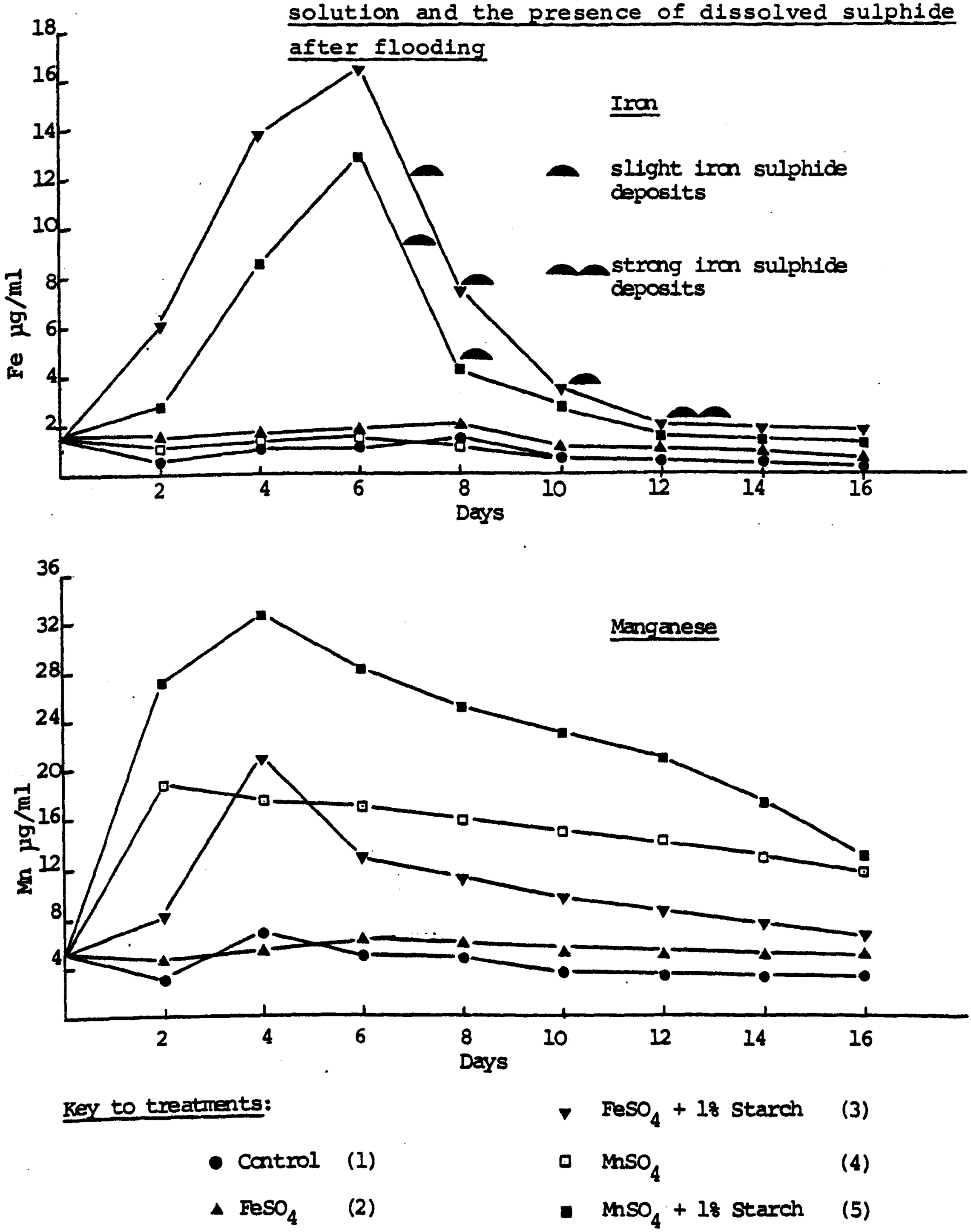
5.2.4.1 Effect of starch and iron and manganese sulphates on the iron and manganese release to solution under anaerobic incubation (Experiment 1)

Figure 5.2.2 shows the soluble Fe and Mn released during the incubation. It can be seen that the control and the iron and manganese sulphate treated soils show no evidence of any iron solubility increase in soil water even after 16 days of submergence, due to the low organic matter content in the soil (0.6% organic matter). None of the soil cultures developed grey or black deposits inside the tubing or on the soil surface, thus indicating little removal of Fe^{++} as FeS.

However, addition of 1% starch in combination with either iron or manganese sulphate applications greatly enhanced iron in the soil solution 8 times and 6 times respectively, as compared with control on the 6th day of flooding indicating that the presence of an organic source in the soil was the main factor in increasing the iron solubility.

After the first 6 days when soluble iron increased, a sharp depression occurred associated with black deposits inside the tubes and on the soil surface. On the 10th day the soils became completely black, due to ferrous sulphide precipitation. After 12 days, iron concentration was the same in all treatments

Fig. 5.2.2 The effect of soil treatments (starch, $FeSO_4$, $MnSO_4$) on iron and manganese release into solution and the presence of dissolved sulphide after flooding



including control, as all soluble iron was precipitated as insoluble black ferrous sulphide.

Manganese reached its maximum solubility on the 4th day after flooding (2 days before iron). Manganese sulphate application to soil cultures enhanced manganese concentration in both the presence and absence of starch. In the absence of starch the manganese increased to three times control values, with 50 mg $MnSO_4.4H_2O$ + 1% starch, manganese concentration quickly increased to 8 times that of control on the 4th day with a decrease to a similar figure to that of manganese sulphate treatment only on the 16th day.

Iron sulphate combined with 1% starch also enhanced manganese solubility, this increase being probably largely due to the starch factor, with a more rapid depression after four days of flooding, while both control and iron sulphate treatments were ineffective in manganese enhancement and soluble manganese values did not change.

Table 5.2.1 The chemical analysis of soils as affected by anaerobic incubation at 30°C

(DTPA extractable µg/g soil)

Treatment	Fe	Mn	Zn	Cu
Unflooded control	2.5	6.5	.70	1.7
Flooded control	4.2	12.3	1.60	2.8
$FeSO_4$	5.0	11.6	1.50	2.9
$MnSO_4$	4.3	20.0	2.0	2.6
$FeSO_4$ + 1% starch	13.3	25.9	3.1	2.7
$MnSO_4$ + 1% starch	11.8	34.3	3.0	2.9

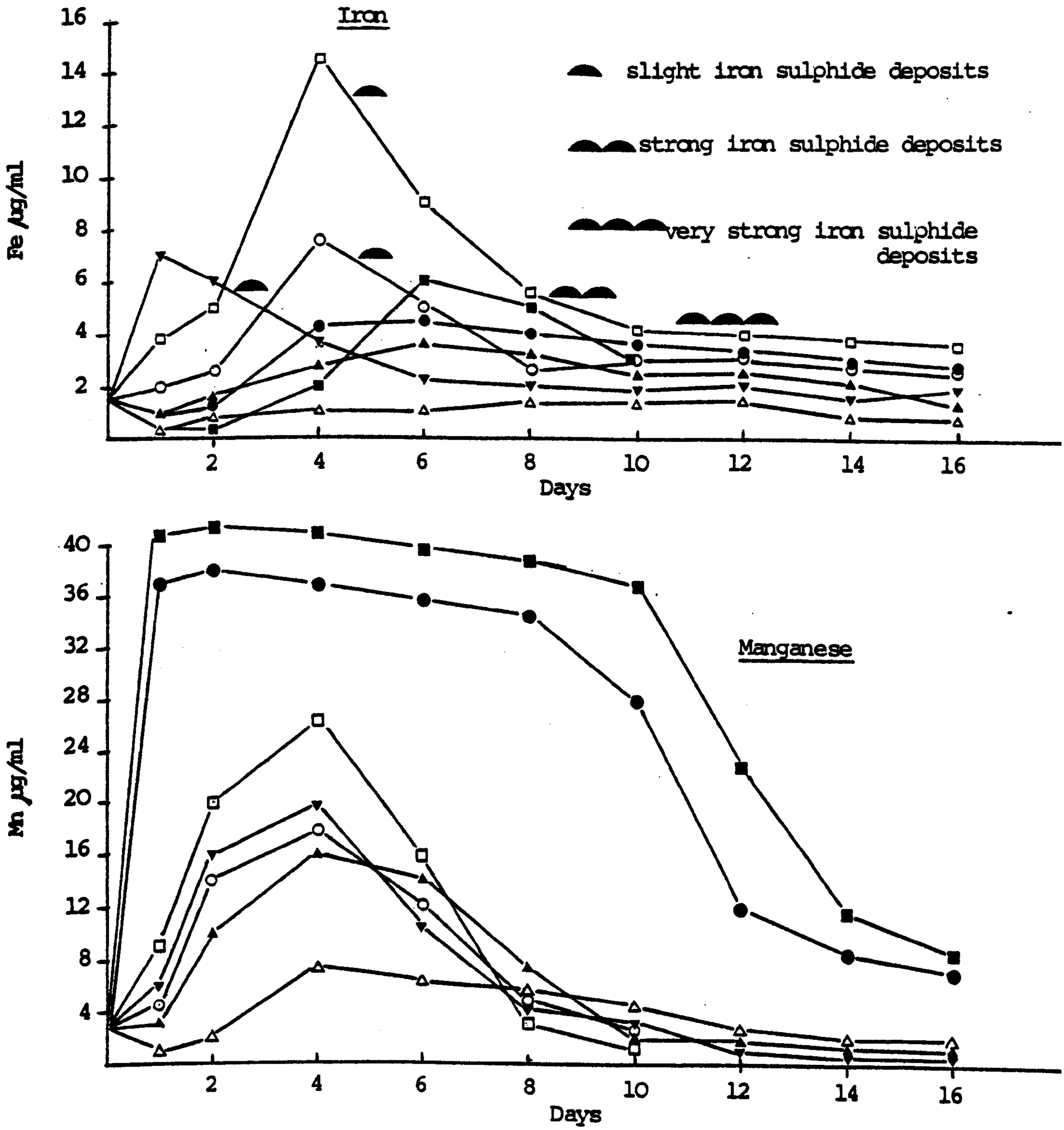
The results in table 5.2.1 show increased D.T.P.A. extractability of Fe, Mn, Zn and Cu after anaerobism. A two-fold increase in Fe, Mn and Zn "availability" was obtained as a result of flooding the soil for 16 days without any organic matter application. The enhancement in iron and manganese caused by supplying starch to increase anaerobism is clearly shown. Their concentrations with both iron and manganese sulphate application in absence of starch were similar to the flooded control treatment.

Copper availability was not affected by starch application to the soil, since similar results were obtained for all flooded soil cultures including control, while zinc extractability was doubled after incubation with starch as compared with incubation in the absence of starch. A control + starch only, but without any Fe + Mn additions should in retrospect have been included in this series. This was included in experiment II.

5.2.4.2 - <u>Effect of dried green manure on iron and manganese release in solution under anaerobic incubation (experiment 2)</u>

The soluble Fe^{2+} and Mn^{2+} in these incubations is shown in fig. 5.2.3. During the first three days, there were no signs of changes in the culture solution for any treatment except a slight grey color developing on the inside of tubing from soils 3, 4, and 6. On the 4th day these three soils developed black surface deposits due to ferrous sulphide precipitation, increasing up to the 10th day of flooding. The iron concentration in solution with these three soils is

Fig. 5.2.3 The effect of soil treatments (green manure, $FeSO_4$, $MnSO_4$) on iron and manganese release into solution and the presence of dissolved sulphide after flooding



Key to treatments:

△ Control (1)

▲ 1% green manure (2)

▼ 2% green manure (3)

○ $FeSO_4$ + 1% green manure (4)

● $MnSO_4$ + 1% green manure (5)

□ $FeSO_4$ + 2% green manure (6)

■ $MnSO_4$ + 2% green manure (7)

attributed to either the high application of organic matter (2%), or to this level combined with 50 mg of iron sulphate. Because of 2% organic matter in particular, both manganese and iron reducing microorganisms which start at the beginning of the experiment, reach fast growth rates by the 4th day. Soils which received manganese and iron sulphate are provided with oxides which can undergo rapid reduction and release into solution as Fe^{2+} and Mn^{2+} complexes. A sharp decrease in soluble iron concentrations occurred between the 4th and 10th day associated with intensive black deposits in both tubes and soils and a drop in soluble Fe^{2+} to minimum on the 10th day with free $S^{=}$ being detected in the solution by the lead acetate test. Clearly when conditions became anaerobic enough for sulphate reduction the Fe^{2+} is rapidly removed from solution as FeS.

Control soil showed no evidence of iron release and also no FeS precipitation up to the end of experiment (16 days) owing to low organic matter content and therefore poor bacterial metabolism in the culture. The soil is rich in gypsum so $SO_4^{=}$ is not limiting.

In general iron release depended on the presence of green manure in the soil. Fresh iron hydroxides were formed in the culture as a result of iron sulphate application and in the presence of organic sources contributed to iron solubility enhancement in the solution. These recently formed surfaces are more favourably attacked by iron reducers, or their metabolic products, than very insoluble native iron oxides present in the soil.

Manganese solubility increased in all cases of

submergence. The magnitude of this increase also depends on both manganese and organic matter application to the soil. The results shown in fig. 5.2.3 indicate that green manure resulted in the release of further amounts of manganese up to the 4th day of submergence decreasing towards the 8th day. At this stage similar manganese concentrations are found in all treatments including control, with the exception of the treatments receiving manganese sulphate combined with both 1 and 2% organic matter. In this maximum manganese solubility, some 30 and 36 times that of control was reached 24 hours after flooding. This solubility was maintained up to the 8th day of flooding after which a rapid decrease occurred with a final concentration of 8 µg/ml at the 16th day being given. This is still higher than in other treatments.

It appears from both starch and green manure procedures that when sulphides are formed, iron and manganese concentration in solution is decreased and moved to the site of FeS and MnS deposition. The concentration of iron in solution at any one time must be the product of iron supply to that solution and its removal. The process of removal is by sulphate reduction and the precipitation of ferrous sulphide. Only where the iron reduction rate exceeds that of sulphate reduction will there be a nett rise in the iron concentration in solution and similarly $S^{=}$ appears in solution only when there is insufficient Fe^{2+}. In this soil, after 10 days of submergence, $S^{=}$ concentration exceeded that of Fe^{2+} with increasing free $S^{=}$ in the solution (detected by lead acetate test) due to the high sulphate content of the soil (1-2% $CaSO_4$ as well as some supply of sulphate with Fe and Mn).

It also appears from the behaviour of iron and manganese

oxides in the soil under flooding that although the soil contents of native oxides are high they are mostly in insoluble compounds. Submergence alone has not been able to increase their solubility under the natural content of organic matter present in soil, but application of adequate quantities of easily decomposed organic material, starch or freshly prepared green manure collected at the early flowering stage, are effective in bringing a large portion of manganese into an available form. The addition of fresh Fe or Mn oxides to the soil (applied as soluble Fe and Mn compounds) and an organic source brought a further increase. Organic matter application alone to the soil induced a slight increase in iron solubility, but when a fresh soluble source of iron ($FeSO_4.7H_2O$) was supplied a considerable increase in iron solubility developed with more Fe^{2+} in soil solution. This could have great value for example in paddy soil where highly soluble/reducible iron oxides are required to poise redox potentials and remove excess $S^{=}$.

Table 5.2.2 The chemical analysis of soils as affected by 16 days of the anaerobic incubation at 30°C

(D.T.P.A. extractable µg/g soil)

Treatment	Fe	Mn	Zn	Cu
Unflooded control	2.5	6.5	.70	1.70
Flooded control	5.0	11.0	1.20	2.10
1% dried green manure	10.1	30.3	4.80	2.30
2% dried green manure	22.3	38.5	6.20	2.80
$FeSO_4$ + 1% dried green manure	15.3	31.4	4.80	2.20
$FeSO_4$ + 2% dried green manure	29.8	36.0	6.60	2.00
$MnSO_4$ + 1% dried green manure	13.5	42.4	5.80	2.60
$MnSO_4$ + 2% dried green manure	23.0	43.2	7.20	2.40

Behaviour of Fe, Mn, Zn and Cu compounds under anaerobic incubation was similar whether the soil was treated with starch or green manure as an organic source. It appears from the data in table 5.2.2 that flooded controls show twice as much D.T.P.A. extractable Fe, Mn and Zn as the original soil before flooding. With application of the green manure before anaerobic incubation, the extractability of these elements was considerably increased and their increase wholly depended on the percentage of organic matter applied to the soil. A further increase in extractable iron and manganese resulted from applications of both iron and manganese sulphates respectively. Zinc extractability was clearly increased by increasing organic matter additions to the soil, presumably via the formation of Zn^{2+} organic matter complexes. Copper extractability only increased slightly on anaerobic incubation in this experiment.

5.2.5 Summary and discussion of the mechanism governing iron and manganese release into solution under anaerobic incubation

The soil used (Deir Zor soil) was agricultural soil containing on the average 0.60% organic matter with very low microbial activity. The presence of free nitrate in the soil environment is very low (traces to 1.0 p.p.m.) due to continued leaching. The soil has high iron and manganese oxide contents. Table 5.2.3 shows the Fe and Mn analysis of the soil before and after anaerobism for 160 hours (Stewart-Jones, W.T. 1980).

Table 5.2.3 The Fe and Mn analysis before and after anaerobism

	Iron (µg/g)			Manganese (µg/g)		
	*DTPA	**HHQ	***DITH	DTPA	HHQ	DITH
Before	7.88	41.6	4940.0	11.4	33.0	242.0
After	64.0	388.0	4160.0	75.0	12.6	172.0

* DTPA - Lindsay and Norvell (1969) extract

** HHQ - Hydroxyhydroquinone extract (Stewart-Jones 1980)

*** DITH - Dithionite extract (Jackson 1958)

The data in table 5.2.3 show the richness of the soil in free iron oxides and significant changes in these oxides after anaerobism affecting their reactivity and solubility in DTPA compared with non-incubated soil (8 times increase Fe, 7 times Mn). The ease of reduction of the products of anaerobic incubation

are also clearly seen. The solid products of anaerobic treatments, be they organic compounds, oxides or sulphides, can clearly readily release Fe^{2+} and Mn^{2+} to a strong reducing agent (HHQ). The soil is naturally rich in sulphate especially at the top soil ($CaSO_4$ is 1.2-2.2% in the top 0-8 cm).

In the field environment O_2 will be the dominant electron acceptor with the microbial metabolism in the soil classified as respiration. Anaerobism in the soil profile and in the plant root medium is associated with a decrease in free oxygen. Anaerobic organisms will develop, but due to the low nitrate concentration in the soil, manganic reducers start directly (a few hours after flooding) releasing more Mn^{2+} in soil solution. Its concentration in solution depends on organic matter content and quality and the form of manganese oxides and their distribution in soil. Soil temperatures will also control microbial activity. Results in figs 5.2.2 and 5.2.3 showed how these factors affected manganese release on flooding. Its concentration remained near to control in the absence of organic matter, but when freshly precipitated manganese oxides were subjected to flooding (50 mg $MnSO_4.4H_2O$/70 g soil) manganese reducers were able to act attacking these new oxide surfaces with greater ease than the native manganese oxides. The same also applied to iron oxides in soil.

The expected sequence of events in the studied soil after flooding is as follows:

1) Dissolved oxygen will be used up within a few hours by aerobic microorganisms (Ponnamperuma, 1972; Wilson and Griffin, 1975).

2) Growth of the aerobes will cease as the Eh falls and

denitrifyers and various hetro-fermenters became more active.

3) Manganic reduction with Mn^{2+} in solution starts.

4) Hetrofermentative organisms become more active metabolizing soluble organic compounds to simple carboxylic acid. Fe^{3+} reduction starts. Mn^{+4} reduction continues.

5) Due to the low reactivity of native iron oxides in soil there will be little poising of Eh and a rapid fall to about -200 millivolts occurs with the accumulation of excess simple carboxylic acids which provide excellent substrates for sulphate reducers.

6) Release of sulphides in solution causes a rapid decrease in both Mn^{2+} and Fe^{2+} in solution by their precipitation as FeS and MnS. Further $S^{=}$ can react with native iron oxides themselves and chemically reduce Fe^{3+} to Fe^{2+} before sulphide formation.

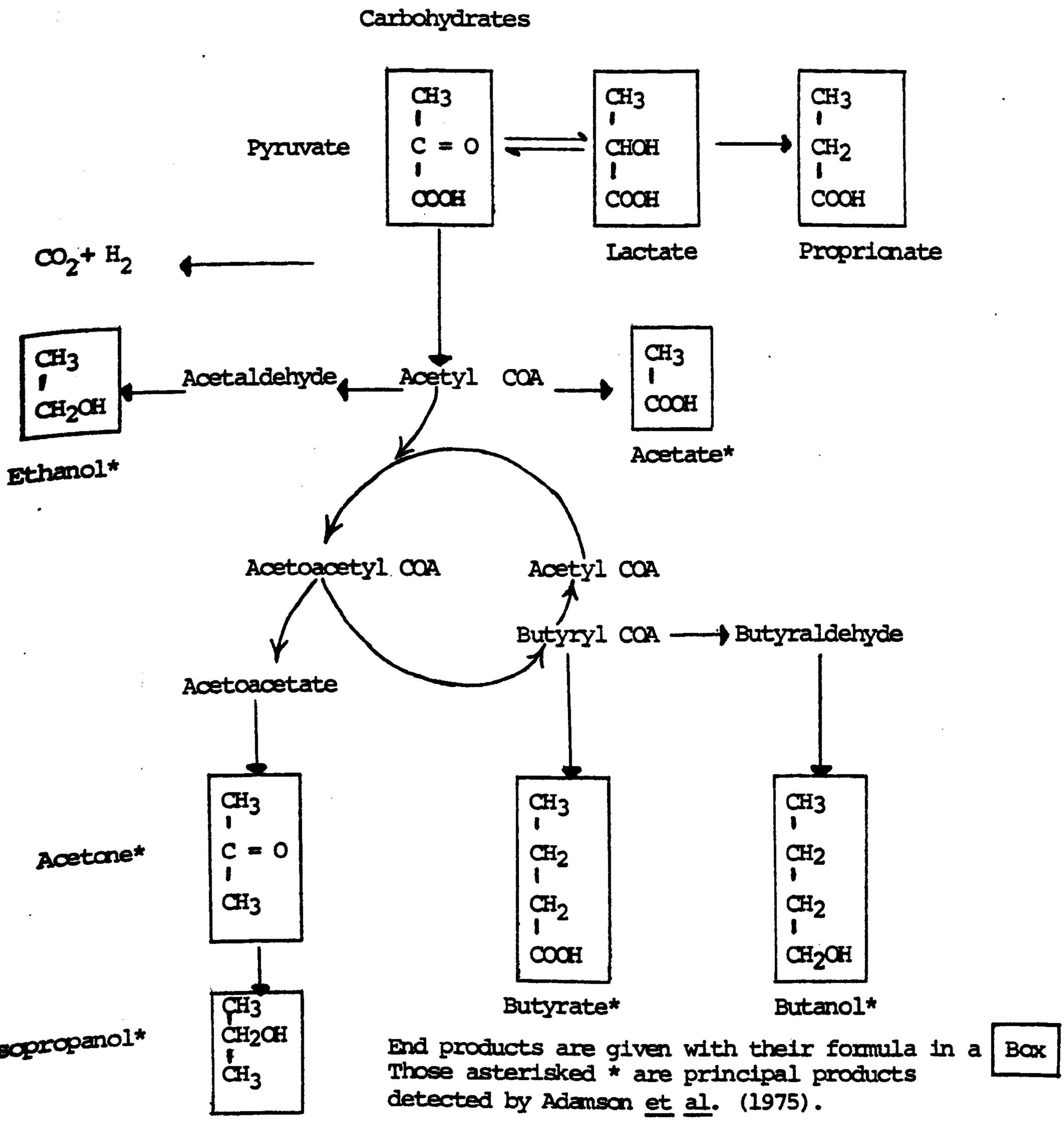
7) By the time the native iron oxides become converted to FeS and the production of $S^{=}$ in solution exceeds Fe^{2+} release free sulphides appear in solution.

8) Excess free sulphides in solution can cause poisoning of sensitive microbes and auto inhibition of the sulphate reducers themselves (Brown et al., 1973). (?)

The microorganisms which thrive in reducing conditions are few: Genus Clostridia and Bacillus and metafermenters Desulfovibrio and Desulfotomaculum are the probable hetro-fermentative organisms (Ottow, 1968; Munch and Ottow, 1977 and Postgate, 1979).

Various soluble small molecular weight organic compounds are the products of hetrofermentation of carbohydrates many

of which can be directly used as substrates by sulphate reducers (Postgate, 1979). Some of these are suggested in the scheme below, which demonstrates the main products of Saccharolytic bacteria in flooded soils (after Yoshida,1975



Many of the products of initial carbohydrate metabolism would prove very effective complexing agents for Fe^{2+} and Mn^{2+}, and soluble Fe^{2+} and Mn^{2+} are probably found in these forms in the middle stages of these anaerobic incubations. When $S^{=}$ appears, more stable FeS and MnS will predominate.

The role of anaerobic metabolism in field soils cannot be easily assessed. No O_2 etc. measurements were made in Deir Zor following irrigation. This is something to study in the future.

However even if such measurements were available they would generally reflect overall soil conditions. In microsites, and especially in parts of the rhizosphere where microbial metabolism was rapid, one might expect quite strongly reducing conditions.

It is therefore probable that laboratory anaerobic incubations for say 4-10 days may reflect to some degree the conditions for Fe and Mn transfer to some roots.

Section 5.3 The influence of phosphorus on the utilization of inorganic iron by cotton plants in water and sand culture

5.3.1 Introduction and aims

Iron deficiency in calcareous soils is a classical problem of plant physiology and ecology. Lindsay (1974) has concluded on the basis of chemical and physical equilibra that the concentration of Fe^{3+} in the soil solution is far too low ($\approx 10^{-10}$M) to meet the demands of growing crops. The sensitivity of higher plants to iron chlorosis varies greatly among natural vegetation, different species of crop plants and even varieties within the same species. Iron sufficiency must, therefore, be based on a special ability of the roots of some plants to take up sufficient Fe^{2+} from a soil in which iron-inefficient plants show iron chlorosis. This can occur by a "2-phase-effect" at the soil/root interface (Jenny, 1961, 1965), an increase in iron solubility by complex-forming organic compounds such as organic acids and amino acids (Scheffer et al. 1965, 1967), humic acids (Badurova et al. 1967), or phenolic substances (Scheffer et al. 1968) or a lower pH and redox potential within the rhizosphere.

Root exudates together with organic material from decaying root-cap cells and root hairs are the reason for the well known high density of microorganisms in the rhizosphere compared with the bulk soil. Scheffer et al. (1967) have illustrated that these exudates are effective in making soluble some low solubility compounds such as iron phosphate (table 5.3.1).

Table 5.3.1 Dissolution of Fe^{2+} phosphate by rhizosphere product of *Sinapis alba* grown for three weeks under sterile conditions. After Scheffer et al. (1967)

	Fe µg/ml	P µg/ml
Control (without addition)	0.7	0.7
Organic acid fraction	2.2	1.6
Amino acid fraction	7.3	3.5

These organic compounds are of great importance in well aerated soils of a high pH, as they form chelates with the inorganic Fe shifting the soil iron equilibrium towards higher amounts of soluble and available Fe^{2+}. Hodgson (1969) and Geering et al. (1969) have reported that in soils with a higher content of organic substances and with higher microbial activity micronutrients like Mn, Zn and Cu are present in the soil solution in concentrations similar to that of solutions of prepared metal chelates.

It can be assumed that in high pH soils with higher amounts of organic matter, the formation of Fe chelates may substantially contribute to the iron nutrition of the plants.

Under iron stress several dicotyledons such as soybean (Brown et al., 1967), sunflower (Venkat Raju and Marschner, 1972) or grapevine (Marschner, 1978) are able to lower the pH of the substrate and increase their roots reducing capacity (Brown and Ambler, 1974) and also release reducing compounds into the

substrate (Ambler et al., 1974 and Marschner et al., 1974).

Monocotyledons on the other hand as well as iron inefficient genotypes of dicotyledons such as tomato (Brown and Ambler, 1974) appear to be unable to lower the nutrient solution pH under iron stress. The uptake of inorganic iron by inefficient plants seems to be inhibited by high phosphate concentrations in the solution (Olsen, 1935; Bell et al., 1962 and Kashirad and Marschner, 1974a). This inhibition by phosphate results mainly from a depression in the solubility of iron hydroxide/phosphate. The adsorption of phosphate on the freshly precipitated iron hydroxide (0.3 P/1.0 Fe on a molar basis; Stamm and Kohlschutter, 1965) as a physical phenomena might also be involved in depressing iron hydroxide solubility.

Azarabadi and Marschner (1979) in their study using combined sand and water culture, with iron deficient nutrient solution circulated through both systems, and using iron hydroxide provided to the sand culture as the only iron source, have reported that any increase in iron solubility in the sand culture must be restricted to the root surface or rhizosphere as no increased iron concentration could be detected in the leachate of the sand culture. It is highly probable that phosphorus depletion around the root in a solid substrate is involved in this rhizosphere effect.

Azarabadi et al. (1979) have concluded that when phosphorus is depleted in the rhizosphere, iron hydroxide can be utilized even at a high redox potential and high pH. These conditions occur in water culture only when phosphorus and iron are supplied separately, and the phosphate supply is extremely low.

The main objectives of this study are to:

a) Investigate the cotton "Alepo 40" variety's ability to utilize inorganic iron in water and sand culture and to follow the influence of variations in both phosphorus and iron concentrations on this to see if the mild development of a rhizosphere in sand culture induces a reduction in the inhibition caused by phosphate on the utilization of inorganic iron by cotton plants.

b) Define the influence of varied levels of P and Fe on Mn, Zn and Cu uptake by plants and their distribution within plant fractions.

c) Determine the toxic and critical levels of iron in cotton leaves above or below which iron toxicity and deficiency chlorosis might appear in the plant.

5.3.2 The procedure

Cotton (Gossypium hirsutum var. Alepo 40) was grown in a water and sand culture system in the greenhouse under two separate ranges of Fe and P concentration (described before page 74) over a period of 28 days.

The nutrient solution reaction was adjusted to pH 6.5 before use. All treatments were duplicated. Fe, Mn, Zn and Cu were determined in plant leaves, stems and roots.

5.3.3 Results and discussion

The yields generally in this experiment are not considered of very great importance, although there are clear differences. The appearance of the plants, and their micronutrient concentrations and distributions within the plant fractions as

affected by the varied ratio of Fe/P application in solutions are of very great significance.

5.3.3.1 Visual appearance of cotton plants as affected by Fe and P concentration in water and sand culture

5.3.3.1.1 Varied concentrations of Fe in water and sand culture

7 days after transferring plants to the glass containers in the greenhouse, abnormal symptoms were first noted on water culture plants that received both 1.0 and 2.0 p.p.m. Fe. The chlorosis first appeared in young leaves (3-4 leaf stage) in the form of light yellowish spots between veins. Progressively the whole plant became chlorotic with reduced growth, the final yield being one-third that of normal plants(photo 5.3.1/A). In sand culture, healthy plants developed and all used concentrations of iron were sufficient to provide adequate iron in contrast to water culture which was in equilibrium with the sand systems in each case, but increased iron applications with a constant phosphate (10. p.p.m.) resulted in a slight increase in plant sizes(photo 5.3.1/B).

5.3.3.1.2 Varied concentrations of phosphate in water and sand culture

All plants in the water culture developed iron deficiency chlorosis. The start and degree of chlorosis on the plants was wholly dependent on phosphate concentration in the solution. The highest one the phosphate applied to culture, the worst the iron deficiency symptoms. The abnormal symptoms were first

Photo 5.3.1 / A Effect of iron treatments in water culture on cotton growth

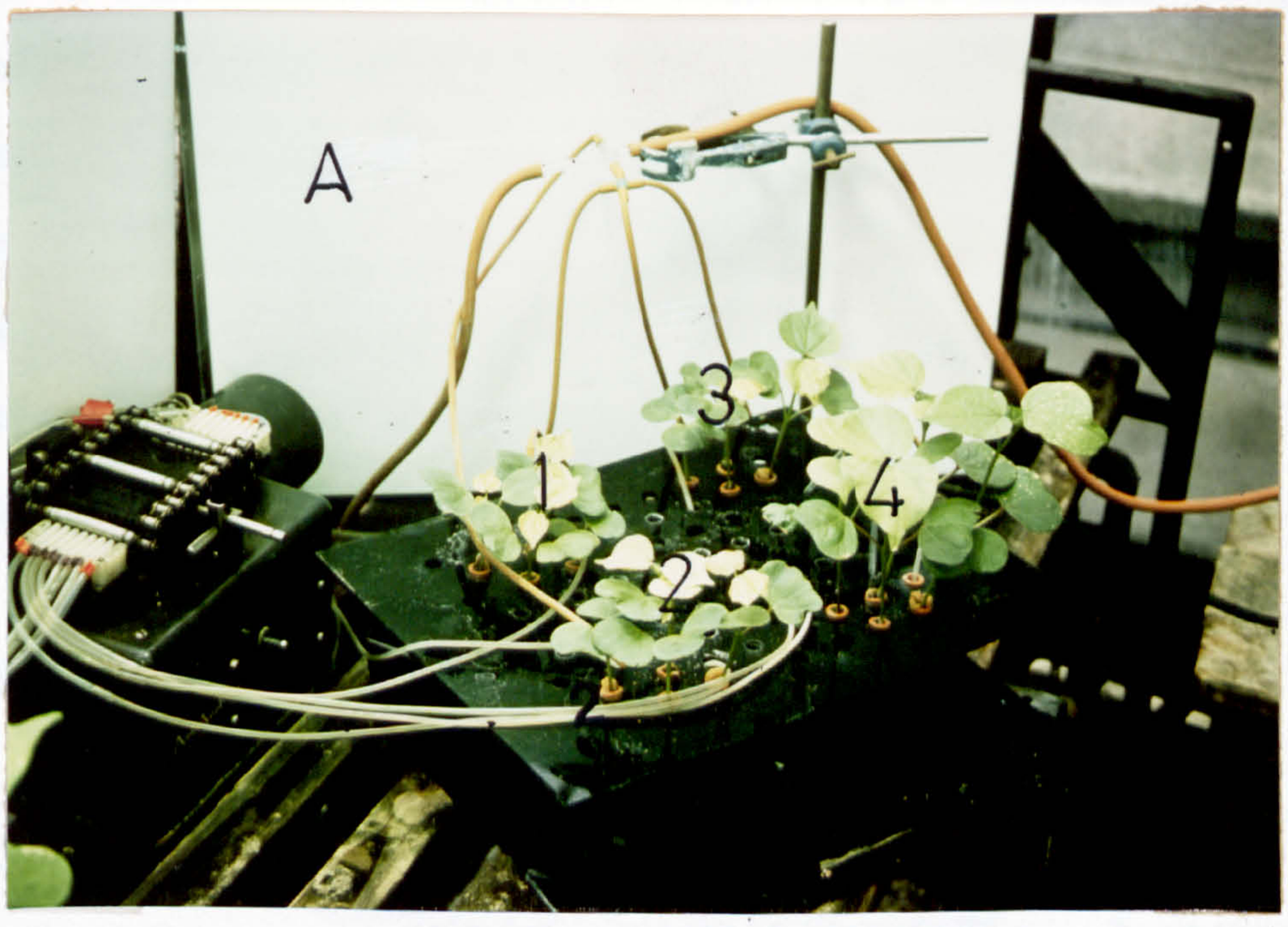
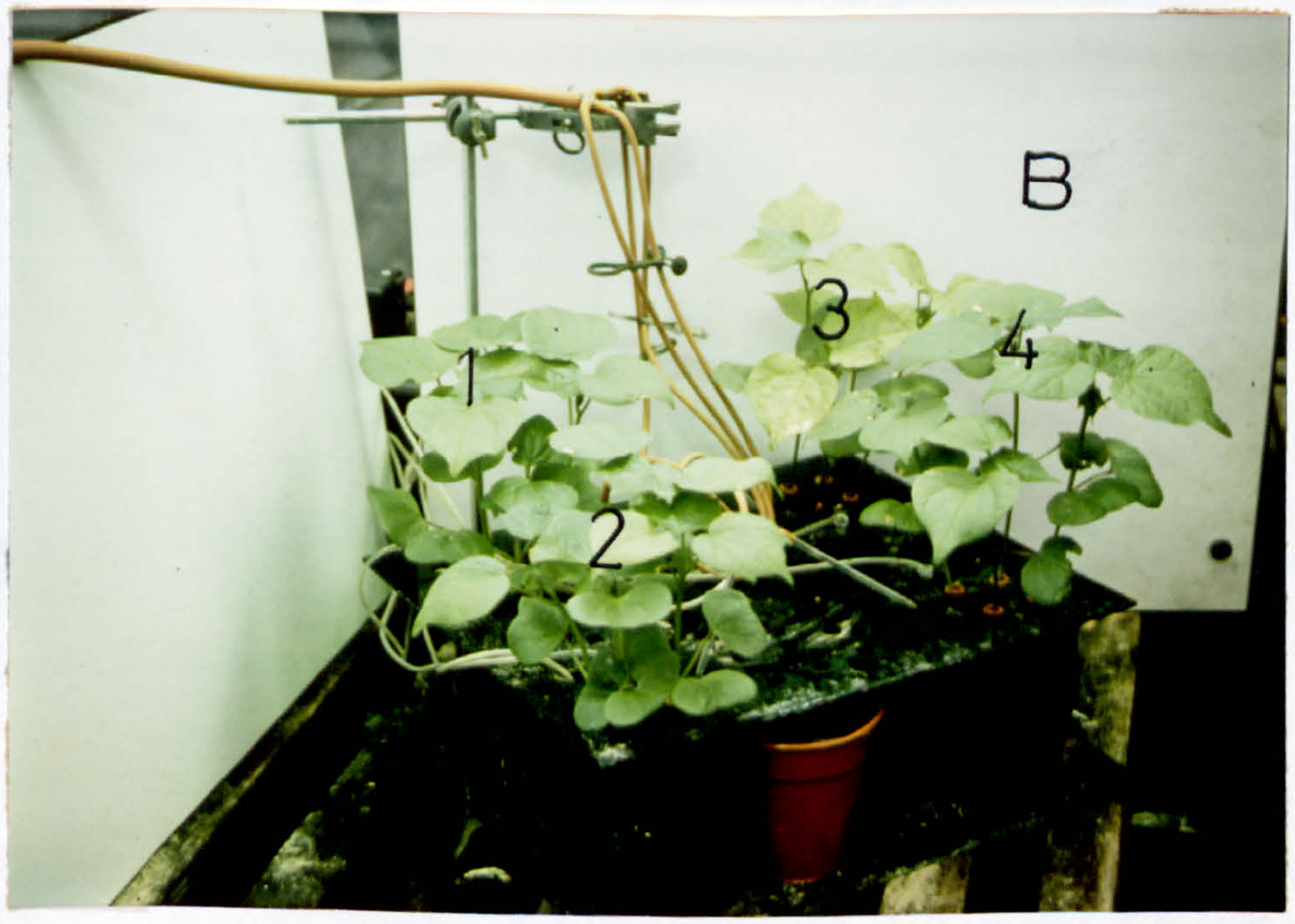


Photo 5.3.1 / B Effect of iron treatments in sand culture on cotton growth



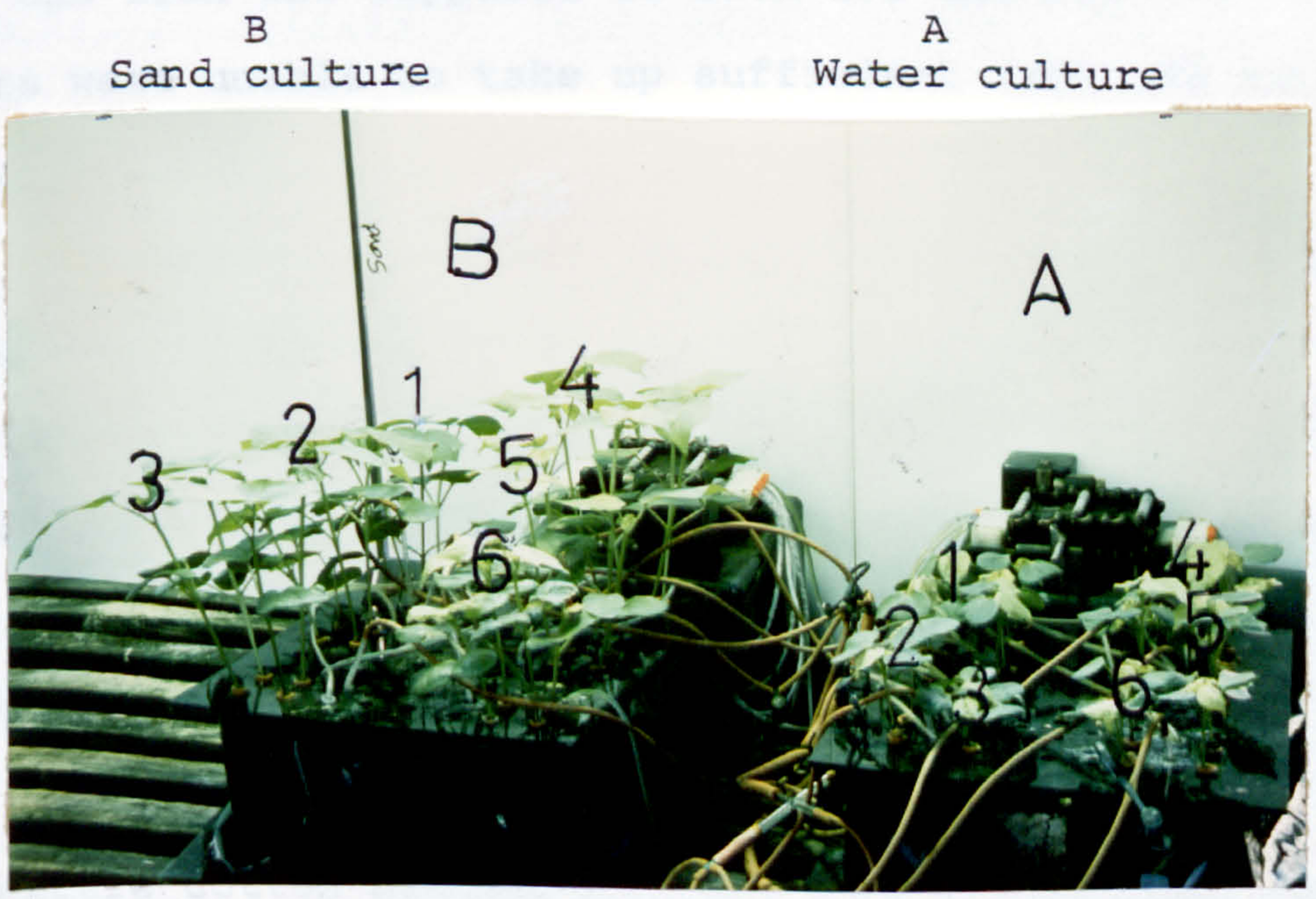
Key to treatments:

1) 1.0 p.p.m. Fe
2) 2.0 p.p.m. Fe
3) 5.0 p.p.m. Fe
4) 10.0 p.p.m. Fe

apparent after 10 days and subsequently became wholly yellowish with severely reduced growth. The final size was about one-fourth that of plants in the sand culture (Fig. 5.3.2/A).

In sand culture, plants receiving lower levels of P up to the 10 p.p.m. level showed no iron deficiency chlorosis symptoms up to harvest. The highest two levels of P (20, 40 p.p.m.) applied to sand culture produced delayed chlorosis on plants (at 21 days) with the usual yellowish colour on the leaves (6-8 leaf stage). The plants seemed smaller compared with healthy plants (Fig. 5.3.2/B).

Fig. 5.3.2



- Effect of P treatments in both water and sand culture on plants' growth.

Key to treatments:

1) 0.5 p.p.m. P
2) 1.0 p.p.m. P
3) 5.0 p.p.m. P
4) 10.0 p.p.m. P
5) 20.0 p.p.m. P
6) 40.0 p.p.m. P

5.3.3.2 Plant growth and micronutrients uptake in water culture

Increasing the iron supply resulted in an increase in the plant growth and the final yield of cotton plants. It is clear from the yield results in table 5.3.3, that dry yield was doubled by increasing initial iron concentration in solution from 1.0 to 10.0 p.p.m. under constant level of phosphate (10 p.p.m. P).

Altering the composition of the growth medium by increasing iron concentration in water culture solutions increased iron uptake by plants under a constant level of phosphorus (10. p.p.m.). When the iron was supplied at both 1.0 and 2.0 p.p.m., the plants were unable to take up sufficient iron and developed severe iron chlorosis after 7 days.

Increasing iron concentration to 5.0 p.p.m. delayed the appearance of iron deficiency chlorosis until after the 12th day (five days later than two lowest levels). Healthy plants developed with the highest iron concentration (10.p.p.m. Fe) with an iron content in the leaf near to the critical level (70 p.p.m. Fe) above which iron deficiency chlorosis disappeared from the plant leaf (85-112 p.p.m. and 57-88 p.p.m. in young and mature cotton blades, respectively. Sabbe and Mackenzie 1973).

Increased iron supply was associated with a slight decrease in the plant stem iron content, while a remarkable increase in the root content resulted suggesting that P/Fe accumulation occurred on and within the root before translocation to the shoots. The data in table 5.3.3 also show that increased iron concentration in the water culture had significant effects on

manganese content of plant fractions and plant Mn concentration decreased with iron increase in the solution. Thus iron deficient and chlorotic plants grown in low iron solutions had higher Mn contents. This is probably due to decreased total growth, and also antagonistic affects in the culture solution allowing more uptake and translocation of manganese especially when one considers the depression in Fe and P concentrations in the culture solution (table 5.3.2) with time as a result of their rapid precipitation while much less severe reductions in Mn, Cu and Zn contents were found under the same conditions.

Table 5.3.2. Variation in nutrient content in a typical culture solution with time

Time/hours after preparation	pH	P	Fe	Mn	Zn	Cu
Original values p.p.m.	6.5	10.0	2.0	2.0	0.5	0.5
2 hours	6.5	5.3	1.3	1.90	0.48	0.40
24 hours	6.4	3.2	0.54	1.90	0.46	0.38
48 hours	6.3	2.4	0.52	1.88	0.41	0.33
72 hours	6.3	2.1	0.40	1.85	0.39	0.25
98 hours	6.3	1.7	0.38	1.83	0.38	0.20
140 hours	6.3	1.4	0.32	1.75	0.37	0.20
164 hours	6.3	1.2	0.32	1.75	0.38	0.20

Table 5.3.3. Effect of increasing supply of iron in water culture on growth and micronutrient concentration of cotton plant

Fe concentration p.p.m.	Yield D.M/g	Leaf				Stem				Root			
		Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
1.0	0.90	**35	365	95	20	119	103	66	21	205	106	206	426
2.0	0.97	**48	340	72	22	90	90	62	25	389	96	204	536
5.0	1.11	*60	333	63	26	75	88	60	24	434	89	212	506
10.0	1.78	70	311	50	27	63	80	58	21	758	69	196	460

* Chlorosis

** Severe chlorosis

(Initial phosphorus concentration 10.0 p.p.m.)

A remarkable decrease in leaf zinc content resulted from increased iron concentration in the culture. Leaf Fe/Zn ratio was 0.37 and 1.4 for 1.0 and 10.0 p.p.m. iron supplied to the solution respectively. The data in table 5.3.3 also show that zinc concentration in both stem and root remained fairly constant and unaffected by increased iron indicating that Fe/Zn interaction probably occurred in the culture solution before uptake by plant roots, and so long as zinc content in culture is constant any further addition of iron induced reduction in plant leaf zinc.

Copper behaved in a similar way to manganese, since iron seemed to have little effect on copper uptake and translocation by the plant, and copper concentration in plant fractions remained similar

for all iron concentrations (table 5.3.3).

The results in table 5.3.4 demonstrate that in well aerated nutrient solutions and a pH near to neutrality, cotton plants were unable to utilize iron (2.0 p.p.m. Fe) at any of the phosphate concentrations used, since all treatments developed chlorosis and the iron content of shoots was considerably depressed by increases of phosphate and showed severe chlorosis (table 5.3.4).

Table 5.3.4. Effect of increasing supply of phosphorus in water culture on growth and micronutrient concentrations of cotton plants

P p.p.m.	Yield D.M/g	Leaf				Stem				Root			
		Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
0.5	0.64	*66	266	66	16	66	71	115	41	329	101	301	584
1.0	0.74	*63	282	70	15	70	70	116	28	424	104	426	615
5.0	0.73	**60	290	72	17	84	68	146	34	528	123	499	639
10.0	0.80	**58	289	64	16	72	99	139	42	614	124	541	665
20.0	0.70	**52	277	67	16	56	79	128	23	745	124	574	740
40.0	0.63	**45	285	64	17	61	77	131	32	913	163	634	723

*Chlorosis

**Severe chlorosis

(Initial iron concentration 2.0 p.p.m.)

There is a slight difference in yield in this experiment (table 5.3.4) compared with the yield recorded (table 5.3.3) for

the first experiment. For example with 10 p.p.m. P and 2 p.p.m. Fe yields are 0.80 and 0.97 g dry matter respectively. These experiments were conducted at different times of the year and although the greenhouse had artificial light and heat there were climatic differences. Any comparison should bear this in mind.

Yields in this experiment (table 5.3.4) show that high levels of P and low levels of P lead to lower yields, in the first case because of severe iron deficiency, and in the second due to phosphate deficiency.

Increased phosphate concentrations had little effect on iron content in stems, while its accumulation in the roots greatly increased. It appears that Fe/P interaction occurs in the solution and also in plant roots. High iron or high phosphate in the rooting medium will bring this about. Under these experimental conditions it is difficult to attain balanced and optimal nutritional conditions for cotton plants, because either iron induces phosphate deficiency or phosphate induces iron deficiency in plants. The low yields particularly at low and high P levels emphasise this.

The plants maintained high contents of zinc in leaves despite increased levels of phosphorus in the water culture solutions. This phenomenon agreed with previous reports by a number of other authors indicating that high phosphate concentrations in plant tissues brought about by increased concentrations in culture solutions induce in the plants an unusually high requirement for zinc despite the maintenance of apparently adequate zinc levels in the plant (Millikan, 1951, 1963; Boawn and Leggett, 1964; Watanabe et al., 1965; Boawn and Brown,

1968). In contrast to plant leaves, zinc in roots increased greatly with a phosphate increase in the solution.

Copper was unaffected by the varied levels of phosphate in the culture solution. Copper content was the same in stems and also in leaves with only a slight increase in the root with phosphate increases.

All phosphate concentrations in water culture solution were ineffective in inducing a decline in manganese uptake or its distribution in various plant fractions.

5.3.3.3 Plant growth and micronutrients uptake in sand culture

In contrast to plants grown in water culture, those grown in sand culture were very well able to utilize inorganic iron even in presence of 10 p.p.m. phosphate in the substrate (table 5.3.5). All plants and for all treatments seemed healthy without showing any iron deficiency symptoms.

Table 5.3.5 Effect of iron concentration on growth and micronutrient content in cotton plants in sand culture

(initial solution phosphate concentration 10 p.p.m.)

Iron p.p.m.	Yield D.M/g (per 4 plants)	Leaf				Stem				Root			
		Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
1.0	2.25	69	337	62	13	54	66	27	13	360	405	172	132
2.0	2.80	78	317	36	13	45	62	18	10	394	410	160	146
5.0	3.20	109	283	29	16	41	60	14	11	428	426	139	194
10.0	3.37	127	234	20	16	40	46	12	10	432	454	123	214

The yield remarkably increased as the initial iron was increased. The high and low P levels (table 5.3.6) tended to decrease yield, showing similar trendsto that of the water culture experiments. These differences in yield for treatments receiving similar initial concentrations of Fe and p, might be due to the same reasons we discussed in the water culture experiments (page 195)

Increased iron application enhanced iron uptake by plants and the amount of iron in the leaf for the lowest iron level plants in sand culture (69 p.p.m.) was similar to those treated by the highest level of iron in water culture (70 p.p.m.).

In contrast to water culture, manganese uptake and translocation depended on the iron level supplied to sand culture, so it is evident in table 5.3.3 that as long as iron content in the leaf or its availability in the root zone is inadequate to meet the plant demands for iron, manganese uptake and translocation did not suffer from iron interference, but in the sand culture where iron availability was sufficient for plant requirements, an antagonistic action took place between iron and manganese inducing a reduction in the manganese content in both leaf and stem with a slight increase in the root. However in sand culture the manganese accumulation in roots was four times that of those bathed in the same solutions but kept only under water culture.

Increasing iron 10 fold in the solution caused a significant depression in zinc content in all plant fractions, but particularly in the leaf and stem. This phenomena tends to confirm an Fe/Zn interaction in the solution before absorption.

Iron application was ineffective in inducing changes in

the copper content in the plant, its levels were similar in leaf and in stem with a slight increase in the root.

In water culture severe chlorosis developed on plants even when phosphate was at minimum (0.5 p.p.m.), while a 20 times increase (10. p.p.m.) in P concentration in the sand culture still allowed healthy plants to develop without showing any iron deficiency symptoms. Further increase in P concentration (20 and 40 p.p.m.) resulted in abnormal plants showing iron deficiency after 3 weeks growth.

Phosphate application had no effect on zinc content in the leaf and in contrast to iron there were lower accumulations in the root compared with water culture under the same levels of phosphate (table 5.3.6).

Table 5.3.6. Effect of phosphorus concentration on growth and micronutrient content in cotton plants in sand culture. (Initial iron concentration 2.0 p.p.m.)

P p.p.m.	Yield D.M/g (4 plants)	Leaf				Stem				Root			
		Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
0.5	1.55	79	356	30	8.0	74	61	30	15.0	214	325	179	328
1.0	1.55	76	340	35	7.8	78	78	29	15.0	222	330	223	393
5.0	2.05	68	312	38	9.0	68	69	19	8.3	238	361	307	366
10.0	2.00	65	310	33	9.3	45	65	17	8.8	254	414	328	381
20.0	1.60	*54	323	28	9.7	44	62	22	12.0	313	517	395	420
40.0	1.12	*50	311	31	10.0	52	70	23	12.0	324	562	367	387

* Chlorosis

The copper contents of both cotton leaf and stem were slightly affected by Fe and P application, but copper concentration in plant roots grown in water culture were twice those in sand culture (tables 5.3.3 and 5.3.5, 5.3.4 and 5.3.6). In water culture, root contents of copper are 3 times and twice those of sand culture for the Fe and P additions respectively. These wide differences in copper uptake and accumulation in roots between the water and sand cultures might be due to:

a) Root exudates in the solid substrates are increased compared with water culture (Barber and Gunn, 1974). These released organic substances if of large molecular sizes, might be involved in strong complexes with copper in the substrate and would thus prevent copper uptake in the sand culture systems.

b) In addition in stirred (aerated) water cultures any local concentrations of exudates/mucilage, such as might be found in rhizospheres in sand would be quickly removed from root surfaces and would not offer a diffusion barrier for Cu^{++} ions.

c) Higher phosphate availability appears to enhance Cu accumulation in roots (Bingham, 1963). This is seen clearly in table 5.3.4. In sand cultures, as already mentioned (Page 187) levels of phosphate will be depleted at the root surface. Thus lower copper contents are expected in sand culture.

5.3.4 Summary of Section 5.3

In a water culture, adjusted to pH 6.5 with nitrogen supplied as nitrate, severe iron chlorosis was observed on the plants. Normal plants could be produced only when high concentrations of iron sulphate were supplied (10. p.p.m. Fe

under these culture conditions when P concentration was constant at the level 10. p.p.m.). Iron chlorosis developed when iron level was 2.0 p.p.m. over a wide range of phosphorus levels reflecting the strong interaction between phosphorus and iron uptake by cotton plants. High manganese and zinc contents were associated with iron deficient plants, and plants in both water and sand cultures showed iron deficiency if the ratio $Fe/Zn < 1$ in the shoots. Increasing both iron and phosphorus to the water culture had little effect on manganese and copper in plant fractions.

In contrast to water culture, plants in sand culture with the same bathing nutrient solution, utilized iron even when its supply was very low (1.0 p.p.m.). Rapid reduction of both manganese and zinc in plant fractions in general and leaf in particular were obtained when further iron was supplied, but copper was not significantly influenced by iron application levels.

Higher phosphate concentrations in the sand culture allowed normal plants so long as $Fe/P > 1$ in the solution, but when this ratio dropped iron chlorosis was found on the plants. Phosphate did not interfere with Mn, Zn and Cu uptake by plants in sand culture.

This experiment shows that surface phenomena or more specifically rhizosphere effects are involved in Fe and P nutrition of cotton plants either because of

(1) P depletion, or

(2) Fe changes in rhizosphere

Moving water cultures (in equilibrium with sand culture) does not allow either localized P depletion and therefore

easier Fe uptake/translocation in roots, or substantial localized changes in iron e.g. reduction followed by complexing of Fe^{2+} from insoluble $Fe(OH)_3$.

The rhizosphere is thus crucial in considerations of these nutrients.

Section 5.4 General discussion and conclusion of the laboratory work

It is clearly evident from the pot experiment using Deir Zor soil that most of the results agree with those obtained from the field.

Green manure clearly enhanced Fe, Mn and Zn D.T.P.A. extractability, and a comparison between pot and field results emphasises the contribution of green manure in the field in increasing availability often to a much greater extent than soil application of the micronutrients.

D.T.P.A. extractability of Fe, Mn, Zn and Cu in the field 30 days after green manure incorporation, and results obtained from the pot experiment, 28 days after 4% green manure application (harvest time) are summarized in table 5.4.1.

Table 5.4.1

	Pot experiment					Field experiment			
	Fe	Mn	Zn	Cu		Fe	Mn	Zn	Cu
Control	4.6	6.0	0.65	3.3	Control	4.1	8.2	0.71	2.20
4% green manure	9.2	23.0	1.0	3.5	Clover crop	5.2	23.0	1.10	2.30
Figure No.	5.1.1	5.1.2	5.1.3	5.1.4	Table No.	4.1.3	4.1.4	4.1.5	4.1.6

Green manure in both experiments seemed ineffective in altering copper extractability in the soil.

Despite the heavy daily irrigation of the pots (300 ml daily) and that initially soluble iron was applied onto the soil ($FeSO_4.7H_2O$), iron extractability increase was restricted to the

top 0-2 cm. There was a slight increase of iron uptake by plants but this was nonsignificant and probably due to fresh iron hydroxide formed in the top of the pot, but with the absence of leaching to distribute iron down towards the active root zone, the iron sulphate application to the soil surface was rather ineffective.

It is likely that more benefit might be obtained if this fertilizer was either mixed with the whole soil volume (pot experiment), or rotovated immediately after application to the field surface. The plant rhizosphere, under calcareous conditions with a high pH and low organic matter content, must come in intimate contact with reactive iron oxides for iron uptake to occur.

In soils with small localized spots of reactive iron oxides, the root system's opportunity of contact with these spots will also be limited, while a soil with a large volume of iron oxide coated surfaces will have greater possibility of plant root contact with these oxides (e.g. from $FeSO_4$ treatment). The extent of recent fresh iron oxide coated surfaces and deposits in the soil depends on the ferrous sulphate crystal size and the influence and speed of irrigation fronts which carry initially soluble Fe^{2+} into the soil.

In Deir Zor soil, it is likely that there is a higher iron oxide content and wider distribution than in some other calcareous soils (e.g. those used by Stewart-Jones 1980) and plant roots are able to contact large surfaces of iron oxides and also to dissolve adequate Fe^{2+} at the soil root interface as a result of root rhizosphere activity. The dominance of Mn^{2+} ions in the rhizosphere in this soil however due to the antagonism of Mn^{2+} and Fe^{2+} either in the soil solution or in the root/shoot after uptake creates unbalanced nutrition and so plants respond to

additional Fe applications.

In the anaerobic incubation experiment, soil iron oxides showed no evidence of reduction after 16 days of anaerobic incubation, and iron concentration in solution remained very low with the natural low soil level of metabolisable organic matter (figs.5.2.2, 5.2.3). When 50 mg of $FeSO_4 \cdot 7H_2O$ in addition to 1% green manure were supplied to the soil (70 g soil), Fe^{2+} came into solution rapidly reaching its maximum after 4 days incubation, after which a sharp reduction occurred with the appearance of black iron sulphide deposits in the culture. Addition of iron sulphate alone to the culture caused no iron solubility increase and its level remained similar to the control soil sample, when no organic microbial substrate was introduced.

This soil is of medium structure and medium permeability. Low organic material associated with low microbial activity means that iron oxide and to a smaller extent manganese oxide surfaces are not attacked, reduced and dissolved in organic complex form, as soil solution redox potential does not drop rapidly under the waterlogged conditions associated with heavy basin irrigation. However when fresh green manure is incorporated, microbial activity in the soil is stimulated and the rapid growth and utilization of O_2 by microbes can lead more rapidly to anaerobic conditions.

The quantities of organic material added to the soil in the pots and the ease with which these materials can be decomposed are of great importance. If insoluble organic materials were the sole substrates available (e.g. cellulose), then microbial activity under anaerobic conditions would probably not readily assist the iron nutrition of the plant, as ~~microbial~~ cellulose

breakdown would be too slow to establish a large population of iron reducers before strict anaerobic sulphate reduction commenced. If the organic source is of the easily decomposable type (e.g. starch) iron reducing bacteria are stimulated, possibly colonizing iron oxide surfaces and enhancing Fe^{2+} concentration (see fig. 5.2.2) in the rhizosphere.

To what extent partially anaerobic microsites persist in the soil contributing to iron plant nutrition is unknown and is well worth study. The rhizosphere, with its own fresh decomposable organic content is clearly of very great importance.

Manganese oxides are more easily colonized by microbes and also attacked by organic acids as the redox potential for manganese reduction is generally lower than that of iron. Despite the increased manganese concentration in soil as a result of green manure, there was no clear evidence of plant response and excess uptake of manganese. All green manure treatments increased iron availability and induced a clear reduction in leaf manganese content and a large increase in Fe/Mn ratio. However any treatment to the soil encouraging manganese solubility must indirectly increase plant demands for iron, so the incorporation of green manure to which iron has been added (e.g. spray in field before ploughing) should be tried.

Soil zinc fertilization is often ineffective in plant zinc nutrition due to rapid fixation. Clay minerals (Bingham et al., 1964; Chu, 1968; and Perkins, 1974), hydrous oxides of iron and alluminium (Chu, 1968; Stanton and Burger, 1970), carbonates (Udo et al., 1970) and soil organic matter (Stevenson and Ardakani, 1972), have all been implicated in this fixation. The degree and strength with which zinc is fixed depends on soil pH, type of soil minerals in the soil, zinc concentration, and the

form in which zinc is applied as well as the amount used (Chu, 1968; Shuman, 1975; Stanton and Burger, 1970).

Kalbasi, M. et al. (1978) have concluded that in calcareous soils, $ZnSO_4$ is usually precipitated as $ZnCO_3$ and $Zn_5(CO_3)_2(OH)_6$.

In Deir Zor calcareous soil in both the field and greenhouse experiments, $ZnSO_4.7H_2O$ fertilizer contributed significantly to zinc availability in soil and caused a clear increase in zinc uptake by cotton plants up to harvesting. Thus either the soil capacity to fix zinc or precipitate is low, or the fresh zinc compounds formed are of high solubility providing adequate zinc to the plants.

Zinc applied to pot soils behaved in a similar way to that in the field with accumulation in the top 0-2 cm horizon, and although the bulk of the plant roots are in the lower layers, the fertilizer enhanced zinc uptake. This suggests some downward movement of soluble zinc compounds.

Phosphate interactions with iron were clearly evident in pot experiments, particularly in the application zone (top 0-2 cm layer) where their insoluble compounds actually precipitate.

P-Fe interaction in soil is considered one of the main problems in iron nutriton of plants. Higher P concentration in the substrate not only decreases the Fe^{2+} concentration by precipitation of iron phosphate, but phosphate also seems able to shift the equilibrium between Fe^{2+} and Fe^{3+} towards Fe^{3+} (Cher and Davidson, 1975).

As Fe^{2+} is the main form of iron available for plants, the presence of phosphate at the root surface or at the uptake sites on the root cells decreases iron availability for plants. O'Connor et al. (1971) have mentioned the importance of root

intersection and diffusion to roots of iron from adjacent oxides (especially those freshly precipitated) as a direct parallel with plant phosphate nutrition. In both cases the effect of root hair development on the limited soil volume in pot experiments will be important (Barley, 1970) especially when iron oxides are low and their distribution likely to be more regional.

It is likely that in pot experiments with the small physical volume of soil and a high density of root exploration, the plant roots were able to contact and intersect more iron oxide particles than would be the case in the field and this is one of the main reasons for the increase in Fe/Mn ratio in the plant leaves in the pot experiment, quite different from the field results. This would point to the desirability of rotovating inorganic iron ($FeSO_4$) into the soil as deep as possible in the field. A combination of 200 p.p.m. P and 10 p.p.m. Zn caused the lowest Fe/Mn ratio, iron deficiency chlorosis was shown on the plants and iron concentration in the leaf dropped near to the critical level (fig. 5.1.7).

Drew and Saker (1975) have concluded that in an iron deficient soil secondary and tertiary lateral root growth could be stimulated by the presence of a reactive iron zone in the manner demonstrated for major nutrients. This phenomenon could be valuable in increasing the importance of ferrous sulphate fertilization in iron nutrition especially for monocot iron uptake.

At a constant pH of 6.5, increasing concentrations of P in the water culture severely depressed the growth of cotton plants and increased iron deficiency symptoms in the plants. The higher the P supply in the culture the more severe was the iron

deficiency chlorosis. In the water culture cotton plants under the lowest concentration of P (0.5 p.p.m.) were unable to utilize adequate inorganic iron at pH 6.5. Azarabadi et al. (1979) reported that corn plants were also unable to utilize iron from $Fe(OH)_3$ at the level of 10^{-5}M P (0.31 p.p.m.) at the same pH level (6.5) and this reveals the similarity of both crops towards iron uptake from water solutions despite corn being an iron inefficient monocot crop while cotton is a dicotyledon, and cotton probably has similar characteristics to tomato.

This high interaction between P and Fe in the water culture makes it difficult to have both Fe and P nutrition at their optima for cotton plants. Their interaction might be by precipitation either in the substrate or on the root surface as well as adsorbed phosphate coating freshly precipitated iron hydroxides (0.3 P/1.0 Fe on a molar basis, Stamm et al., 1965).

Kashirad et al. (1973) have studied the role of pH in plant utilization of iron in water culture. They concluded that corn plants grown at low pH can obtain an adequate supply of iron from inorganic Fe^{3+} compounds even in the presence of phosphate. This confirms the plant rhizosphere role in iron nutrition by pH lowering of the substrate. The cotton root under the pH used (6.5) in water culture was ineffective in obtaining iron and plants under all phosphate concentrations showed severe iron deficiency.

In contrast to water culture, in sand culture healthy cotton plants developed although the same solution was circulated. Three different features probably contributed.

(1) In sand due to the increased surface area as compared with water culture, the equilibrium between the iron precipitated and iron in solution is more rapidly adjusted. Azarabadi

(1977) reported that phosphate concentration in the sand culture decreased to 60-70% at equilibrium corresponding to a P/Fe ratio of 0.3/1.0 in the iron precipitates.

(2) Plant roots increase their exudation in solid substrate compared with aqueous solutions (Barber and Gunn, 1974) and this phenomenon might be involved directly in iron solubility in sand culture either by complexation or via enhanced microbial activity associated with the lowering of redox potential and increased Fe^{2+} availability (Trolldenier, 1971). Anton et al. (1965) have however suggested that the contribution of microorganisms in iron nutrition can often be overestimated. They found that alfalfa was able to lower pH and to utilize iron even in sterilized substrate.

(3) Iron uptake from Fe^{3+} oxides in the sand culture must be restricted to the root surface where they are in intimate contact. The increased iron uptake by plants with rhizospheres in sand presumably near these insoluble oxides, associated with an absence of detectable iron in the leachate supports this strongly (Anton et al., 1965; Azarabadi et al., 1979). The iron released from its oxides is taken up directly. However root ability to take up iron depends on the behaviour of phosphate compounds, and in sand substrate the phosphate concentration is probably depleted around the root as has been shown in soils (Lewis and Quirk, 1967; Bhat and Nye, 1973).

These conditions can occur in water culture only when P and Fe are supplied in separate containers. Iron for absorption should be in solution in water and this can only be achieved if there is rhizospheric pH drop and the simultaneous release of reductants for example in tomatoes (Brown et al. 1977b) and in sunflowers (Marschner et al., 1974).

Kashirad and Marschner (1974b) have shown that iron reduction did not occur when the rhizospheric pH was buffered

or when H^+ ions were neutralized and the ability of bean roots to release reductants was largely inhibited by a pH greater than 4.5 (Ambler et al., 1971). In a constant pH substrate, therefore, plants unable to lower pH are not able to release sufficient reductants and finally cannot obtain iron from Fe^{3+} precipitates.

Monocotyledons, plants such as maize and sorghum, are iron inefficient and are unable to lower their solution pH and release reductants as readily as dicotyledons (Brown, 1972).

From the above discussion it might be concluded that the presence of phosphate in the rhizosphere exerts a very important role in iron solubility and uptake by roots. In the water culture despite the intimate contact between cotton roots and iron hydroxide deposits as a result of their precipitation directly on the roots, plants were unable to absorb and to translocate adequate iron to the shoots for normal growth, and this probably was due to the fact that high levels of phosphate were adsorbed on these hydroxide surfaces and the free and faster diffusion of the phosphate anions in solution made the 'rhizosphere' always rich in phosphate.

This sort of interaction might be taking place also within the root after absorption of P and Fe preventing iron translocation to shoots to cover plant demands for iron. The results obtained indicate that P-Fe interaction after uptake seems to be of less importance as compared with what actually occurs in the substrate including root surfaces.

All results revealed very strong reduction of zinc uptake by iron applied to the soil and also iron uptake reduced by zinc applied to the soil under the varied levels of phosphate. Their concentration in both stem and root remained constant probably

because Fe-Zn interactions mostly take place in the substrate before absorption.

P inhibited both Fe and Zn in the shoots. An increase in P increased Fe concentration in roots and stem while only a slight increase of Zn occurred in roots. These results also reflect P-Fe interactions in root and stem in addition to substrate, while P-Zn inhibition occurs mostly in the substrate or at the adsorption stage inside the plant.

The experiment using green manure and calcareous soil in the greenhouse is of particular significance. Iron supplied by the organic complexing activity of green manure or its products was clearly quite different from iron supplied from ferrous sulphate additions in that little Fe accumulated in roots. Even on the addition of phosphate little Fe was trapped in roots. Natural organic chelate sources of iron are thus much more mobile and 'active' within the plant.

Clearly further field work should investigate this phenomenon.

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