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SOME ASPECTS of SOIL EXAMINATION in the FIELD

TECHNIQUES and APPLICATION

A B S T R A C T.

(1) A number of colorimetric methods suitable for field as well as laboratory use were developed and adapted for the analysis of Morgan extract of soils.

A small lightly-constructed shaker and centrifuge were designed to suit field work.

(2) The effect of different moisture levels of soils on the extractability of different plant nutrients by Morgan's reagent was studied. Five different soils varying in pH, organic matter content and texture were incubated (28°C) at three different moisture levels (25%, 50% and 75% of moisture holding capacity.) Iron, manganese, aluminium, magnesium, sulphates, phosphates and potassium were determined and the fluctuations in their extractability with time were examined.

(3) Perennial rye grass was grown in acid washed sand, and also in soil diluted with sand to study the mineral deficiency symptoms in relation to levels of nutrient supply and to the efficiency of the methods of analysis developed. Very clear visual deficiency symptoms of iron, manganese, magnesium and sulphur were obtained from the sand cultures and confirmed by recovery tests in the plants. In the soil-sand mixture, however, deficiency symptoms were observed only in the case of magnesium and sulphur while chemical analysis (of soil

solution obtained by laboratory lysimiters and Morgan extracts) showed clearly the lower levels of these elements in the untreated soil.

Iron, manganese, aluminium, magnesium sulphates, phosphates potassium and nitrogen were studied and deficiency levels are compared with the existing data. Also the effect of iron, manganese, magnesium and sulphur deficiency on the mineral composition of the grass is reported.

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A C K N O W L E D G E M E N T S

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CHAPTER I

INTRODUCTION. REVIEW OF LITERATURE

OBJECT OF RESEARCH

Chapter I

INTRODUCTION - HISTORICAL REVIEW

OBJECT of RESEARCH

The use of chemical analysis to assess soil fertility dates back to the nineteenth century. Davy[1], was one of the earliest scientists to call attention to soil analysis when he wrote "If land be unproductive and a system of ameliorating is to be attempted, the sure method of obtaining the object is to determine the cause of its sterility which must necessarily depend upon some defect in the constitution of the soil which may be easily discovered by chemical analysis".

Early workers, having established the fact that plants obtain an important portion of their food from soils, made efforts to evaluate soil fertility on the basis of total nutrient content. Despite the fact that poor correlation was obtained between the data of such analysis and crop response to fertilisers in the field, these analyses have served many useful purposes in the development of soil science.

The discovery of the principle of base exchange by Way[2], marks the beginning of the modern era of soil chemical analysis. Since then, in the search for the so-called "available" or "exchangeable" state of nutrients, chemists turned their efforts to developing methods of extraction and determination of such portions of nutrients in soils which could meet the

immediate requirements of the growing plants. There is evidence that Daubeny[3], suggested the terms "active" and dormant" to differentiate between the easily and difficultly soluble constituents in soils and proposed the use of carbonic acid to distinguish between the two categories.

It was obvious from the literature that phosphorus, potassium and nitrogen attracted the attention of workers more than the other nutrients. Liebig[4], investigated the availability of phosphorus and potassium on Rothamsted soil, using dilute hydrochloric, nitric and acetic acids for extraction. Dyer[5], suggested the use of citric acid for phosphate extraction ; his choice came as a result of an examination of a wide variety of root saps of plants. He reported that soils with less than 0.01% soluble P_2O_5 probably need phosphate fertilisation.

The use of neutral salts such as ammonium chloride[6], and ammonium acetate[7] for leaching of the available bases in soils became common at the beginning of the present century. Strong mineral acids have been used by several workers[8] for the purpose but the main objection to these is that they virtually bring into solution more nutrients than can be regarded as available. Other conventional methods of soil analysis include electrodialysis, water extraction and soil solution studies. Electrodialysis was used as early as 1905 to investigate the rate of solution of phosphate from soil[9]. This technique was used by many workers[10] specially in studies of phosphorus and potassium but the real impetus was not reached until 1926 when Mattson[11]

introduced the 3-chambered cell. Purvis and Hanna[12] used a Marsden cell[13] with smaller dimensions and replaced water with 0.05 N boric acid solution thus increased the rate of release of bases so that electrodialysis was accomplished within twenty minutes. Cornfield[14], working with soils from long term manurial trials concluded that electrodialysis is a reliable method for extracting available plant nutrients from soils. He compared electrodialysis with well known chemical extractants (Truog, citric Morgan) and found significant correlation between the methods.

Workers devised numerous techniques to correlate nutrients extracted with pure water, with the growth of plants. Schloesing[15], seems to be the first who conducted such investigations. He used fresh soil and poured on it water coloured with carmine, collected the effluent and analysed it for several nutrients and organic matter. Pierre and Parker[16], suggested the use of collodion bags for diffusion of water-soluble constituents from the soil. Other techniques involve the extraction of the soil solution either under pressure[17] or dispersion of soil solution in a large volume of inert liquid as paraffin[18] or glycol[19] or ethyl alcohol.

The main difficulty in analysing the soil solution is the great dilution of nutrients existing in it, this requiring very sensitive analytical techniques.

In contrast to the long conventional methods of soil analysis, the development of rapid techniques to assess the soil fertility and to help in fertiliser recommendation found its place

in agricultural science some thirty years ago. The main object of such techniques is to examine a large number of soils in the shortest possible time and at minimum expense. Gravimetric and volumetric procedures for soil analysis are not sensitive enough for the determination of such minute quantities as are concerned. Often the experimental error is considerable and significant differences between fertile and infertile soils are not clearly shown. On the other hand, the use of colorimetric methods and in certain cases the turbidimetric techniques in analysis of soil extracts do reveal such significant differences. The "rapid" or "field tests" owe their existence to the intelligent device of spot tests and suitable extractants. It is in the interest of the farmer and the most important task of the agricultural chemist to determine the nutritional need of a crop in the field to obtain them highest possible yield with the least expense. Three aspects should be examined.

(1) The problem is to be diagnosed.

(2) The degree of deficiency is to be determined and

(3) the proper amount of fertiliser is assessed to give the desired yield.

The diagnosis of the problem :- When all other factors of plant growth are normal, two main procedures are followed to help in making a correct diagnosis in the field :-

1st : Chemical analysis of soil by rapid methods.

2nd : Use of plants as means of soil examination (deficiency symptoms if existing.

Chemical analysis of soil in the field depends largely on sampling, types of extractants and analytical methods used. Sampling seems to be a rather challenging problem but with some common sense one can obtain a representative sample.

Different extractants were used for the purpose. Among the early field tests were those for phospherus in the United States. Spurway[20], tested the water-soluble and dilute nitric acid-soluble phosphorus in the soil by testing a drop of the soil extract with a solution of m ammonium molybdate in nitric acid, using a tin pencil to develop the colour. Bray[21], introduced a similar technique but he tested the phospherus in the presence of soil. Truog[22], extracted the soil with dilute sulphuric acid and developed the colour with stanneus chloride and ammonium molybdate solution in sulphuric acid. Morgan[23] published a bulletin in which he included a system of soil examination for pH, phospherus, nitrogen (ammonia and nitrate), aluminium, calcium and sulphur. He used different extractants for the different constituents, and by means of coloured charts estimated various extractable constituents with the exception of potassium. He devised the "Morgan soil test block" for leaching the soil.

Spurway[24], used a very dilute solution of acetic acid (0.023N) approximately one part of soil to six parts of distilled water (acidified with acetic acid) for extraction, shaking for one minute and filtration, Nitrate-nitrogen, phospherus, potassium, calcium, carbonate, pH, ammonia-nitrogen, nitrite, magnesium, iron, aluminium, manganese, sulphate chloride, sodium were examined

colorimetrically and again he used coloured charts for his determinations.

Morgan[25], introduced one of the most widely used extractants for diagnostic and advisory work. This is a mixture of acetic acid and sodium acetate highly buffered at pH 4.8. It is prepared by dissolving 100 gms. of sodium acetate trihydrate in a litre of distilled water containing 30 ml. of glacial acetic acid. The mos important characteristics of this system is that it is highly buffered, it has an unchanged solvent action while extracting, and extracts are suitable for colorimetric and turbidimetric techniques, it extracts quantities of available nutrients which are within the sensitivity range of the analytical methods in force, and the amounts of nutrients extracted are correlated with response of different crops over a wide range of soils and its resistance to biological decomposition at least for a time long enough to carry out the required tests.

For extraction, Morgan suggested that a teaspoon of soil (approximately 5 gms.) is placed in a funnel with 10 ml. of the extracting reagent to percolate through it, moistening with water will be necessary if the soil is not readily wetted by the extractant. This is more or less one of the complete systems for soil examination as it includes all the nutrients, most of the harmful constituents of the soil, pH, organic matter, and lime requirement.

Since then, several workers have modified and used Morgans or similar ones to determine more than one constituent in a single extract e.g. Hester[26] used the same analytical techniques

as Morgan but employed an extracting solution with a total acetic acid concentration of 0.2N buffered with sodium acetate at pH 5. Thomas[27], used the Morgan system but made many minor variations.

Another technique used for rapid tests was applied by Bray[28], using a neutral salt solution of 25% sodium perchlorate but Morgan[29], believed that extraction with neutral salt solution fails to bring out into solution measurable amounts of available phosphorus, aluminium and iron.

Baver and Brumer[30], used 0.3N HCl for extraction and the ratio of soil to extractant 1:2.

It is clear from the foregoing that a variety of extracting agents have been used for rapid tests. These could be classified into 4 main groups :-

1- Very dilute acid whose action is similar to water e.g. the Spurway type (0.028N acetic acid.

2- The fairly concentrated neutral salt solution type used by Bray[21] and Peech[31].

3- The strong mineral acid solution which is sometimes buffered at a certain pH e.g. used by Truog[27],[32], Bray[8] Spurway[24],(using 0.135N HCl), Baver and Brimer[30],using 0.3N HCl while Miles[33] used a buffered perchloric acid extractant.

4- The weakly ionised organic acid buffered with its sodium salt or Morgan[25] type used by Hester[26], Peech[31] and many others.

Peech[34] reported that these different systems may be so

related that a factor may be employed to convert values obtained from one system to another.

Many workers[35] improved and standardised the rapid microchemical tests to attain the highest possible accuracy while others[36], compared these rapid techniques with the long conventional laboratory methods. Peech and English[35] called attention to the use of specific reagents for colorimetric analysis and the need for careful examinationm of interferences. Anderson and Noble[36], made a comparative study on widely different soils using different systems of quick tests and reported that :-

(a) Results by two observers with the same set on a particular soil were in fairly good agreement.

(b) These tests are of valuable aid for diagnostic purposes if interpreted by well-trained agronomists who are familar with the lands

With the development of the rapid tests, naturally there was development of apparatus to meet the speed, and accuracy of required for such tests. In the field, several testing kits have been designed for examining the different soil nutrients, making use of coloured charts or of permanent discs for comparison.

Constable and Miles[37], made several improvements in the working techniques such as pipetting, extracting, filtering to increase the efficiency of the laberatory production.

Plants growing in the field furnish another means for testing the supplying power of soils under natural conditions and thus throw some light on the availability of nutrients. Comparative study[38], and analysis of healthy and "abnormal" or "unhealthy" samples often leads to correct diagnosis particularly if coupled with a soil test.

Different organs of plants are sampled for analysis for different nutrient elements[39] e.g. nitrogen test (NO_3-N) is usually made on the main stem or leaf midrib, while in wheat, oats, barley and rye the main stem is used. Phosphorus in maize is determined on leaf midribs near the ear, while in graminae leaf tissue near the centre of the plant.

Carlens[40], suggests making the test on the sap obtained from plant samples collected after maceration, extraction with 2% acetic acid and decolourisation with charcoal.

Nicholas[41], examined plant tissue in the field by extraction with Morgan's reagent and application of colorimetric and turbidimetric techniques making use of a Lovibond Comparator. He also stresses the comparative study of plants in suspected fields as this facilitates the diagnosis of deficient or toxic cases in situ. The Long Ashton Field Kit he designed, contains a hydrometer balance for weighing in the field.

Comparative studies in the field are of great value for diagnosis even if the shortage of an element is not severe enough to cause visual symptoms. In acute cases however, when nutrients are critically low, plants may be stunted in growth, show

signs of sickness on leaves, stems, fruit and disintegration of various parts of the plants. These symptoms of nutrient deficiencies furnish the quickest and cheapest method of diagnosis in the field. Many atlasses, (42),(43) and (44), guides and publications with coloured plates are available to help in identification of these symptoms in the field.

However, correct diagnosis requires good powers of observation and very careful discrimination. Also it is important but not easy to exclude other possible causes of poor growth or sy symptoms that look like mineral deficiencies as a result of other agencies as for example, wind, frost, heavy rain, drought, overliming, insect damage and virus diseases. Wallace(43), classified the nature of mineral deficiencies into two categories, the simple type in which symptoms are clear and decisive in contrast to the complicated type which do not afford conclusive evidence. He classified certain plants under the name of INDICATOR PLANTS, which are sensitive to mineral deficiencies give very clear and decisive symptoms and suggested making use of them in the field under investigation in case of confusion. The literature on effects of interfering factors in mineral deficiency symptoms is tremendous and can be illustrated by only a few examples. Boas(49), claimed that 1 - 2 Kgms. of borate/hectare removed all drought signs from a field ofn red clover despite eight weeks of continuous heat. Camp(46), found that the availability of zinc varies with the pH, being lower as the pH rises, the critical point being between 5.5 and 6.5.

Barchad[47], noticed variation in molybdenum conteht in several plants at different seasons in different localities. Fluctuations in available nutrients in soils (particularly nitrogen which accumulates in Spring-time) has been examined by several workers. Harmer[48], states that it is possible that plant responses to copper are greater in hot dry summers than inn cool moist ones. Hewitt[49], has shown that iron deficiency can be induced in a crop growing on a soil containing too high a concentration of metallic ions e.g. cobalt, copper, chromates, zinc, manganese and lead can all cause plants to show symptoms of iron deficiency as well as those of specific toxicity of individual metals. Wallace[43], pointed out that eel worm, wire worm and cut worms injure roots and stems resulting in the appearance of yellow, red, purple tinting similar to nitrogen, phosphorus and potassium deficiencies. He found also that some virus diseases produce leaf patterns which can easily be confused with mineral deficiency e.g. virus yellow of sugar beet which is similar to magnesium deficiency.

Interpretation of data from soil and plant chemical analysis :-

This is the most delicate operation in the whole system of soil examination, requiring wide experience, good acquaintance with the soils examined, their physical and chemical properties. Morgan[29], comments in this connection "sound judgment through agronomic training and much experience in the application of tests on soils of known performance are essential to the

diagnostic interpretation of soil tests in terms of soil management practices." He adds that " the practical value of the tests is limited chiefly by the qualifications of the person who is responsible for translating the data into amounts and kinds of fertilizers, manures, limes and other soil amendments that are most likely to be effective in promoting profitable production."

Plant tissue tests on the other hand, require a sound understanding to the functions of the element tested, the forms in which it exists in plant tissues and its assimilation. The plant tissue tests are valuable in indicating the plant nutrient which is limiting the growth.

It is very clear that soil and plant "quick tests" are only indicative and diagnostic and far from being quantitative. No general rule could be laid down for interpretating the data of such tests in terms of fertiliser requirement. A knowledge of the physical and chemical conditions of the examined soil e.g. texture, compactness, permeability, aeration, temperature, water supply, pH, lime requirements, cropping history, organic matter content will also be needed.

The primary step however, is the establishment of techniques for the chemical testing of the soil or plants, which can be standardised to agreed levels of accuracy and are not liable to interfere under the wide range of conditions in which the tests may be expected to be made both in the field and in the

laboratory. Effective diagnosis and the assessment of the remedial measures then becomes a matter of coupling data so obtained with local knowledge of soil types, conditions etc.

The analytical methods sought in the course of this work, (Chapters III, IV, V and VI, VII) were developed to deal with Morgan extracts of soil. Morgan's extractant was chosen for the present studies partially because of its very general adaptation in many countries thus facilitating comparative study and partly because among the extractants used for the purpose it permits examination of a particularly wide range of nutrients in one single extract. It is extremely difficult if not impossible to find an extractant suitable for all soils or all elements. Piper[50], remarked in this connection " it is improbable that any simple form of analysis can simulate the conditions existing in the soil throughout the period of actual plant growth and so give a reliable measure ofm availability under all conditions)".

However, the successful use of a chemical test depends mainly on the careful calibration of the results of the test with responses of different crops to application of fertilisers on different soils. Bray[51], on the other hand, describes the requirements for a successful soil test more optimistically and specifically. He points out three definite principles for such a test :-

(1) The extracting solution and the procedure used should

extract the total amount (or a proportional part) of the available form or forms of a nutrient from soils having variable properties.

(2) The amount of nutrient in the extract should be measured with reasonable accuracy and speed.

(3) The amounts extracted should be correlated with the growth and response of each crop to that nutrient under various conditions.

The successful use of Morgan's procedure for diagnostic and advisory work is now reported from many countries.

It is thought that one of the main factors contributing to some failures in establishing a correlation between soil chemical analysis and crop response in the field is the inefficiency and inaccuracy of some of the chemical methods applied particularly in the so-called "quick" tests. The analytical methods developed and modified in the course of this investigation are mainly colorimetric and intended for use in rapid tests of soils in the field as well as in the laboratory. Procedures and apparatus required have been simplified as far as possible. All these methods have been established initially, using the Biochem Absorptiometer or the Tintometer for standardisation and finally permanent colour standards, whenever suitable, have been made by Messrs Tintometer Ltd. The main advantage of these methods of analysis may be summarised as follows :-

(1) They are relatively simple techniques, requiring simple apparatus. A technician with a reasonable training can carry out such examination using these techniques fairly accurately.

(2) Another clear advantage of these tests whether used in laboratory or in the field is the rapidity with which they may be carried out. Rapid decisions as to fertiliser requirements or in diagnosis of deficiencies in growing crops are obviously valuable.

(3) All the methods described are standardised for Morgan extracts, thus enabling the investigator to examine numerous nutrients in one extract. The effects of Morgan's extractant on the colour formation was thoroughly studied so that in each, maximum quantities of the Morgan reagent which may be used in the tests without any interference or undesirable side reactions have been determined. This decides the size of aliquot of extract used in the tests.

OBJECT OF RESEARCH :-

The present work was conducted :-

1st. To contribute to the refinement and development of techniques which are reasonably accurate and simple and could be used in the field as well as in the laboratory investigations.

2nd. To study the effect of different moisture levels on the extractability of different plant nutrients. The general trend in soil analysis is always to air dry the soils before their examination. In the field, soils are always under different moisture levels and if on-the-spot tests are required, would the test be affected by the moisture content, and to what extent ?

3rd. To study the deficiency symptoms of certain nutrients in perennial rye grass and its mineral composition as affected by such deficiencies. This experiment was utilised to examine the efficiency of the testing methods developed. Moreover, a correlation between Morgan extractable nutrients, soil solution contents and uptake of nutrients by plants was investigated.

CHAPTER II

Analytical Methods

Apart from the methods established in this work to suit the analysis of Morgan extracts of soil it was necessary to use other methods in supplementary work and for later experiments in the use of the methods of analysis. These methods are in detail below :-

1 - Soil Analysis.

Samples for Soil Solution :-

The rye grass experiment was designed so that two pots from each treatment, one Kilogram Capacity each, were utilised for soil solution displacement, Morgan extraction for availability test and moisture content determination.

Displacement of Soil Solution :-

The Alcohol displacement method described by Parker[52] and modified in this laboratory was adopted. A battery of Bakelite tubes were used for the purpose. Glass cloth support was treated with hydrochloric acid and rinsed thoroughly with distilled water. It has no effect on the pH of the displaced soil solution.

Analysis of the Soil Solution :-

The Moisture content of the soil used was determined on oven dry basis with a ten gram sample of the fresh soil.

Micro-chemical methods of analysis were used and all data from soil solution analysis are expressed in parts per million of oven dry soil to obtain comparable results.

Measurement of pH :-

The Cambridge glass electrode pH meter was used in the case of coloured solutions while the Levibond Comparator was used in the case of colourless clear solutions.

Organic Carbon :-

Walkley and Black[53] Method.

Mechanical Analysis :-

The hydrometer method using Calgon (sodium hexametaphosphate) in 0.1N sodium hydroxide was adopted.

Determination of Iron :-

The thiocyanate method modified as described in CHAPTER V was used.

Determination of Manganese :-

The leucomalachite green method developed in this laboratory for the purpose and described in detail in CHAPTER IV of the thesis was used.

Determination of Aluminium :-

The "Aluminon"( )method was used. It was noticed in a preliminary investigation at the early stage of this work that different "Aluminon" batches differ both in sensitivity towards aluminium and in the stability of the lake. Accordingly, it was decided to use one and the same sample throughout the whole investigation.

Determination of Magnesium :-

The Titan yellow method adapted by Tobia[54] was used.

Determination of Sulphate:-

The turbidimetric method adapted by Jarrett[55] was used.

Determination of Phosphate :-

Stannous method as modified by Yuen and Pollard[56] was used.

Determination of Potassium :-

Flame photometer was used.

Morgan Extracts :-

The Morgan[29] test of nutrient availability was adopetd. When coloured solutions were obtained, purified activated charcoal was used for decolourisation, this was rarely the case. Aliquots of the filtrate were used for the following determinations :-

Iron :- Thiocyanate as modified and described in detail in CHAPTER V of the thesis, page 99.

Manganese :- Leucomalachite green method described in CHAPTER IV, page 55

Aluminium :- Aluminon method as above. The solution was neutralised before analysis using 0.2N NaOH.

Magnesium :- This was determined by the colorimetric method of Cornfield and Pollard[57] using Titan yellow as modified in this laboratory by the author.

Sulphate :- This was determined by a newly developed colorimetric method using barium chloranilate. The method is described in detail in CHAPTER III, page 32.

Phosphate :- α-naphthol sulphonic acid method.

Potassium :- Flame photometer.

2 - Plant Analysis.

The cuttings were made by stainless steel scissors. The samples were dried in an electric oven at a temperature of 70 - 80°C. The samples were then mixed thoroughly, crushed by hand and then in an agate mortar. The metal mill was avoided for fear of iron contamination.

Fe, Mn, Al, Mg, P, K, N were determined after wet ashing with sulphuric-acid-hydrogen peroxide as described by Yuen and Pollard[58].

Fe was determined by o-phenanthroline method described by Peech and English[59].

Mn was determined by Yuen's method[60].

N was determined by direct Nesslerization.

Al, Mg, P and K were determined by the same methods as for Morgan extracts.

Sulphate :- A separate subsample was taken for this determination. The method described by Butters and Chenery[61] was adopted.

:ADAPTION OF ANALYTICAL METHODS:

FOR USE IN SOIL EXTRACTS BY THE MORGAN'S

PROCEDURE

"WHEN YOU CAN MEASURE WHAT YOU ARE SPEAKING ABOUT AND EXPRESS IT IN NUMBERS, YOU KNOW SOMETHING ABOUT IT, AND WHEN YOU CANNOT MEASURE IT, WHEN YOU CANNOT EXPRESS IT IN NUMBERS, YOUR KNOWLEDGE IS OF A MEAGRE AND UNSATISFACTORY KIND. IT MAY BE THE BEGINNING OF THE KNOWLEDGE, BUT YOU HAVE SCARCELY IN YOUR THOUGHT ADVANCED TO A STAGE OF A SCIENCE."

- LORD KELVIN.

CHAPTER III

COLORIMETRIC DETERMINATION of SULPHATES in MORGAN SOIL EXTRACTS by means of BARIUM CHLORANILATE

Despite the fact that sulphur stands among the essential plant macro-nutrients, soil chemistry and pedologists do not often determine the sulphate content in routine analysis of soil or in advisory work. This is partly due to the fact that sulphate-deficient soils are uncommon, and partly to the fact that sulphur is frequently added to the soil in fertilisers and manures and in rainfall. However, a knowledge of the sulphate status of the soils is desirable if not necessary especially in arid and semi-arid regions or in saline soils in which high contents of sulphates and other salts may interfere with the growth of plants.

Gravimetric methods for determining sulphate are not suitable for field work for obvious reasons. Several workers (55) (62), developed and improved turbidimetric methods for sulphate determination in soils but when applied to soil extracts such techniques frequently are subject to interference by other soil components e.g. from colloidal organic matter, especially if Morgan extracts are usedfor extraction. Little(63) pointed out that shaking with charcoal decolourises the extract but does not remove the organic matter completely. Hesse(64) claimed that the organic matter interferes in turbidimetric methods of sulphate determinations in two ways :- 1st : at low sulphate concentrations, organic matter may act as a protective colloid and cause low results or 2nd : at high sulphate concentrations when it co-precipitates and leads to erroneously high determinations. He suggests the precipitation of organic matter using

5% ferric chloride and 40% sodium hydroxide prior to the determination of the sulphate turbidimetrically to obtain more reliable accurate results.

The sulphate ion gives few coloured reactions and this probablyn explains why gravimetric, titrimetric and turbidimetric sulphate determinations received more attention than colorimetric techniques. Marsden and Pollard[65] developed a very sensitive method for sulphate determination in soil extracts using benzidine hydrochloride. The technique is lengthy and not suitable for field work.

Recently, Lambert, Yasuda and Grotheer[66] used the insoluble thorium borate-amaranth dye reagent for colorimetric determinations of the sulphate but the method suffers from interference of ions likely to be present in soil extracts.

Bertolacini and Barney[67] made use of the reaction between solid barium chloranilate and sulphate ions to liberate the highly coloured chloranilic acid ion for determining sulphates in oils. The test solution is passed first through a resin column to remove the cations which interfere with the determination. Tyner[68], studied the properties and reactions of chloranilic acid (2,5 dichloro, 3,6 dihydroxy-p-quinone) in an attempt to determine calcium in plant material. He reported that cations interfere with the colour of the chloranilic acid either by forming soluble complexes as in the case of iron (when present in low concentrations), or by forming precipitates as in the case of copper, manganese, barium, strontium thus reducing

the residual concentration of the chloranilic acid without affecting its basic colour or spectral transmission characteristics.

In the original method, Bertolacini and Barney pretreated the test sálution by passing it through a resin column (Dowex 50 x 80, 12 - 50 mesh), to remove the cations tha taking an aliquot which contains up to 40 mgms sulphate and developing the colour in a 100 ml. volumetric flask and separating the excess barium chloranilate and the precipitated barium sulphate by centrifuge or filtration. They reported that the maximum sensitivity of the reaction is at pH 4 and used 0.05M potassium hydrogen phthalate as a buffer. They found that the raection is sensitive in 5% alcoholic medium.

Inn trying to develop a colorimetric method for sulphate examination, the above method was followed substituting Zeo-carb 225 (hydrogen-form) for Dowex 50 x 8.

Two main points were investigated:-

1st the possibility of applying this method for sulphate determination in the presence of Morgan's reagent.

2nd the effect of interfering anions and the elimination of them and, at the same time, keeping the procedure and apparatus sufficiently simple for field use.

The Standard solution was prepared by dissolving 1.814 gms of potassium sulphate, analytical reagent, in a litre of distilled water, then passing it through a resin column of Zeo-carb 225 (H-form). The first 40 to 50 mls. were rejected and the next

let was collected in a clean dry flask and used for preparing the standard curve. The detection of the change in pH using wide range B.D.H. pH papers, helped in observing the arrival of the decationised solution. A resin column of Zeo-carb 225 ofm 20 cm height and 1.5 cm in diameter gives over 500 mls. of this solution before it needs regeneration. It was observed on the other hand that a column of the same resin, of the same dimensions, gives only 25 to 27 mls. if Morgan's reagent was passed through it.

To each standard 1 ml. of 0.05M phthalate solution was added. The adsorption was measured by the HILGER BIOCHEM ABSORPTIOMETER after choosing the proper filter. The zero reading was adjusted in every case with a blank determination.

Preparation of Resin Columns :-

Tubes of 35 cm. length and 1.5 cm. diameter, fitted at one end with a tap to, control the flow of the effluent are prepared (preferably 25 ml. burettes). The ends near the tap is plugged with glass wool of 1.0 to 1.5 cm. in height to support the resin column. The resin is soaked in about twice its volume of 2N hydrochloric acid (69) for half to one hour with occasional stirring, then transferred to the prepared tubes. Water is passed upwards through the bed to wash away very fine materials and air bubbles. Care should be taken to see that the rate of flow of the water should not be so high as to carry away the main bulk of the resin column. This backwashing is continued

till the water leaving the tube is clear when the surplus water is drained downward through the bed to the level of the top of the bed. ONE bed volume (i.e. 35 mls. in this case) of 2N - hydrochloric acid is next passed through the column in 10 to 15 minutes. The effluent should be strongly acid as tested by methyl orange or B.D.H. pH papers. The acid is drained to bed level and excess acid is washed out till 100 mls. of the effluent requires less than 1 ml. of N/50 alkali to neutralise using methyl orange as indicator. Experience in this laboratory however, showed that if the effluent gives a negative test with silver nitrate, it is safe to use the resin column. The column is now ready for use.

Regeneration of resin columns :-

Tests on the treated effluent (using B.D.H. paper) show that its acidity due to the exchange rises to a maximum which is maintained until the capacity is exhausted as indicated with the fall of the acidity of the treated solution. Regeneration is then carried out using hydrochloric acid 1.25 bed volume (i.e. roughly 45 ml in this case) of 1.5N hydrochloric acid.

Through this work, for each sample of Morgan extract, a resin column was used once and then either regenerated or replaced by a new resin column as the Morgan extractant is very exhaustive to such resin columns due to its high sodium content.

Experimental :-

It was reported(64) that ammonium ions do not interfere

if present in solution up to the concentration ofm 250 γ/ml. This was not confirmed especially in presence of Morgan's solution pre-treated in resin columns; very high blanks were obtained. The adjustment of the effluent pH to 4 required higher concentrations than 250 γ/ml. ammonia especially when working with as limited a volume as 10 mls. It was decided therefore, to use 1 ml of phthalate solution (0.05M) with a view to raising the pH of the medium.

Choice of Filter :-

This test was made by running sulphate solutions containing 50, 200, 500, 1000, 2000, micrograms of SO_4 into centrifuge tubes marked at the 10 ml. level. One ml. of 0.05M phthalate, 2 ml. of Morgan's solution passed through the column and 5 ml. of alcohol (absolute) were added mixing after each addition. The tube was filled to the mark with distilled water and the contents mixed thoroughly again. From the tip of a thin-bent glass rod (2mm in diameter), 0.03 gm. of barium chloranilate was added. The tube was shaken for 5 minutes, then centrifuged for another 5 minutes at 2500 r.p.m. Readings were taken immediately after preparation and results are shown in Table I, (page 37) suggest clearly the use of the green filter OGI.

Effect of Morgan's Solution :-

To see the effect of Morgan's solution as compared with water solutions on pH and on the nature of the colour developed

two sets of standards ~~were made~~ covering the range from 50 µ/gm to 1000 µ/gms SO_4 (i.e. 5 to 100 ppm SO_4") were prepared and one set was made with the addition of 2 ml. of Morgan solution passed through the column and the other set was made with water only. The results are shown in Table 2, p. 38

It is noticed that the intensity of the colour has increased in the presence of Morgan's solution and at all concentrations of sulphate tested. From the curves (Fig. I) it is evident in both sets of standards the reaction follows Beer's law closely.

Stability of the Colour :-

To test the stability of the colour readings of a set of standards in the presence of 2 ml. of Morgan passed through resin column were taken immediately (after 10 minutes), half an hour, one hour 3 hours and 15 hours after preparation. Results are shown in Table 2, p. 39.

It is observed that the colour is very stable, no change whatsoever could be traced in at least 15 hours from the time of preparation.

Recovery :-

The recovery of amounts of sulphate added to standard solutions or soil extracts of predetermined sulphate content was examined. Results are shown in Table 4, p. 40 Recovery ranged from 98% to 102%.

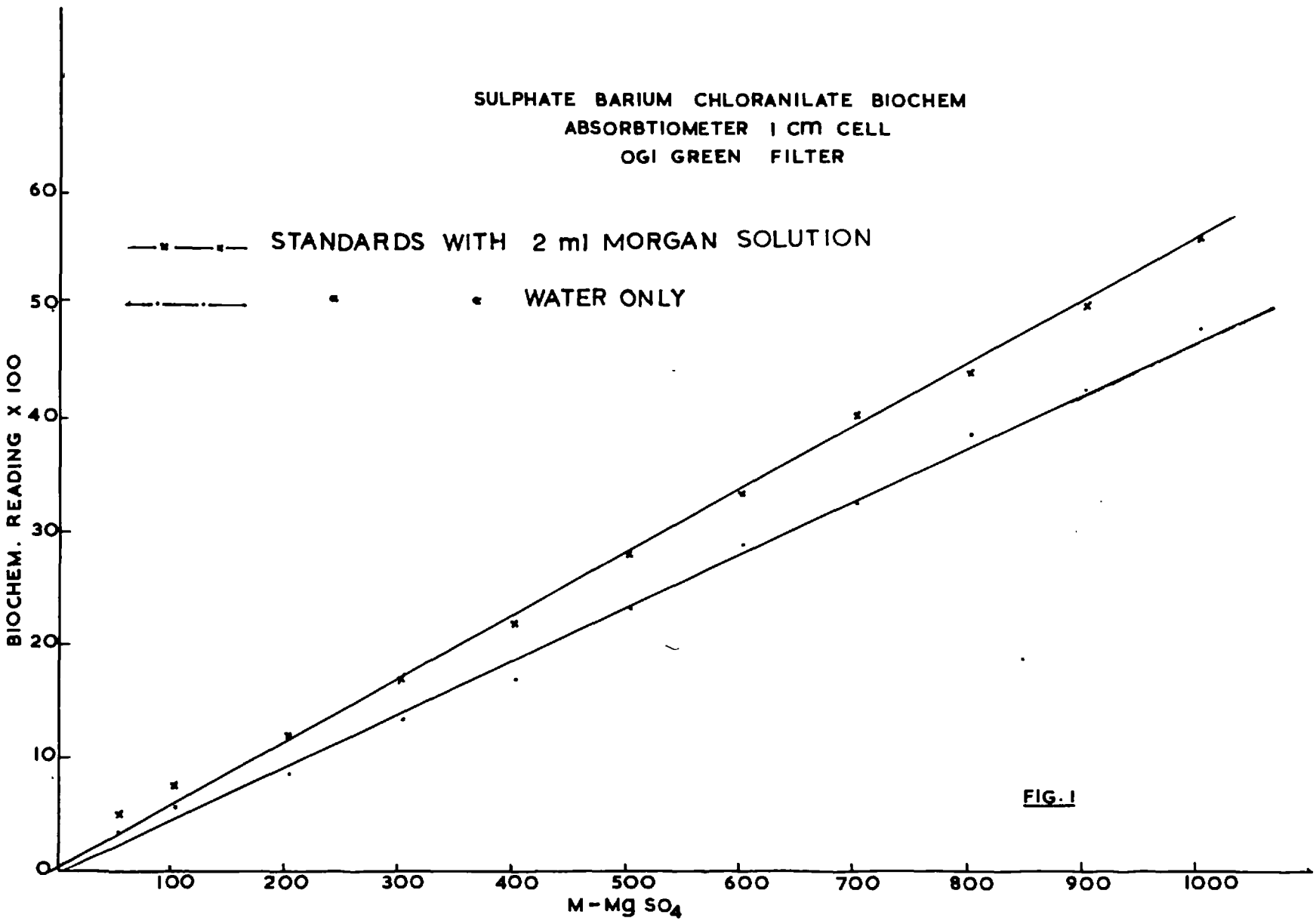

SULPHATE BARIUM CHLORANILATE BIOCHEM
ABSORBTIOMETER 1 cm CELL
OGI GREEN FILTER
STANDARDS WITH 2 ml MORGAN SOLUTION
WATER ONLY
BIOCHEM. READING X 100
0
10
20
30
40
50
60
100
200
300
400
500
600
700
800
900
1000
M-Mg SO4

FIG. 1

Effect of Interfering Anions.

The serious interference of cations which are likely to be present in soil extracts with the colour of chloranilic acid liberated during the sulphate determination is eliminated by removing these cations using ion exchange resin columns. It was decided therefore, to test the interference of anions only. Solutions of the desired concentrations were made and passed through the resin column - rejecting the first 40 to 50 ml. and receiving sufficient quantities for the test in clean dried tubes.

All these tests were made by the following procedure :-

In a centrifuge tube marked at 10 ml. 2 ml. of Morgan's solution passed through the resin column were placed and mixed with the required quantity of sulphate and foreign anion to be tested. Then 1 ml. of 0.05M potassium hydrogen phthalate and 5 ml. of absolute alcohol were added and the tube was filled to the 10 ml. mark with distilled water, the solution being well mixed after each addition. 0.03 grm. of solid barium chloranilate was added and the tube was shaken for 5 minutes, centrifuged at 2500 r.p.m. for 5 minutes and readings were taken by the biochem absorptiometer. Results are shown in Tables 5, 6, 7, 8 and 9, p.41-43. The quantity of barium chloranilate added is roughly estimated by the quantity removed on the tip of a glass rod (2 mm. thickness) with a slight bend at its end dipped in the solid reagent. Excess of barium chloranilate in moderate amounts does not affect the determination as it will be removed

together with the precipitated barium sulphate by centrifuging but too great an excess might lead to high blanks despite that the solubility of barium chloranilate in 1:1 ethyl alcohol-water is only 5.2×10^{-5} mols per litre(71).

The effect of nitrates, phosphates, chlorides, carbonates, bicarbonates was neglible in concentrations as high as 1000 µgms (i.e. 100 p.p.m.) in each case with quantities of sulphates up to 800 µgms. (micro grams).

With the higher concentrations than 800 µgms. sulphate the anions interfered and the interference became more serious the higher was the concentration of sulphate. Thus the safe limit of the suggested method applied to SO_4 determination is between 50 - 800 µgms. i.e. 5 to 80 p.p.m. sulphate if the recommended procedure is to be followed.

Notes on the Method :-

(1) To test the effect of organic matter, determinations were made on two samples of a Morgan soil extract. To one of them the reagents were added directly without removing the organic matter. The other sample was treated with H_2O_2. No significant effect of the destruction of organic matter was apparent.

(2) No attempt was made to remove other anions as they are nominally present in amounts insufficient to interfere.

(3) For field work, a small shaker with a capacity of 42 tubes and hand driven centrifuge were designed and made in the College workshop. This apparatus designed is light (weight 13 - 15 pounds) and is shown in Plate I.
The shaker is hexagonal, carries 7 centrifuge tubes on each side. Each row of tubes is fixed by a long bar which holds them in position while shaking.
The centrifuge is designed with a head to take 20 tubes and to be capable of 2000 r.p.m.

PLATE I :- "SHAKER AND CENTRIFUGE DESIGNED FOR FIELD WORK."

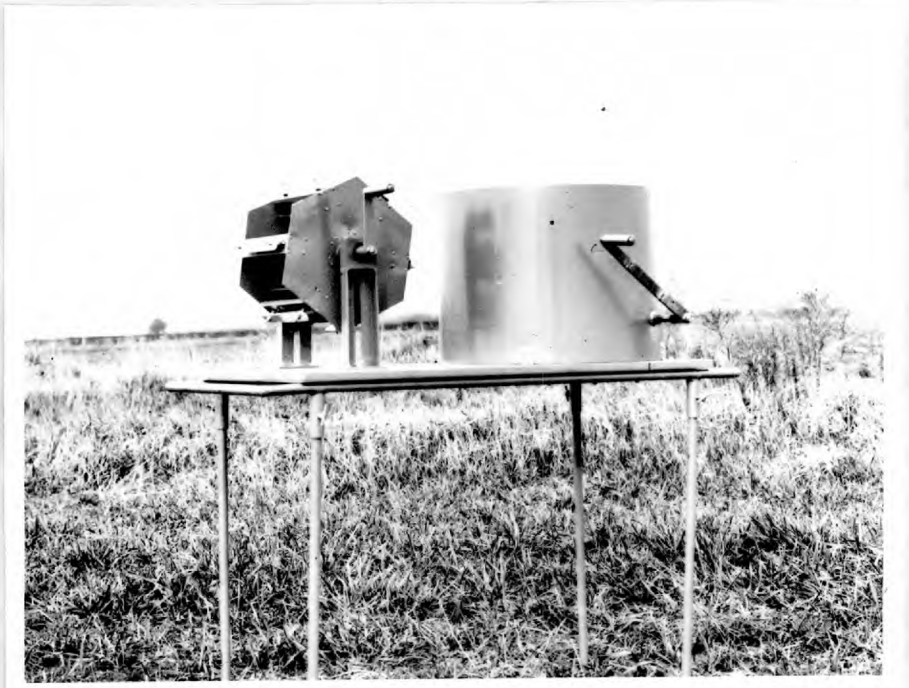


PLATE 1.

The Method Finally Adapted.

Apparatus :-

Biochem Absorptiometer (Adam Hilger).

1 cm. cell.

Green filter OG1.

Or

Lovibond Comparator with tubes.

Sulphate disc 5 - 80 p.p.m. range with screen.

Reagents :-

1- Zeo-carb 225 resin (H-form) (16 - 52 mesh) :- Take aliquot enough for the column required and soak it in double its volume of N-HCl for 1/2 - 1 hour with occasional stirring. Transfer to the column and wash it chloride free.

2- Standard sulphate stock solutions 1000 p.p.m. SO_4 Dissolve 1.814 gms. of potassium sulphate K_2SO_4 (A.R) in one litre of distilled water. Pass through a resin column of zeo-carb 225 (H-form) of 20 cm. in height and 1.5 cm. in diameter. Reject the first 40 - 50 ml. and collect the effluent in a dry clean flask.

3- Morgan reagent :- Dissolve 100 gms. of sodium acetate trihydrate (A.R.) in 500 ml. of distilled water. Transfer to one litre flask (volumetric) containing 30 ml. glacial acetic acid (A.R.) fill to the mark with

water, mix thoroughly. Pass aliquot of this through the resin column and collect it for use.

4- Barium chloranilate solid.

5- Ethanol (absolute).

6- 0.05 M potassium hydrogen phthalate solution :-
Dissolve 10.211 gms. of potassium hydrogen phthalate in ONE LITRE of distilled water.

7- B.D.H. wide range pH paper.

Method of Procedure :-

A- Calibration Curve :-

Run from a burette volumes of sulphate solution containing from 50 - 800 micro-grams of sulphate into a centrifuge tube marked at 10 ml. Add 2 ml. of Morgan's solution passed through the resin column, 1 ml. of phthalate solution, 5 ml. of alcohol and fill to the mark with distilled water mixing thoroughly after each addition. From the tip of a thin glass rod, add roughly 0.03 gm. barium chloranilate, stopper with a polythene top (rubber and cork are to be avoided) shake for 5 minutes, centrifuge at 2500 r.p.m. for another five minutes. Measure the absorption in the Biochem Absorptiometer or match with the disc.

The relation between the absorptiometer readings and the amount of sulphate in micrograms is then expressed graphically (Fig. 2).

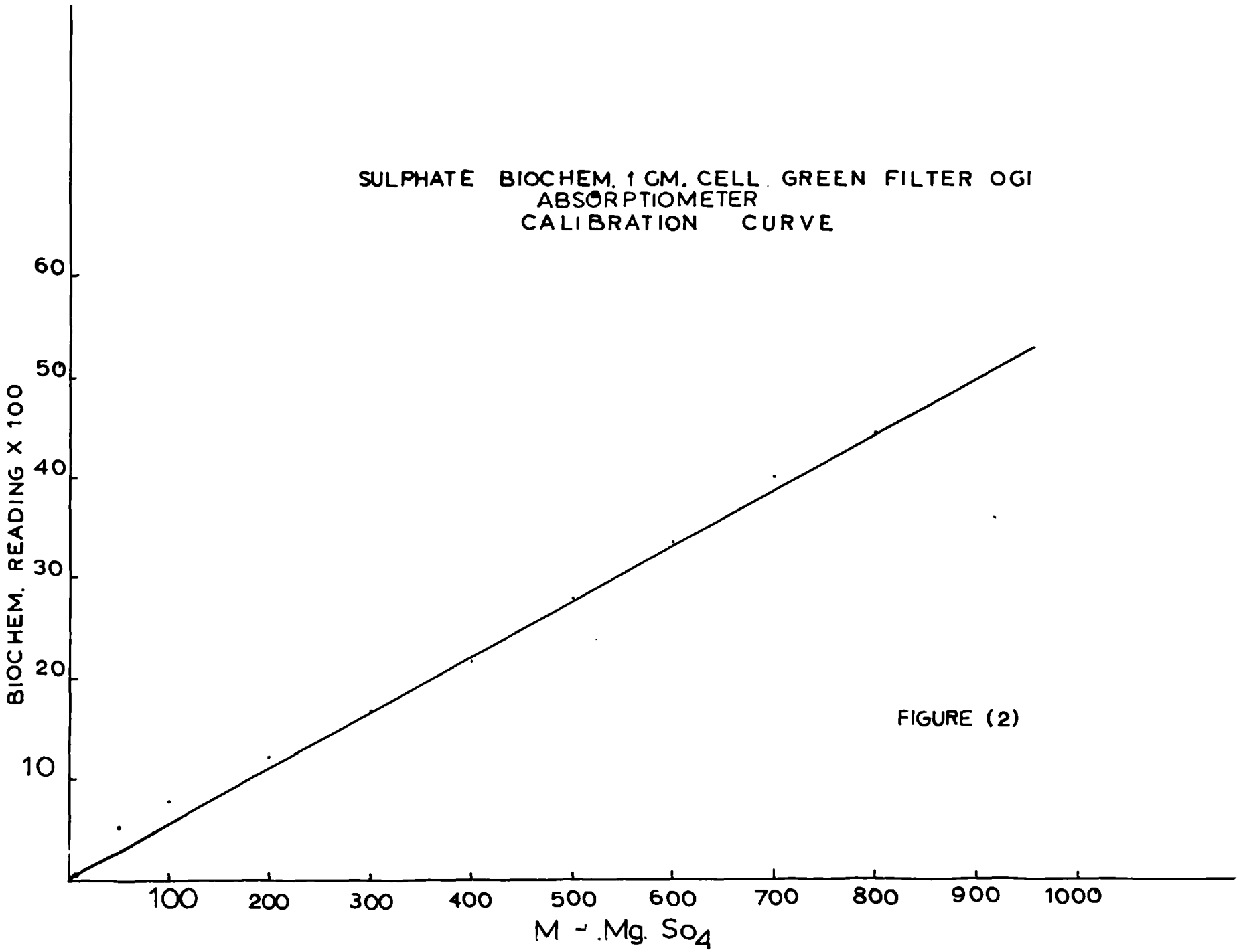

SULPHATE BIOCHEM. 1 CM. CELL GREEN FILTER OGI
ABSORPTIOMETER
CALIBRATION CURVE
BIOCHEM. READING X 100
60
50
40
30
20
10
100
200
300
400
500
600
700
800
900
1000
M - .Mg. So4

FIGURE (2)

B- Unknown Solutions :-

Shake 20 gms. of soil with 40 ml. of Morgan reagent and filter. Pass the extract through the resin column. Detect the removal of the cations by the change in the pH of the effluent using wide range pH papers (the pH of the effluent drops to 2) - reject the first 15 ml. and collect the next 2 ml. in a centrifuge tube and continue as in the procedure.

The amount of sulphate in the test solution is determined either by referring to the calibration curve or by matching, using the Lovibond Comparator, and the suggested disc.

SUMMARY

A colorimetric method for the determination of sulphate in Morgan extracts is worked out. The method is based on the highly coloured chloranilic acid ion liberated when solid barium chloranilate reacts with the sulphate solutions in a 50% alcoholic solution.

The serious interference by the cations is eliminated by prior treatment of the solution with ion exchange resin.

The anions which are likely to be present in normal agricultural soils (e.g. nitrates, phosphates, chlorides ...etc.) do not interfere to the 1000 microgram level on the determination of 800 micrograms of sulphate.

A coloured disc was made to cover the range from 50 - 800 μgms SO_4 i.e. 5 - 80 ppm to be used in the field.

A combined shaker and hand-driven centrifuge were designed for field work, easy to transport due to its light weight and convenient for determinations in the field.

The reaction is rapid, the colour produced under the experimental conditions described is stable for at least fifteen hours. The main modifications from the original method :-

1- The method suggested does not require preliminary adjustment of the pH.

2- The use of Zeo-carb 225- Hydrogen form instead of Dowex 50 x 8.

3- Morgan extracts were used instead of water extracts.

The method is accurate, reliable and of special value for the field work as well as laboratory investigations.

TABLE I

Choice of Filter :-

Micrograms SO_4	Absorptiometer Readings X 100		
	Dark Blue Filter OB1	Light Blue Filter OB2	Green Filter OG1
50	1.0	2.0	5.5
200	3.5	5.5	7.5
500	8.5	17.5	28.0
1000	20.5	45.5	56.0
2000	48.0	96.0	125.0

TABLE 2.

Standard Curves :-

Micrograms SO_4	Absorptiometer readings X 100	
	With distilled water only (average of 4 readings)	In the presence of 2 ml. of Morgan reagent passed through resin column. (average of 4 readings)
50	3.5	5.0
100	5.5	7.5
200	8.5	12.0
300	13.5	17.0
400	17.0	21.5
500	23.0	28.0
600	29.0	33.5
700	32.5	40.0
800	38.5	44.0
900	42.0	50.0
1000	48.0	56.0

TABLE 3.

Stability of Colour :-

Micrograms SO_4"	Absorptiometer readings X 100 (each determination is an average of 2 readings)				
	10 minutes	30 minutes	ONE hour	3 hours	15 hours
50	5.5	5.0	5.5	5.5	5.5
100	7.5	7.5	7.0	7.5	7.5
200	12.5	12.5	12.0	12.0	12.0
300	17.5	18.0	18.0	17.5	17.0
400	21.0	21.5	21.5	21.5	21.0
500	28.5	28.5	28.0	28.0	28.5
600	33.5	33.5	34.0	34.0	33.5
700	40.5	40.5	40.0	40.5	41.0
800	44.5	45.0	45.5	44.5	44.5
1000	56.0	56.0	56.5	56.0	56.5

TABLE 4

Recovery of sulphates from mixtures of standard sulphate and Morgan extracts of soil passed through the resin column independently and then mixed prior to the determination.

Test Solution	Microgram SO_4"		
	Theory	Found	% Error
2 ml. Harlington soil extract.	-	225	-
" " " " + 100 µgms SO_4"	325	305	-6.1
2 ml. Buxted soil Morgan extract.	-	160	
" Buxted soil " + 200	360	375	+ 4.1
2 ml. Silwood soil Morgan ext.	-	120	
" " " + 100 µgms SO_4"	220	210	- 4.5
100 µgms SO_4"	100	98	- 2.0
" " " + 100 µgms SO_4"	200	205	+ 2.5

TABLE 5.

Effect of Interfering Anions.

1- **Effect of Nitrates :-**

Micrograms NO_3^-	Absorptiometer Readings X 100				
	Micrograms $SO_4^=$				
	0	100	500	800	1000
0	-	7.5	28.5	44.0	56.5
100	-	7.5	28.0	44.5	56.5
500	-	7.5	29.0	44.0	57.5
1000	-	8.0	28.5	44.5	58.5
1500	-	9.0	29.5	46.5	61.5

TABLE 6

2- **Effect of Phosphates :-**

Micrograms $PO_4^{'''}$	Absorptiometer Readings X 100				
	Micrograms $SO_4^=$				
	0	100	500	800	1000
0	-	7.5	28.0	43.5	56.0
20	-	7.5	28.0	43.5	56.0
50	-	8.0	28.5	44.0	56.5
100	-	8.0	28.5	44.0	56.5
200	-	7.5	28.0	45.0	58.0
500	-	7.0	28.0	44.5	57.0
1000	1.0	8.0	29.5	44.5	58.5

TABLE 7.

3- Effect of Chlorides :-

Micrograms Cl'	Absorptiometer Readings X 100				
	Micrograms SO_4''				
	0	100	500	800	1000
0	-	8.0	27.5	44.0	56.5
100	-	8.5	27.0	43.5	56.0
500	-	8.0	28.0	43.0	57.0
1000	-	8.0	28.5	43.5	57.5
1500	2.5	9.5	29.5	45.5	58.5
2000	4.5	11.0	31.5	47.5	59.5

TABLE 8.

4- Effect of Carbonates :-

Micrograms CO_3''	Absorptiometer Readings X 100				
	Micrograms SO_4''				
	0	100	500	800	1000
100	-	7.5	28.0	43.5	57.0
500	-	7.5	27.5	44.0	56.0
1000	-	7.5	27.5	44.5	58.5
1500	2.5	9.0	30.0	46.5	59.5

TABLE 9.

5- Effect of Bicarbonates :-

Micrograms Carbonates	Absorptiometer Readings X 100				
	Micrograms SO_4"				
	0	100	500	800	1000
0	-	7.5	27.5	43.0	56.5
500	-	7.5	27.0	44.0	57.0
1000	-	8.0	28.0	43.5	57.0
1500	1.5	9.5	30.0	45.5	59.5
2000	3.5	11.0	31.5	46.5	61.0

Comparative study of sulphate content of Morgan soil extracts using gravimetric, turbidimetric methods and the suggested method was made to see how would the method suggested stand among the other methods in force. Results are in Table 10.

TABLE 10.

Comparison between gravimetric and turbidimetric techniques and the suggested Colorimetric method.

ppm SO_4" (even dry basis) each figure-average of 2 determinations

Morgan Soil extract tested.	Gravimetric method.	Turbidimetric method.	Suggested method
Buxted	14.2	13.7	16.8
Harlington	25.8	28.6	25.2
Silwood	12.0	19.5	12.5
Slough	13.0	13.2	13.6

A coloured disc was made covering the range from 50 - 800 µgms. SO_4"(i.e. 5 - 80 ppm. SO_4")-checked and used to compare the data with the Biochem Absorptiometer Table 11.

TABLE 11.

Sulphate content of soils as determined with the Biochem Absorptiometer and by Lovibond Comparator.

Soil	Comparator Disc.	Values obtained from the curves using the Biochem.
		ppm. SO_4"
Harlington	20	22.2
Silwood	10	11.6
Buxted	15	16.2
Garden	40	36.0
E. Pakistan (Paddi soil)	5	6.0

CHAPTER IV.

"Colorimetric Determination of Manganese in Soil Extracts using Leucomalachite green"

Introduction :-

Since the introduction of the periodate oxidation method by Willard and Greathouse, it has been applied to the manganese determination in both soil extracts and plant material. This is largely due to the fact that the method is simple fairly accurate and relatively free from interference. For very low manganese contents, however, the method is not sensitive enough Moreover, the method involves heating to boiling point which is not suitable for field work. 72, 73, 74, 75.

Several workers, reported the detection of minute quantities of manganese using p.p'-tetramethyldiaminodiphenyl methane - known also as tetrabase or methane base or Arnold's base.

Harry[76], [77], was the first to use this reagent for the determination of manganese quantitatively; ammonium persulphate (25% solution) was usedas an oxidant. He reported that the colour obtained varies with the manganese content of the test solution from pale blue at low concentration to purple with high concentration. Nicholas[78], studying manganese deficiency in plants, reported that he successfully used the tetramethyldiaminodiphenylmethane for detecting manganese deficiency in cereals, the formaldoxime method was not sensitive enough for the purpose. He used a saturated potassium periodate for oxidation and measured the blue colour initially formed with the tetrabase. The colour produced, however, was too bright to match and unstable. Moreover, Yuen[79] reported that at low temperatures (below 10°C), a

superficial mist forms on the walls of the optical cells and leads to errors in absorptiometry.

Cornfield and Pollard[80] applied the methane base for determining manganese in soil extracts and measured the colour ten minutes after the addition of the reagent but the colour formed was unstable and its rapid change made it difficult to obtain accurate measurements.

The use of a chemically related compound to the methane base, the "Leucomalachite green" or tetramethyl diamino triphenylmethane was reported by a number of workers determining manganese in biological materials. Schwarz, Fisher and Hagemann[81], used this compound in determining manganese in butter. They developed the colour in lactic acid medium and measured the colour after ten minutes from the addition of the colour-forming reagent. Gates and Ellis[82] determining manganese in small samples of blood serum and other tissues used p-p'-tetramethyldiaminotriphenylmethane and reported that it is approximately ten times as sensitive as the diphenyl compound. They used potassium periodate in a mixture of nitric and phosphoric acids at 100°C for oxidation of manganous ions and measured the yellow colour produced with p-p'-tetramethyldiaminotriphenylmethane at 80°C.

Recently, Yuen[79], made use of the same compound for the determination of manganese in plant digests. He reported that the results obtained are in good agreement with the permanganate method with a precision of approximately ± 5%. The principle

of the method is the oxidation of manganous ions to permanganic acid at room temperature using potassium periodate for the purpose. The reaction is made to proceed in acetate-acetic acid buffer solution at 3.35 pH and use is made of the colour produced as a result of the oxidation of the leucomalachite green by the permanganic acid formed to determine minute quantities of manganese. The present study was undertaken with a view to using this reaction in the determination of manganese in Morgan extracts of soils and soil solutions with a form suitable for field work. Three problems were investigated :-

(1) The possibility of the use of leucomalachite green as a colour-forming agent in the presence of Morgan's reagent.
(2) The effect of the interfering ions on the colour formed in the presence of Morgan's solution.
(3) The stability of the colour.

The tests were made with manganese standards containing 0.03 to 1 micrograms of manganese. All the trials were made in 10 ml. tubes with a view to making a permanent coloured disc to be used in the field determinations. The Hilger Biochem Absorptiometer was used with a red Chance filter OR_2 to establish the method. From a preliminary investigation it was found preferable to change the concentration of the leucomalachite green to 0.04% instead of 0.1% as the latter gave very high blanks.

Effectb of Morgan reagent on the colour formed:-

The following reagents prepared as follows were used for

this investigation :-

1 Manganese Standard Stock Solution :- 0.203 gms of $MnSO_4$. $4H_2O$, A.R. are dissolved in water containing 2 ml. of conc. H_2SO_4 diluted to one litre. This solution contains 50 micrograms of manganese/ml.

2 Standard Solution"A" :- 2 ml. of the above solution are diluted with distilled water to one litre. This solution contains 0.1 microgram manganese/ml.

3 Standard Solution "B" :- 50 ml. of Solution "A" are diluted to 250 ml. with distilled water. This solution contains 0.02 micrograms/ml.

4 Morgan's Reagent :- 100 grms of sodium acetate trihydrate A.R. are dissolved in 800 ml. of distilled water, 30 ml. of glacial acetic acid A.R. are added and the volume made up to one litre.

5 Buffer Solution :- 50 grms of sodium acetate trihydrate A.R are dissolved in one litre of distilled water containing 400 ml. of glacial acetic acid A.R.

6 Potassium Periodate Solution :- 0.2% solution = 1 grm of KIO_4 dissolved in 500 ml. of distilled water.

7 Leucomalachite green :- 0.04% solution = 0.1 grm leucomalachite green dissolved in 250 ml. of distilled water containing 1.25 ml. of conc. HCl.

Effect of Morgan's Reagent on the Colour developed :-

To test the effect of Morgan's reagent on the colour developed, four sets of standards were prepared, the first with no Morgan reagent, the second with 0.1 ml. Morgan reagent, the third with 0.3 ml. Morgan reagent and the fourth with 0.5 ml. of Morgan reagent. To each solution tested, 1 ml. of buffer was added, 1 ml. of 0.2% potassium periodate, mixed thoroughly after each addition with a thin glass rod bent at its end to aid mixing and the tubes were left to stand for 10 minutes at room temperature (23°C), then 0.5 ml. of the leucomalachite green solution was added and the tubes were filled to the mark with distilled water, and the contents mixed thoroughly. Readings were taken 45 minutes later. The absorptiometer was adjusted to give a zero reading with the "blank" test. The results are in Table 12 and Fig. 3, 4, 5 and 6. It is observed that Morgan's reagent up to 0.3 ml.per test does not affect the colour formation or stability. The colour developed with 0.4 and 0.5 micrograms of manganese in the absence or presence of Morgan' reagent was too fugitive for satisfactory measurement when developed in the 10 ml. tubes. On the other hand, the presence of 0.5 ml. of Morgan's reagent caused an appreciable decrease in colour intensity. With more than 0.5 micrograms of manganese, the solutions developed a deep violet to pink colouration. It was decided then to use only 0.1 and 0.3 ml. of Morgan's reagent in the following tests and the quantities of manganese were limited to 0.5 micrograms in test solutions.

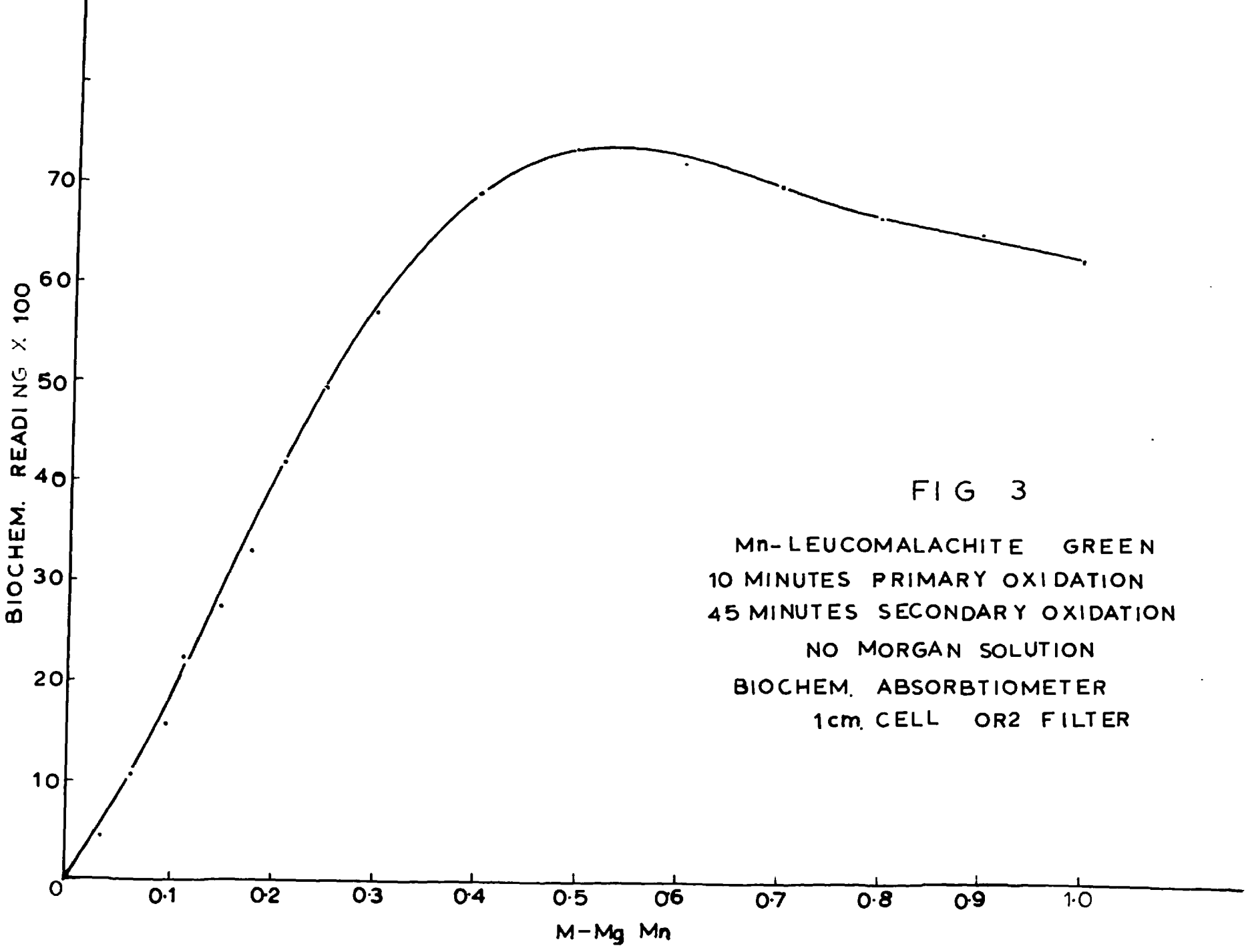


FIG 3

Mn-LEUCOMALACHITE GREEN
10 MINUTES PRIMARY OXIDATION
45 MINUTES SECONDARY OXIDATION
NO MORGAN SOLUTION
BIOCHEM. ABSORBTIOMETER
1cm. CELL OR2 FILTER

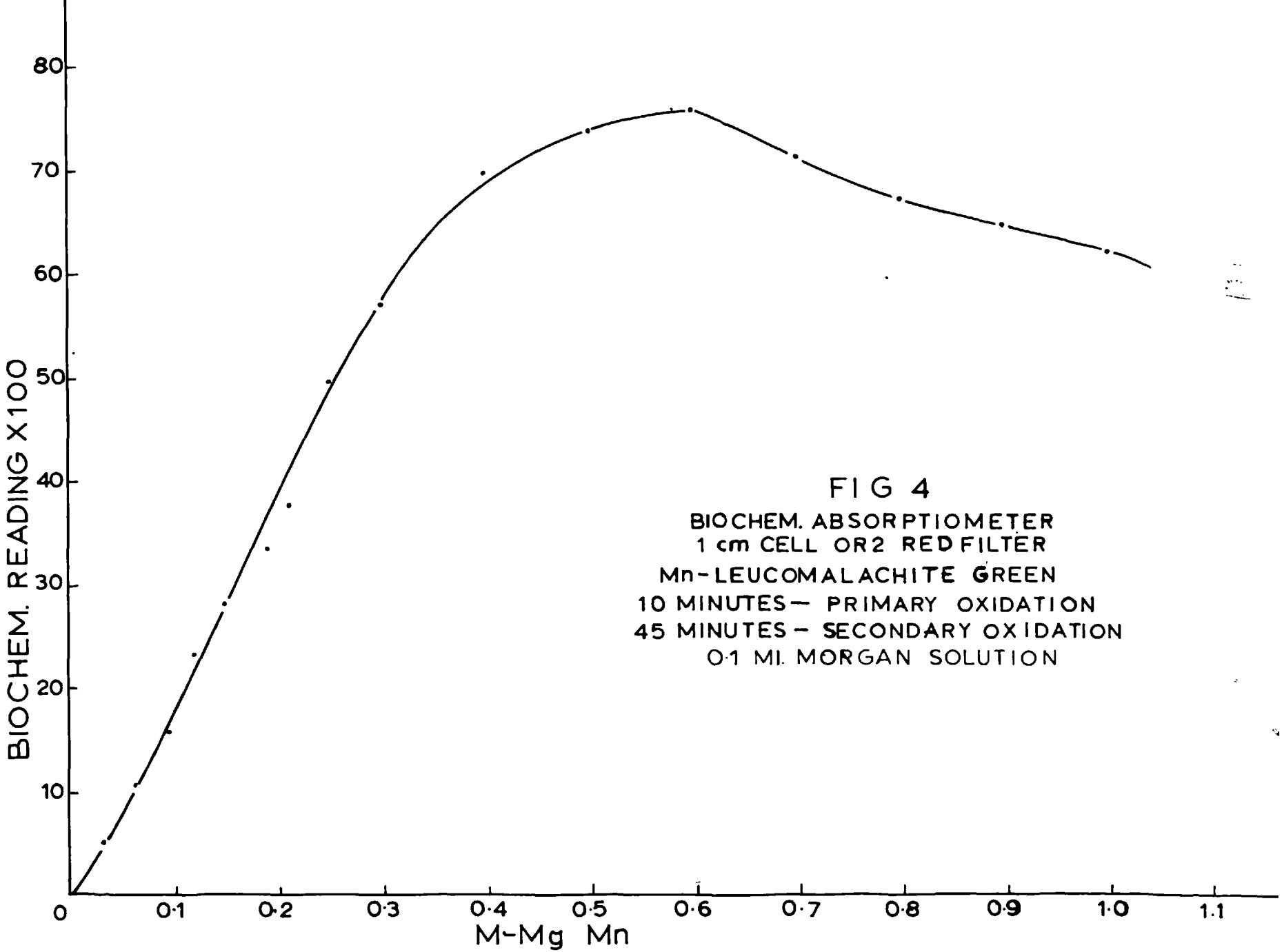


FIG 4

BIOCHEM. ABSORPTIOMETER
1 cm CELL OR2 RED FILTER
Mn-LEUCOMALACHITE GREEN
10 MINUTES— PRIMARY OXIDATION
45 MINUTES – SECONDARY OXIDATION
0·1 MI. MORGAN SOLUTION

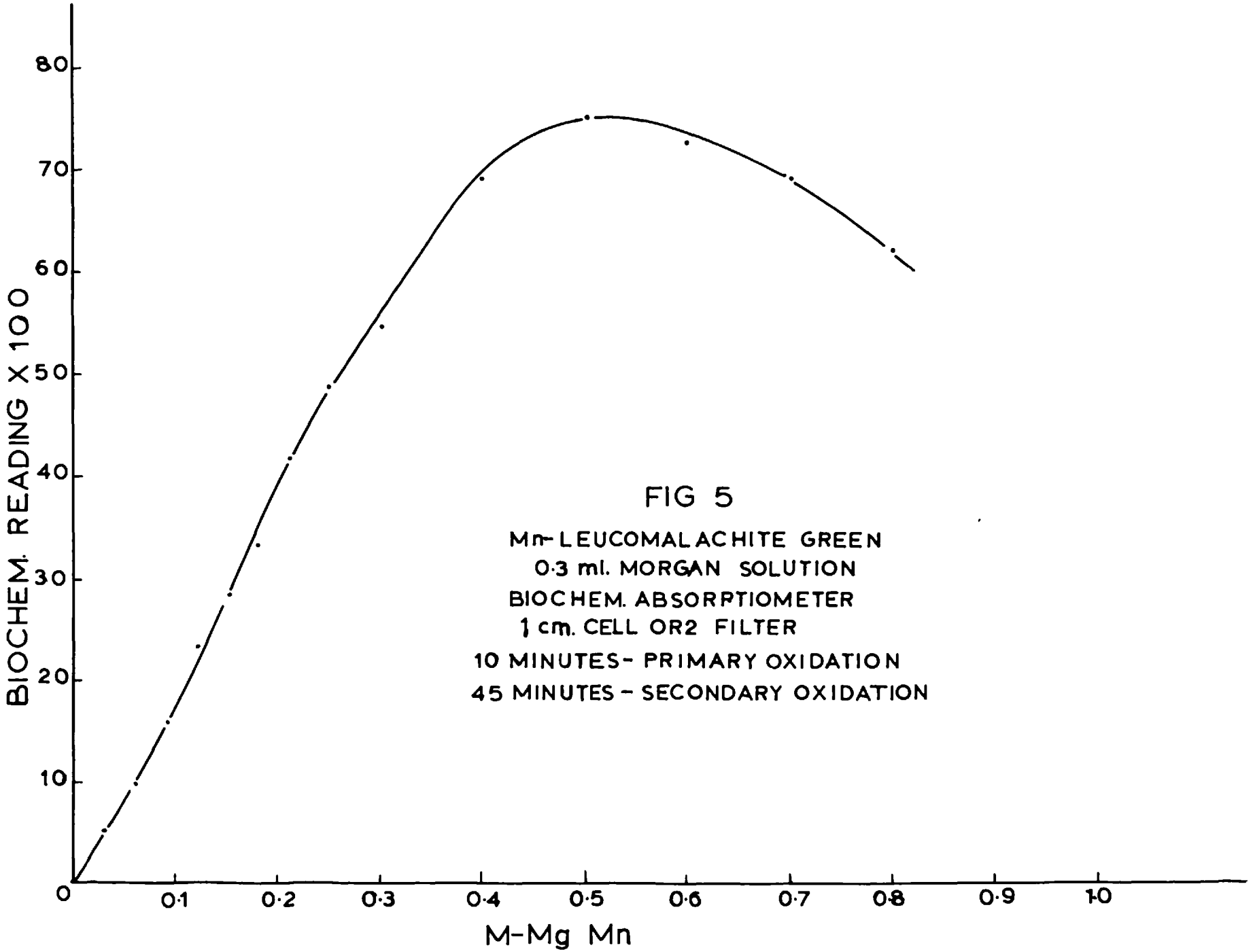


FIG 5

Mn-LEUCOMALACHITE GREEN
0·3 ml. MORGAN SOLUTION
BIOCHEM. ABSORPTIOMETER
1 cm. CELL OR2 FILTER
10 MINUTES- PRIMARY OXIDATION
45 MINUTES - SECONDARY OXIDATION

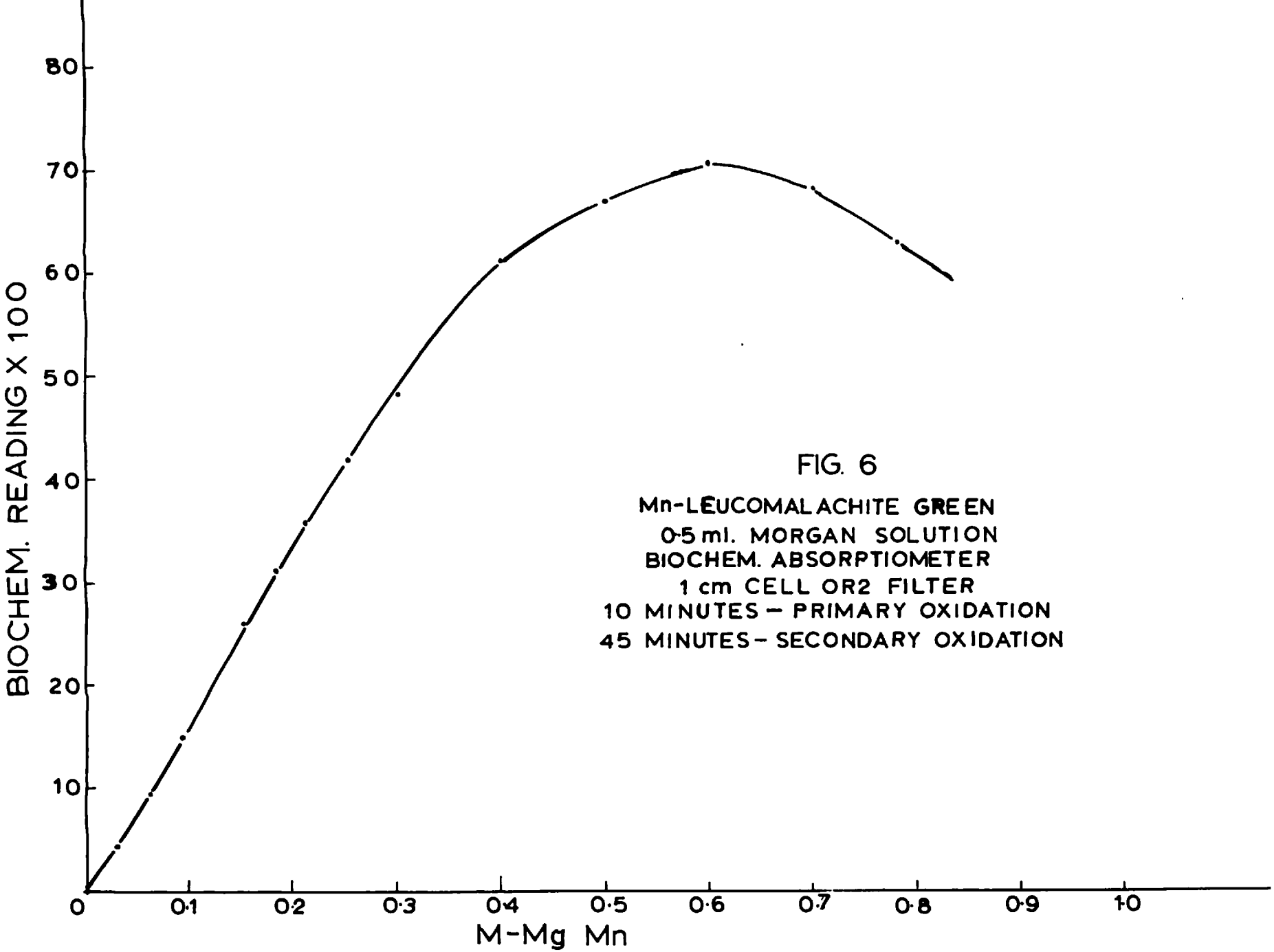


FIG. 6

Mn-LEUCOMALACHITE GREEN
0·5 ml. MORGAN SOLUTION
BIOCHEM. ABSORPTIOMETER
1 cm CELL OR2 FILTER
10 MINUTES – PRIMARY OXIDATION
45 MINUTES – SECONDARY OXIDATION

Effect of time of Primary and Secondary Oxidation on the stability of the Colour formed in the presence of Morgan's Reagent:-

Fixing the time of the primary oxidation (i.e. the oxidation of the manganous ions to permanganic acid with the periodate) at 10 mimutes, the effect of time of secondary oxidation was tested at 30, 45, 60 and 80 minutes to find out the shortest time needed for this oxidation to ensure the formation of a stable colour. In thid experiment, standards containing from 0.03 to 0.3 micrograms of manganese were used. The sensitivity of the method suggested the test should be made in the presence of 0.1 ml. of Morgan's reagent with a view to using 0.1 ml. of the soil extract. Results are shown in Table 13 & Fig 4. It is observed that the colour formation increases considerably till the forty-five minutes limit and seems to settle afterwards as no appreciable increase took place in the last thirty five minutes of the experiment (from 45 - 80 minutes). It was decided to take readings at 45 mimutes after mixing with leucomalachite green.

Effect of time of primary oxidation on the stability of the colour formed in the presence of Morgan's Reagent:-

Fixing the time of the secondary oxidation at 45 minutes, the effect of time of primary oxidation was examined. The same experimentb was repeated but adding the leucomalachite green 10, 20 and 30 minutes after the addition of the buffer and periodate to the standards in the presence of 0.1 ml. of Morgan's reagent. Results are shown in Table 14, p. 63. It is quite clear from the

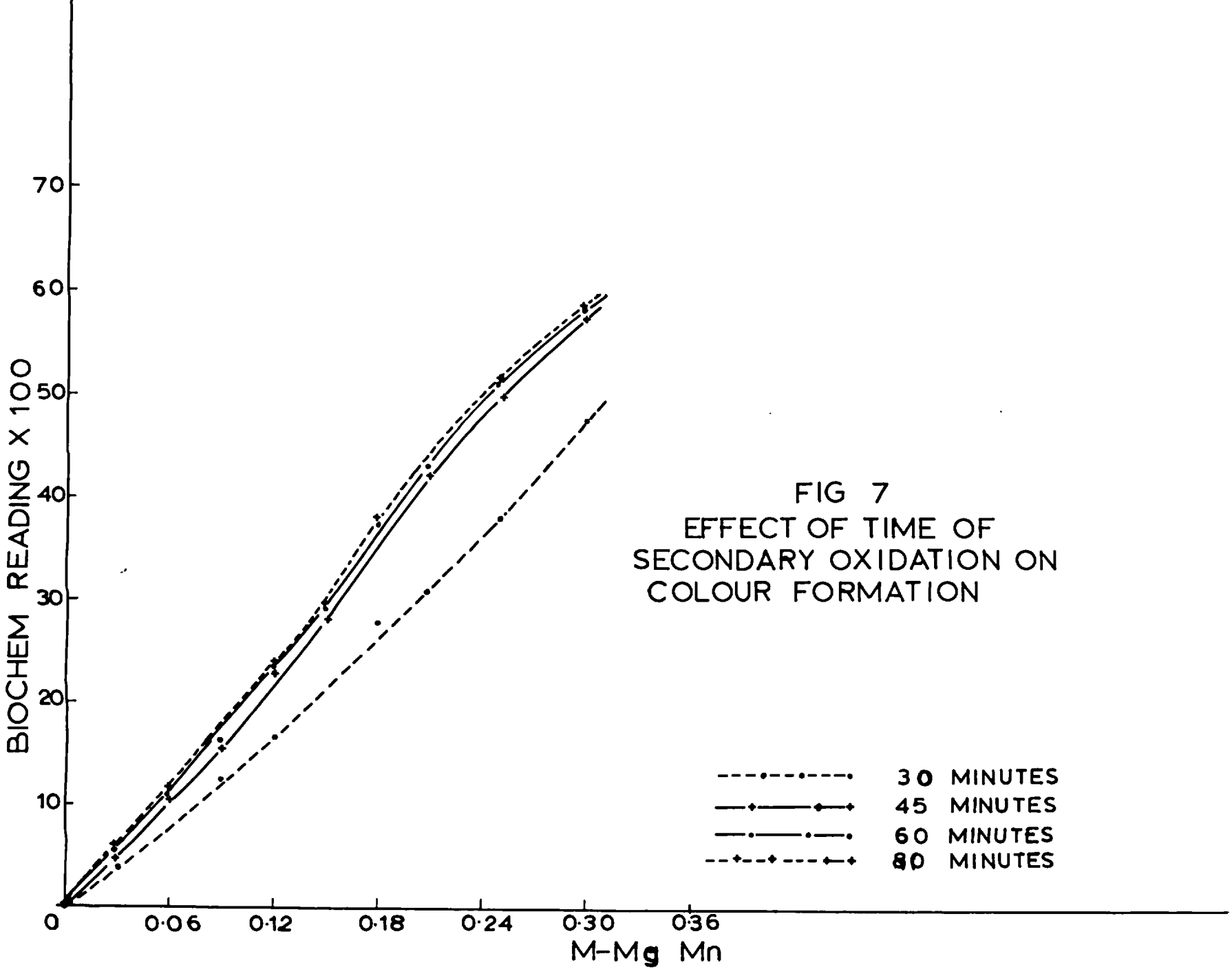


FIG 7
EFFECT OF TIME OF SECONDARY OXIDATION ON COLOUR FORMATION

results that no advantage could be gained by extending the time for the primary oxidation beyond ten minutes.

Interferences :-

1 - Tungsten :- At the earliest stages of this work, tungsten was thought to be the cause of the appearance of the pale violet colouration. Sandell[83] reported that tungsten gives a very similar colour with leucomalachite green. Sodium tungstate was used to clarify this point and it was found that up to 150µgms of tungstate had no effect on the colour formed with 0.3 µgms manganese. Moreover, when tungstate alone was examined, it gave no colour with leucomalachite green. Table 15, p.64. With the progress of the work, it was noticed that the violet and pink colours are always associated with comparatively high manganese content in the soil. Also, the standard solutions above the 0.5µgm level showed such colouration and with 0.8 µgm manganese the whole solution turned pink, in some cases before the addition of the leucomalachite-green solution and in others immediately after the addition of the colouring reagent. It was concluded that the maximum quantity to be determined in the presence of 0.1 ml. of Morgan reagent is only 0.5 µgm manganese.

2 - Effect of Acetates = (Morgan Reagent) :-

Standards containing 0.1, 0.3 and 0.5 ml. of Morgan solution were examined, Table 12, p.61, and the data obtained showed clearly that only 0.1 ml. - 0.3 ml. had no effect can be used, on the colour of the standards as compared with the standards without Morgan solution. The set of standards containing 0.5 ml.

Morgan solution showed an appreciable decrease in the colour. 0.1 ml. of Morgan reagent contains 7.41 mgm acetate and thus up to 22.2 mgm acetate added would not affect the determination if the recommended procedure were to be followed.

3 - Ferric and Ferrous Iron :-

To test whether ferric and ferrous iron interfere with the determination, two sets of standards were prepared adding to each 0.1 ml. of Morgan'reagent and different amounts of iron both in the ferric and ferrous state, Table 16 shows the results. The results show that ferric iron interferes if it is present in concentrations as low as 10 µgms. Ferrous iron on the other hand, shows more serious interference; the presence of over 6 µgms of ferrous iron causes a considerable reduction in the colour.

All trials to eliminate the effect of ferric iron using fluorides, oxalates or citrates were unsuccessful. Due to the fact that only 0.1 ml. of Morgan extract of soil is enough for the determination owing to the great sensitivity of the method, this interference is not as serious as it appears. When applying the method to the determination of manganese in soil- extracts it was necessary in several cases to dilute the soil-extract 10 times or 50 times or sometimes 100 times. This greatly reduces the interference of iron or even eliminates it altogether.

Interference of Aluminium :-

A set of manganese standards containing 0.03, 0.12 and 0.3 µgms manganese was used, to each 0.1 ml. of Morgan's reagent

was added and varied quantities of aluminium as aluminium sulphate were used . The results are shown in Table 17, p.66. Aluminium interferes in the determination of manganese, the interference increases with manganese concentration. The presence of twenty micrograms of aluminium causes an appreciable increase in colour of the 3 standards used and 15 µgms of aluminium is taken as the maximum amount to be present without introducing any measurable interference with the precision of the suggested method in the presence of Morgan reagent. Taking into consideration that only 0.1 ml. of the soil extract is to be taken for analysis, such high quantities of aluminium are not likely to be present in Morgan extracts of soils under normal field conditions.

Application to Soils :- Twelve soils of different pH were examined for their Morgan extractable manganese. The pH of the soils used ranged from 4.0 to 8. In a preliminary investigation it was noticed that the colour did not reach its maximum until after 80 minutes from mixing with leucomalachite green, and it was decided to increase the time of the primary oxidation to twenty minutes and the time of the secondary oxidation to eighty minutes, whereby very stable colours were obtained. It seems possible that reducing agents existing in soil extracts may cause this retardation in the presence of process of oxidation of the manganous ions and in turn that of leucomalachite green to malachite green. 0.1 & 0.3 ml. of Morgan extracts of soil were utilised in the determination of extractable manganese. Data obtained

are compared with determinations made by the "permanganate" method. Data are shown in Tables 18 and 20 & 69. The results of the soil analysis show consistently that when 0.3 ml. of Morgan extract is taken for analysis, values obtained are lower than when 0.1 ml. of the extract is used. The difference was so clear that it was decided to use 0.1 ml. of the spil extract for analysis.

Recovery of added known amounts of Manganese to Morgan soil extracts and to standard solutions :-

The recovery tests were made on soil extracts with comparatively low manganese content to avoid the appearance of the pink colouration caused by higher concentration than 0.5 µgms of manganese. 0.1 ml. of the extract was pipetted into a 10 ml. tube and diluted with distilled water to 5 or 6 ml. One ml. of the buffer and 1 ml. of the periodate were added. After 20 minutes leucomalachite green was added and readings were taken 80 minutes later using a 1 cm. cell and 802 filter. The results are shown in Table 19.

At this stage it was decided to make a coloured disc covering a range from 0.03 - 0.3 micrograms of microgram manganese. The disc was checked against a set of standard solutions and it was approved for use. Due to the brightness of the colour v produced, a dulling screen is used with it.

"The Method as Finally Adapted"

Apparatus :-

Biochem Absorptiometer.

One-cm. cell.

Red filter OR2.

or Lovibond Comparator.

Manganese disc with dulling screen covering the range from 0.03 - 0.3 micrograms manganese .

Comparator tubes marked at 10 ml.

Reagents and Solutions :-

(1) Morgan Reagent :- Dissolve 100 gms of sodium acetate trihydrate(A.R.) in 500 ml. of distilled water, transfer to a litre volumetric flask. Add 30 ml. of glacial acetic acid (A.R.) and dilute with water to the mark.

(2) Stock Manganese Solution (if Biochem absorptiometer is to be used):- Dissolve 0.203 gms of $MnSO_4.4H_2O$ (A.R.)in one litre of distilled water containing 2 ml. of concentrated H_2SO_4 (A.R.). This solution contains 50 µ/gms Mn/ml.

(3) Standard Solution "A" :- From the stock solution pipette 2 ml. accurately into a one-litre volumetric flask and complete to the mark with distilled water. This solution contains 0.1 microgram Mn/ml.

(4) Standard Solution "B" :- From solution "A" pipette 50 ml. into a 250 ml. volumetric flask and complete to the mark

with distilled water. This solution contains 0.02 micrograms Mn/ml.

(5) Buffer Solution :- Dissolve 50 gms sodium acetate trihydrate (A.R.) in a litre of distilled water containing 400 ml. of glacial acetic acid (A.R.).

(6) Potassium Periodate :- Prepare 0.2% periodate solution by dissolving 1 gm of KIO_4 (A.R.) in 500 ml. of distilled water. This solution must be prepared freshly every week.

(7) Leucomalachite green Solution :- Prepare 0.04% solution by dissolving 0.1 gm of leucomalachite green in 250 ml. of distilled water containing 1.25 ml. of concentrated HCl (A.R.). This solution should be prepared freshly every five days.

Procedure :-

A. Calibration Curve :- Run from a burette volumes of the dilute manganese solutions (use Standard Solutions A and B prepared) containing from 0.03 - 0.3 μ/grm into the 10 ml. tubes. Add to each standard 0.1 ml. Morgan reagent. Dilute with water up to 6 ml., add 1 ml. of buffer and 1 ml of periodate solution. Mix thoroughly and leave to stand for 20 minutes. Add 0.5 ml of leucomalachite green, complete to the mark and mix thoroughly. Take a reading in

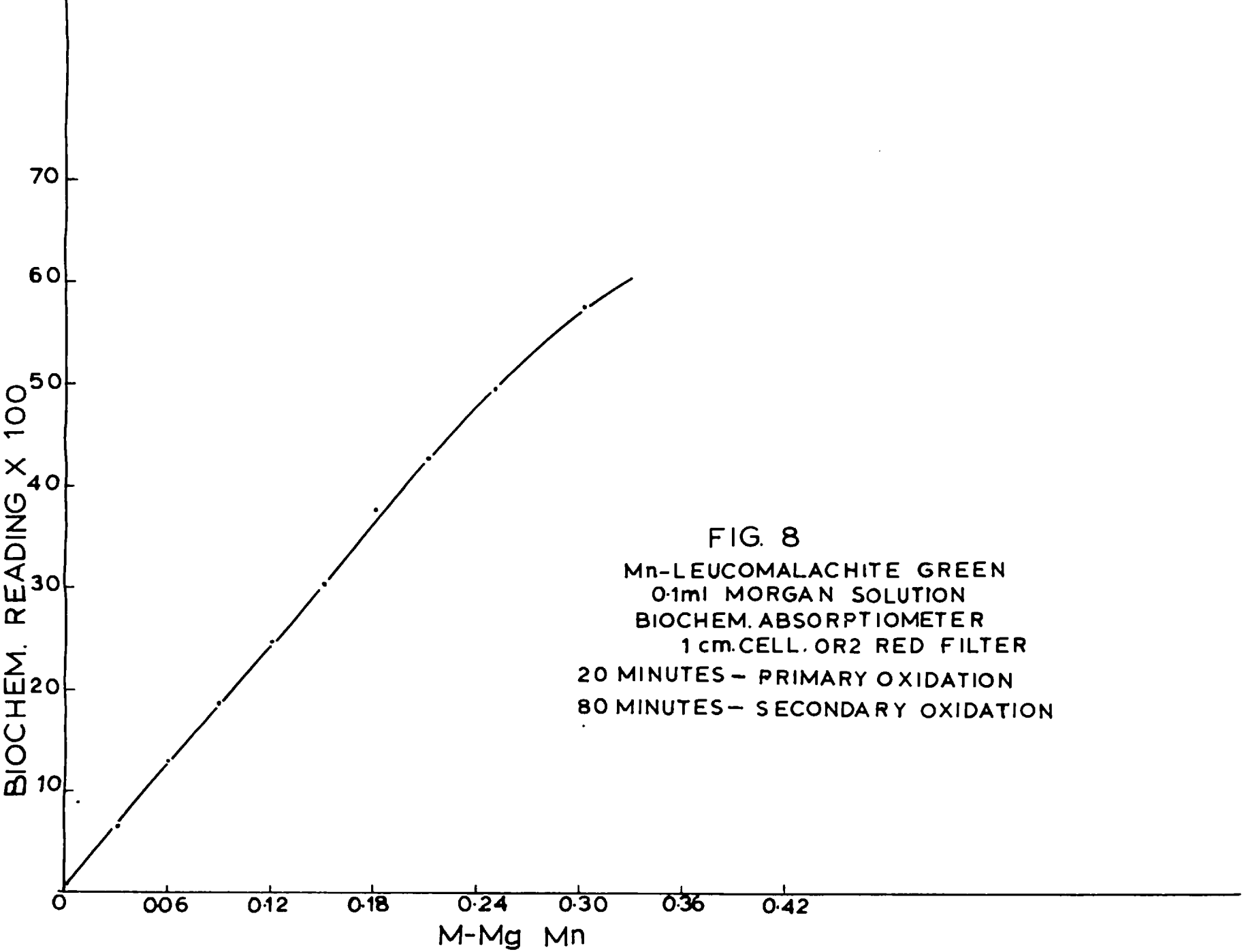


FIG. 8

Mn-LEUCOMALACHITE GREEN
0·1ml MORGAN SOLUTION
BIOCHEM. ABSORPTIOMETER
1 cm. CELL. OR2 RED FILTER
20 MINUTES – PRIMARY OXIDATION
80 MINUTES – SECONDARY OXIDATION

the Biochem absorptiometer using a 1 cm. cell and red filter OR2 after 30 minutes.

For field tests use the Lovibond Comparator and the suggested disc.

B. Unknown Solutions :-

Pipette accurately 0.1 ml. of the Morgan extract (10 gms of soil shaken vigorously for one minute with 20 ml. of Morgan reagent, filtered through a No. 1 Whatman filter paper and collected in a dry flask) into a 10 ml. tube, dilute with water to five or six ml., add buffer, periodate, mix thoroughly and stand for twenty minutes, add 0.5 ml. leucomalachite green, take absorptiometer readings. The amount of manganese in the test solution is determined by referring to the calibration curve (see ~~curve 7~~ Fig. 8). Alternately, match against the permanent colour standards using the suggested disc and daylight, a north light is preferable whenever possible.

Notes on the Method :-

(1) It was noticed that if the tubes used were not rinsed with chromic-sulphuric acid mixture before the determination, no colour would be developed. It seems possible that the cleaning mixture activates the glass surface and has something to do with the reaction. The maximum time recorded from the time of rinsing in this laboratory is 6 - 8 hours after which the colours become irregular if developed. Accordingly, advice is given to rinse the tubes with chromic acid preferably

immediately before the test and not more than 6 hours before the test is done.

(2) Effect of organic matter and Colour of the Extract on determinations:-

Due to the sensitivity of the suggested method (the original extract is diluted at least 100 times) it was obvious that even in the highly coloured extracts no interference would be expected. However, the apparent instability of of the colour formed in the presence of the soil extracts which did not appear while working with standard solutions may be due to the presence of some reducing compounds in the organic matter extracted with Morgan's reagent. Also it is known that soil humus may also work as an oxidation-reduction system and this might affect the mechanism of the reaction. Unfortunately, the mechanism of the reaction of production of malachite green from the leucomalachite green is not well established. Swain[84] however, attempted to explain it. They suggest that the mechanism is similar to that of oxidising agents of high potentials. They obtained evidence that carbinol is not the intermediate stage in the conversion of leucomalachite green to the dye in aqueous solutions at room temperature using ceric sulphate as an oxidising agent. Accordingly, they suggested that the leucomalachite green is oxidised directly to the malachite green.

and Hedberg,

It is possible that this is also the case while oxidising the ~~leuco-base~~ manganese to the dye by potassium periodate to permanganate, ~~being intermediate~~ This and, in turn, oxidising leucomalachite green to malachite green. The speed with which the dye is formed on the addition of the leuco-base, suggests the improbability of the formation of an intermediate stage.

(3) Effect of Temperature :-

The reaction is affected by wide differences in temperature but it was found that between 20°C & 30°C there is no appreciable effect on the colour formed. Below 20°C however, the colour developed comparatively slowly and above 30°C the colour developed rapidly and irregularly. A series of tests on both standard solutions and soil extracts were made at 15°C, 20°C, 25°C, 30°C and 35°C. 0.1 ml. of Morgan extracts of the soils or the required standard to which 0.1 ml. of Morgan reagent is added was pipetted into 10 ml. tubes. One ml. of buffer and one ml. of periodate were added after dilution to six ml. with water, mixing thoroughly after each addition. The tubes were left in water baths (250 ml. beakers were used) for 20 minutes and then leucomalachite green solution was added and readings were taken after 80 minutes. Results are shown in Tables 21 and 22, p 70

SUMMARY.

A sensitive method for the determination of manganese in Morgan extracts has been adapted for use in diagnosing manganese-deficient soils.

The only serious interference seems to come from ferrous and ferric salts but due to the sensitivity of the method to manganese, iron interference is reduced to a great extent by dilution. The methods is sensitive to 0.03 - 0.5 μ/gm.Mn and 10 μ/gm. Fe^{+3} were needed to cause interference on the recommended procedure. Accordingly, the concentration of the iron should be at least 20 times that of Mn in the extract to cause serious interference.

Wide variations in temperature affected the colour formationbut standard solutions and Morgan extracts did not show serious error when the temperature was raised from 20°C to 30°C. Temperature seems to increase the rate of the reaction but not the intensity of the colour.

The maximum time for the primary oxidation is 20 minutes and for the final oxidation is 80 minutes. The colour was stable for two hours after the 80 minutes limit and no change could be traced in the colours during these two hours. After this time however, the colour started to fade.

The method was also very useful in studying manganese levels in different soils solutions.

TABLE 12.

Effect of Morgan's Solution:-

Test Solution microgram Mn.	Absorptiometer Readings x 100 (each reading is an average of 4 samples).			
	No Morgan added	0.1 ml. Morgan added	0.3 ml. Morgan added	0.5 ml. Morgan added
Blank (0.0)	3.0	3.0	4.0	4.5
0.03	5.0	5.0	5.0	4.5
0.06	11.0	10.5	9.5	9.5
0.09	16.0	15.5	15.5	15.0
0.12	23.0	23.0	22.0	21.5
0.15	28.0	28.0	27.5	26.0
0.18	33.5	33.0	34.0	31.5
0.21	42.5	42.5	41.5	36.0
0.25	50.0	49.5	48.5	42.0
0.30	57.5	57.0	54.5	48.5
0.40	70.0	70.0	69.0	61.5
0.50	75.0	74.0	75.0	67.0
0.60	73.0	76.0	72.5	71.0
0.70	71.0	71.5	69.0	68.5
0.80	68.0	67.5	62.0	+
0.90	66.5	65.0	+	+
1.00	64.0	62.0	+	+

N.B. :- All the samples marked + showed violet to pink colour and readings werenot taken.

TABLE 13.

Effect of time of 2 dy. oxidation on the stability of the colour in presence of Morgans Reagent.

Test Solution (each with 0.1 ml. Morgan reagent)	Readings after 30 minutes	45 mins.	60 mins	80 mins.
Blank	2.5	3.0	3.0	3.5
0.03 µ/gm. Mn.	4.0	5.0	5.5	5.5
0.06 " "	7.5	10.5	11.0	11.5
0.09 " "	12.5	15.5	16.0	16.5
0.12 " "	16.5	23.0	23.5	23.5
0.15 " "	25.5	28.0	28.5	29.0
0.18 " "	28.0	37.5	37.5	38.0
0.21 " "	31.5	42.5	42.0	42.5
0.25 " "	38.0	50.0	50.0	51.0
0.30 " "	47.5	57.5	58.0	58.5

It is observed that the colour formation increases considerably till the forty-five minutes limit and seems to settle afterwards as no appreciable increase took place in the last thirty-five minutes of the experiment. It was decided to take readings at forty-five minutes after mixing with leucomalachite green.

TABLE 14.

Effect of time of 1 ry. oxidation on the stability of the colour formed in the presence of Morgan's Reagent.

Test Solution	Absorptiometer Readings x 100 (each reading is an average of 4 samples).		
	10 minutes for primary oxidation	20 minutes	30 minutes
Blank.	3.5	3.5	3.5
0.030 µ/gm. Mn.	5.0	5.0	5.0
0.060 " "	11.0	10.5	11.0
0.090 " "	16.0	16.5	16.5
0.120 " "	23.5	24.0	25.0
0.150 " "	28.0	29.0	28.0
0.180 " "	38.5	39.0	39.0
0.210 " "	43.0	42.0	43.0
0.250 " "	50.0	51.0	50.5
0.300 " "	57.0	58.0	57.0

TABLE 15.

Effect of Tungstate :-

Using Sodium tungstate.

Tungstate added µ/gm.	Absorptiometer Readings x 100 (each reading is an average of 2 samples.		
	0.03 µ/gm. Mn.	0.12 µ/gm.Mn.	0.3 µ/gm.Mn.
0.0	6.0	23.0	56.0
10.0	5.5	24.0	56.0
20.0	5.5	23.0	57.0
50.0	6.5	23.0	56.0
75.0	6.0	23.5	57.5
100.0	5.0	24.0	56.0
150.0m	5.5	23.0	56.0

TABLE 16.

Interference of Ferric and Ferrous Iron :-

Iron Added	Absorptiometer Readings x 100 (each reading is an average of two determinations).		
	0.03 µ/gm. Mn.	0.12 µ/gm. Mn.	0.3 µ/gm. Mn.
Ferric iron :-			
0 µ/gm.	5.5	23.0	58.0
2 "	5.5	23.0	57.5
4 "	5.0	23.5	58.0
6 "	5.5	23.0	57.0
8 "	4.5	24.0	58.0
10 "	4.0	22.0	56.0
15 "	3.5	19.0	54.0
20 "	2.5	15.0	50.0
Ferrous iron :-			
0 µ/gm.	5.0	23.0	57.0
2 "	5.0	23.0	58.0
4 "	4.5	23.5	57.5
6 "	3.5	21.5	51.0
8 "	3.0	19.0	48.0
10 "	2.5	17.5	45.5
15 "	----	----	----
20 "	----	----	----

N.B.:- Concentrations above 20 µ/gm. of ferric iron caused turbidity in the solutions. The test is carried out in the presence of 0.1 ml. Morgan reagent in each manganese standard solution.

TABLE 17.

Interference of Aluminium :-

Aluminium added.	Absorptiometer Readings x 100 (each figure is an average of two determinations).		
	0.08 µ/gm. Mn.	0.12 µ/gm. Mn.	0.3 µ/gm. Mn.
0. µ/gm. Al.	5.0	23.5	57.0
1. " "	5.0	23.0	57.5
2. " "	5.5	24.0	57.0
4. " "	5.0	23.5	58.0
8. " "	5.0	23.5	57.5
12. " "	5.5	24.0	58.0
15. " "	5.0	25.0	58.0
20. " "	6.5	25.5	59.5
25. " "	6.0	26.0	60.0
30. " "	6.5	27.5	61.5

TABLE 18.

Application of Method to analysis of Morgan Soil Extracts :-

Morgan Extractable Mn expressed as p.p.m. oven dry soil (each determination is an average of 2 samples.

Soil Tested	pH.	Original Soil Extract.		Ten times Dilution.		Fifty times Dilution.		Average	
Soil-I		0.1ml.	0.3.ml	0.1.ml.	0.3.ml.	0.1ml.	0.3ml.	0.1ml.	0.3ml
Soil I	5.5	7.4	6.6	7.0	5.6	7.5	5.8	7.3	6.0
" II	6.0	4.5	3.8	4.0	2.9m	3.6	3.2	3.8	3.0
" III	6.5	+	+	8.3	6.8	8.0	6.6	7.9	7.0
" IV	7.0	5.0	4.2	4.0	3.6	4.5	3.0	4.5	3.6
" V	7.0	+	+	8.0	6.6	7.5	6.0	7.7	6.3
" VI	6.0	1.0	1.2	1.4	1.2	---	---	1.2	1.2
" VII	6.5	+	+	42.0	39.2	46.0	40.0	44.0	39.6
" VIII	4.0	+	+	30.0	27,0	30.0	24.6	30.0	25.8
" IX	8.0	+	+	+	+	130.0	120.0	130.0	120.0
" X	5.5	+	+	16.6	12.0	14.0	12.0	15.3	12.0
" XI	5.0	+	+	12.0	10.0	12.0	11.6	12.0	10.8
" XII	4.5	2.4	2.0	3.0	2.6	2.8	2.0	2.7	2.2

N.B. :- Solutions marked + showed a deep violet to pink colouration indicating higher concentrations of manganese. Such solutions should be diluted 10 or 50 times as required.

DILUTION SHOULD ALWAYS BE MADE WITH MORGAN'S SOLUTION.

TABLE 19.

Recovery of added known amounts of Manganese to Standard Solutions and to Soil Extracts.

Test Solution	µ/gm. Manganese. Theoretical	Found	Error %
Soil extracts:-			
Soil I	—	0.38	—
" " +0.12 µ/gm.Mn.	0.50	0.48	- 4.0
Soil II	—	0.225	—
" " +0.10 µ/gm.Mn.	0.325	0.325	0.0
" " +0.21 µ/gm.Mn.	0.535	0.520	- 2.8
Soil IV	—	0.250	—
" " +0.12 µ/gm.Mn.	0.375	0.375	0.0
" " +0.09 µ/gm.Mn.	0.259	0.256	- 1.1
Soil VI		0.050	
" " +0.03 µ/gm.Mn.	0.080	0.078	- 2.5
" " +0.12 µ/gm.Mn.	0.170	0.170	0.0
" " +0.21 µ/gm.Mn.	0.260	0.265	+ 1.9
Standard Solutions :-			
0.03 µ/gm.Mn.	0.130	0.124	- 4.6
0.03 + 0.09	0.120	0.125	+ 4.5
0.12 " "	0.220	0.220	0.0
0.12 + 0.03µ/gm.Mn.	0.123	0.125	+ 1.6
0.21 " "	0.310	0.300	- 4.6

TABLE 20.

Comparison between the Periodate Method and the suggested Leucomalachite green Method.

Soil used	Morgan Extractable Manganese (O.D. basis).	
	Periodate Method	The suggested Leucomalachite green Method.
Soil I	6.8	7.4
" II	4.0	4.5
" III	7.5	8.3
" IV	4.5	4.0
" V	9.3	8.0
" VI	---	1.2
" VII	46.0	42.0
" VIII	32.0	30.0
" IX	130.0	130.0
" X	15.0	16.6
" XI	11.3	12.0
" XII	3.8	3.0

TABLE 21.

Effect of Temperature :- Readings of Standard Solutions :-

Test Solution	Absorptiometer Readings x 100				
	15°C	20°C	25°C	30°C	35°C
0.03 µ/gm.Mn.	2.5	3.5	4.5	6.0	8.0
0.12 µ/gm.Mn.	19.0	21.5	24.0	26.0	28.5
0.30 µ/gm.Mn.	49.0	54.5	56.0	58.0	59.5

TABLE 22.

Effect of Temperature :- Determination of Mn in Soil Extracts.

Test Solution	p.p.m. Mn. in Soils (Oven Dry Basis).				
	15°C	20°C	25°C	30°C	35°C
Soil I	6.0	7.1	7.6	8.4	9.2
Soil II	2.9	3.2	3.6	4.0	4.6
Soil IV	3.6	4.5	5.1	5.4	5.7
Soil VI	1.0	1.2	1.4	1.4	1.6

CHAPTER V

'COLORIMETRIC METHODS for DETERMINATION of IRON in MORGAN EXTRACTS of SOILS'

A : THIOCYANATE METHOD using an ORGANIC EXTRACTANT.

B : THIOCYANATE METHOD using an ORGANIC STABILISER.

C : O-PHENANTHROLINE METHOD.

Introduction :-

The thiocyanate method for iron^{+3} determination is the oldest and one of the most convenient techniques generally accepted in analytical practice. It is very likely that Berzelius (85) was the first to use the distinctive reaction between ferric ions and thiocyanate ions. It was also suggested by Ossian (86) for iron analysis. The main disadvantage of the method however, was the instability of the colour of the complex formed which tends to fade rapidly on exposure to light. Since then, efforts of different workers have been concentrated on trials to stabilise the colour of the ferric thiocyanate complex to ensure accurate determination of the iron.

Two principal schools of thought originated in the course of these studies :-

1st : Stabilising the colour in situ by adding a "stabilising reagent" e.g. Stokes and Cain (87) suggested thiocyanic acid reagent stabilised with mercuric thiocyanate. They oxidised the iron with potassium persulphate. Other workers (88) used hydrogen peroxide and preferred it to potassium permanganate for the same purpose. Baily (89) recently, used acetone-methyl ethyl ketone mixture to stabilise the thiocyanate complex in aqueous medium and was able to determine up to 0.020 mgm Fe^{+3} in 100 ml. solution.

2nd : The second school of thought favours the extraction

of ferric thiocyanate with an organic liquid immiscible with water, the separation of the organic layer and its direct use for colour comparison e.g. Bernard and Drekter [90] used a mixture of the monobutyl ether of ethylene glycol and ethyl ether. The extracted thiocyanate colour was intensified and it was stable for twenty-four hours.

One of the main factors which contributes to the instability of the ferric thiocyanate complex in water is its abnormally high dielectric constant (80). In order to stabilise the thiocyanate complex, liquids of lower dielectric constants are added to the reacting medium so that the dielectric constant of the mixture becomes comparatively low. Ionization of the thiocyanate complex is thus minimised or even inhibited for a period sufficient to obtain an accurate measurement of the colour. Alternatively, the thiocyanate complex is extracted in organic liquids of comparatively low dielectric constant, thus avoiding dissociation of the complex.

The present study was undertaken to develop a reliable method for the determination of iron in Morgan extracts of soils. Two successful trials were made :-

1st : Extraction of the ferric thiocyanate complex with a mixture of equal volumes of amyl alcohol and amyl acetate.

2nd : Addition of a mixture of methyl ethyl ketone and acetone to the reaction medium, thus stabilising the colour in situ.

The method adapted here is based on the reaction of ferric iron with thiocyanate (which should be present in excess to increase the colour intensity), in the presence of mineral acid which serves to suppress the hydrolysis of the iron complex. Hydrochloric acid is usually used instead of sulphuric or nitric acid. There is evidence[91] that sulphates are more effective than chlorides in preventing the development of the colour and accordingly, hydrochloric acid was used for adjustment of the pH to avoid hydrolysis of iron ($Fe + 3H_2O \rightleftharpoons Fe(OH)_3 + 3H^+$). Nitric acid may contain traces of nitrites which form nitrosyl thiocyanate NO.CNS, the colour of which is very similar to that of iron thiocyanate and would introduce appreciable error if present in measurable quantities.

The stability of the resulting complex is affected by pH, temperature, light, and properties of the extractant or "stabiliser" applied as claimed by Lister and Rivington [93].

A : "Colorimetric Determination of Iron in Morgan Extracts of Soils by the Thiocyanate Method using an Organic Extractant.

In these studies the Biochem Absorptiometer and Tintometer were used for colour measurements.

Stock Iron Solution : 0.1 g/l.

The test solutions were iron standard solutions prepared in amounts such that each five ml. will give :- 2, 4, 6, 8, 10, 12.5, 15, 20 or 25 µgms of iron.

Ammonium thiocyanate :- 7.5 M. Solution.

Potassium permanganate : 0.1 N Solution.

Hydrochloric acid :- Approximately 5 N. Solution.

Amyl alcohol - Amyl acetate (v/v) : was used as an extractant.

Choice of Filter :- To select an appropriate filter for use with the Biochem Absorptiometer, a set of standards was prepared as above. Five ml. aliquots were pipetted into separating funnels with one ml. of hydrochloric acid and one drop of potassium permanganate followed by ten ml. of the amyl alcohol-amyl acetate the funnels were shaken vigorously by hand for 1 - 2 minutes and left to stand till the two layers separated (5 - 10 minutes). The aqueous layer was decanted and the organic layer transferred

to a one-cm. optical cell. Three filters were examined, the zero being adjusted in every case with a blank determination. Results are shown in Table 23, p104.

It is clear from the readings that the light blue filter gives rather the widest range of colour and thus was used throughout this investigation whenever amyl-alcohol-amyl acetate mixture was used for extraction of the complex. Readings were taken immediately after separation i.e. 20 minutes from the time of addition of the ammonium thiocyanate.

Stability of the Colour extracted :-

The same experiment was repeated to test the stability of the colour both in the shade and in direct sunlight. Two sets of standards were prepared, one of which was kept in the shade in the laboratory and the other set was put on the window sill of the laboratory. It was a bright sunny day (13.7.1959). Temperatures averaged 23°C in the laboratory and 25.5°C in the open. Results recorded in Tables 24 and 25 p105 show clearly that the standards kept in the shade were stable for at least ONE hour while those put in the sunlight were not stable for more than half an hour. However, all readings obtained in further work were taken immediately after separation of the organic layer i.e. twenty minutes from the time of the addition of the thiocyanate reagent. It is observed that light affected the colours of thiocyanate to a greater extent from the higher than from lower iron concentration.

Effect of Morgan's Solution on the Colour developed :-

Another two sets of standards were prepared, one with Morgan's solution and the other with distilled water so that one ml. of each will give the required quantity of iron to be tested (i.e. 2, 4, 6, 8, 10, 12.5, 15, 20 and 25 µgm. iron/ml.) The desired aliquot of iron was pipettedv into separating funnels adding the amount of Morgan's solution or water to complete the volume to five ml. The set of standards with five ml. Morgan's was taken from the previous experiment. The colours were produced as above in the presence of 0.1, 3 and 5 ml. of Morgan's solution. In each case, the zero was checked against blanks containing the appropriate amount of Morgan's solution. Results are recorded in Table 26, p106. These results show that the presence of up to 5 ml. of Morgan's solution in the reaction medium did not introduce any appreciable interference with the development of the colour. Fig. 9 shows the relation between iron concentration and absorptiometer readings using standards containing five ml. of the Morgan's solution.

Effect of added Hydrochloric acid on the Colour formation :-

Morgan's solution is buffered at pH 4.8 and it was necessary to find the volume of hydrochloric acid (5N approximately) needed to lower the pH to 1.5 - 2.5 which is the favourable pH for the determination to prevent ionization of the Fe complex. It was found that 0.75 ml. of the 5N hydrochloric acid are required

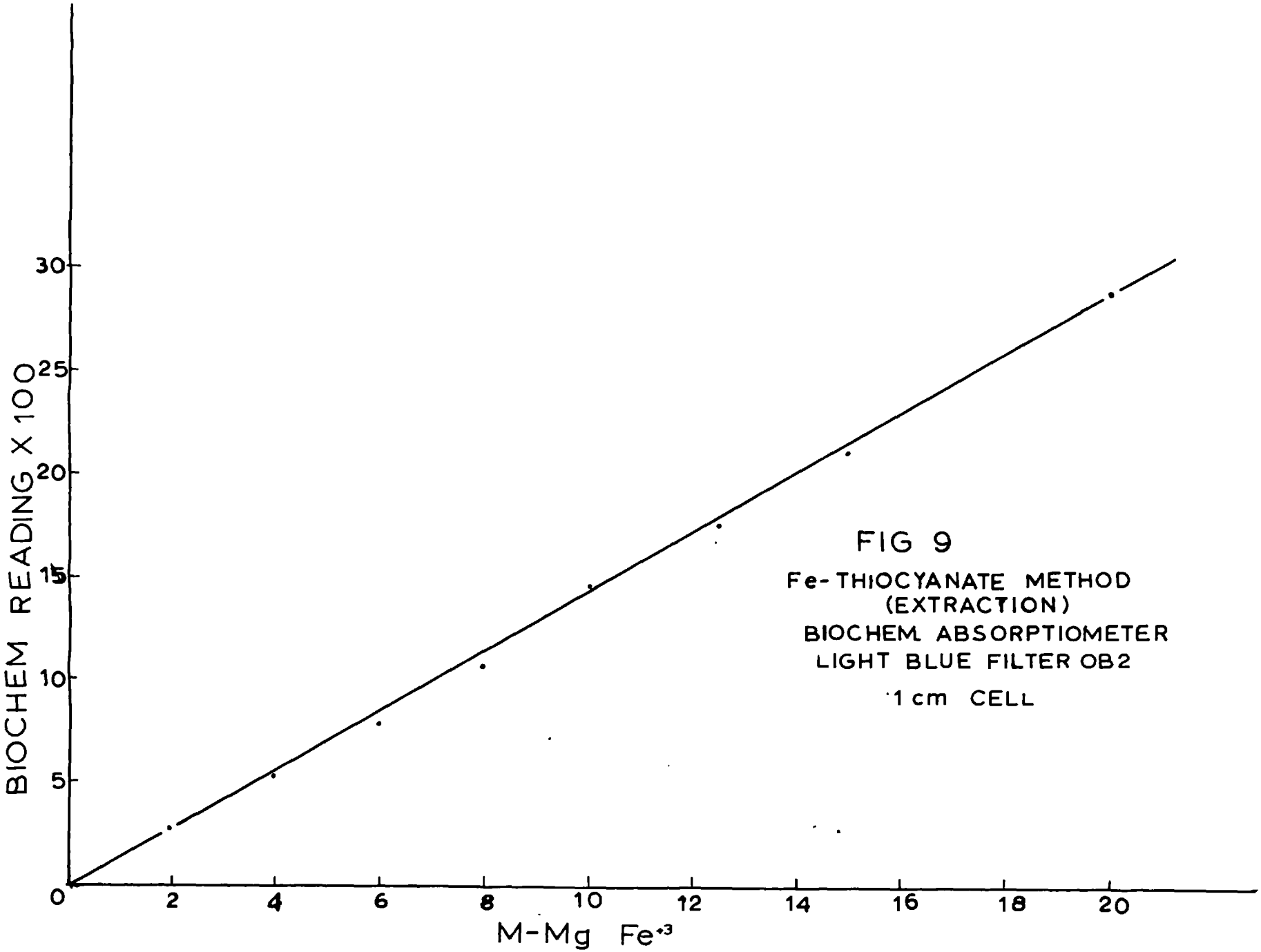


FIG 9

Fe-THIOCYANATE METHOD
(EXTRACTION)
BIOCHEM. ABSORPTIOMETER
LIGHT BLUE FILTER OB2
1 cm CELL

to change the pH of five ml. of Morgan's solution to 1.67. In practice however, some elasticity in the quantity of acid added may be assumed without appreciable error in the determination. Because this method was devised for both field and laboratory work, the amount of hydrochloric acid needed to give maximum sensitivity of the test was measured. The results are shown in Table 27, *p107*. The colour intensity decreased with increase in the amount of acid used. For each five ml. of Morgan's solution it was decided to add 1.5 ml. of hydrochloric acid (5N), due regard being paid to the excess of acid required to prevent interferencies particularly of the phosphates.

Effect of added Potassium permanganate on the colour of the Thiocyanate Complex :-

The effect of potassium permanganate on the colour of the complex formed in Morgan's solution using standard solution and soil extracts WAS EXAMINED. The soils chosen for this study vary mainly in the organic matter content namely, Harlington soil with organic matter 2.3%, Silwood soil with 3.5% and Slough soil with approximately 10% organic matter. The latter is a waterlogged soil and rich in organic matter. The Morgan extracts of these soils were observed to have a gradation in colour, Harlington soil extract being lightest, Silwood soil extract more deep yellow in colour, Slough a deep yellow.

The required standards made in Morgan' solution were pipetted into separating funnels (5 ml. from each) and from the

Morgan soil extracts five ml. were used in each case. To each test solution 1.5 ml. of hydrochloric acid, different volumes of potassium permanganate were added, followed by five ml. of thiocyanate solution and ten ml. of amyl alcohol-amyl acetate mixture. The colour was extracted and readings were taken both in the Biochem absorptiometer, using a 1-cm. cell and light blue filter OB1 twenty minutes after preparation, and in the Tintometer to trace any change in the nature of the colour extracted.

The results are shown in Tables 28, 29, 30, 31, 32 and 33, p108, 109 & 110.

It is evident that 0.1 ml of 0.1N $KMnO_4$ had no effect on the colourv extracted either in the case of standard solutions or Morgan extract of soils.

It was also observed that the solutions which did not receive any permanganate gave initially higher readings than those which received up to 0.3 ml. of the permanganate solution. These solutions which did not receive any permanganate however, were unstable and lost their colour in less than half an hour even when they were kept in the shade.

Tintometer readings on the other hand, showed that the yellow and red units increased gradually in the standard solutions which received permanganate while in soil extracts the red units were more or less constant (when figures obtained from each soil are compared between themselves) and there was a gradual increase in the yellow component of the extracts. This was further investigated by obtaining Absorptiometer readings of the soil extrac

using a dark blue filter OB1 and a 1 cm. cell and the thiocyanate. The coloured soil extracts of the previous three soils were used to clarify this point. Five ml. of each extract were used and the results are shown in Table 34. The data of this experiment suggested two possibilities :-

1st : either the colouring material in soil extracts is ~~partially~~ extracted by the organic layer during the extraction of the thiocyanate or

2nd : The organic layer decolourises the soil extracts.

By shaking the soil extracts with all reagents except thiocyanate it was found that the amyl alcohol-amyl acetate mixture decolourised the soil extracts as the organic layer gave practically no reading on the Biochem Absorptiometer.

Interferences :-

The most common interferences in this method are due to phosphates, fluorides, acetates, citrates and nitrites. Fluorides are not likely to be present under normal conditions in quantities to cause any interference. The method as adjusted tolerates up to 5 ml. of Morgan's solution with no interference whatsoever. Phosphates, citrates and nitrites were examined as follows:-

Three standard solutions of iron containing 2, 10 and 20 µgm Fe^{+3} in Morgan's solution so that 5 ml. will give the

required Fe concentration, were placed in separating funnels with 1.5 ml. of HCl and 0.1 ml. of 0.1N $KMnO_4$ followed after thorough mixing by the desired quantity of the foreign anion to be tested. Five ml. of ammonium thiocyanate and 10 ml. of the organic extractant were added. The extraction of the thiocyanate complex was carried out as usual and readings were taken after 20 minutes from preparation.

Results are in Tables 35, 36n and 37, p 112 & 113.

Interference of Phosphates :-

The presence of 500 µgm of phosphate did not cause any interference but there was a considerable decrease in colour in the presence of 1000µ/gm of phosphate added as NaH_2PO_4. In Morgan extracts such quantities of phosphates (100 p.p.m. phosphates) would be considered extremely high and very unlikely to be present in Morgan extracts of soil even after a recent addition of phosphatic fertilisers. Results are shown in Table 35.

Interference of Citrates :-

Citrates to the concentration of 1000 µgm added as sodium citrate did not cause any measurable interference in the colour. Results are shown in Table 36.

Nitrites :-

20 µgm of nitrites caused an increase in the colour extracted and the effect was more evident with low iron concentrations

(2 µgm) and almost negligible at at the 10 and 20 µgm Fe^{+3} levels. Twenty µgm/5ml extract is equivalent to 4 p.p.m. and such high concentrations of nitrites would be toxic under normal agricultural conditions. Results are shown in Table 37, p113.

Recovery tests :-

The recovery of added amounts of ferric iron to standard iron solutions in the presence of five ml. of Morgan reagent and to soil extracts was examined. The results are shown in Table 38, p114.

The results show clearly that the method as adapted affords perfect evidence for complete recovery. It is seen that this method is extremely sensitive and very reliable. The extraction of the colour from the reacting medium immediately after its preparation prevents almost every interference. The recoveries are between 99.5% and 101.0% in both soil extracts and standard solutions.

At this stage a standard colour disc was made, checked and used in determination of iron in soil extracts. The data obtained is compared with those of the absorptiometer determinations Table 39, p115.

The Method as finally Adapted.

Apparatus :-

Biochem Absorptiometer.

1 cm. cell.

Light Blue Chance Filter OB2 or

Comparator and Comparator Tubes.

Iron Disc (Thiocyanate Method).

Reagents :-

1. Morgan's Extractant Solution :- Dissolve 100 grams sodium acetate trihydrate (A.R) in one litre of distilled water containing 30 ml. of glacial acetic acid (A. R.).
2. Potassium permanganate N/10 solution :- Dissolve 3.16 gms $KMnO_4$ (A. R.) per litre of distilled water. Keep in a dark bottle with glass stopper.
3. Hydrochloric acid 5N solution :- Measure 423 ml. of HCl (A.R.) Sp. Gr. 1.18 in a litre flask and complete to the mark with distilled water.
4. Ammonium thiocyanate 7.5M. solution :- Dissolve 570 gms (A. R) in a litre of distilled water.

This solution is left for twenty four hours before use after preparation. It should be kept in a dark bottle with glass stopper to exclude light and kept in a cool place. Experience in this laboratory showed that it keeps unchanged under

these conditions for at least six months.

5. Amyl alcohol and Amyl acetate mixture 1:1 by volume.

6. Standard Iron Solution :-

0.1 gm pure iron wire/l. Suitable aliquots were diluted with Morgan's solution to give standards containing from 2 to 25 µgm Fe/5 ml.

Procedure :-

1- Calibration Curve :- (if absorptiometer to be used.)
Pipette 5 ml. of each of the standards above prepared in a se separating funnel, acidify with 1.5 ml. of the hydrochloric acid, add 0.1 ml of the permanganate, mixing thoroughly after each addition. Add 5 ml. ammonium thiocyanate followed by 10 ml. of the amyl alcohol-amylacetate mixture, shake vigorously for 2 minutes and leave to stand for the organic layer to separate from the aqueous layer (7 to 10 minutes) required for complete separation). Decant the aqueous layer and transfer the organic layer to a 1-cm. cell and take readings. Fig. 9 shows the calibration Curve.

2- Unknown Solutions :-

Pipette five ml. of the Morgan soil extract (prepared by shaking 20 gms of soil with 40 ml. of Morgan solution for 1 minute vigorously and filtration through Whatman filter paper (No. 1) into a separating funnel, add 1.5 ml.of hydrochloric acid, 0.1 ml. of $KMnO_4$ and

5 ml. of the thiocyanate followed by 10 ml. of amyl alcohol-amyl acetate mixture. Proceed as for the calibration curve. The amount of iron is found by referring to the calibration curve. The Comparator could be exchanged for the absorptiometer and a ~~met~~ north window used whenever possible is preferred for matching with standards.

The standard colour disc is marked 2, 4, 6, 8, 10, 12.5, and 25 , these being the actual amounts of iron producing the colour in the test. Accordingly, the colour is matched against 20 when 5 ml. of the solution is taken for the test, the concentration of the iron will be 4 p.p.m..

If the colour produced is deeper than the standards, the test may be carried out on a smaller aliquot of the original extract after dilution with Morgan's reagent to five ml. Alternatively, the thiocyanate extracted may be diluted with amyl alcohol-amyl acetate mixture to produce an appropriate colour within the range of the method.

SUMMARY.

A sensitive method for the determination of iron (total as ferric) in Morgan extracts of soils and in soil solutions is presented.

The distinctive colour of the thiocyanate complex formed is extracted immediately after its formation using an amyl alcohol-amyl acetate (1:1 v/v) mixture.

The colours produced are stable for one hour in the shade (i.e. away from direct sunlight) and for half an hour in direct sunlight.

The most important modifications of the original method are :-

1- Application to Morgan extracts of soils.

2- The volume of 5N hydrochloric acid added was increased to 1.5 ml.

3- The volume of 0.1N potassium permanganate added was increased to 0.1 ml. instead of one drop (0.009 ml.) This increased the stability of the colour.

The ions which are present in soil extracts and which are likely to interfere with the determination were examined and the method as suggested is not subject to these interferences.

Recoveries are very high, ranging from 97.4 to 100%.

A standard colour disc was made covering the range of 2 - 20 micrograms Fe i.e. 0.4 to 4 p.p.m. Fe if five ml. are

taken for analysis. In cases in which the iron concentration exceeds the above, a convenient dilution is made before the measurement of the colour. The disc was used to determine iron content in Morgan extracts of soils and results are compared with those obtained from the absorptiometer, Table 39.

The method is valuable in determining iron in displaced soil solutions, the only precaution being the adjustment of the pH to 1.5 - 2.5 before the determination.

B Colorimetric Method for the Determination of Iron. Thiocyanate Technique using an Organic Stabiliser.

This method is basically similar to the previous method except that in order to simplify its operation in the field, the possibility was examined of stabilising the thiocyanate complex in situ i.e. without extracting it in the organic solvent. Thus saving time and reducing the amount of apparatus to be carried. The stabiliser here was ethyl methyl ketone + acetone (2:1 v/v). The test solutions used were standard iron solutions of the same concentrations as used previously. Tubes of twenty ml. capacity were used instead of the separating funnels. Five ml. of the standard solutions were pipetted into the tubes, 1.5 ml. of 5N hydrochloric acid were added, one drop of 0.1N $KMnO_4$ and three ml. of ethyl methyl ketone mixture, shaking thoroughly after each addition. It was noticed that when the thiocyanate was added, the colour produced differed considerably from that extracted with amyl alcohol-amyl acetate using the Biochem Absorptiometer, the effectiveness of the two blue filters OB1 and OB2 was compared. Readings were obtained thirty minutes after preparation. The Absorptiometer was adjusted to zero reading as usual. Results are shown in Table 40 from which it appears that the dark blue filter is better. It was used subsequently throughout this investigation.

Effect of Morgan's Solution on the Colour formed :-

To test the effect of Morgan's solution on the colour formed, two sets of standards were made, one with distilled water and the other in Morgan's solution so that five ml. of each will give the required quantity of iron to be tested. One drop of $KMnO_4$, 1.5 ml. of HCl and after mixing thoroughly, three ml. of the stabiliser were added. Finally, five ml. of the thiocyanate were added and the solutions were mixed thoroughly and left to stand for thirty minutes after which, readings were taken and shown in Table 41.

It is evident from the results that the presence of up to five ml. of Morgan's solution did not interfere with the colour formation or intensity.

Effect of various quantities of the stabiliser on the colour :-

Keeping the volume constant at 14.5 ml., different volumes of the stabiliser were added to standard iron solutions prepared so that one ml. gives the required quantities of iron to be tested. 0.3 ml. of HCl (5N) was added (to lower the pH of the test solution to 1.5 - 2.5) together with one drop of $KMnO_4$ and mixed thoroughly. Water and varying proportions of stabiliser were then added to make the volume up to 14.5 ml. taking into account the five ml. of the thiocyanate reagent to be added. Readings were made after 30 minutes and blanks were always used to

adjust the zero reading before reading the standard solution. Results are shown in Table 42 p118 and suggest that the effect of the stabiliser was to intensify the colour, the proportion being of similar order for the four levels of iron tested. It was thought advisable to use seven ml. of stabiliser instead of the three ml. first used.

Effect of time on colour formation and stability :-

The test was repeated using five ml. of standard iron solution in Morgan's solution, containing the quantity of iron to be tested, 1.5 ml. of HCl, one drop of $KMnO_4$ and 7 ml. of the stabiliser being added followed by 5 ml. of the thiocyanate. Readings were taken after 10, 30, 60, 90, 120 minutes from preparation.

Results are given in Table 43, 119

The colour of the thiocyanate complex formed in the presence of the stabiliser does not form instantaneously as in the case of amyl alcohol-amyl acetate extraction method but takes approximately 30 minutes to develop. The colour developed is stable in the shade for at least two hours if the suggested procedure is followed.

A standard curve was prepared in the presence of five ml. of Morgan's solution and seven ml. of stabiliser to be used for iron determination in soils. Readings were taken thirty minutes after preparation using a 1-cm. cell and dark blue filter

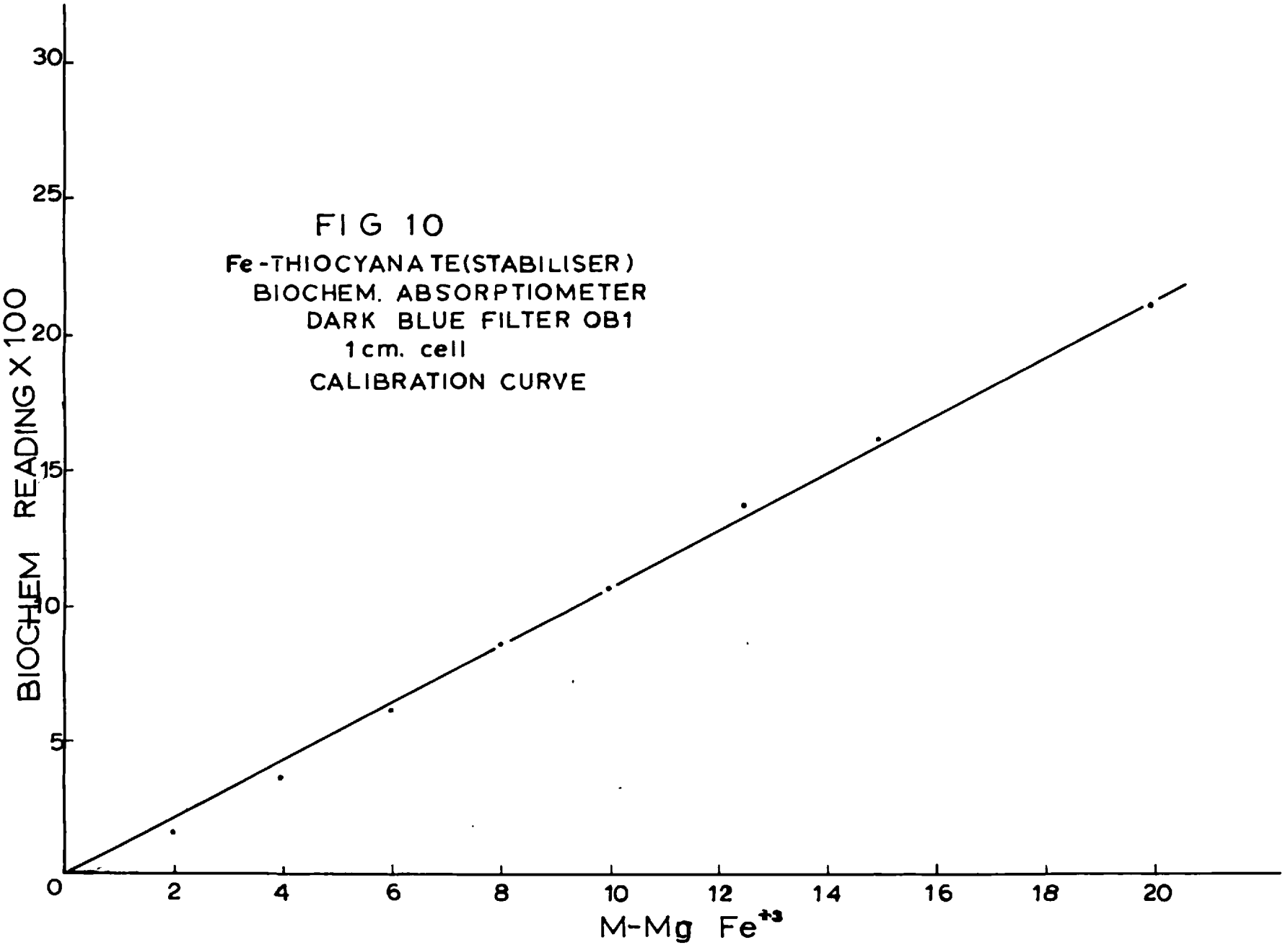
OBi. Results are given in Table 44, p120. Fig. 10 shows this calibration curve.

Interferences :-

This test was made by preparing the standard iron solutions in Morgan's solution so that four ml. will give the required iron standard. The interfering ions were dissolved in Morgan's solution to provide the required quantity of the foreign ion in 1 ml. of test solution. With this arrangement it was possible to examine the effect of foreign ions on the thiocyanate colour formed in the presence of 5 ml. of Morgan's solution. The effect of phosphates, aluminium, calcium, magnesium were examined and the results are shown in Tables 45, 46, 47 and 48, p121 & 122.

Effect of increasing quantities of $KMnO_4$ on the colour developed :-

The effect of different volumes of 0.1N $KMnO_4$ used was tested and it was clear from the results (Table 49 p123) that up to 0.3 ml. of the permanganate do not introduce any interference with the readings. This serves the purpose if total Fe would be determined in soil extracts as ferric. It is presumed that such a volume of permanganate is capable of oxidising the ferrous iron to ferric iron if 5 ml. aliquot is taken for analysis as suggested. At this stage of the work a disc was made according to the final method adopted.


FIG 10
Fe-THIOCYANATE(STABILISER)
BIOCHEM. ABSORPTIOMETER
DARK BLUE FILTER OB1
1cm. cell
CALIBRATION CURVE
BIOCHEM READING X 100
0
5
10
15
20
25
30
2
4
6
8
10
12
14
16
18
20
M-Mg Fe+3

Comparison between the thiocyanate method involving the extraction of the colour with amyl alcohol-amyl acetate mixture and the technique using ethyl methyl ketone-acetone mixture as stabiliser was made and results are shown in Table 50, p124.

Method Finally Adapted.

Apparatus :-

Biochem Absorptiometer.

1-cm. cell.

Dark Blue Filter OB1 or

Comparator and Comparator Tubes.

Standard Iron Disc (thiocyanate - ethyl methyl ketone-acetone method.

Reagents :-

1- Morgan's Solution.
2- $KMnO_4$ 0.1N solution.
3- HCl (5N approximately).
4- Ammonium thiocyanate 7.5M solution.

) prepared as before.

5- Ethyl methyl ketone-acetone mixture = 2/1 by volume.

6- Standard iron solution :- prepared as before.

7- Standard iron solutions in Morgan's solution :- a set of standards prepared in 100 ml. volumetric flasks, using Morgan's solution for dilution so that five ml. of each will give 2, 4, 6, 8, 10, 12.5, 15 or 20 µgm of iron.

Procedure :-

A- Standard Curve :- (if Absorptiometer is to be used) Pipette 5 ml. of each of the standard solutions into a 20 ml tube, add 1.5 ml. of HCl, one drop of $KMnO_4$ (up to 0.3 ml. will not affect the colour), mix thoroughly after each addition. Add 7 ml. of ethyl methyl ketone-acetone, mix thoroughly and the add 5 ml. of the thiocyanate. Stand for 30 minutes and take readings. Draw a curve showing the relation between the iron concentration and Absorptiometer readings. Fig. II.

Unknowns :-

Take 5 ml. of the Morgan extract of soil, add 1.5 ml. of the acid, one drop of $KMnO_4$, mix thoroughly after each addition. Add 7 ml. of the ethyl methyl ketone-acetone mixture. Stand for 30 minutes after which take readings using a 1.cm. cell and dark blue filter OB1 in the Biochem Absorptiometer. The iron concentration in the test solution is calculated by reference to the calibration curve.

Alternatively, fill the Tintometer tube with the blank and place it in the left-hand side of the Comparator, place another tube with the test solution in the right-hand side and match against the permanent colour using a north window whenever possible.

SUMMARY.

A sensitive method is described for the determination of iron using the thiocyanate method. The colour produced is stabilised in situ using ethyl methyl ketone-acetone mixture. The colour is stable for at least two hours in the shade and over half an hour in the sunlight.

The method is quick and almost free from interference of other ions present in soils under natural field conditions.

C- "Determination of Iron using O-phenanthroline:-

Introduction :-

Walden, Hammett and Chapman[94] claimed that the ferrous ortho-phenanthroline complex was discovered by Blau in 1898.

Saywell and Cunningham[95] determined the iron colorimetrically in fruit juices and wines by the o-phenanthroline method after wet ashing with sulphuric-perchloric mixture and reduction of the ferric iron to the ferrous state using hydroxylamine hydrochloride solution. They observed that the method was superior to thioglycollic acid method having a relatively more intense colour for a given iron concentration and a greater working sensitivity.

Hummel and Willard[96] used the same reagent for determining iron in other biological materials (food and blood...etc.) They claimed that the ferric iron was effectively reduced to the ferrous state by a solution of hydroquinone in a sodium acetate-acetic acid buffer of pH 4.5. They also observed that the interference of the large quantities of calcium and phosphates present in the metabolic materials they analysed could be obviated by making the test at pH 3.0 - 4.0, at which acidity maximum colour is developed.

Fortune and Mellon[97] made an extensive study of the factors which may affect the formation of the o-phenanthroline

ferrous complex.

In the present work a trial was made to adapt the method to iron analysis in Morgan extracts of soil with a view to developing a quick technique for field tests.

Effect of Morgan's solution on the ferrous o-phenanthroline complex formed :-

The test solutions in this experiment are ferrous ammonium sulphate standards containing 10, 20, 30, 40, 50, 60, 80, 100 and 120 µgm Fe/ml. From these, four sets were prepared, one without Morgan's solution, the other three containing one, three or five ml. of Morgan's solution. The desired quantity of iron to be tested is pipetted into a ten ml. tube (Comparator tubes were used), mixed thoroughly with the desired volume of Morgan's solution and diluted with distilled water to 6 ml. if necessary. One ml. of 10% hydroxylamine hydrochloride is added and the tube left at room temperature for 10 minutes. Then 1 ml. of buffer (made by mixing equal volumes of 3N HCl and 5N ammonium acetate) and 0.2 ml. of 0.5% ortho-phenanthroline solution in 50% alcohol are added. The tubes are filled to the mark with distilled water and the contents are mixed thoroughly. Readings are taken in the Biochem Absorptiometer using a 1.cm. cell and dark blue filter OB1 after twenty minutes. The Absorptiometer zero readings are adjusted against distilled water in each case, then the readings of the blank in each case is recorded and the Absorptiometer is set at zero against the blank of the appropriate series

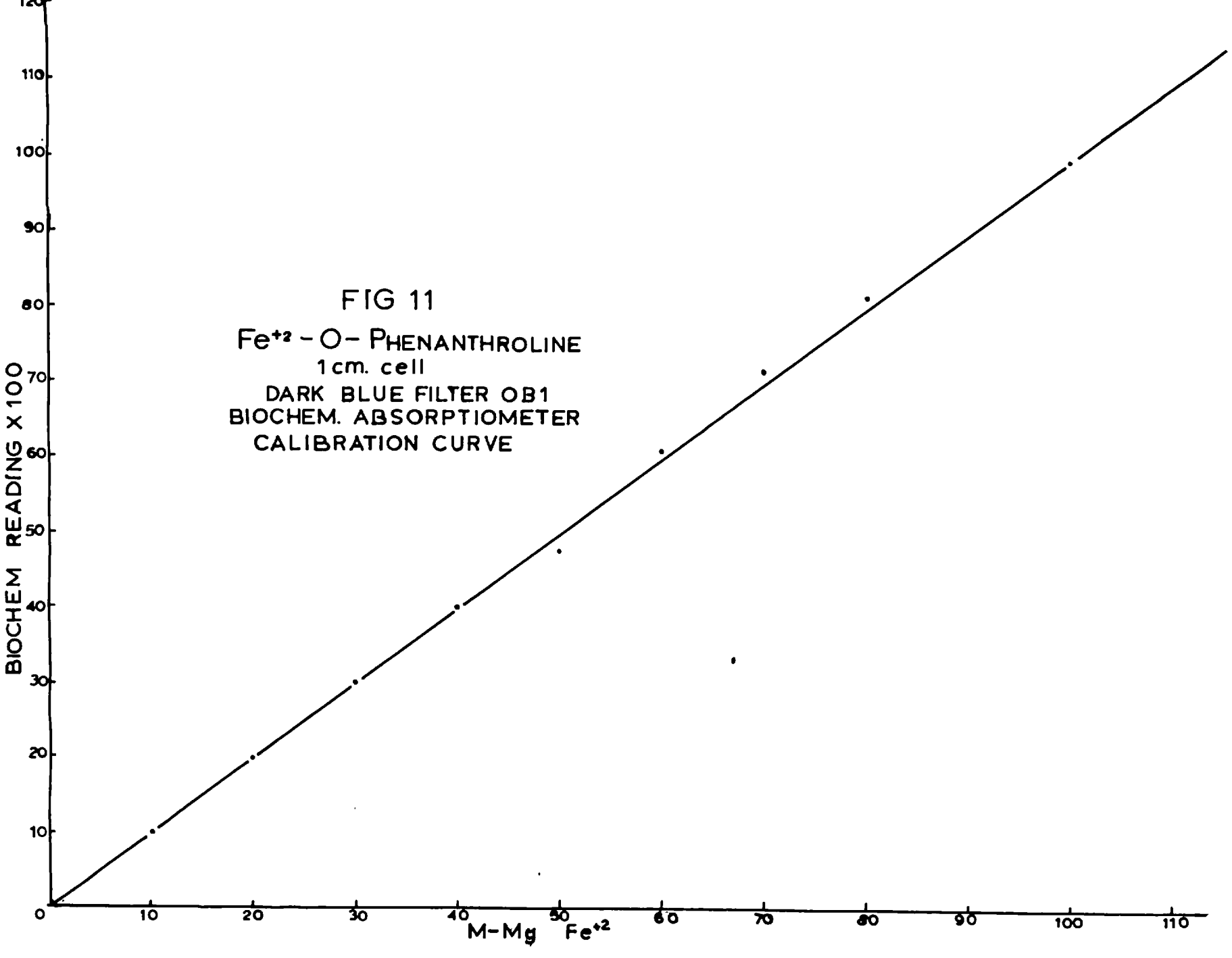
before the readings are taken. Results in Table 51, p/25 show clearly that the presence of up to five ml. of Morgan's solution in the reaction medium did not introduce any effect on the colour formation. The calibration curve (Fig. II) is prepared with standards containing 5 ml. of Morgan's solution.

Adjustment of the volume of o-phenanthroline solution added :-

In a preliminary study of this method, it was noticed that the addition of 0.1 ml. of the o.phenanthroline solution to the standards did not provide a clear distinction between the different levels of iron, especially at higher levels than 60 µgm Fe. The doubling of the quantity of this reagent i.e. the addition of 0.2 ml. (instead of 0.1 ml. in the original method) gave a better opportunity for differentiation.

Effect of Temperature :-

Three sets of standards were prepared as usual and the determinations were made at three different temperatures, 20, 25, and 30°C. The test solutions, reagents used and the cell in which the readings to be taken were kept at the required temperature in a water bath maintained at that particular temperature. Immediately before readings, the cell was taken out, wiped with filter paper and used. Results are shown in Table 54, p/28. There is a slight increase in the colour with the rise


FIG 11
Fe+2 - O - PHENANTHROLINE
1 cm. cell
DARK BLUE FILTER OB1
BIOCHEM. ABSORPTIOMETER
CALIBRATION CURVE
BIOCHEM READING X 100
0
10
20
30
40
50
60
70
80
90
100
110
M-Mg Fe+2

in temperature and the effect is greater the higher the concentration of the iron in the test solution.

Substitution of hydroxylamine crystals for the 10% solution :-

Hydroxylamine hydrochloride solution is unstable and it is always necessary to prepare it freshly before use. An attempt was made to use the crystals (0.1 gm. for each determination) instead ofm using the solution. The 0.1 gm. could be measured roughly on the tip of a glass spoon made in the laboratory from a glass rod of four mm. in thickness. The readings are in Table 52 (126) and show two sets of standards treated similarly except in that one set received one ml. of 10% hydroxylamine hydrochloride while the other received 0.1 gm. of the hydroxylamine hydrochloride crystals approximately. There was substantially no difference in colour between the two series. The only precaution however, is to ensure the complete dissolution of the solid reagent.

Stability of the ferrous o-phenanthroline complex in presence of Morgan's solution :-

To test the stability of the colour formed in presence of Morgan's solution, the test was repeated in presence of 5 ml. of Morgan's solution. To each standard solution, 1 ml. hydroxylamine hydrochloride 10% solution was added, mixed thoroughly and

left to stand for ten minutes. One ml. of buffer and 0.2 ml. of o-phenanthroline solution were added and the tubes filled to the mark and the contents mixed thoroughly. Readings were taken with the Biochem Absorptiometer after 20 minutes, 40 minutes, one hour, three hours and fifteen hours. Results are shown in table 53 and indicate the striking stability of the colour of this complex for at least fifteen hours. p 127.

Interferences :-

The likely interference of phosphate in soil extracts was examined. The test was made by adding increasing amounts of phosphate to standard iron solutions in the presence of five ml. of Morgan's solution. Table 55 shows that up to 200 µgm phosphate (i.e. 20 p.p.m. PO_4 in final volume) has no effect on the colour but larger quantities caused a reduction in the colour intensity. p 129.

Effect of organic matter :-

Organic matter under natural conditions has no effect on the determination. However, it was found necessary to decolorise the extracts of soils relatively rich in organic matter. Decolorisation using activated charcoal was found to introduce an error (a decrease of 7 - 10%) in the results, very likely partly due to adsorption of a portion of the iron in the solution and

partially due to that of the yellow coloured organic matter. A permanent colour disc which could be used for field work was then prepared. The disc covers a range of 10 - 140 µgm. Fe^{+2} i.e. 2 - 28 p.p.m. Fe^{+2} in the original soil extract if five ml. are used in the test.

The Method as Finally Adapted.

Apparatus :-

Biochem Absorptiometer.

One-cm. cell

Dark Blue Filter OB1.

or alternately

Comparator and Comparator Tubes.

Standard Iron Colour Disc.

Reagents :-

(1) Morgan's Solution.

(2) Hydroxylamine hydrochloride :- 10 gms of Analar $NH_2OH\,HCl$ are dissolved in 100 ml. of distilled water.mixed thoroughly.

(3) Hydroxylamine hydrochloride crystals (for use in the field).

(4) O-phenanthroline Solution :- 0.5% solution in 50% alcohol.

(5) Buffer solution :- Equal volumes of 2N HCl and 5N ammonium acetate (Prepared from Analytical grade reagents) are mixed thoroughly.

(6) Standard Ferrous Iron Solution :-

0.7023 gms of ferrous ammonium sulphate $Fe(NH_4)_2(SO_4)_2$ $6H_2O$ is weighed accurately and dissolved in water with the addition of 20 ml. of 0.6N HCl. This is transferred quantitatively to a litre volumetric flask which is filled with distilled water. This solution contains 100 p.p.m. Fe i.e. 100 micrograms iron/ml.

Procedure :-

A- Calibration Curve :- (Absorptiometer).

From a micro-burette, run solutions containing the required amount of iron, into a tube, add five ml. of Morgan's solution and mix thoroughly. Add 1 ml. of 10% hydroxylamine solution, mix and stand for 10 minutes at room temperature (20 - 30) °C. Add 1 ml. of buffer and 0.2 ml. of o-phenanthroline solution, mixing thoroughly after each addition. Complete the tubes to the 10 ml. mark, mix again and take readings in the Biochem Absorptiometer after twenty minutes using a 1-cm. cell and dark blue filter OBi.

B- Unknowns :-

In a 10 ml. tube, pipette five ml. of Morgan Extract of soil (prepared by shaking soil with Morgan' solution, Wt/vol.1:2 for 1 minute and filtering), add 0.1 ml. of 10% hydroxylamine HCl solution and mix thoroughly, or alternately, add 0.1 gm. of

hydroxylamine hydrochloride, dissolve completely and mix thoroughly with the help of a thin glass rod (2 m.m. in diameter bent at its end to effect complete mixing). Leave to stand for 10 minutes, add 1 ml. of buffer, 0.2 ml. of o-phenanthroline solution and complete to the 10 ml. mark with distilled water, mixing thoroughly after each addition. Take readings in the Absorptiometer after 20 minutes. The amount of iron in the solution can be obtained by referring to the Calibration Curve. Alternately, match the colour against the permanent glass standard in the disc using north daylight whenever possible. A blank should always be prepared from all the reagents added to the unknown and placed in the left-hand compartment thus falling behind the permanent glass standards.

If the colour produced from the sample is more intense than the highest in the disc, the test may be repeated using a smaller aliquot of the filtrate and diluting to five ml. with water or Morgan's solution. Develop the colour as above described and calculate the iron concentration.

Recovery Test :-

Recovery of ferrous iron added to standards and to soil extracts was tested by the suggested technique. Table 56 shows that iron can be determined with an accuracy of ± 2.1%, p130. The test solutions used were five ml. of Morgan soil extracts or

standard solutions in the presence of five ml. of Morgan's solution.

Determination of both ferrous and total iron using the Method :-

Eight air-dry soils were examined for their ferrous and ferric iron contents using the suggested method. The ferrous iron was determined immediately after extraction with Morgan's solution using the normal procedure except that no hydroxylamine hydrochloride solution was added. As soon as the extractant was obtained, a five ml. aliquot was placed into a 10 ml. tube, one ml. of buffer was added and mixed, followed by 0.2 ml. of o-phenanthroline solution and the colour was developed.

Another five ml. aliquot was taken from each soil extract and the total iron was determined by the reduction of the ferric iron to the ferrous state. The difference between the two values obtained is taken as the ferric iron. Results are in Table 57 and show that from 88.9 - 100% of the iron is present in the ferric state under air-dry conditions, p131.

SUMMARY.

The o-phenanthroline method is adopted for the determination of iron (ferrous and ferric) in Morgan extracts of soils. The presence of up to five ml. of Morgan's solution did not cause any interference in the development or the stability of the colour and the colours are stable for at least fifteen hours if the procedure described is followed.

Phosphates in amounts up to 40 p.p.m. (PO_4'') in soil extract did not interfere. Such high values of phosphates are unlikely to be present in Morgan extracts of soils.

The temperatures in the range 20 - 30°C caused no appreciable increase in the colour of the complex formed and the increase at higher iron concentrations did not exceed the experimental error.

The method was adopted to field tests and permanent colour standards were made for the purpose. Hydroxylamine hydrochloride solution being unstable is better replaced by the solid reagent. The quantity required being easily measured with sufficient accuracy on a marked length of a thin glass rod. O-phenanthroline solution in a well-stoppered dark bottle may be kept unchanged for at least six months.

The method is accurate, quick, almost free from interference and is very promising for field work.

Table 23.

Choice of Filter :-

Test Solution	Absorptiometer Readings X 100		
	Light Blue Filter CB2	Dark Blue Filter CB1	Green Filter CG1
2 micrograms Fe^{+3}	3.0	3.0	2.0
10 micrograms Fe^{+3}	14.5	12.5	11.5
25 micrograms Fe^{+3}	36.5	30.5	30.0

Table 25.

Set of Standards kept in direct Sunlight :-

Test Solution	Absorptiometer Readings X 100 (Mean of two readings)			
	15 minutes	30 minutes	60 minutes	120 minutes
2 micrograms Fe^{+3}	3.5	3.5	2.5	1.5
10 micrograms Fe^{+3}	14.5	14.0	12.5	10.5
25 micrograms Fe^{+3}	36.5	36.0	32.5	25.0

Table 24.

Set of Standards kept in the Shade :-

2 micrograms Fe^{+3}	3.0	3.5	3.0	2.5
10 micrograms Fe^{+3}	14.0	14.5	14.5	12.5
25 micrograms Fe^{+3}	36.0	35.5	35.5	31.5

Table 26.

Effect of Morgan's Solution on Colour :-

Test Solution	Absorptiometer Readings X 100 (each reading is the mean of duplicates)			
	Standards with NO Morgan solution	in presence of 1 ml. Morgan soln.	in presence of 3ml. Morgan soln.	in presence of 5ml. Morgan soln.
2 µgm Fe^{+3}	3.0	2.5	3.5	3.0
4 µgm Fe^{+3}	6.0	5.5	7.0	5.5
6 µgm Fe^{+3}	9.5	9.0	9.0	8.0
8 µgm Fe^{+3}	11.5	11.0	12.0	11.0
10 µgm Fe^{+3}	14.5	14.5	15.0	15.5
12.5 µgm Fe^{+3}	18.5	18.0	18.5	18.0
15.0 µgm Fe^{+3}	22.0	22.0	22.5	21.5
20.0 µgm Fe^{+3}	29.0	29.0	30.0	29.0
25.0 µgm Fe^{+3}	38.0	38.5	38.5	38.0

Table 27.

Effect of added hydrochloric acid on the colour formed :-

Volume of acid added	Absorptiometer Readings X 100		
	2 µgm Fe^{+3}	10 µgm Fe^{+3}	20 µgm Fe^{+3}
None	4.5	16.0	31.0
0.5 ml.	4.0	15.5	29.5
1.0 ml.	3.5	15.0	29.0
1.5 ml.	3.5	14.5	29.0
2.0 ml.	3.0	13.5	29.0
3.0 ml.	2.5	12.0	28.0
4.0 ml.	1.5	12.0	28.0

Table 28

Standard Solutions:-

4 µgm of ferric iron in 5 ml. of Morgan's solution.

0.1N $KMnO_4$	Absorptiometer Readings x 100	Tintometer Readings (average of two determinations)	
	Average of two determinations.	Yellow units	Red units
0 ml.	6.5	0.9	1.2
0.05 ml.	5.5	0.8	1.0
0.1 ml.	5.0	0.8	1.1
0.2 ml.	5.5	0.8	1.2
0.3 ml.	6.0	1.0	1.6
0.5 ml.	7.5	1.2	3.0

Table 29.

15 µgm of ferric iron in 5 ml. of Morgan's solution.

0.1N $KMnO_4$	Absorptiometer Readings x 100	Tintometer Readings (average of two determinations)	
0. ml.	24.0	2.5	8.5
0.05 ml.	22.0	2.3	7.7
0.1 ml.	22.5	2.1	7.8
0.2 ml.	22.5	2.2	8.0
0.3 ml.	24.5	2.4	8.2
0.5 ml.	26.0	2.7	8.8

Table 30.

20 µgm of ferric iron in 5 ml. of Morgan's solution.

0.1N $KMnO_4$ added	Absorptiometer Readings x 100	Tintometer Readings (average of two readings)	
	Average of two readings	Yellow units	Red units
0. ml.	31.5	2.9	10.0
0.05 ml.	30.0	2.6	9.8
0.1 ml.	29.0	2.4	9.6
0.2 ml.	29.5	2.4	9.4
0.3 ml.	31.0	2.8	10.1
0.5 ml.	33.0	3.0	10.6

Table 31.

Soil Extracts :- Harlington Soil Morgan Extract (1:2 ratio soil : Extractant - shaking for 1 minute and filtration) - 5 ml. were used for this test.

0.1N $KMnO_4$	Absorptiometer Readings x 100	Tintometer Readings (average of two readings)	
	Average of two readings	Yellow units	Red units
0. ml.	8.0	1.3	3.0
0.05 ml.	8.0	1.2	3.0
0.1 ml.	8.5	1.3	3.1
0.2 ml.	8.5	1.4	3.0
0.3 ml.	9.0	1.4	3.1
0.5 ml.	9.5	1.7	3.0

Table 32.

Silwood Soil Morgan Extract :-

0.1N KMnO4 added	Absorptiometer Readings x 100	Tintometer Readings	
	Average of two determinations.	Yellow units	Red units
0. ml.	10.5	1.3	3.4
0.05 ml.	10.0	1.1	3.3
0.1 ml.	10.0	1.1	3.4
0.2 ml.	10.0	1.2	3.3
0.3 ml.	10.5	1.3	3.5
0.5 ml.	11.5	1.4	3.5

Table 33.

Slough Soil Morgan Extract :-

0.1N KMnO4 added	Absorptiometer Readings x 100	Tintometer Readings	
	Average of two determinations.	Yellow units	Red units
0. ml.	52.5	5.6	18.5
0.05 ml.	51.0	5.1	18.0
0.1 ml.	53.5	5.3	19.0
0.2 ml.	52.5	5.3	18.1
0.3 ml.	55.0	5.6	19.0
0.5 ml.	56.5	5.9	19.2

Table 34.

Effect of added $KMnO_4$ on the Colour of Soil Extracts :-

Test solution	Absorptiometer Readings x 100. Slough	Silwood	Harlington
Original extract	64.0	15.5	6.5
0. ml. $KMnO_4$	24.5	5.0	colourless(no reading obtained)
0.05 ml. $KMnO_4$	23.0	Colourless)no reading obtained)	" " "
0.1 ml. $KMnO_4$	20.0	" " "	" " "
0.2 ml. $KMnO_4$	18.0	" " "	" " "
0.3 ml. $KMnO_4$	16.0	" " "	" " "
0.5 ml. $KMnO_4$	12.5	" " "	" " "

Table 35.

Interference of Phosphates :-

Phosphate added micrograms	Absorptiometer Readings x 100		
	2 µgm Fe^{+3}	10 µgm Fe^{+3}	20 µgm Fe^{+3}
10	3.0	16.0	29.0
20	3.5	16.0	30.0
50	3.0	15.0	30.0
75	3.0	15.5	29.5
100	3.5	15.0	29.0
200	3.0	16.0	29.5
300	4.0	15.5	30.0
500	3.5	15.0	29.5
1000	2.5	13.5	26.0

Table 36.

Interference of Citrates :-

Citrates added micrograms	Absorptiometer Readings x 100		
	2 µgm Fe^{+3}	10 µgm Fe^{+3}	20 µgm Fe^{+3}
10	3.5	15.5	30.0
20	3.0	16.0	29.5
50	3.0	16.0	29.0
100	3.5	15.0	29.0
150	4.0	15.5	30.0
200	3.5	16.0	29.0
400	3.5	15.0	29.5
800	3.0	15.5	29.0
1000	3.5	15.5	30.0

Table 37.

Interference of Nitrites :-

Nitrites added micrograms	Absorptiometer Readings x 100		
	2 µgm Fe^{+3}	10 µgm Fe^{+3}	20 µgm Fe^{+3}
2	3.5	16.0	29.5
4	3.0	16,0	29.0
6	3.0	16.0	29.0
8	3.5	15.5	30.0
10	3.0	15.5	30.0
15 b	4.0	15.5	30.0
20	5.5	16.5	29.5

Table 28.

Recovery Test :-

Test Solution=	µgm Fe^{+3}		
	Theory	Found	Error %
5 ml. of Harlington Soil	---	6.0	---
" " " + 8 µgm Fe^{+3}	14.0	14.0	0.0
" " " + 12.5 " "	18.5	18.4	- 0.1
5 ml. of Silwood Soil	---	7.8	---
" " " + 2 " "	9.8	10.0	+ 2.0
" " " + 8 " "	15.8	15.6	- 1.3
" " " + 12.5 " "	19.5	20.0	+ 2.6
2 µgm. of Fe	2.0	2.0	0.0
" " " + 6 " "	8.0	7.8	
" " " + 8 " "	10.0	10.0	0.0
10 " "	10.0	10.0	0.0
10 " " + 2 " "	12.0	11.8	- 1.6
10 " " + 6 " "	16.0	16.0	0.0
10 " " + 8 " "	18.0	18.2	+ 1.1

Table 39.

Comparison between values obtained for Iron Determination using the Biochem Absorptiometer and the suggested disc : using the same Soils as in the Sulphate and Manganese tests.

Test Solution	p.p.m. Fe^{+3} (oven dry basis)	
	Biochem Absorptiometer.	Disc.
Soil I	2	2
Soil II	3.6	4
Soil III	1.3	1.2
Soil IV	1.2	1.2
Soil V (Harlington Soil)	1.2	1.2
Soil VI (Silwood Soil)	1.6	2.0
Soil VII	1.6	2.0
Soil VIII	1.7	1.6
Soil IX	1.0	1.2
Soil X	3.4	3.0
Soil XI	3.6	4.0
Soil XII	(was diluted 5 ti-8.8 mes i.e. 1 ml. of Morgan soil extract with 4 ml. Morgan Solution and concentration calculated.	8.0

Table 40.

Choice of Filter :-

Test Solution.	Absorptiometer Readings x 100	
	Dark Blue Filter OB1	Light Blue Filter OB2
2 µgm Fe^{+3} in 5 ml. Morgan's solution.	2.0	1.0
10 µgm Fe^{+3} in 5 ml. Morgan's solution.	10.5	8.5
25 µgm Fe^{+3} in 5 ml. Morgan's solution.	30.5	24.5

Table 41.

Effect of Morgan' solution on the Thiocyanate Colour :-

IRON present	Absorptiometer Readings x 100 (each figure is an average of two readings).	
	No MORGAN solution (i.e. Standards made in water).	5 ml. of Morgan's solution present.
2 micrograms Fe^{+3}	2.0	2.0
4 " " "	4.5	4.5
6 " " "	6.5	6.5
8 " " "	8.0	8.5
10 " " "	10.5	10.5
12.5 " " "	13.5	13.0
15.0 " " "	16.5	16.0
20.0 " " "	21.5	22.0
25.0 " " "	28.5	29.0

Table 42.

Effect of various quantities of Stabiliser on the colour formed :-

	Absorptiometer Reading x 100(average of duplicates)				
	Amount of Stabiliser added.				
Fe in test solution (μ gms.)	0	1 ml.	3 ml.	5 ml.	7 ml.
2	1.5	2.0	2.5	2.0	2.0
10	6.5	8.0	8.5	9.5	10.5
15	11.5	12.5	14.0	15.0	16.5
20	14.0	16.0	18.0	19.5	21.5

Table 42.

Effect of Time on Colour Formation and Stability :-

Test Solution	Absorptiometer Readings x 100 (each figure is an average of two determinations.)				
	10 minutes	30 minutes	60 minutes	90 minutes	120 min.
2 µgm Fe^{+3}	1.5	2.0	2.0	2.0	2.0
6 " "	5.5	7.5	7.5	7.5	7.5
10 " "	11.0	12.0	12.0	12.0	12.5
15" "	17.5	19.0	19.0	19.5	19.8
20 " "	22.5	26.0	26.0	26.5	26.5

Table 44.

Standard solution	Absorptiometer Readings (average of two readings).
2 µgm Fe^{+3}	2.0
4 " "	4.0
6 " "	6.0
8 " "	8.5
10 " "	10.5
12.5 "	13.5
15.0 "	16.0
20.0 "	21.0

Table 45.

A - Effect of Phosphates :- ($Na\ H_2\ PO_4$)

Phosphate added micrograms PO_4.	Absorptiometer Readings (average of two readings) x 100		
	2 micrograms Fe^{+3}	10 micrograms Fe^{+3}	20 micrograms Fe^{+3}
0	2.0	11.5	21.5
100	2.0	11.5	22.0
200	2.0	11.5	22.0
500	2.5	11.0	21.0
1000	2.0	11.0	21.5
1500	2.0	10.5	19.0

B - Effect of Aluminium (potash alum). Table 46.

Aluminium added Micrograms Al.	Absorptiometer Readings(average of two readings) x 100		
	2 micrograms Fe^{+3}	10 micrograms Fe^{+3}	20 micrograms Fe^{+3}
0	2.0	11.5	22.0
2	2.0	11.0	21.5
4	2.0	11.0	22.0
10	2.0	11.0	21.0
20	2.5	12.0	22.0
30	2.0	12.0	22.0

Table 47.

C - Effect of Calcium :- (calcium chloride.)

Calcium added micrograms	Absorptiometer Readings x 100.		
	2 µgm. Fe^{+s}	10 µgm. Fe^{+s}	20 µgm. Fe^{+s}
0	2.0	11.5	22.0
200	2.5	11.0	21.5
400	2.0	11.5	22.0
800	2.0	11.0	22.0
1000	2.0	11.0	21.0

Table 48.

D - Effect of Magnesium :- ($MgSO_4$ $7H_2O$).

Magnesium added micrograms	Absorptiometer Readings x 100.		
	2 µgms. Fe^{+s}	10 µgms. Fe^{+s}	20 µgms. Fe^{+s}
0	2.0	11.5	22.0
50	2.0	11.0	21.0
100	2.5	11.0	21.5
150	2.0	11.0	22.0
200	2.0	11.5	22.0
400	2.0	12.0	21.5
500	2.0	11.5	21.5

Table 49.

Effect of increasing quantities of $KMnO_4$ on the colour developed

$KMnO_4$ added in mls.	Absorptiometer Readings x 100 (average of two readings.)		
	2 µgms. Fe^{+3}	10 µgms. Fe^{+3}	20 µgms. Fe^{+3}
0	2.5	11.5	21.5
0.05	3.0	11.5	22.0
0.1	2.5	11.0	22.5
0.2	2.0	11.5	22.0
0.3	2.0	11.0	22.0

At this stage of the work a disc was made according to the final method adopted.

Table 50.

Comparison between the Thiocyanate Method involving the extraction of the colour with amyl alcohol/amyl acetate mixture and the technique using ethyl methyl ketone/acetone mixture as Stabiliser :- (Absorptiometer readings).

Test Solution	Thiocyanate - Extraction with amyl alcohol) amyl acetate mixture.	Thiocyanate - Stabilising the colour in situ by ethyl methyl ketone/acetone/ mixture.
Soil I	2.5	2.8
" II	4.0	4.2
" III	1.4	1.4
" IV	1.2	1.2
" V (Harlington)	1.2	1.2
Soil VI (Silwood)	1.6	1.6
Soil VII	1.8	1.8
" VIII	1.7	1.4
" IX	1.0m	1.0
" X	3.5	3.6
" XI	3.8	4.1
" XII	8.8	9.2

Table 51.

Effect of Morgan's solution on the Colour of the o-phenanthroline Complex :-

Test Solution	Absorptiometer Readings x 100 (each figure is an average of two readings).			
	No Morgan's solution.	1 ml. Morgan's solution.	3 ml. Morgan's solution.	5 ml. Morgan's solution.
Blanks	0.0	0.0	0.5	1.5
10 micrograms Fe^{+3}	9.0	9.0	9.5	10.0
20 " " "	19.5	19.5	19.5	20.0
30 " " "	30.0	30.0	30.5	30.0
40 " " "	40.5	40.0	41.0	40.0
50 " " "	52.0	51.0	51.5	51.0
60 " " "	62.0	62.0	62.0	61.0
70 " " "	72.0	73.0	73.0	72.0
80 " " "	83.0	83.5	83.0	82.0
100 " " "	100.0	100.0	100.0	100.0
120 " " "	120.0	120.0	120.0	120.0

Table 52.

Effect of form of hydroxylamine hydrochloride added :-

Test Solution	Absorptiometer Readings x 100.	
	Standards with 1 ml. of 10% hydroxylamine hydrochloride solution.	Standards with 0.1 gm. of hydroxylamine hydrochloride crystals.
10 µgms Fe^{+2}	10.0	9.5
40 µgms Fe^{+2}	41.0	40.0
80 µgms Fe^{+2}	81.5	83.0
120 µgms Fe^{+2}	120.0	120.0

Table 53.

Stability of ferrous o-phenanthroline complex in presence of Morgan's solution.

Test Solution	Absorptiometer Readings x 100 after :-				
	20 minutes	40 minutes	One hour	Three hours	Fifteen hr
10 micrograms Fe^{++}	9.5	9.5	9.0	9.5	9.5
30 " "	30.0	30.0	30.5	31.0	30.5
60 " "	61.0	62.0	61.0	61.0	61.0
80 " "	80.0	80.5	81.0	80.0	81.5
120 " "	120.0	120.0	120.0	120.0	120.0

Table 54.

Effect of Temperature :-

Test Solution	Absorptiometer Readings x 100 (each reading is an average of two determinations)		
	20°C	25°C	30°C
10 µgms. of Fe^{+2}	9.0	9.5	10.0
50 " " "	51.0	51.0	52.0
100 " "	100.0	110.0	115.0

Table 55.

Interference of Phosphates :-

Phosphate added (PO_4) µgms.	Absorptiometer Readings x 100(average of two readings)		
	10 µgms. Fe^{+2}	50 µgms. Fe^{+2}	100 µgms. Fe^{+2}
10	9.5	51.0	100
50	9.0	51.0	100
100	9.5	50.5	105
150	10.0	51.0	100
200	9.5	50.0	110
250	10.0	50.5	99.5
500	8.5	49.0	96.0

Table 56.

Recovery Test :-

Test Solution	Micrograms Fe.		
	Theory	Found	Error %
Soil I	13.5	--	--
" +10 µgms.Fe^{+2}	23.5	23.0	- 2.1
" +40 " "	53.5	53.5	0.0
" +80 " "	93.5	93.5	0.0
Soil III	8.0	--	--
" + 10 µgms. Fe^{+2}	18.0	18.0	0.0
" + 40 " "	48.0	49.0	+ 2.0
" + 80 " "	88.0	88.5	+ 0.5
Soil VI	10.0	--	--
" + 10 µgms.Fe^{+2}			
" + 40 " "	50.0	50.0	0.0
" + 80 " "	90.0	91.5	+ 1.6
Soil VIII	7.5	--	--
" + 10 µgms.Fe^{+2}	17.5	17.5	0.0
" + 40 " "	47.5	47.0	-1.0
" + 80 " "	87.5	88.0	+ 0.6
10 µgms.Fe^{+2} + 20 " "	30.0	30.0	0.0
+ 50 " "	60.0	61.0	+ 1.6
+ 80 " "	90.0	89.0	- 1.1

Table 57.

Determination of Ferrous and Ferric Iron in Soils :-

Soil used	Total Iron µgm in5 ml.of soil extract.	Ferrous Iron µgm in 5 ml.of soil extract.	Ferric Iron µgm in 5 ml. of soil extract.	% Ferric Iron
Soil I	13.5	0.8	12.7	94.07
" III	8.0	0.5	7.5	93.65
" IV	9.0	1.0	8.0	88.90
" V	5.5	0.25	5.25	95.56
" VI	10.0	1.0	9.0	90.0
" VII	6.5	0.5	6.0	92.3
" VIII	7.5	0.25	7.25	96.67
" IX	5.0	--	5.0	100.0

CHAPTER VI.

Adaptation of the Titan Yellow Method for determination of Magnesium in Morgan extracts of Soils.

Introduction :-

Magnesium hydroxide exhibite a notable capacity for adsorbing certain organic dyestuffs[98]. With some dyestuffs, of the azo and anthraquinone series, there is a difference in colour between the adsorption product and the non-adsorbed dyestuff. Since the adsorption is instantaneous, the reaction affords an excellent test for magnesium.

Kolthoff[99] observed that magnesium imparts an orange or red colour to alkaline solutions of titan yellow and this formed the basis of a colorimetric technique for magnesium determination.

In Morgan's system of quick tests for soils, two reagents for magnesium determination were used, namely, titan yellow and p-nitrobenzenazo-resorcinol. The titan yellow test was also used by Baver and Bruner[30] while Gillan[100] applied it for magnesium determination in waters.

Cornfield and Pollard[57] made use of the titan yellow test and determined magnesium in plant digests.

The most important interfering ions in the test are calcium, manganese, aluminium, phosphate and to a smaller extent, iron. The attempts to eliminate such interferences are summarised below :-

1- Separation of the interfering ion by precipitation.
2- Complexing the interfering ion thus rendering it ineffective.
3- Addition of compensating solutions.

Hirschfelder and Serles[101], determining magnesium in blood, separated calcium by precipitation as oxalate prior to the determinationn of magnesium. Becka[102], on the other hand, carried out the determination without the removal of calcium although Kolthoff[99] had shown that the presence of calcium alters both the intensity and nature of the colour produced by magnesium. Baver and Bruner used sucrose to obviate the calcium interference through complex formation. Gillam also made a similar trial.

Peech and English[35] have shown that the addition of sucrose partly eliminated the calcium interference if the magnesium concentration is less than 20 p.p.m. With higher concentrations of magnesium, calcium decreased the colour even in the presence of sucrose. They eliminated calcium interference by the addition of a "compensating solution" containing sufficient calcium to produce maximum intensification of the lake. They used the same procedure to prevent aluminium interference.

Mannite was used by Peech[103] in preventing the interference of small amounts of manganese, while Gillam separated iron and aluminium as phosphates but did not attempt to remove manganese. He used a solution of hydroxylamine hydrochloride to prevent the colour fading of the lake which occurs by oxidation in alkaline solution. Peech attributes the beneficial effect of hydroxylamine hydrochloride reported by Gillam to the action of this reagent in preventing manganese interference rather than any direct effect on colour fading of the lake.

Recently, Mikelsen, Toth and Prince[104] separated calcium and the heavy metals as insoluble tungstates. This is based on the fact that only the alkali and magnesium tungstates are soluble. Mikkelsen[105] and Toth, after studying a large number of dye stuffs found that Thiazol yellow (sodium 2-2 disulphonate of methylbenzolethiazole) was the best for magnesium determination. The sensitivity range of this compound (obtained from general aniline works) is 0 - 30 p.p.m. magnesium as reported by these two workers as compared with 0 - 15 p.p.m. magnesium for titan yellow (obtained from Dr. Grubler and Co., Germany), 0 - 10 p.p.m. magnesium (obtained from Eastman Kodak and Co., P4453), 0 - 8 p.p.m. magnesium (obtained from Eimer and Amend, B 37), 0 - 5 p.p.m. magnesium (obtained from Eastman Kodak and Co., Clayton yellow 770), Titan yellow (often known as Clayton yellow in Britain) is the reagent commonly used in this country.

The present study of the method was undertaken to adapt the method for magnesium determination in Morgan soil extracts. Morgan's solution is buffered at pH 4.8 and it was necessary to adjust the volume of the alkali added to give a satisfactory colour which is stable for a sufficient period for measurement.

From a preliminary study of the method it was observed that titan yellow from different sources differed considerably in the production of the final colour of the lake formed. Accordingly, in all the workm done in this investigation, one sample of titan yellow was used throughout.

Two main points were investigated :-

1st : Effect of Morgan's extractant on the colour formed before and after its neutralisation.

2nd : Recovery of added magnesium to standard solution and to soil extracts.

The method is initially as follows :-

Reagents :-

(1) Compensating solution : 13.9 gms. calcium chloride, 0.96 gms. potassium dihydrogen phosphate and 0.88 gms. potassium alum. are dissolved in water with the addition of two ml. of concentrated HCl. The solution is transferred to a liter flask and diluted to the mark with distilled water.

(2) Mannitol : 2.5% aqueous solution.

(3) Titan yellow : 0.05% aqueous solution.

(4) Sodium hydroxide 3N solution : 120 gms/l.

Procedure :-

An aliquot of Morgan soil extract containing up to 60 µgms. magnesium (usually 1 - 2 ml.) is pipetted into a 25 ml. tube marked at 21 ml. The solution is neutralised by sodium hydroxide, one ml. of the compensating solution and one ml. of mannitol were added and the solution diluted to 21 ml. with distilled water, mixed thoroughly and left in a water-bath at 20 - 21°C for 15 minutes. Then 2 ml. of titan yellow were added with mixing followed by two ml. of 3N sodium hydroxide. The contents of the tube were mixed thoroughly and left to stand for 10 minutes ± 1 minute. Readings were taken in a Biochem Absorptiometer

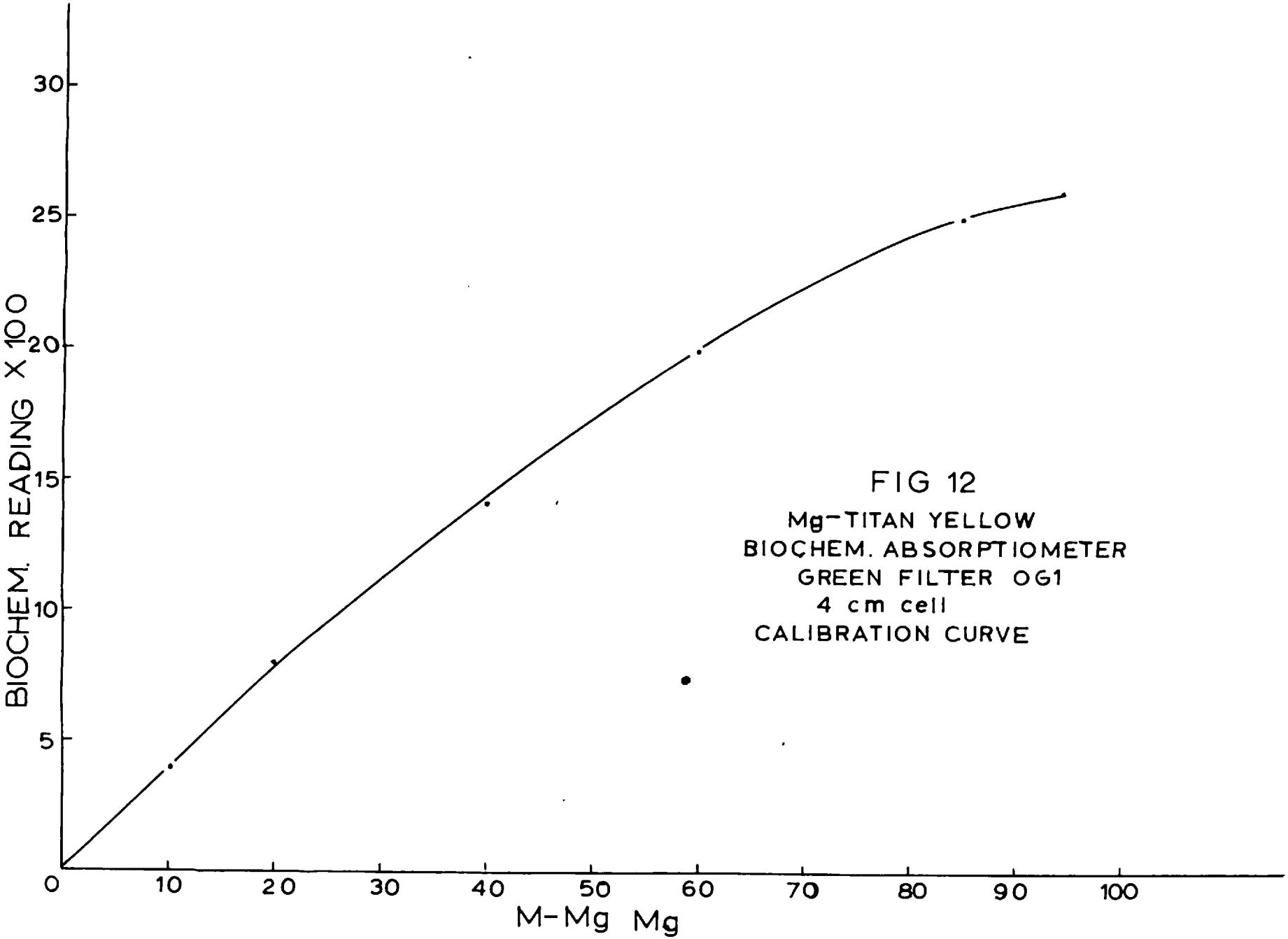


FIG 12

Mg-TITAN YELLOW
BIOCHEM. ABSORPTIOMETER
GREEN FILTER OG1
4 cm cell
CALIBRATION CURVE

using a 4 cm. cell and OG1 green filter.

Effect of Morgan's Extractant :-

Solutions containing 10, 20, 40, 60 and 80 µgms. of magnesium were pipetted into tubes marked at 21 ml., and one ml. of compensating solution was added followed by one ml. of mannitol. The contents were mixed thoroughly and diluted to 21 ml. with distilled water and then mixed again and kept at 20°C for 15 minubes. Two ml. of titan yellow were added with shaking followed by two ml. of sodium hydroxide and the solution was thoroughly mixed, left in the water-bath at 20°C for 10 minutes, after which readings were taken.

Five sets of standards were examined, one was prepared with water only (no Morgan solution was added), 2 sets with one ml. of Morgan solution added and 2 sets with two ml. of Morgan solution added. The colour was developed in the presence of Morgan's solution without previous neutralisation with sodium hydroxide solution,and after neutralisation. The results are shown inn Table 58, p/37

It is clear from the results that the acidity of Morgan's solution (pH 4.8) prevents the complete colour formation if it is not neutralised before the determination. When Morgan solution was neutralised on the other hand, no difference could be traced between standards prepared with water only and in the presence of one or two mls. of Morgan's solution. Figure 12 shows the calibration curve.

Recovery :- Table 59 shows that recoveries ranged from 94 - 103%, p/37.

Table 58.

Effect of Morgan's Solution on Lake formation of Titan Yellow :-

Test Solution	No Morgan Solution added	Absorptiometer Readings x 100			
		One ml. of Morgan Soln.		Two ml. of Morgan Soln.	
		Before neutralisation.	After neutralisation	Before neutralisation	After neutralisation.
10 µgms. Mg.	4.0	3.5	4.0	3.5.0	4.0
20 "	8.0	4.0	8.0	6.0	7.5
40 "	14.0	9.5	13.5	11.5	14.5
60 "	20.0	12.5	19.5	17.0	20.0
80 "	25.0	22.5	25.0	21.5	25.0

Table 59.

Recovery Test :-

Test Solution Morgan extract.	Theory	Found	Error
1 ml. Buxted Soil	--	15.5	--
+ 10 µgm. Mg.	25.5	24.0	- 2.0
1 ml. Harlington Soil	--	27.0	--
+ 20 µgm. Mg.	47.0	47.5	+ 1.0
2 ml. Silwood Soil	--	7.5	--
+ 40 µgm. Mg.	47.5	47.5	0.0
2 ml. Garden Soil	--	12.5	--
+ 10 µgm. Mg.	22.5	21.0	- 6.3
2 ml. E. Pakistan Soil	10.6	--	
+ 10 µgm. Mg.	20.6	20.6	0.0

CHAPTER VII

A Field Test for Potassium in Morgan Soil Extracts using Cobaltinitrite.

Introduction :-

Since the introduction of the cobaltinitrite method for the determination of potassium by Adie and Wood[106] early this century, it has been extensively studied and used by a great number of workers. This is due to the fact that the method is rapid, economical and adaptable to semimicro techniques. The only faster and more flexible method for potassium determination is by the flame photometer.

The cobaltinitrite method depends primarily on the differencies in solubility of cobaltinitrites of sodium and potassium and would have been ideal but for the isomorphism of the sodium and potassium salts.

Volk[107] studied the effect of different concentrations of sodium in the precipitation medium on the domposition of the sodium potassium cobaltinitrite precipitated. He observed That the higher concentration of the precipitating reagent (within certain limits, 10% concentration in the final solution being optimum) the more accurate are the results. He also observed that with the 10% limit the precipitate contained a lower percentage of potassium but was less subject to changes in composotion.

Brown et al[108] succeeded in developing a rapid technique for the potassium determination in soil extracts by precipitating it as cobaltinitrite, oxidising it with excess of ceric sulphate and back titrating the excess ceric sulphate using a o-phenanthroline complex as an indicator.

Tinsely[109] made an extensive study of the possibility

of applying the method for the determination of potassium in many biological and agricultural materials such as blood, milk and different soil extracts.

Kramer[110] has demonstrated that sodium, calcium, magnesium, barium, strontium, zinc, iron, sulphate, nitrate and phosphate ions do not interfere in the determination of potassium by cobaltinitrite. Ammonia however, introduces appreciable error if present in considerable quantities. This interference could be obviated by addition of formaldehyde or hypochlorite.

Addition of different alcohols is very common in this technique asa this decreases the solubility of the precipitate.

The present work was based initially on Tinsely's technique and was undertaken with the view th the approximate estimation of potassium in Morgan extracts of soils using a technique sufficiently simplified to permit a rough estimate of fertiliser requirement in the field by an unexperienced worker. The test is sensitive enough to differentiate between low, medium and high levels of potassium corresponding to levels of 10, 25, and 50 p.p.m. K in the soils.

The method described here was first established in the laboratory using a Biochem Absorptiometer. After several trials the following technique was adopted and simplified for field work as described later.

Reagents :-

(1) Morgan Solution :- 100 gms. of sodium acetate trihydrate

(A. R.) are dissolved in 500 ml. of distilled water in a litre volumetric flask. 30 ml. of glacial acetic acid are added and the mixture is diluted with distilled water to the mark and shaken thoroughly before use.

(2) Sodium Cobaltinitrite
(3) Sodium nitrite
} (A. R.)

(4) Glycol :- 2% aqueous solution (v/v).

(5) iso-propyl alcohol and ethyl alcohol mixture V/V :- mix 100 ml. of each of the alcohols and shake thoroughly before use.

(6) Standard potassium solution :- Dissolve 1.907 gms. of potassium chloride in a litre of Morgan's solution. Dilute aliquots with Morgan's solution to prepare solutions containing 5, 12.5 and 25 µgms. K/ml. i.e. 10, 25, and 50 µgm/K/2 ml.

Apparatus :- Flat bottom tubes 75 m.m. in length and 15 m.m. internal diameter.

A grid made by drawing 2 lines 1 m.m. in thickness and 1/8" of an inch apart with indian ink on a semi-transparent paper kept betweenn two glass plates (photographic plates were used) or alternately a plastic plate half white and half black (supplied by Tintometer Ltd., Salisbury.

Method developed in the laboratory :-

In a tube of the above dimensions, 0.12 gm. of sodium cobaltinitrite and 0.12 gm. of sodium nitrite were weighed and dissolved in 3 ml. of 2% glycol solution. Five ml. of iso-propyl-ethyl alcohol mixture were added and 2 ml. of Morgan soil extract blown in (the soil extract is prepared by shaking 2o gm. of soil with 40 ml. of morgan solution and filtered through Whatman filter paper).

The tube was shaken thoroughly for 30 seconds and left to stand for 40 minutes. The unknowns were compared with the standards prepared simultaneously containing 10, 25 and 50 µgm. K. The test is made as follows :-

Take an empty tube of the above dimensions (75 m.m. length and 15 m.m. inner diameter with a flat bottom) and place it on the grid, pour the solution in just enough to obscure the lines Measure the height each time . By comparing the heights of the different standards (low, medium and high) with those of the unknowns, the status of the potassium in the soil is predicted.

Experimental :-

The Biochem Absorptiometer was used in all these tests to establish the applicability of the method to soil extracts. The method was finally tested with five soils varying in content of Morgan extractable potassium.

Stability of the ~~colour~~ cloud formed.

The test solutions were standard potassium solutions

containing 5, 10, 15, 20 or 25 µgm. K/ml. so that when 2 ml. are used give 10, 20,30, 40 or 50 µgm.K i.e. 10, 20, 30, 40 or 50 p.p.m. K with respect to soil if the ratio soil to Morgan solution used on extraction is 1:2 (e.g. 10 gm. of soil extracted with 20 ml. of Morgan's solution).

0.12 gm. of sodium cobaltinitrite and 0.12 gm. of sodium nitrite were weighed in flat bottom tubes of the above dimensions, dissolved in 3 ml. of 2% glycol, 5 ml. of amyl alcohol mixture added and 2 ml. of the standard were blown in. The mixture was then shaken for 30 seconds. Readings were taken 5 minutes, 20 minutes, 40 minutes and 60 minutes from the time of preparation using the Biochem Absorptiometer and red filter OR2. In order not to disturb the cloud formed, readings were taken in the special tubes (1 cm.diameter) which fitted in a plastic cone used for such purposes. A blank was prepared with set of standards and was used to adjust the zero readings of the absorptiometer before taking readings from standards. Results are shown in Table 60 and graphically (those taken at 40 minutes) in Fig.13.

The results show that the cloud formation increases gradually, reaches its maximum at 40 minutes after which the stability of the cloud declines as shown by readings taken after 60 minutes, p 146.

<u>Height of Standard Solutions at which lines of the grid become invisible</u> :-

The same experiment was repeated and the grid was used to measure the height of the liquid required to obscure the lines.

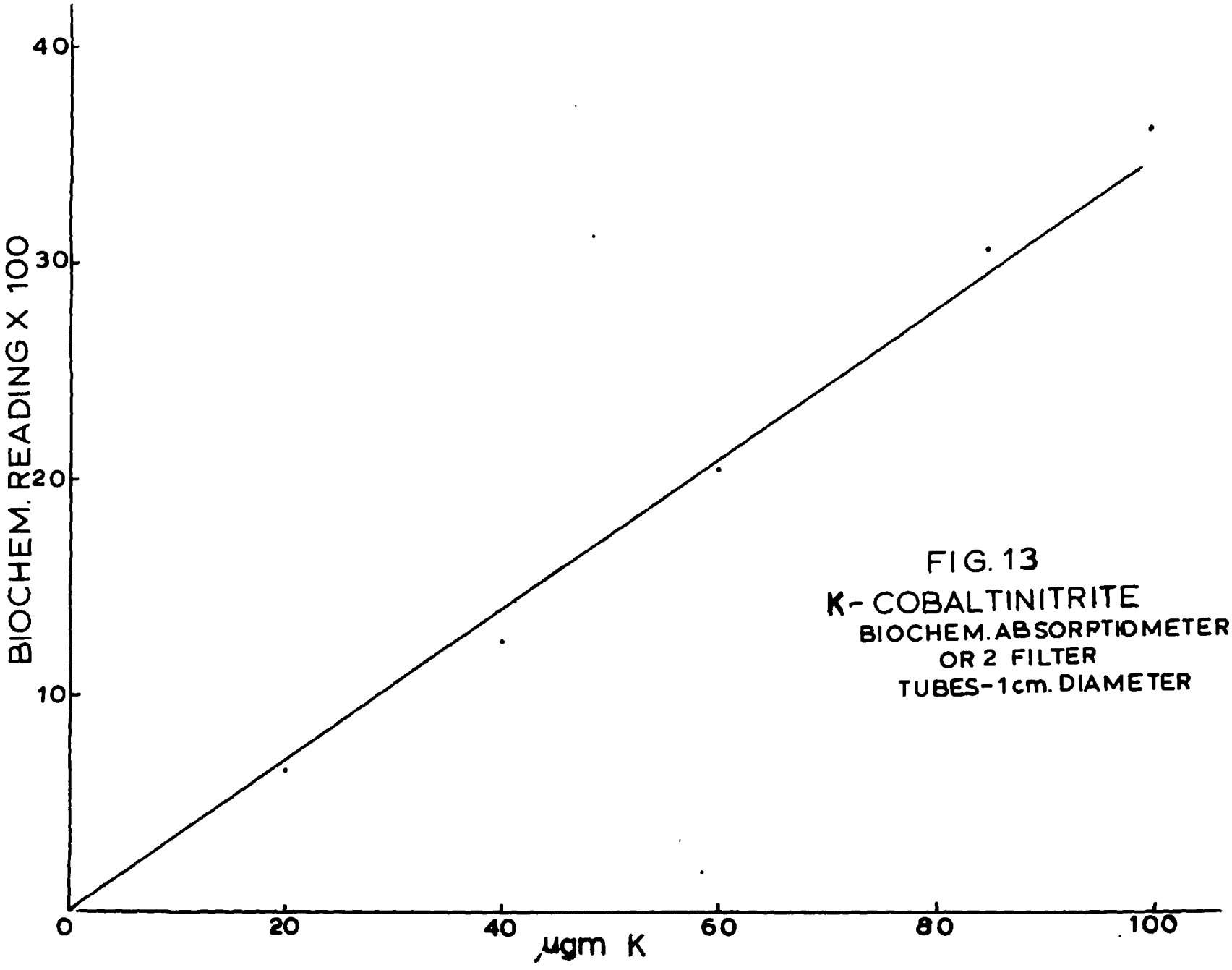


FIG. 13

K- COBALTINITRITE

BIOCHEM. ABSORPTIOMETER

OR 2 FILTER

TUBES-1cm. DIAMETER

Results are shown in Table 61, p146.

Application to Soil :-

Five different soils were tested for their Morgan extractable potassium as follows :- Ten grams of air-dry soil were extracted with twenty ml. of Morgan's solution by shaking for 1 mi and filtering through a No. 1 Whatman filter paper.

The mixture of sodium cobaltinitrite and sodium nitrite (0.12 gm. each) was dissolved in 3 ml. of 2% glycol, 5 ml. of alcohol mixture were added and 2 ml. of the soil extract were blown in and the whole mixture was shaken vigorously for thirty seconds. Readings were taken after 40 minutes and are shown in Table 62, p147.

Adaption of the test for field work :-

It was agreed that the cobaltinitrite as well as the nitrite sodium salts could be prepared in tablet form for field work. In the field, the soil is either weighed by a rough balance on a sheet of polythene or estimated by measuring it on a spoon which carries a mark showing roughly the volume of soil required for the test (usually 5 or 10 grams). The tube used for the test is marked at the 3rd ml., 8 ml and 10ml.levels. The above test for potassium was simplified for field work as follows:

Fill the tube to the 3 ml. mark with the glycol solution, dissolve in it one tablet of sodium cobaltinitrite and another of sodium nitrite. When the tablets are completely dissolved,

add 5 ml. of the alcohol mixture, mix thoroughly and add two ml. of the soil extract. Shake vigorously for thirty seconds and after 40 minutes standing put the tube used for the determination on the grid and look through the depth of the liquid ; if the lines are clear, then the potassium status of the soil is low. On the other hand, if the lines are not visible, take another tube and pour in solution till the lines are just obscured. Measure the height.

From the work done in the laboratory, it was found that soils containing less than 10 p.p.m. Morgan extractable potassium when tested, do not obscure the lines of the grid at all with the use of the whole volume of the liquid used in the test. With soils which contain 25 p.p.m. Morgan extractable potassium the lines become invisible at 1.5 - 2.0 cm. height, while the soils which give higher values than 25 p.p.m. Morgan extractable potassium the lines are obscured at a lower height (0.5 - 1.5 cm.)

N.B. It was found that up to 50 µgms. ammonium added as ammonium chloride did not affect the readings when tested in the presence of the three levels of potassium above mentioned.

Table 60.

Stability of the cloud formed :-

Test Solution µgm. K.	Absorptiometer Readings X 100			
	5 minutes after preparation.	20 minutes	40 minutes	60 minutes
20	5	5.5	6.5	5.0
40	9.0	11.5	12.5	10.5
60	15.5	18.0	20.5	17.5
80	23.5	28.5	31.5	29.0
100	29.5	33.5	36.0	33.0

Table 61.

Standard Solutions :-

Test Solution µgm. K.	Absorptiometer Readings X 100	Height of the solution just enough to obscure the visibility of the line in cm.
20	6.5	3.8
40	12.5	3.2
60	20.5	2.3
80	31.5	1.7
100	36.5	1.2

Table 62.

Application of the Method to Soil Extracts :-

Soil extract used.	Absorptiometer Readings X 100.	Height of the liquid in the tube just enough to obscure the lines of the grid.	Remarks as to K Status.
Harlington Soil.	27.0	Less than 1 cm.	High
Silwood Soil.	9.5	4 cm.	Low
Burted Soil.	19.5	2.3	High
Garden Soil.	22.0	1.2	High
E. Pakistan Soil.	5.0	4.4	Low

CHAPTER VIII

"Studies on the Effect of Different Moisture Levels of Soils on Mineralisation and Extractability of Nutrients."

INTRODUCTION :-

Mineralisation under different moisture levels :-

Mineralisation is the process of breakdown of complex compounds into simpler ones finally into simple inorganic compounds or elements which are utilised by plants or the soil fauna. The most important agents which play the major role in the mineralisation process are the micro-organisms inhabiting the soil. Almost every element is subject either directly or indirectly to the action of these organisms. Iron, manganese, aluminium, magnesium, sulphur, phosphorus, potassium, calcium, zinc, cobalt, nickel, carbon, nitrogen, hydrogen and oxygen compounds in the organic and inorganic forms are acted upon by bacteria, fungi, algae and other members of the soil fauna. The net result is the formation of inorganic compounds which are utilisable by the growing plants or by the soil inhabitants. Other agents which take part in the process of mineralisation are either physical or chemical in distinction from the biochemical processes above mentioned. It is a well established fact that activities of the micro-organisms are affected by temperature, moisture, pH of the medium, light, oxygen supply and in general, any factor which affects the activity of such organisms will affect mineralisation processes.

This work was undertaken to study two main points :-

1st :- The mineralisation of iron, manganese, aluminium,

magnesium, potassium, sulphur, and phosphorus at different moisture levels.

2nd :- The effect of air-drying the soil from different moisture levels on the extractability of these elements , using Morgan's solution.

A quick survey of the forms of these elements in the soil reveals the fact that iron is present in the soils as the minerals, hornblende, biotite and chlorite. The iron in the minerals is largely in the ferrous state and on weathering, most of the iron is changed to the ferric state and forms different iron compounds. In nature, the kind of parent material and the conditions under which it is weathered influence the valency state, distribution and kinds of iron compounds formed.

Manganese, on the other hand, occurs in some ferro-manganese minerals and other complex silicates and as the oxides and hydrated oxides. A certain amount of iron and manganese occurs in soils in organic forms which during mineralisation are converted into soluble salts. These salts may be utilised by plants or micro-organisms inhabiting the soil. Under normal agricultural conditions, these two elements are not likely to be lost by leaching especially if well-aerated conditions prevail and the insoluble higher oxides are formed.

Aluminium is one of the most abundant elements in soils especially in the clay fractions where it occurs in combination with the silicates. It seems likely that mineralisation of

aluminium is not readily brought about as in the case of iron and manganese. Magnesium is introduced into the soil in considerable quantities in the form of inorganic materials such as dolomite or rock phosphate, often used as fertilisers or in the organic matter added to the soil. Ames[111] reported that magnesium compounds are less resistant than those of calcium to the action of nitric and sulphuric acids formed in the process of nitrification and sulphur oxidation.

Sulphur is added to soils as ammonium and potassium sulphate or sometimes in the form of elemental sulphur to change the soil pH. Often, other different fertilisers contain an appreciable quantity of sulphates e.g. grades of superphosphates, one of the most commonly used fertilisers, were reported by Mehring, and Lundstrom[112] to contain as high as 8.09% sulphur. Sulphur is also added to soils in the form of organic materials such as manures and plant residues. Moreover, rain contributes to the sulphur content of the soil sometimes in appreciable quantities. The changes which organic sulphur compounds undergo during mineralisation processes are governed largely by prevailing conditions of the soil e.g. moisture content, aeration and also the nature of the sulphur compound. According to Waksman[113], the organic matter is first decomposed by heterotrophic bacteria, fungi, actinomycetes and the sulphur bearing fraction is liberated. In the presence of sufficient air (oxygen), the sulphur is changed to sulphate ($SO_4^{=}$)

having first been reduced by hydrogen sulphite and then oxidised. the sulphurous and sulphuric acids produced in the soil as a result of sulphur mineralisation exert a solvent action upon other minerals. Under anaerobic conditions however, the reduction processes predominate and sulphates are reduced to elemental S and H_2S if such conditions prevail.

Phosphates are present in normal soils in the form of inorganic as well as organic compounds. The inorganic compounds include phosphates of potassium, sodium, calcium, magnesium, aluminium, iron and manganese. The organic compounds include the phosphorus of plant and animal residues as nucleic acids, lecithin and phytin. When organic matter is mineralised, the phosphorus is liberated in the form of inorganic salts which may be reassimilated by the same or other micro-organisms and synthesised into microbial protoplasm. Meanwhile, insoluble organic phosphates may be made soluble by the products of metabolism of micro-organisms, such as carbon dioxide and various organic and inorganic acids.

A large number of micro-organisms including various heterotrophic bacteria, fungi and actinomycetes are capable of decomposing organic phosphorus compounds. A part of the potassium supply of mineral soils comes from the minerals e.g. orthoclase, microcline, muscovite and biotite. A certain quantity of the potassium exists in the "fixed" form i.e. very difficult to replace. Another portion of the potassium may be held as a constituent of the organic matter particularly if an appreciable

quantity of fresh plant residue is present. Potassium minerals differ considerably in their degree of weathering, microcline being very resistant to weathering agents, thus gives up its potassium very slowly; orthoclase is less resistant than microcline. The potassium of muscovite and biotite, on the other hand, is fairly available especially if the minerals are in the finely divided state as reported by Denison(114).

Potassium is usually added to the soil in the form of soluble inorganic salts such as potassium sulphate or potassium nitrate or in some localities, insoluble form such as marl or an insoluble form as manure. The activities of bacteria may lead to an increase in the available potassium as by the decomposition of organic matter and by the formation of acids which liberate the potassium from its minerals. Microbial activity may also lead to a decrease of the available potassium through a process of assimilation and transformation into organic materials. According to Ames(111) the liberation of potassium in the soil is brought about by the salts formed gatherather than by the direct action of acidity on insoluble potassium compounds although he found that both nitrification of organic and inorganic compounds and oxidation of sulphur in the soil increased the amount of water-soluble potassium.

Thus, plant nutrients in general are present in either the inorganic or organic form and the organic compounds are acted on by different micro-organisms inhabiting the soil, and the nutrients are mineralised and i.e. liberated in forms available to

the plant. The process of mineralisation whether chemical or biological, requires moisture which should be present in the medium which is the soil. Little attention was given to the effect of different moisture levels on mineralisation and mobilisation of plant nutrients.

Morgan's solution was used in the present study to extract the soil, keeping the ratio, soil to extract 1:2.

It is general practice to air dry the soil before it is subject to chemical analysis. In the field tests however, the soil may be used in its original form (moist) and a sub-sample taken for moisture determinations in order to express the results on one and the same basis. Undoubtedly, soils in the field vary considerably in their moisture content and it is of interest to investigate the effect of the air drying of soils on the status of the nutrients and on their extractability using Morgan's solution.

Experimental :-

Five soils were collected to the depth of 6", air dried, sieved through a 2 m.m. sieve before use. A short description of the soils used is give below :-

(1) Buxted Spil :- This soil is collected from a farm near Buxted in Sussex. It is greyish in colour, acidic, with a pH of 5.28, It is comparatively rich in organic matter. It is with a very low clay but high silt and sand percentage as

revealed by the mechanical fractionation of the mineral portion of the soil.

(2) Garden Soil :- This soil was collected from an allotment in Harrow, Middlesex. The soil is dark, brownish, neutral, having a pH of 7.14. This soil contains 26% clay, 38% silt and 31% sand. This, together with the high percentage of organic matter accounts for the high water holding capacity.

(3) Harlington Soil :- This soil was collected from a plot under grass in the College Field Station. The soil is light-brownish in colour and is slightly alkaline in reaction, with a pH of 7.56. This soil is exceptionally rich in the most of the nutrients studied.

(4) Silwood Soil :- The soil was collected from Silwood Park, Research Station, from a plot which is ploughed annually and is under grass. The soil is very sandy (81.5% sand), dark in colour with 3.56% organic matter.

(5) East Pakistan Soil :- This soil was brought from Dacca University Farm to the department to be used for other purposes. Its reddish-brown colour suggests that it is lateritic. Compared with other soils studied, this soil is very poor in plant nutrients studied, however, it is notably high in manganese. The soil is acidic, ph 5.89, very poor in organic matter (0.51%) and with 17.5% clay,

29.2% silt and 51.5% sand-

The rest of the analysis of these soils is given in Table 63. The Morgan extractable iron, manganese, aluminium, magnesium, potassium, sulphate and phosphate were determined in each soil before the incubation of the soil (as described below) and results are given in the table of the corresponding soil (Tables 64 - 68).

Incubation :- The effect of three different levels of soil moisture namely, 25%, 50% and 75% of the moisture holding capacity on the mineralisation and extractability of nutrients on air drying of the soil were studied. Plastic flower pots having a total capacity of 300 gms. of soil were used. Each pot was numbered, weighed accurately (with a piece of glass cloth previously treated with HCl, rinsed till it was chloride free and dried at 60°C.) to hold the soil in position. Half of the soil was weighed into the pot and mixed with half the volume of the water required to bring it to the M.H.C. Then the other half of the soil was added and mixed with the rest of the water required to bring the soil to the desired water level. The pots, with the moist soil were then incubated at 28°C and the loss of water from each pot was replaced by weighing each pot independently and adding distilled water till the original weight was regained.

Sampling and Extraction :- The incubated soil was sampled at three week intervals and extracted with Morgan's solution in the wet condition i.e. at the moisture level at which these soils were maintained. In order to extract the soil at a moisture

content at which the pots had been kept, the incubated soils were brought to the required moisture level as usual and the soil was sampled by weighing out fifty grams for analysis,

The amount of water required to dilute a Morgan solution of double the usual concentration to the 1:2 ratio with respect to the oven-dry soil was calculated, taking into account the amount of moisture present in the sampled moist soil.

Four samples were analysed in the twelve weeks (at 3weekly intervals.) After the fourth sampling, the soil remaining in the pots was spread on polythene sheets in the constant temperature room to dry, then extracted with Morgan solution, as usual a sub sample was taken for air dry moisture and all the results were expressed as p.p.m. on oven-dry basis.

Results :-

The soils under investigation were analysed in the air-dry condition at the beginning of the experiment (i.e. before incubation). After twelve weeks of incubation, the remaining soils in the pots were air dried and the Morgan extractable nutrients were again determined. The results are given in Tables 64 - 68.

The difference between Morgan extractable elements before and after incubation was taken as the amount mineralised in twelve weeks. The difference is expressed as Percent of the original amount extracted from the corresponding soil before incubation.

The results are shown in Table 69.

Table 70 shows the difference, per cent, in extractability of the elements studied when using the same soil in the wet condition, and after air drying. The figures express the increase or decrease per cent in the extracted elements on the basis of the air dry soil. All the results are expressed on oven-dry basis to enable comparative study.

Discussion of Results :-

Mineralisation :- It is observed that in general, the extractability of all elements studied is greater the higher is the moisture content. Iron and manganese showed wide variations at the different moisture contents of soils. An extreme example is met with in the Buxted soil. The Morgan extractable manganese of the air-dry soil before incubation was 40 p.p.m. on oven-dry basis. After six weeks of incubation at 75% M.H.C., the Morgan extractable manganese increased to 420 p.p.m. on oven-dry basis (i.e. the extractable manganese increased 10 1/2 times in 6 weeks incubation at 75% M.H.C. at 28°C. This is not the case with the lower levels of moisture where changes observed were far below this value.

Iron behaved similarly in this respect in general, relatively large quantities of iron are mineralised at the higher water content. In the same soil (Buxted) and at the same moisture level (75% M.H.C.) the soil which originally gave 2.9 p.p.m. Morgan extractable iron when extracted in the air-dry condition,

gave 70 p.p.m. Morgan extractable iron after incubation. It seems possible that moisture affects the elements inn twé ways :-

1st :- At higher moisture levels and a high percentage of the pore space is filled with water, and oxygen, becomes less available, and thus conditions approach the anaerobic conditions more than at the low moisture levels, especially when the finer fractions of the soil (namely clay and silt) predominate. Under such conditions, more of the two elements tend to exist in the lower state of valencies, both of which are more soluble than the oxidised form.

2nd :- At higher moisture levels more of the soluble forms will mobilised and dissolved than at lower moisture levels due to the abundance of moisture.

Aluminium followed more or less the same trend as iron, more aluminium appearing in solution at the higher moisture levels. Mineralisation was more rapid at the 75% M.H.C., particularly in acid soils, as compared with the two lower levels of moisture. In the same Buxted soil, the Morgan-extractable aluminium at the beginning of the experiment (before incubation) was 5.24 p.p.m aluminium as against 40.6 p.p.m. Morgan-extractable aluminium after six weeks of incubation at 75% M.H.C. The behavior of iron, manganese and aluminium in this manner in such a soil may be attributed partly to the acid reaction of the soil and partly

to the high percentage of silt which possibly contributed partly to the partially anaerobic condition when the soil was incubated at 75% M.H.C.

In neutral or alkaline soils studied (garden soil, pH 7.14 and Harlington soil, pH 7.56), the picture was essentially the same in the sense that comparatively larger quantities of iron, manganese and aluminium were mineralised at higher moisture levels, notably at the 75% M.H.C. level. However, it was very clear from the results that the fluctuations were relatively smaller and in no case reached those in acid soils at any level of the moisture in any of the soils studied, e.g. in the garden soil, Morgan-extractable iron was 5.04 p.p.m. at the beginning of the experiment (i.e. before incubation) and the highest value recorded was 12 p.p.m. at 75% M.H.C. after six weeks of incubation. Manganese in the same soil behaved in a very similar manner as originally it was 3.5 p.p.m. and after six weeks of incubation it rose to 11.4 p.p.m. at 75% M.H.C., while aluminium was 2.6 p.p.m. and became 8.0 p.p.m. at the same moisture level after the same time of incubation. The same picture was obtained in the Harlington soil which is more towards the alkaline side than the garden soil. Thus it is concluded that mineralisation of iron, manganese and aluminium is dependent on the reaction of the soil and their mobilisation and activity under acid conditions, by far exceeds their activity under alkaline conditions.

Magnesium :- Magnesium mineralisation generally increased with

the moisture level. Comparison of the data obtained at each moisture level shows that mineralisation increases with time and then becomes more or less constant. The case was not the same however, in Silwood soil and the E.Pakistan soil where the mineralisation in the first and second moisture levels (25% and 75% M.H.C.) was higher than at the third level. In the Silwood soil which is a very sandy soil (81.6% sand) with very low clay (5.2%) and silt (7.9%) the original native magnesium was washed out to a great exteht, the value of Morgan-extractable magnesium being low compared with that of other soils. Thus the increase in magnesium obtained during incubation is presumably from the organic matter rather than from the mineral fraction of the soil. The mineralisation of the organic matter in this soil was higher at the 25% and 50% M.H.C. levels than at the 75% level. This irregularity in this soil was noticed not only in the case of magnesium but also in the case of iron and phosphate especially towards the end of the experiment i.e. at the 3rd and 4th sampling Possibly the breakdown of organic matter which proceeds more rapidly in presence of sufficient oxygen is restricted at the higher moisture content due tov the more complete filling of the pore spaces with water.

In the E.Pakistan soil however, the case is the reverse as the organic matter content of this soil is extremely low (0.5%) and the contribution of the mineralised magnesium comes from the mineral part of the soil mainly.

Sulphate :- Generally there was a gradual increase in Morgan extractable sulphate with the increase of moisture level. The increase in the extracted sulphate became, in most cases, greater with the progress of the experiment. An increase in the sulphate is almost always associated with a considerable increase in most of the other elements studied. The effect being greater, the higher the moisture status of the soil. This statement could be explained by the following examples from the results of thid experiment :-

a :- In Barted soil, The morgan-extractable sulphate in the air-dry condition at the beginning of the experiment was 8.75 p.p.m. SO_4" (oven-dry basis), while aluminium was 5.24, magnesium 130, phosphate 2.5 and potassium 60 p.p.m. In the first sampling after 3 weeks incubation at 75% M.H.C., the aluminium increased to 40.5, magnesium to 200 p.p.m., phosphate to 20 and potassium to 124 p.p.m. (oven-dry basis) while the increase of the sulphate was to 41.25 p.p.m.

b :- In the Garden Soil, In the second sampling (i.e. after 6 weeks from incubation), also at the 75% M.H.C., the Morgan-extractable- SO_4" increased from 23.75p.p.m. in the air-dry condition, to 31.24 after 6 weeks incubation and aluminium increased from 2.6 to 8.0 p.p.m., magnesium from 285 to 550 p.p.m., phosphate from 5.0 to 17.5 p.p.m. (oven-dry basis).

c :- Harlington Soil :- The same picture is maintained. It

is worth mentioning however, that in most cases an increase in Morgan-extractable-sulphate was always associated with an increase in aluminium and potassium values. Where the sulphate increase was great, there was a notable increase in almost all the elements studied. The explanation given to this phenomenon is that the final product of the sulphur mineralisation is sulphureus and sulphuric acids which exert a remarkable solubility effect on the rest of the elements, bringing them into solution as shown by analysis of Morgan extracts.

Phosphates :- The phosphates became more soluble the higher the moisture content particularly at the 75% M.H.C. In most of the soils however, very little increase is gained by increasing the moisture level from 25% to 50% M.H.C. It seems possible that the mobilisation of the phosphate is related to the pH of the soil; Harlington soil being slightly alkaline (pH 7.56) showed one remarkable increase in Morgan-extractable phosphates in all the samples analysed and the net gain in phosphate content at the end of the incubation period (12 weeks) was roughly three times the original value, i.e. from 10.6 to 44 p.p.m. (Table 66.) In Buxted soil on the other hand, which is an acid soil (pH 5.28) the net gain was only one and a half times the original value. from 2.5 to 4.0 p.p.m. Moreover, in the Garden Soil, which is almost neutral in reaction (pH 7.1), there was a net gain of twice the original value (5 increased to 15 p.p.m.). The data suggest that mineralisation of phosphates is favoured more

by a slightly alkaline or neutral soil than an acidic one. It is worth mentioning however, that the Harlington soil contains slightly less organic matter than the Buxted soil (2.34%)in Harlington soil and 2.65% in Buxted soil), whereas, the Harlington soil contains 10.3% more clay, 10% less silt than the Buxted soil. Thus, it is reasonable to attribute the differences in mineralised phosphates to the pH of the soil rather than to the mineral or organic fraction.

Potassium :- Potassium showed great uniformity in its mineralisation, in all the different soils. There was a very steady increase in the values of the Morgan-extractable potassium in all the stages of the experiment at different moisture levels. The increase is gradual from 25% level to the 50% level to the 75% level. It was observed however, in most of the cases, that the increase in mineralisation from the 50% level to the 75% level is almost always higher than between the 25% and 50% levels. This becomes clearer by examination of the curves of potassium of the corresponding soil.

Mineralisation Curves :-

Generally speaking, these curves could be divided into 3 different parts :-

1st :- Wetting Period :- Wetting the soil resulted in an increase of all elements in all soils with the exception of magnesium and, in a few instances iron and manganese e.g. in the case of

KEY

(FOR FIG 14 — FIG 48)

—+—+—+—+—+— 25% M.H.C.

---•---•---•---•--- 50% M.H.C.

—•—•—•—•—•— 75% M.H.C.

M.H.C. Moisture Holding Capacity

1: BUXTED SOIL

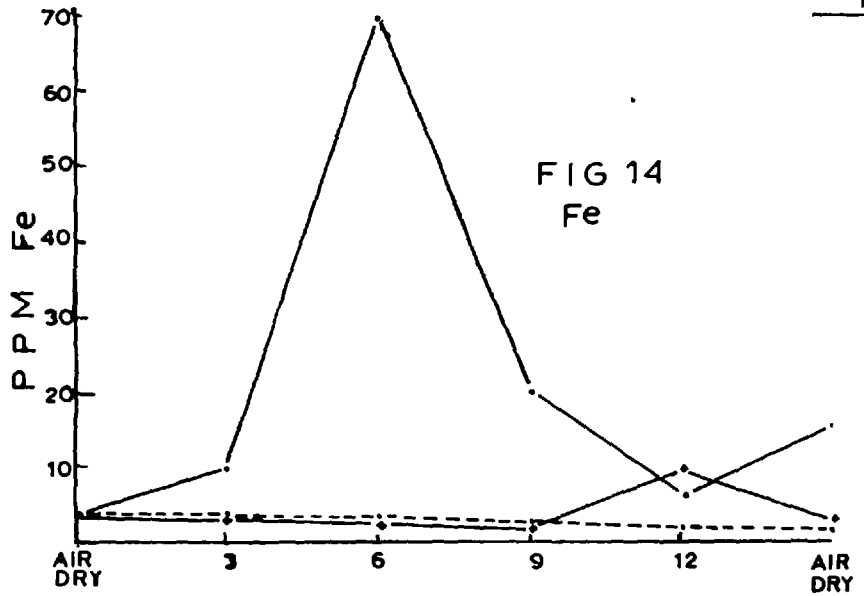


FIG 14
Fe

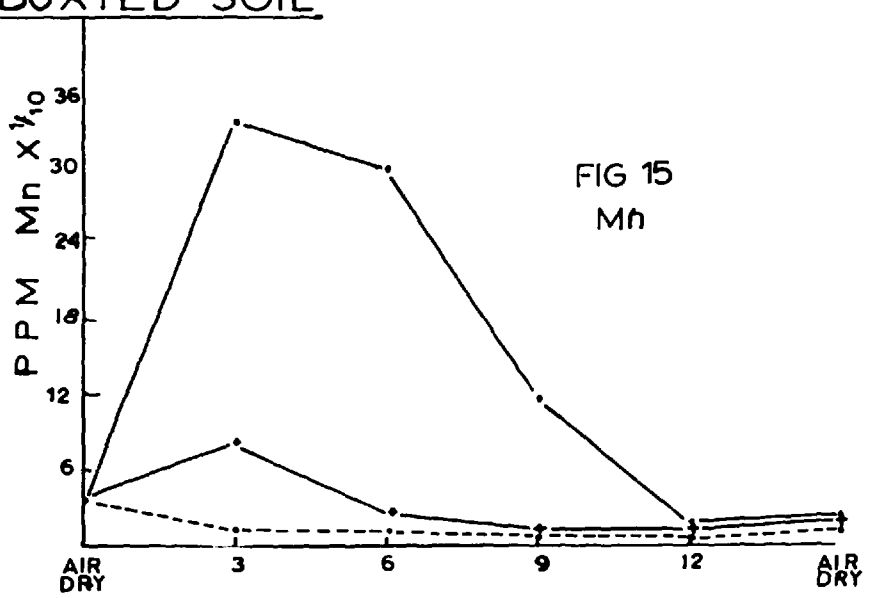


FIG 15
Mn

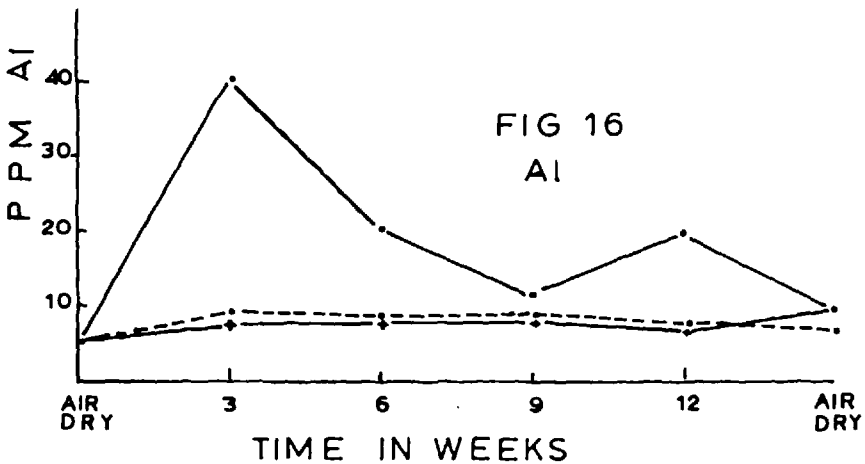


FIG 16
Al

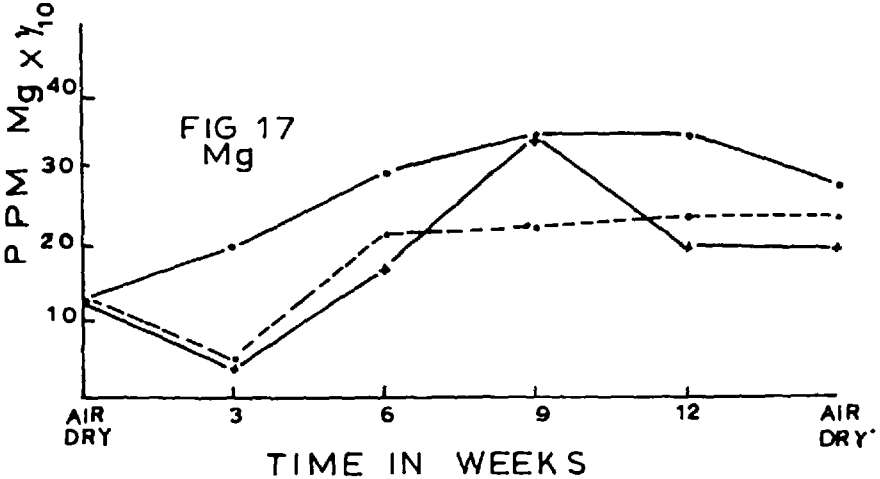


FIG 17
Mg

BUXTED SOIL (CONT.)

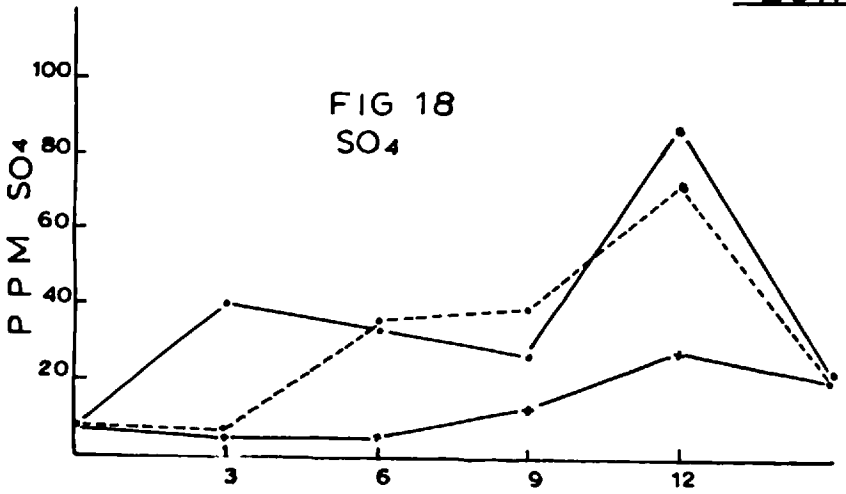


FIG 18
SO_4

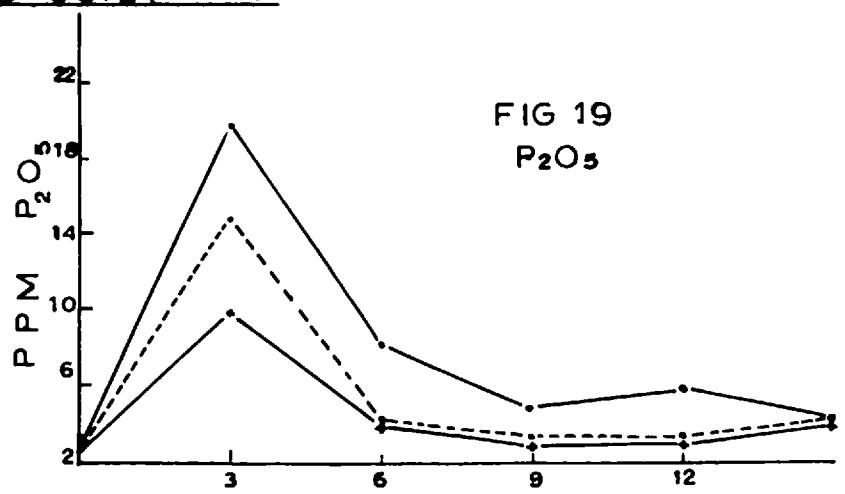


FIG 19
P_2O_5

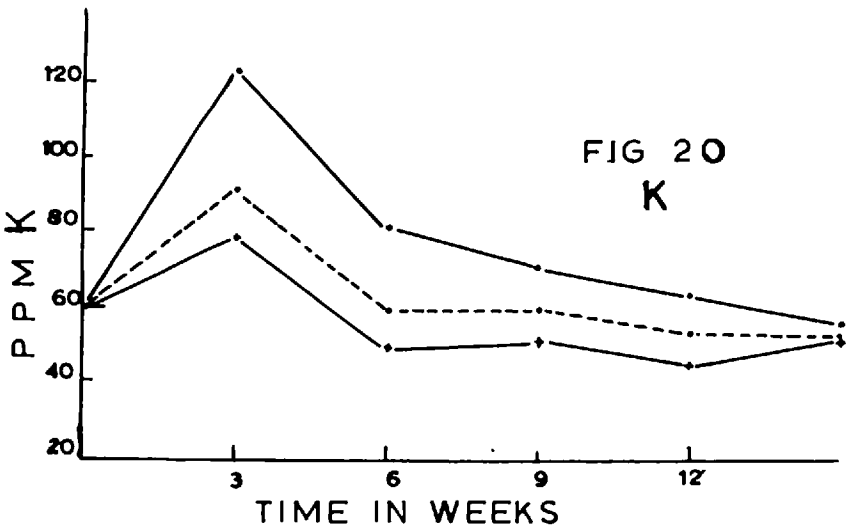


FIG 20
K

2: GARDEN SOIL

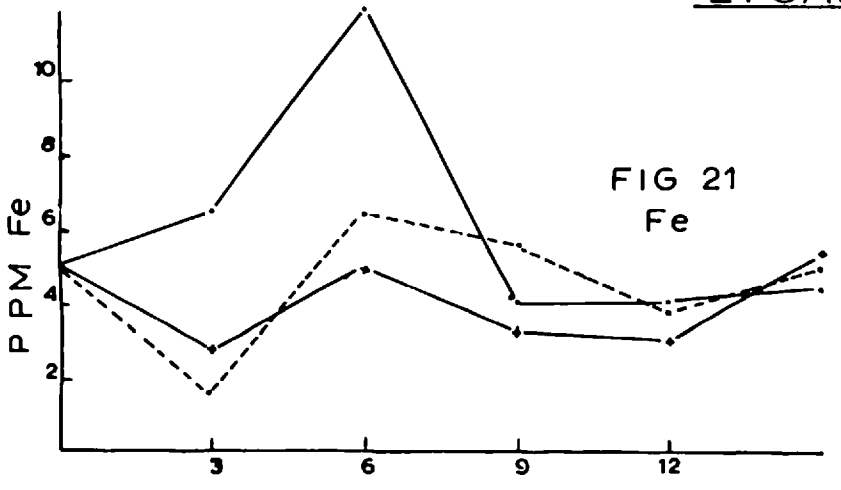


FIG 21
Fe

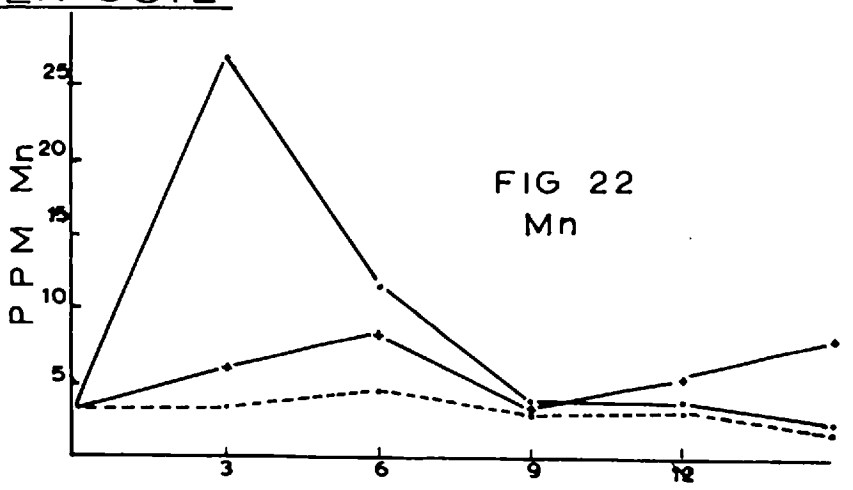


FIG 22
Mn

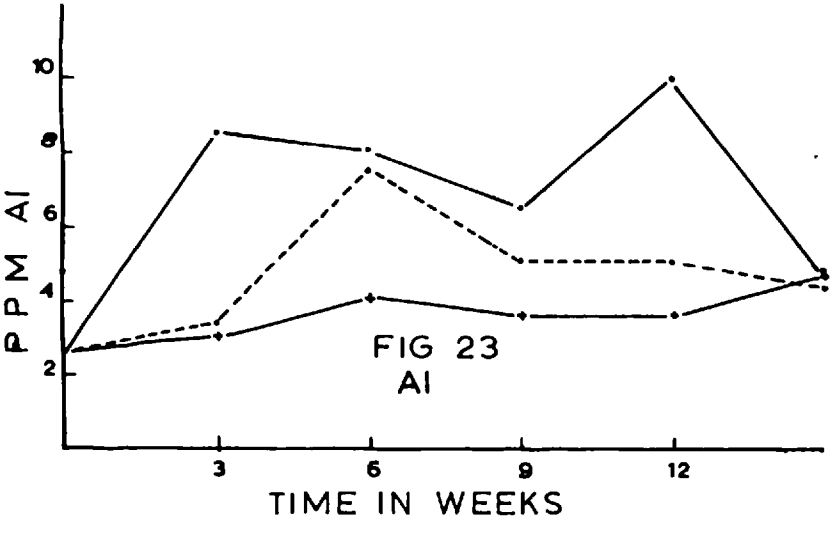


FIG 23
Al

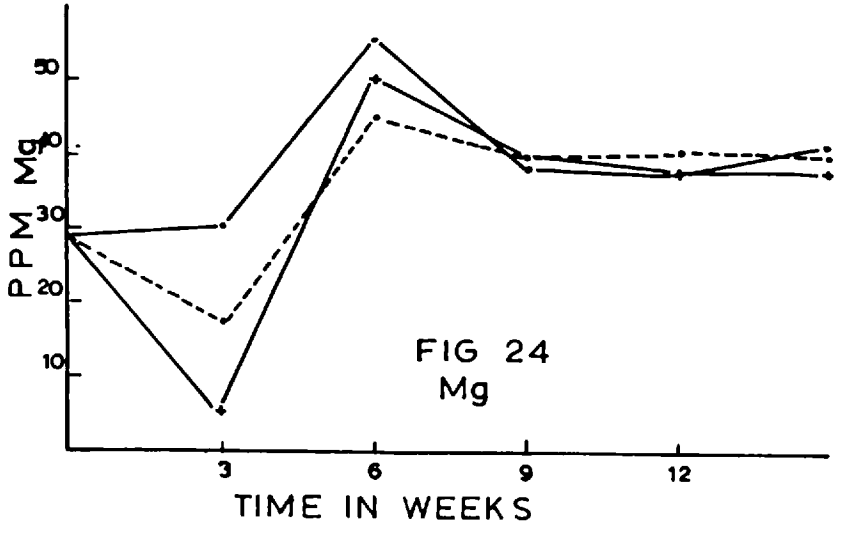


FIG 24
Mg

GARDEN SOIL (CONT.)

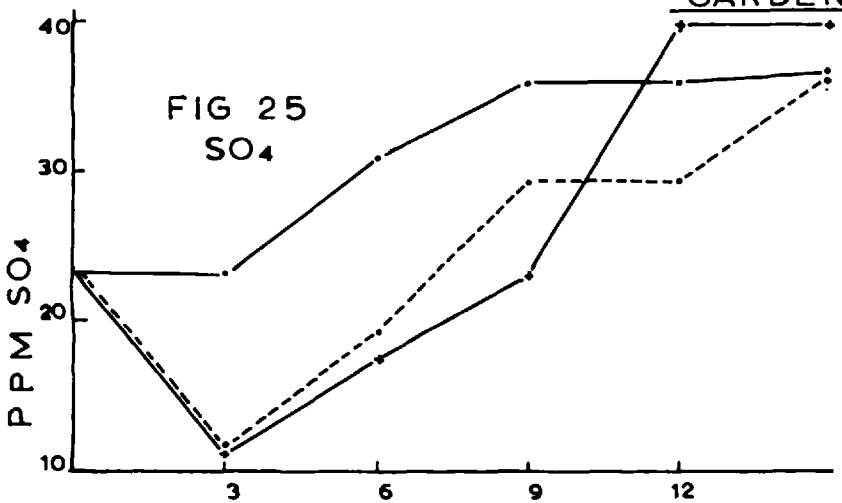


FIG 25
SO_4

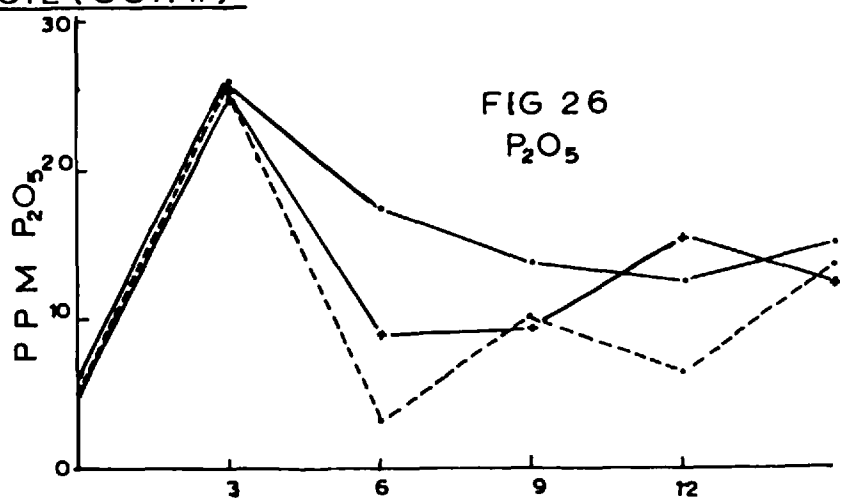


FIG 26
P_2O_5

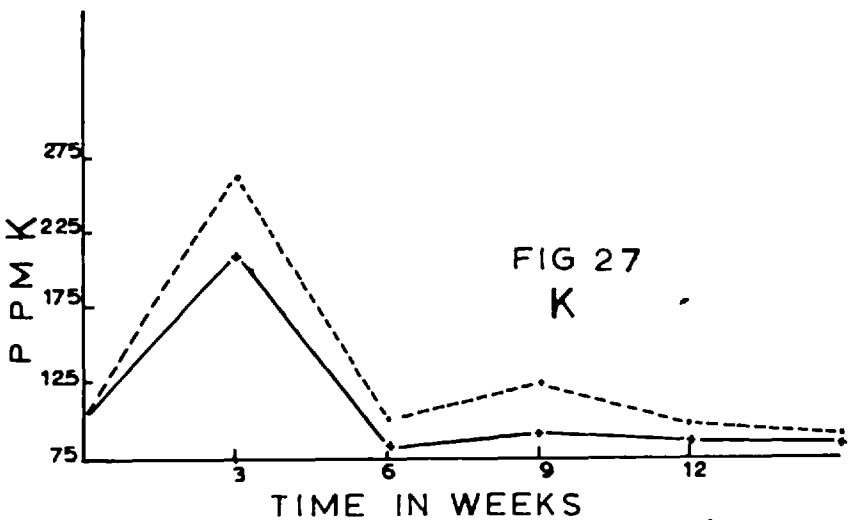


FIG 27
K

3: HARLINGTON SOIL

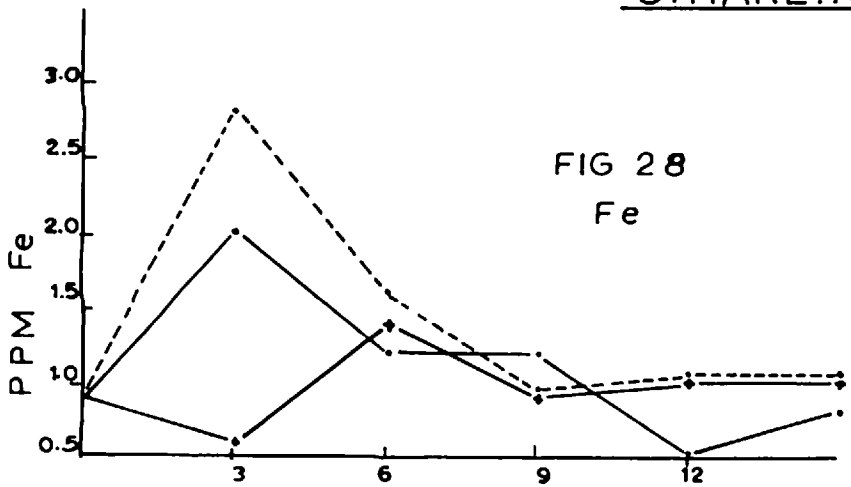


FIG 28
Fe

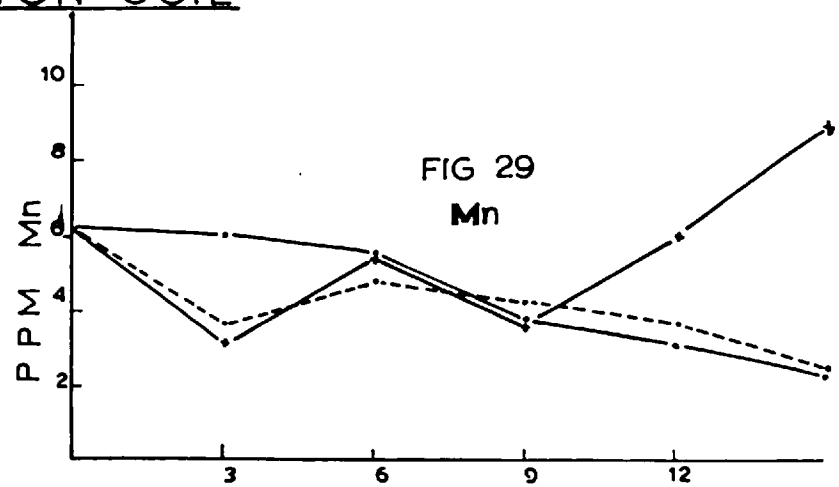


FIG 29
Mn

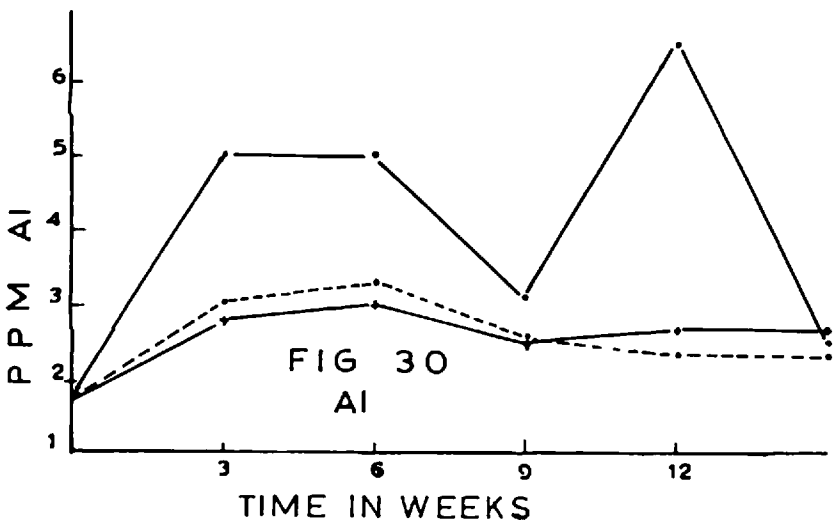


FIG 30
Al

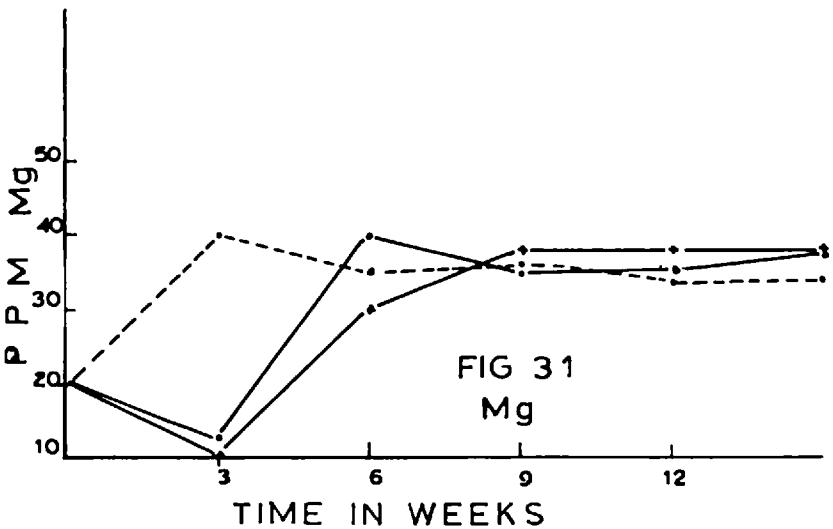


FIG 31
Mg

HARLINGTON SOIL (CONT.)

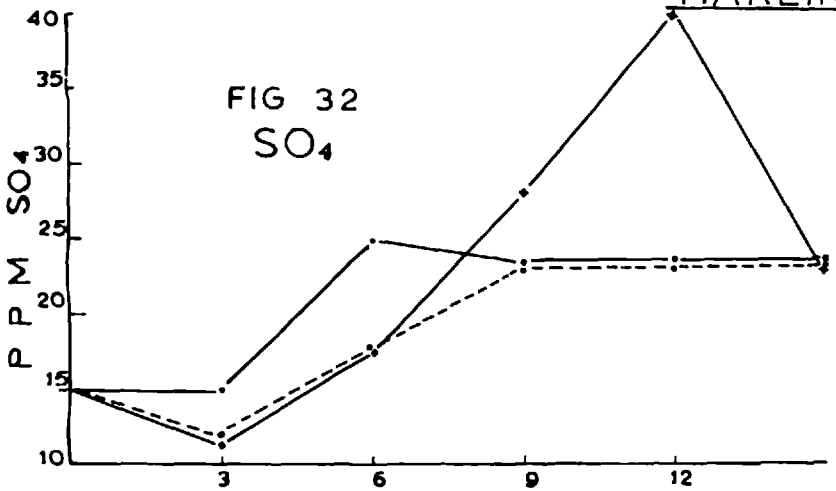


FIG 32
SO_4

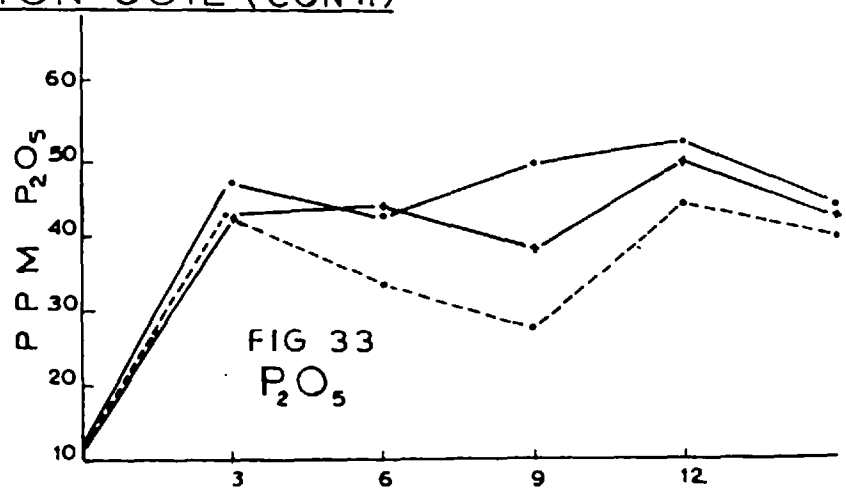


FIG 33
P_2O_5

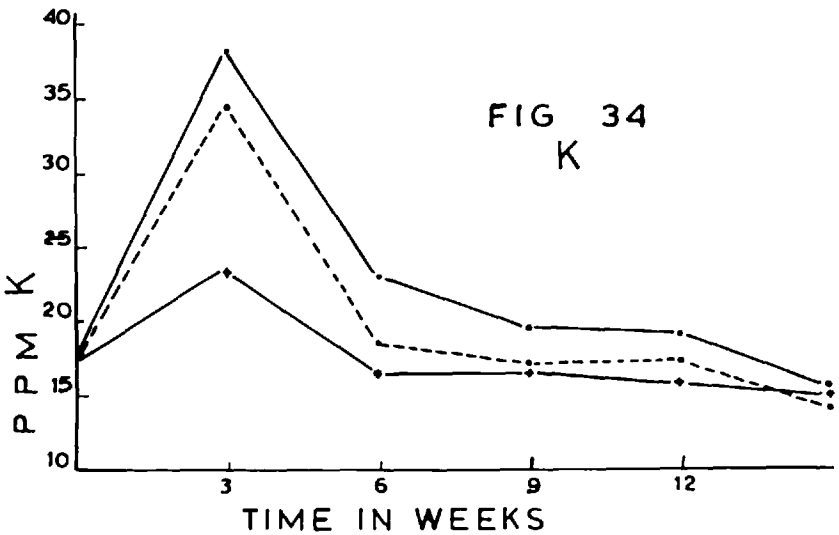


FIG 34
K

4: SILLWOOD SOIL

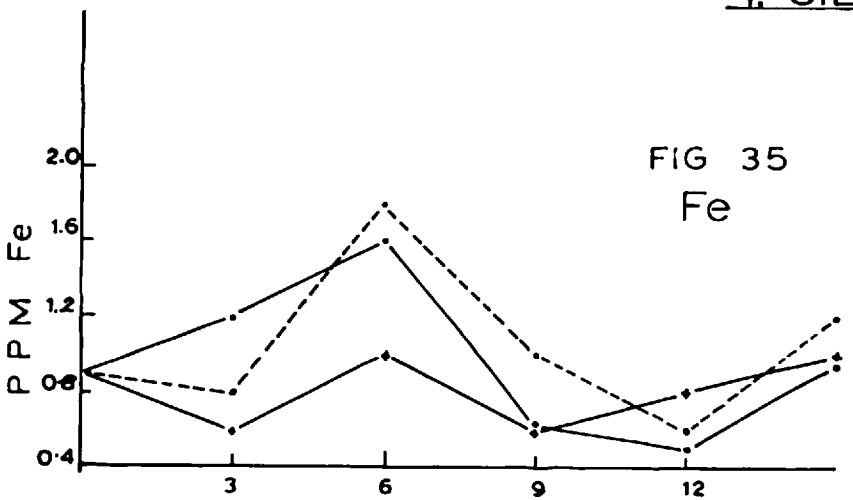


FIG 35
Fe

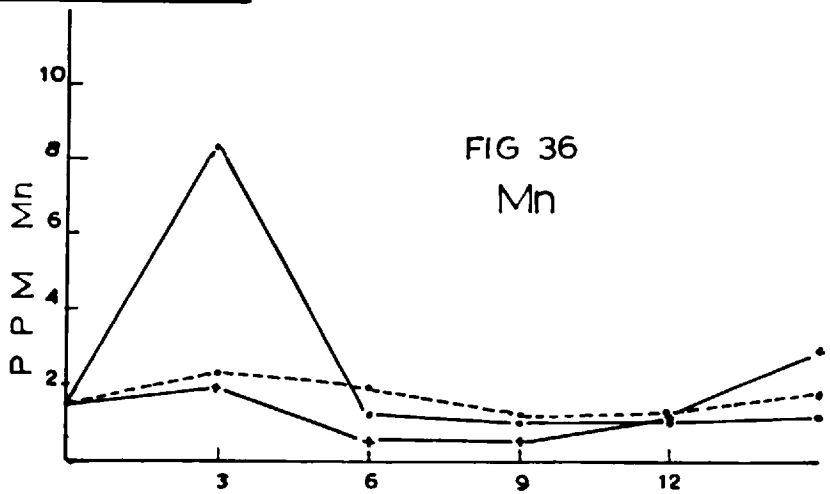


FIG 36
Mn

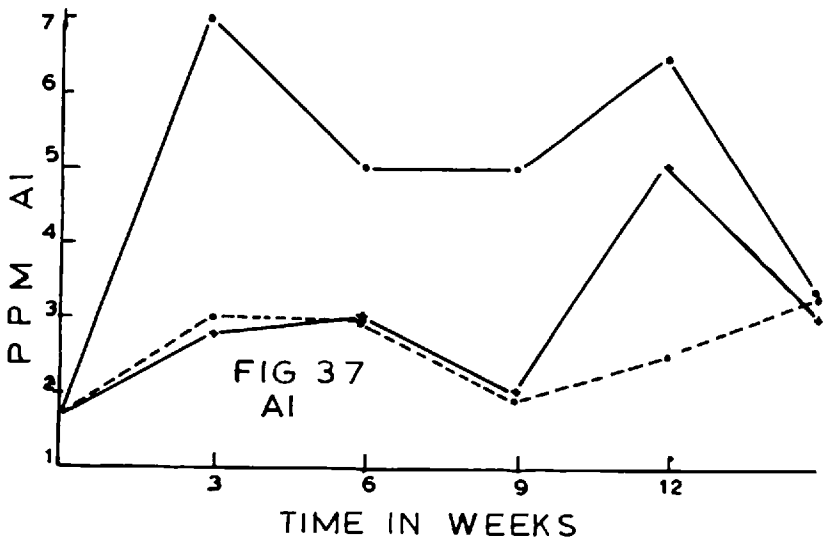


FIG 37
Al

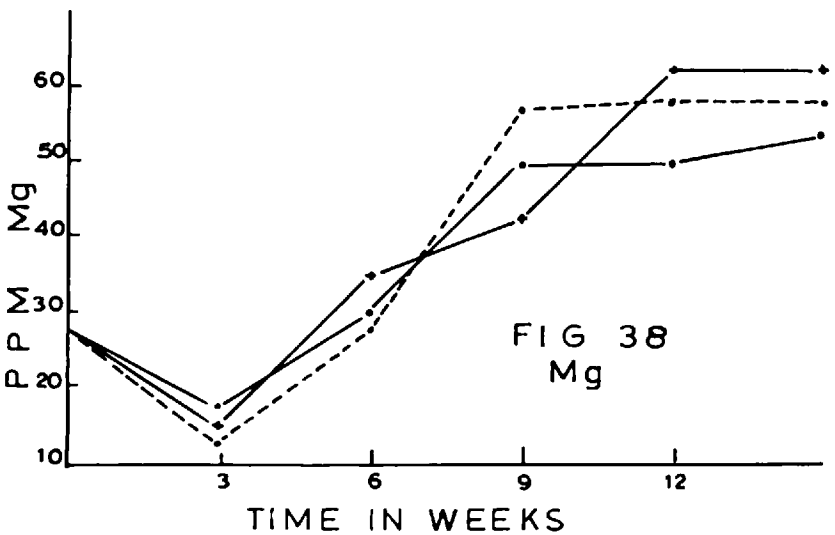


FIG 38
Mg

SILLWOOD SOIL (CONT.)

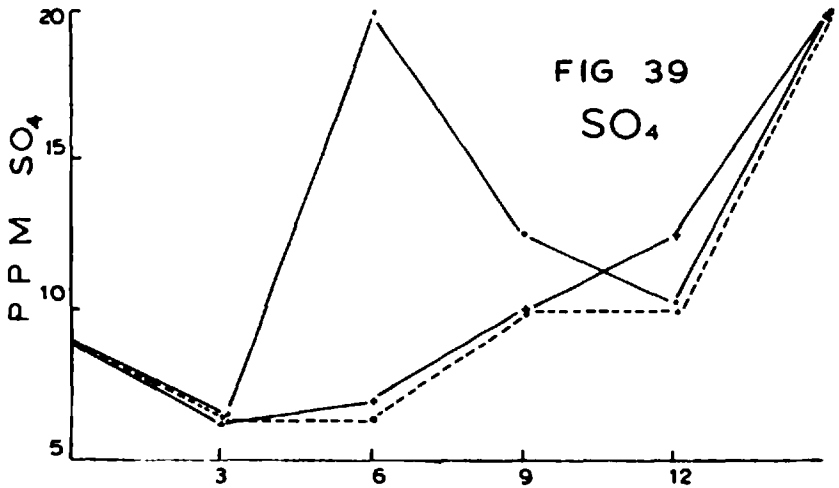


FIG 39
SO_4

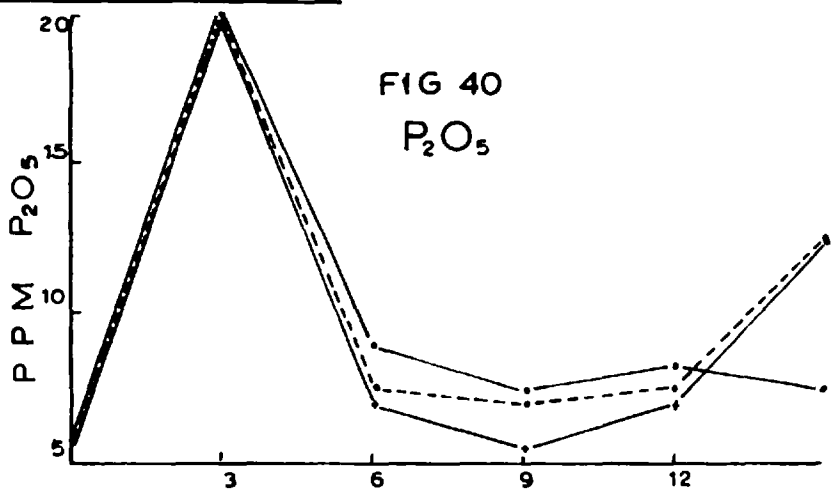


FIG 40
P_2O_5

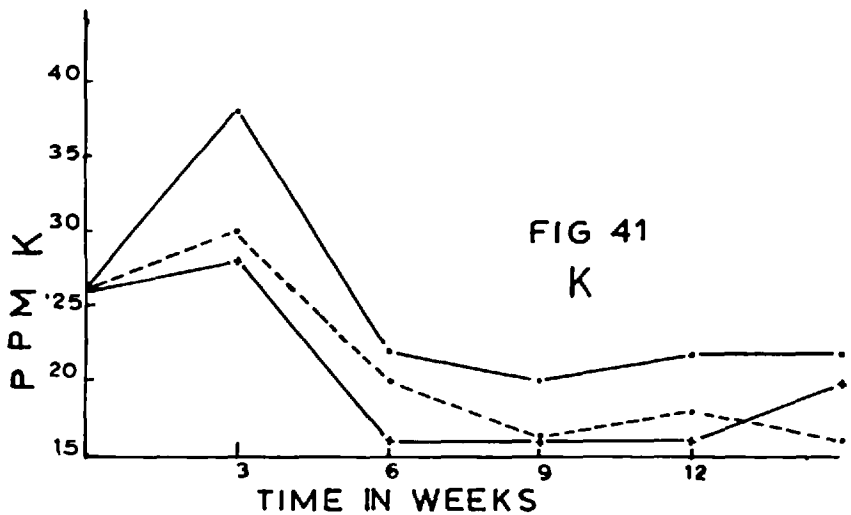


FIG 41
K

5: E. PAKISTAN SOIL

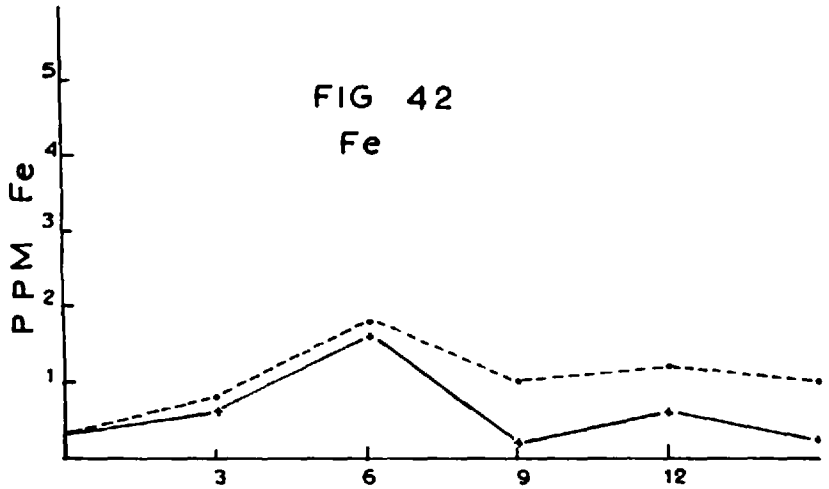


FIG 42
Fe

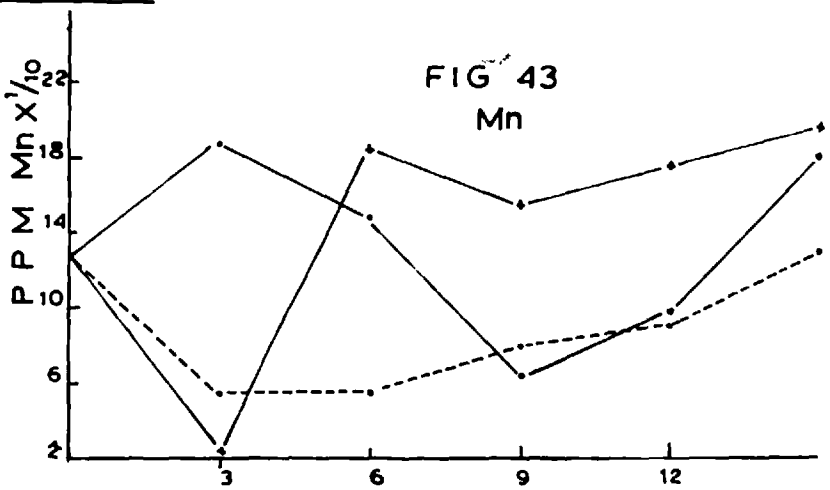


FIG 43
Mn

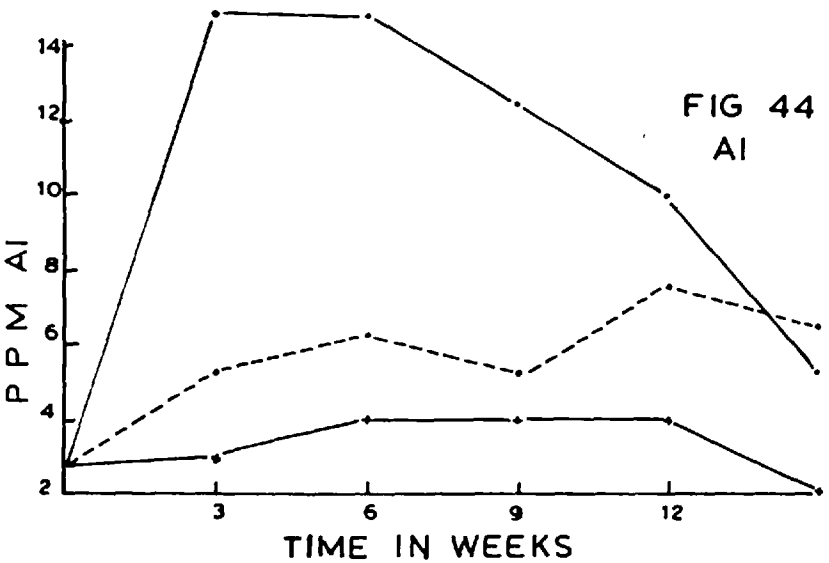


FIG 44
Al

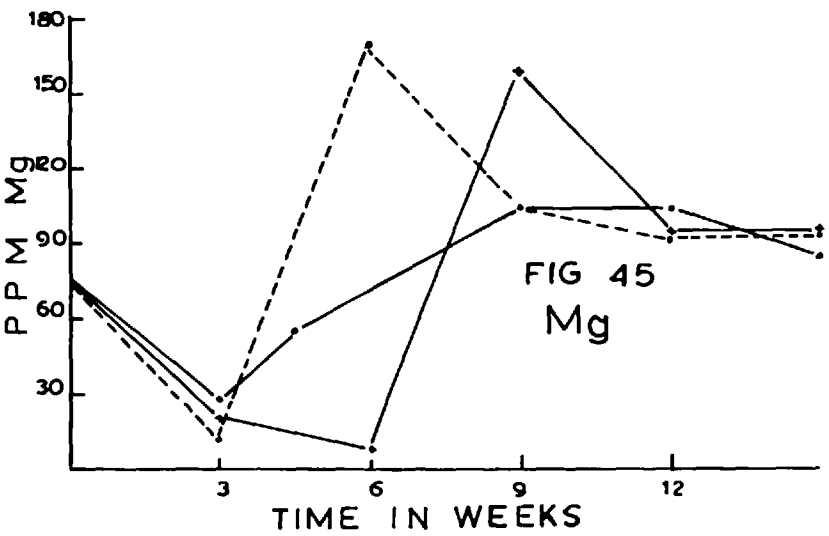


FIG 45
Mg

E. PAKISTAN SOIL (CONT.)

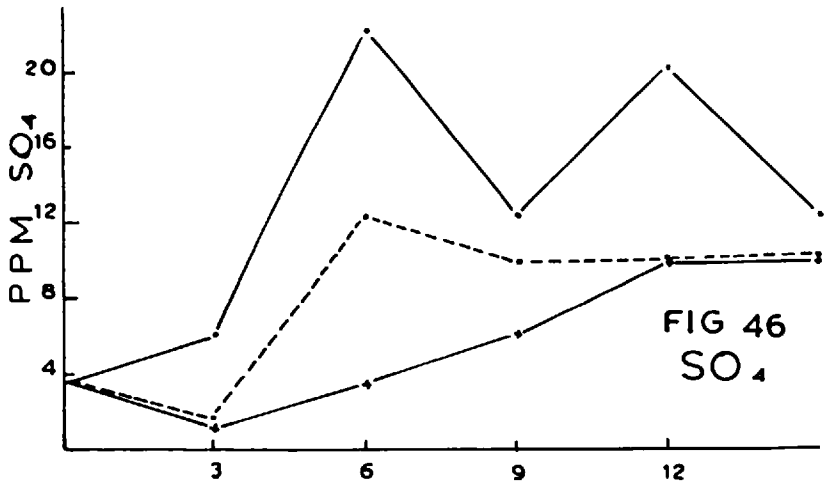


FIG 46
SO_4

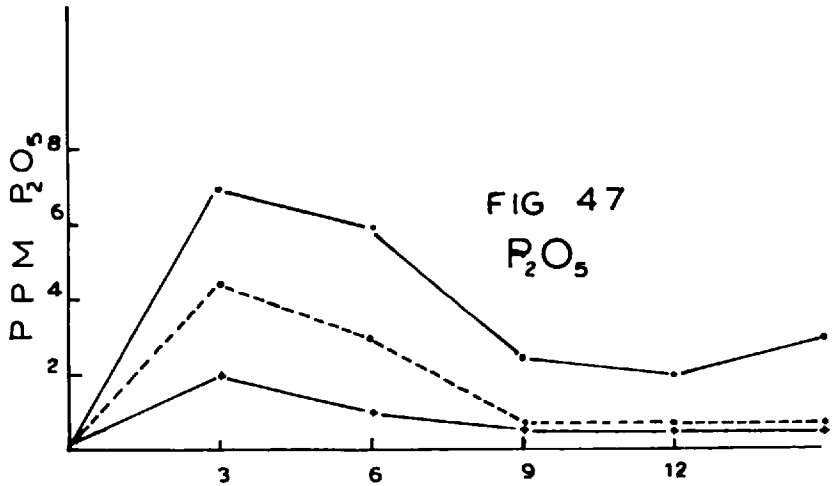


FIG 47
P_2O_5

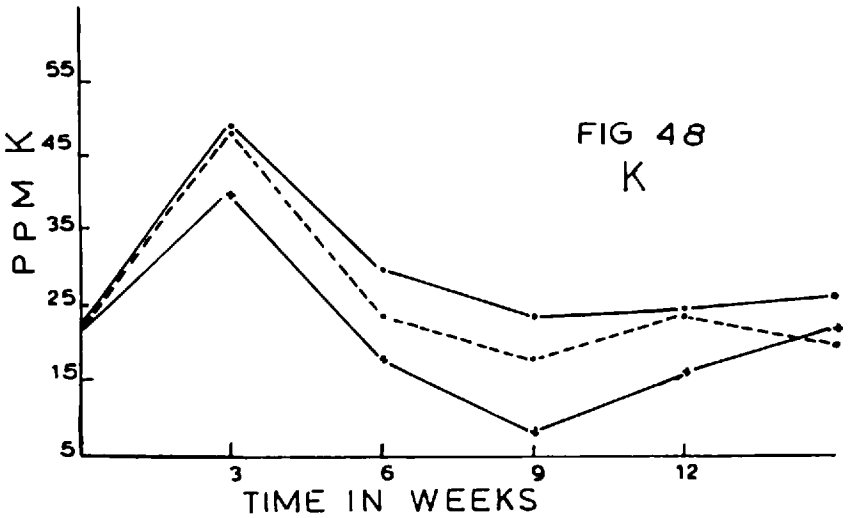


FIG 48
K

manganese, 50% level in Buxted soil and iron (25% and 50% levels) in the garden soil, iron and manganese in Harlington soil in the same two levels where a decrease in Morgan-extractable-iron and manganese was recorded if compared with the original values obtained before incubation. In no case however, was a decrease in Morgan extractable values recorded on wetting the soil and maintaining it at the 75% M.H.C. level.

Magnesium on the other hand, behaved differently and was the only element studied which, on wetting, showed a decrease in Morgan-extractable values in a few cases. The behavior of magnesium in such a manner could not be explained.

This suddad increase in mineralisation on wetting of the soils could be explained as follows :-

A :- Wetting the soil stimulates the micro-organisms to act upon the organic matter present in soils.

b :- On wetting, the process of slaking the minerals is brought about. This could affect the mineralisation of certain elements particularly potassium.

After the third week, there wasalmost always a sharp decrease in the values of the elements mineralised except in a few cases (e.g. in sulphate).

Magnesium in contrast, showed in most cases, an increase after the third week of incubation i.e. after the first sampling. This could be explained by the fact that magnesium minerals are not as soluble as some other minerals of the soil.

2nd :- The second part of the curves represents the next two samplings (at 9th and 12th weeks) after incubation when the mineralisation reached a sort of equilibrium and more or less a steady level of the "available" form is obtained; this level being dependent upon the chemistry of the element studied, moisture level, pH of the soil and the mineral or organic portion of the soil, e.g.

Iron and manganese on the other hand, showed the most drastic fluctuations particularly at the 75% moisture level, more especially under acid conditions than under neutral or alkaline conditions, Aluminium, to a great extent, followed the same trend of iron and manganese.

3rd :- The third and last part of the curve shows the fluctuation (increase or decrease) of the values obtained on air drying the soil after twelve weeks of incubation and extracting it in the air-dry condition.

The net gain of the soil in elements (i.e. mineralised) is taken as the difference between Morgan-extractable values obtained at the beginning of the experiment (as air-dry soil before incubation) and these obtained at the end of the experiment (i.e. from air-dried soil after incubation). This difference is expressed as percent of the original values. The question remains open, to what extent these nutrients mineralised and mobilised at different moisture levels are utilised by plants. Several workers have tried to answer this question and only a

few examples are cited here.

Archibald, Nelson and Bennett[115], studied the chemical composition of field grown grasses under different manurial treatments and reported a close correlation between amounts of rainfall and phosphorus content of the test plants, the higher the rainfall the higher was the uptake of the phosphate. Daniel and Harper[116] undertaking similar studies drew the conclusion that phosphate uptake increases with the increase of rainfall while calcium decreased. Metwalli and Pollard (117), made an attempt to study the uptake of nutrients by barley plants grown under different moisture levels in a glasshouse pot experiment and pointed out that there was an increase in uptake of nutrients and dry matter when the soil moisture increased. Very recently, Kilmer[118] et al, studied the effect of different moisture levels on the concentration of twelve nutrient elements in field-grown forage crops and reported that the phosphorus content in all the plants tested increased as the soil moisture increased but other elements investigated were not consistently affected.

It should be noted however, when taking the data of such experiments into consideration, the difference in conditions between incubated soils cited here and soils supporting plants growing. The nutritional balance will be continually changing due to absorption of the nutrients by the plants.

Effect of air drying the soils on the extractability of the elements studied :- Despite the fact that results often obtained did

not furnish a very conclusive evidence, except in certain cases, of the effect of air-drying on the extractability of these elements studied, however, the results obtained indicate that :- in the case of iron and manganese, air-drying resulted in an increase in the Morgan-extractable quantities of both of these elements. In the case of manganese, it was noticed that air-drying always resulted in higher quantities than obtained by the wet analysis of wet soil. It was interesting to observe that this is always the case in soils which possess an acid reaction but not in neutral or (more definitely) in alkaline soils, e.g. air-drying the soilresulted consistently in an increase in Morgan-extractable-manganese in Buxted, Silwood and East Pakistan soils all of which are acidic in reaction. In contrast, Garden and Harlington soils, Morgan-extractable-manganese decreased on air-drying. It was also observed that the decrease in extractable manganese occurred only in the 50% and 75% levels whereas, there was an increase at the 25% M.H.C.

Sherman and Harmer[119] in this connection, found that air-drying or heating the soil samples resulted in an increase in the exchangeable manganese by considerable amounts. They cited an example in which the exchangeable manganese increased from 4 p.p.m. to 80 p.p.m. on air-drying. According to Fujimoto and Sherman[120], drying seems to release the divalent manganese(Mn^{++}) from higher oxides.

Aluminium :- Aluminium extracted from any air-dried soil studied

was in general, less than that extracted from the corresponding wet sample maintained in the wet condition. Except in a few cases, there was an increase in the Morgan-extractable aluminium e.g. in Buxted soil when the sample at 25% level was extracted it gave 6.9 p.p.m. aluminium and on air-drying it yielded 9.3 p.p.m. aluminium and this phenomenon was clearer in extracts of acid soils than in the neutral or alkaline soils.

Magnesium was the element least affected by the process of air-drying the soil. At the low moisture levels studied, in this work, (the 25% and 50% M.H.C. levels) no effect of air-drying was apparent in any of the soils studied. At the highest moisture elevl (the 75% M.H.C.) however, there was a difference which in no case exceeded ± 25% of the value obtained from the air-dry soil.

Potassium :- Air-drying the soil before extraction often resulted in a different value from that obtained on extracting the corresponding sample which was maintained at any of the moisture levels studied. From the results may be concluded that the effect of air-drying is influenced by the pH of the soil in the case of potassium. In acid soils, air-drying increased the Morgan-extractable-potassium, particularly in those soils incubated at the lowest moisture level. On the other hand, neutral or slightly alkaline soils (e.g. Garden soil and Harlington soil) showed consistently a decrease in the Morgan-extractable-potassium on air-drying.

Lawton (as cited by Black[122], demonstrated that more non-exchangeable potassium is released to the exchangeable form upon incubation under acid than under neutral conditions. Attoe[122] reported that air-drying the soil decreases the content of exchangeable potassium in soils treated with soluble potassium salts and increases it in soils not so treated. The decrease in the potassium added to soils is generally accepted to be brought about by a process of fixation in the clay lattice layers.

Phosphates :- There was an increase in Morgan-extractable phosphates on air-drying acid soils. In neutral or alkaline soils, the extractability of phosphates was either unaffected or decreased on air-drying as in the case of the Garden and Harlington soils.

Sulphates :- There was a general tendency of the sulphate extractability to decrease with air-drying, the only exception being the Silwood soil which is acidic in reaction, comparatively high in organic matter and the most sandy soil of the soils examined. This increase may be accounted for by the possibility of the mineralisation of an additional amount of sulphur on air-drying, especially the first day where the moisture content was high and there was ample oxygen supply and favourable temperature (23°C.).

SUMMARY :-

Five soils differing in texture, organic matter content and reaction, were incubated at three different moisture levels (namely, 25%, 50% and 75% of the M.H.C.) at 28°C. The fluctuations in Morgan-extractable iron, manganese, aluminium, magnesium, potassium, phosphate and sulphate with time, were studied. At the end of twelve weeks incubation at the constant moisture levels, soils were air-dried and analysed for Morgan-extractable elements above mentioned. The changes in extractability of these elements as a result of air-drying were studied. It was observed that :-

1- Iron, Manganese and Aluminium :- The extractability of these three elements was higher at the upper moisture levels, usually increased with time of incubation; aluminium being less affected than iron or manganese, manganese being most active with iron being intermediate. The extractability of iron and manganese and to a certain extent, aluminium, particularly manganese, increased when when air-drying acid soils, but a negative effect was observed when soils were neutral or alkaline.

2- Magnesium :- Magnesium was the most uniform of all the elements studied in its extractability. The drastic decrease which took place on wetting the soil (notably at 25% and 50% M.H.C.) was followed by a sharp increase in Morgan-

extractable values of this element after which it seemed a state of equilibrium must have been attained at each of the moisture levels studied. Magnesium was the element least affected by air-drying the soil. No appreciable change was observed in Morgan-extractable-magnesium on air-drying the soils studied from the 25% and 50% moisture holding capacity levels. Such changes, however, occurred on air-drying the soil from the upper level of moisture (75% M.H.C.) and even then these changes were small and in no case exceeded ± 25% of the Morgan-extractable-magnesium from the corresponding air-dried sample.

3- Potassium :- There was a steady increase in Morgan-extractable-potassium with rising moisture levels. Air-drying caused an increase in the Morgan-extractable in acid soils; in neutral or alkaline soils, there was always a decrease.

4- Sulphate :- The extractability of sulphates increased the higher the moisture level is; however, there were few exceptions recorded, e.g. in the 3rd sampling in Buxted soil, the sulphate extracted at the 50% M.H.C. was appreciably higher than that extraced at the 75% level.

An increase in Morgan-extractable-sulphate was almost always associated with an increase in Morgan-extractable quantities of other elements studied, particularly aluminium and potassium.

In general, a decrease in sulphate-extractability resulted from air-drying, except in the case of Silwood soil, a very sandy soil with appreciable organic matter content (3.67%)

5- Phosphate :- Mobilisation of phosphates under acid conditions was greater than under alkaline or neutral conditions, probably due to the soluble iron and manganese phosphates under acid conditions.

On air-drying, Morgan-extractable-phosphates from acid soils increased as compared with the values obtained from the corresponding soil maintained at a certain moisture level. This was not the case under neutral or alkaline conditions where the phosphate extracted was equal or even less than those obtained from analysis of wet soils.

The results obtained from this experiment indicate that air-drying introduces a variation in the extractability of the nutrients studied. A suggestion is made that analysis of the fresh soil (field moisture) might lead to better correlation between their extractability and uptake by plants.

Table 63.

Physical and Chemical properties of the Soils Studied :-

Soil	Moisture Holding Capacity.	Organic Matter.	pH	Air-dry Moisture % O.D basis.	Clay.	Silt.	Sand.
Buxted	55.4	2.65	5.28	1.57	8.20	40.70	47.50
Garden	67.0	4.14	7.14	4.67	26.0	38.0	31.0
Harlington	48.4	2.34	7.56	1.65	18.5	30.2	50.4
Silwood	40.8	3.57	5.94	1.17	5.2	7.9	81.5
E.Pakistan	42.7	0.51	5.89	1.27	17.5	29.2	51.5

I Burtad Soil. Table 64

M.H.C = Moisture Holding Capacity. (p.p.m. O.D. basis)

	Fe	Mn	Al	Mg	SO_4"	P_2O_5	K
Air-dry Soil at the beginning of the experiment.	2.9	40.0	5.24	130 .0	8.75	2.5	60.0
1st Sampling :- 3 weeks after incubation.							
25% M.H.C.	2.45	80.0	7.7	40.0	6.25	10.0	80.0
50% M.H.C.	2.0	7.2	9.7	45.0	6.25	15.0	92.0
75% M.H.C.	10.0	330.0	40.5	200.0	41.25	20.0	124.0
2nd Sampling :- 6 weeks after incubation.25%	2.6	21.0	8.0	172.5	6.25	4.0	50.0
50% M.H.C.	2.4	9.0	7.3	220.0	37.5	4.0	60.0
75% M.H.C	70.0	420.0	21.5	300.0	35.0	8.75	82.0
3rd Sampling :- 9 weeks after incubation							
25% M.H.C.	2.0	9.0	8.0	350.0	12.5	3.0	52.0
50% M.H.C.	1.8	6.6	8.0	225.0	40.0	3.0	60.0
75% M.H.C.	20.0	114.0	11.5	350.0	26.25	5.0	72.0
4th Sampling :- 12 weeks after incubation.							
25% M.H.C.	10.0	9.0	6.9	200.0	28.75	3.0	46.0
50% M.H.C.	1.8	9.0	7.6	240.0	71.5	3.0	54.0
75% M.H.C.	7.0	12.0	20.0	350.0	37.5	6.0	64.0
Air-dry Soil at end of experiment.							
25% M.H.C.	3.2	18.0	9.3	200.0	20.25	4.0	52.0
50% M.H.C.	2.4	9.0	7.0	240.0	20.25	4.0	52.0
75% M.H.C.	2.0	12.0	9.3	280.0	20.25	4.0	56.0

Table 65.

II Garden Soil.

Condition of soil when extracted :-	Fe	Mn	Al	Mg	SO_4"	P_2O_5	K
Air-dry soil at the beginning of the experiment.	5.04	3.5	2.6	285	23.75	5.0	102
1st Sampling :-							
25% M.H.C.	2.45	6.0	3.0	55.0	11.25	20.0	210
3 weeks from 50% M.H.C.	1.6	3.6	3.3	175	11.25	20.0	264
incubation 75% M.H.C.	6.5	27.0	8.5	300	23.75	20.0	-
2nd Sampling :-							
6 weeks after incubation.							
25% M.H.C.	5.0	8.4	4.0	500	15.0	9.0	86.5
50% M.H.C.	6.4	4.8	7.5	450	18.75	3.0	100
75% M.H.C.	12.0	11.4	8.0	550	31.25	17.5	-
3rd Sampling :-							
9 weeks after incubation.25%	3.2	3.6	3.6	400	25.5	9.5	90
50% M.H.C.	5.6	3.6	5.0	400	28.75	10.0	125.8
75% M.H.C	4.0	3.0	6.5	380	36.25	13.75	80
4th Sampling :-							
12 weeks after incubation.							
25% M.H.C.	3.0	5.4	3.6	380	40.0	15.5	84.0
50% M.H.C.	3.8	3.0	5.0	400	28.75	6.5	96.0
75% M.H.C.	4.0	3.6	10.0	380	36.25	12.5	-
Air-dry Samples at end of experiment.							
25% M.H.C.	5.4	7.8	4.6	380	40.0	12.5	84
50% M.H.C.	5.0	1.8	4.3	400	36.25	13.5	86
75% M.H.C.	4.4	1.8	4.6	410	36.25	15.0	94

Table 66.

III Harlington Soil.

Condition of soil analysed.	Fe	Mn	Al	Mg	SO_4"	P_2O_5	K
Air-dry soil at beginning of the experiment.	0.89	6.2	1.7	200	15.0	10.6	170
1st Sampling :- 3 weeks after incubation.25%	0.60	3.1	2.8	100	11.25	42.5	238
50% M.H.C.	2.8	3.6	3.0	400	11.25	42.5	344
75% M.H.C.	2.0	6.0	5.0	115	15.0	47.5	380
2nd Sampling :- 6 weeks after incubation. 25% M.H.C.	1.4	5.6	3.0	300	17.5	44.0	164
50% M.H.C.	1.6	4.8	3.3	275	17.5	33.5	182
75% M.H.C.	1.2	5.4	5.0	400	25.0	43.0	228
3rd Sampling :- 9 weeks after incubation. 25% M.H.C.	0.80	3.6	2.5	380	28.5	38.5	164
50% M.H.C.	0.80	4.2	2.5	380	23.0	27.5	168
75% M.H.C.	1.2	3.6	3.0	350	23.0	46.0	196
4th Sampling :- 12 weeks after incubation. 25% M.H.C.	1.0	6.0	3.3	380	40.0	50.0	160
50% M.H.C.	1.0	3.6	2.3	370	23.0	44.6	174
75% M.H.C.	0.5	3.0	6.5	350	23.0	52.5	192
Air-dry Samples :- end of the experiment. 25% M.H.C.	1.0	9.0	3.3	380	23.0	42.5	150
50% M.H.C	1.0	2.4	2.3	370	23.0	40.0	142
75% M.H.C	0.8	2.4	2.5	380	23.0	44.0	152

Table 67.

IV Silwood Soil.

Condition of soil analysed.	Fe	Mn	Al	Mg	SO_4''	P_2O_5	K
Air-dry soil at beginning of the experiment.	0.89	1.5	1.7	27.5	8.75	5.0	26
1st Sampling :- 3 weeks after incubation.25%	0.6	2.0	2.8	15.0	6.25	20.0	28
50% M.H.C.	0.8	2.4	3.0	12.5	6.25	20.0	30
75% M.H.C.	1.2	8.4	7.0	17.5	6.25	20.0	38
2nd Sampling :- 6 weeks after incubation. 25% M.H.C.	1.0	0.6	3.0	35.0	7.0	7.0	16
50% M.H.C.	1.8	2.0	3.0	27.5	6.25	7.5	20
75% M.H.C.	1.6	1.2	5.0	30.0	21.25	9.0	22
3rd Sampling :- 9 weeks after incubation. 25% M.H.C.	0.6	0.6	2.0	42.5	10.0	5.5	16
50% M.H.C.	1.0	1.2	2.0	57.5	10.0	7.0	16
75% M.H.C.	0.6	1.2	5.0	50.0	12.5	7.5	20
4th Sampling :- 12 weeks after incubation. 25% M.H.C	0.8	1.2	5.25	62.5	12.5	7.0	16
50% M.H.C.	0.6	1.2	2.5	58.5	10.0	7.5	18
75% M.H.C.	0.5	1.2	6.4	50.0	18.6	8.5	22
Air-dry samples :- End of experiment 25% M.H.C.	1.0	3.0	3.0	62.5	20.25	12.5	20
50% M.H.C.	1.2	1.8	3.3	58.5	20.25	12.5	16
75% M.H.C.	1.0	1.2	3.3	54.0	20.25	7.5	22

Table 68.

V E. Pakistan Soil.

Condition of soil subject to analysis	Fe	Mn	Al	Mg	SO_4"	P_2O_5	K
Air-dry soil at beginning of expt.	0.3	130	2.65	75.0	3.75	trace	21.4
1st Sampling :- 3 weeks after incubation.							
25% M.H.C.	0.6	24.0	3.0	20.0	1.25	2.0	40.0
50% M.H.C.	0.8	56.0	5.3	12.5	1.25	4.5	48.0
75% M.H.C.	11.6	189	15.0	29.0	6.25	7.0	48.0
2nd Sampling :- 6 weeks after incubation.							
25% M.H.C.	1.6	186	4.0	8.5	3.75	1.0	18.0
50% M.H.C.	1.8	57.0	6.3	172.5	12.5	3.0	24.0
75% M.H.C.	11.6	150	15.0	57.5	22.5	6.0	30.0
3rd Sampling :- 9 weeks after incubation.							
25% M.H.C.	0.2	156	4.0	160.0	6.25	0.5	8.0
50% M.H.C.	1.0	81.0	5.3	105.0	10.0	0.5	18.0
75% M.H.C.	130	63.0	12.5	105.0	12.5	2.5	24.0
4th Sampling :- 12 weeks after incubation.							
25% M.H.C.	0.6	177	4.0	95.0	10.0	0.5	16.0
50% M.H.C.	1.2	90	7.6	95.0	10.0	0.5	24.0
75% M.H.C.	27.5	99	10.0	105.0	20.25	2.0	24.0
Air-dry soil :- End of experiment.							
25% M.H.C.	0.2	198	2.0	95.0	10.0	0.5	22.0
50% M.H.C.	1.0	130	6.5	95.0	10.0	0.5	20.0
75% M.H.C.	15.2	180	5.3	85.0	12.5	3.0	26.0

Table 69.

Mineralisation of the Elements studied.

(Difference between 1st and last air-dry samples(12 weeks period) expressed as percentage of the first.)

Soil	Fe	Mn	Al	Mg	SO_4"	P_2O_5	K
Buxted							
25% M.H.C.	+10.4	-55	+ 77.5	+ 53.8	+131.4	+ 60	- 25
50% M.H.C.	-17.1	- 77.5	+ 33.5	+ 80.8	+131.4	+ 60	- 25
75% M.H.C.	-31.0	- 70.0	+ 77.5	+115.3	+131.4	+ 60	- 50
Garden							
25% M.H.C.	+7.2	+122.9	+ 76.9	+ 33.3	+ 68.8	+150	- 17.6
50% M.H.C.	-0.8	-48.6	+ 69.2	+ 40.4	+ 52.6	+170	-15.7
75% M.H.C.	-12.4	- 48.6	+76.9	+ 43.9	+ 52.6	+200	- 7.9
Harlington							
25% M.H.C.	+12.3	+ 54.1	+ 94.1	+ 90.0	+ 53.3	+306	-11.8
50% M.H.C.	+12.3	-61.2	+ 35.3	+ 85.0	+ 53.3	+268	-16.5
75% M.H.C.	-10.1	- 61.2	+ 47.0	+ 90.0	+ 53.3	+315	-10.6
Silwood							
25% M.H.C.	+12.3	+100.0	+ 76.4	+127.2	+131.3	+150	- 23
50% M.H.C.	+34.8	+ 20.0	+ 94.1	+112.7	+131.3	+150	-38.5
75% M.H.C.	+12.3	- 20.0	+ 94.1	+ 96.3	+131.3	+150	-15.4
E.Pakistan	-33.3	+ 92.2	-22.6	+ 22.6	+166.6	---	+ 2.8
25% M.H.C							
50% M'H'C.	+166.6	----	+107.5	+ 22.6	+166.6	---	- 6.5
75% M.H.C.	+4966.6	+ 74.8	+100.0	+.13.3	+240.0	---	+21.5

Table 70.

Changes in Extractability of Elements Studied on air-drying expressed as % of the values obtained from air-dried soils.

Soil	Fe	Mn	Al	Mg	SO_4''	P_2O_5	K
Buxtad 25%	-212.5	+ 50.0	+ 25.8	—	- 42.0	+ 25.0	+ 11.5
50% M.H.C.	+ 25.0	—	- 8.6	—	-200.0	+ 25.0	- 3.8
75% M.H.C.	-250.0	—	-115.0	- 25	-330.2	- 50.0	-14.3
Garden							
25% M.H.C.	+ 44.4	+ 30.7	+ 21.7	—	—	- 24.0	- 0.0
50% M.H.C.	+ 24.0	- 66.6	- 16.3	—	+ 20.7	+ 51.8	- 11.6
75% M.H.C.	+ 9.0	-100.0	-117.3	+ 7.3	—	+ 20.0	——
Harlington							
25% M.H.C.	—	+ 33.3	—	—	- 73.9	- 17.6	- 6.6
50% M.H.C.	—	- 50.0	—	—	—	- 11.5	- 22.5
75% M.H.C.	+ 37.5	- 25.0	-160.0	+ 7.9	—	- 19.3	- 26.3
Silwood							
25% M.H.C.	+ 20.0	+ 60.0	- 75.0	—	+ 48.1	+ 44.0	++20.0
50% M.H.C.	+ 50.0	+ 33.3	+ 24.2	—	+50.6	+ 40.0	- 12.5
75% M.H.C.	+ 50.0	—	- 97.0	+ 7.4	+ 50.6	-13.3	—
E.Pakistan							
25% M.H.C.	+200.0	+ 10.6	-100	—	—	—	+ 27.3
50% M.H.C.	+ 20.0	+ 30.7	- 17.0	—	—	—	- 20.0
75% M.H.C.	+ 80.9	+ 45.0	-88.7	- 23.5	-62.0	+ 33.3	+ 7.7

CHAPTER IX.

POT CULTURE EXPERIMENT USING PERENNIAL RYE GRASS (LOLIUM perenne)

1. Studies on the visual symptoms caused by magnesium, sulphur, iron or manganese deficiency and the possibility of making use of such deficiencies in the field to examine the supporting power of soils.
2. The effect of these deficiencies on the mineral composition and uptake of nutrients by the starved grass.
3. Correlation between Soil Solution Content, Morgan extractable nutrients and uptake of plants.

INTRODUCTION :-

The relationship which exists between the mineral composition of plants and soils supporting them has attracted the activities of many workers in agriculture since early stages of the development of scientific agriculture. The main object of these workers was and still is to find out what is the level of nutrients in a particular soil at which the optimum yield of a particular crop is obtained. Previously, carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium and to a certain extent, magnesium and sulphur known as "macro-nutrients", required by plants in relatively big quantities, attracted most of the efforts of these workers. The problem increased in complexity with the establishment of the essentiality of iron, manganese, copper, zinc, boron and molybdenum, the so-called micro-nutrients, required by plants in smaller quantities or traces and hence, the old terminology, trace elements. Moreover, other elements were observed to have beneficial effects on certain crops, at least under certain sets of experimental conditions and were claimed by certain schools of thought to be essential to growing plants, e.g. Kroyer et al[123], claimed that chlorine is an essential element and were able to obtain characteristic visual symptoms of wilt, chlorosis, necrosis and unusual bronze discolouration on the leaves of tomato plants grown in chlorine deficient solutions. The symptoms disappeared on addition of chlorine

to the nutrient solutions confirming their observations. On analysing leaves of tomato plants suffering from acute chlorine deficiency, they reported that such leaves contained 250 p.p.m. chlorine on dry matter basis. Ozane[124], found that subterranian clover (Trifolium subterraneum L.) showed chlorine deficiency symptoms when grown in nutrient solutions deficient in this element or in soils poor in chlorine and supplied with complete nutrient solutions deficient in this element. Sodium was observed to have beneficial effects on a variety of crops. Harmer and Benne[125], claimed that sodium has caused vigorous development of barley, carrot, cotton, flax and tomato only when potassium is deficient. The same authors found that even in presence of potassium in sufficiency, addition of sodium has beneficial effects on the growth of celery, table and sugar beet and turnips. Recently, however, Brownwell and Wood[126], obtained evidence that sodium is ann essential micronutrient for Atriplex vesicaria.

Plant physiologists found it necessary to adopt criteria by which the indispensability of an element can be judged and ruled out that the essentiality of an element is established only if :-

a Lack of this element results in injury, aberrant development and ultimately death of the plant grown in culture media using a technique for refining the culture and nutrients used.

b Before a certain element can be finally accepted as essential for the life and healthy growth of green plants generally, its essentiality for a wide variety of species representing a number of different families of the plant kingdom must have been demonstrated.

c Demonstration that no other element of similar chemical properties can substitute that particular element is necessary.

Undoubtedly, the economic importance of these nutrients will depend on whether or not growth in the field is limited by their deficiency. Plants differ markedly in their nutritional requirements and in tolerance to mineral deficiencies. A plant suffering from a nutrient deficiency does not necessarily show any visual symptoms except probably that its growth is not as good as that of other healthy plants.

Apart from directly measurable criteria of growth, visual symptoms have the advantage of interpretation in the field without needing any equipment. When such symptoms exist, they furnish the most cheap and quick diagnosis of the lacking "available" element. The deficiency of a nutrient may occur at any stage of the life of a plant and cause an unbalanced nutritional condition which might affect the rate of growth, yield or often both.

Under field conditions, however, it is unlikely that the complete absence of one or more of the essential elements above listed would occur. The element causing the trouble may be

present in the soil but held up in an unavailable form in one way or another which is not readily taken by the plant. There is every reason to classify such an element as deficient at least as far as the growing plant is concerned.

To make use of the visual symptoms, one should have a wide knowledge of the plants growing on his land. He should be acquainted with life history of these plants, their habitats and behavior at the different stages of growth. He should also possess a good power of discrimination to differentiate between symptoms caused by different climatic conditions as rain, drought, temperature, wind and other factors such as insect damage, diseases and symptoms which result from mineral deficiency. Detailed description of mineral deficiency symptoms of the different crops is a useful tool for diagnostic purposes. There remains an assessment of the right quantity of that element to to be added in a suitable form which could be utilised by the crop usually as foliage spray or when added to the soil.

Physiologically, iron and manganese seem to be involved in nitrogen metabolism. Nicholas(127), (128), has shown that copper is essential for nitrogen reduction but there is reason to believe that iron, manganese, zinc and molybdenum are involved at one stage or another. Kessler(129), has shown that iron is concerned in some way with nucleic acid metabolism while De Kock and Morrison claim that iron is connected with metabolism of amino acids.

Manganese on the other hand, apart from being connected with nitrogen reduction, appears to have a function in photosynthesis. Manganese has always been involved in oxidation-reduction phenomena. According to Sommers and Shive[132], manganese is respossible for the oxidation of ferrous iron to the physiologically inactive ferric form. The several observations that iron is present in chlorotic leaves in quantities higher than green healthy ones lends support to Sommers and Shives's theory which explains the iron deficiency as manganese toxicity and manganese deficiency as iron toxicity.. The school of thought originating from this theory bases its consideration of chlorotic leaves suspected of either iron or manganese deficiencies on the iron/manganese ratio of soluble forms rather than on the absolute value of either.

Magnesium is the only mineral constituent of the chlorophyll molecule and in this respect it is different from the rest of the elements generally accepted as essential for plant life. The chlorophyll molecule contains 2.7% magnesium. This element is known to play a role in phosphate metabolism and thus in the respiratory mechanism.

Sulphur is known to be a constituent of certain amino acids which build the proteins in plants e.g. cystine,

b a constituent of certain plant hormones e.g. thiamine and biotin and

c the mustard oil glucosides e.g. sinigrin.

Magnesium and sulphur in contrast with iron and manganese are easily translocated to the different organs of plants. In the case of deficiency translocation of iron and manganese from older leaves with ample supply is not readily effected.

Visual symptoms of iron, manganese, magnesium and sulphur previously described in the members of Graminae :-

Iron deficiency symptoms :- Wallace[43], described iron deficiency in young wheat plants as severe chloresis of leaves, beginning at the tips which finally results in their death. In cereal crops, iron deficiency shews as intervenal chlorotic stripes as in wheat and oat leaves. Hoffer described nitrogen, phosphorus, potassium, magnesium, calcium, boron and zinc deficiencies in maize but no mention was found by the author in connection with iron deficiency in other small grains such as wheat, oats, barley or rye.

Manganese deficiency symptoms :- Are typically displayed in oats leaves due to the sensitivity of this plant to this deficiency. Wallace describes manganese deficiency symptoms in wheat leaves as intermittent intervenal chlorotic streaks. According to the same author, manganese defisiency symptoms in oat leaves show as irregular greyish-brown lesions which coalesce and result in leaf collapse. These symptoms are known as grey speck symptoms. Albert[133], in the United States observed a manganese deficiency in oats growing on heavily limed soil. He

describes the symptoms under these conditions as the bright yellow colour which break down at their basal ends and die slowly. The tip ends remained yellowish-green and alive. Walsh[134], studying the susceptibility of grasses to manganese deficiency found that perennial and Italian rye grasses grown on a soil-sand mixture developed manganese deficiency symptoms a month after germination ; the younger leaves developed a chlorotic appearance accompanied by severe tip withering. Spraying the chlorotic manganese plants with soluble manganese salts restored the green colour of the leaves and the symptoms disappeared entirely. It is observed that iron and manganese deficiencies show first in the younger leaves as the supply of these two elements become restricted while the older leaves were not affected. This is explained by the fact that little or no translocation of iron or manganese occurs in the tissues of plants.

Magnesium
Manganese deficiency :- Wallace describes magnesium deficiency in cereal crops :- " Leaves are slightly pale green, older leaves yellowing specially near margin, aldo these marginal and intervenal brown streaks and leaves die off prematurely." Thornton in the United States studied the magnesium deficiency symptoms in maize grown in controlled nutrient cultures and describes magnesium starved plants as stunted in growth with leaves showing a distinct mottling of lighter yellowish-green patches.

Sulphur deficiency :- According to Wallace, sulphur deficiency

was not recorded in England. It is possible that many factors contributed to the masking of sulphur deficiency or rather inadequacy in the different crops and retarded the research in the sulphur status of soils and plants; the early workers determined sulphur in plant ash and often gave values lower than the actual values, thus erroneously established that sulphur is required in quantities smaller than the actual.

b Sulphur is almost always present in varying amounts in artificial fertilisers; nitrogen, potassium and to a certain extent magnesium are often applied in the form of sulphates. Organic manures ofter added to the soil also contain sulphur in different organic forms.

c Rain contributes to the soil sulphur.

Thus it is evident that considerable amounts of sulphur are returned to the soil mainly mixed with other ameliorating agents which are almost always credited with the beneficial effects on growing crops while the sulphur contribution is ignored or overlooked. Thus ammonium sulphate contains approximately 20.5% nitrogen and 24% sulphur but still its beneficial effects on crops are attributed to nitrogen rather than sulphur.

It is evident from the literature that sulphur has not been studied as extensively as many other plant nutrients. The possibility of inadequate supplies of sulphur in soils, however, is becoming more generally recognised. Methods of sulphur determination in soil extracts and plant material have been

improved by several workers and also the mineralisation of organic sulphur has been investigated. Walker[135], recently surveyed the situation in Australia and New Zealand and collected evidence of many instances in which responses of grass to sulphur dressings occurred. He suggests that two sets of conditions under which sulphur response are likely to occur :-

a The accumulation of organic matter particularly under high producing pastures where legumes are relied upon as the main source of nitrogen and

b Under intense cropping where heavy dressings of nitrogen and other nutrients are applied.

White[136], studied the mineralisation of sulphur and nitrogen in sulphur-deficient soils and suggested that mineralisation and immobilisation of sulphur in a soil may follow a pattern similar to that of nitrogen. The present work was conducted with a view to :-

1 : To study the visual symptoms caused by iron, manganese, magnesium and sulphur on perennial rye grass (Lolium perenne), and the possibility of using these symptoms in the field for diagnostic purposes.

2 : The effect of these deficiencies on the mineral composition of the grass grown on sand culture and soil mixed with sand.

3 : To investigate the possibility of the existence of a relation

between soil solution content, Morgan extractable nutrients and plant uptake.

The test plant chosen was a grass for the following reasons:-

I Grasses are almost always growing on agricultural land either as a part of the agricultural rotation or as "intruders" thus furnishing an ever ready material for field observation.

II : Grasses either alone or often mixed with each other or with legumes, form the bulk of pasture cover upon which grazing cattle depend mainly for their feeding. There is a rising necessity to maintain a healthy growing crop to support these cattle which, due to increasing population became restricted to a more limited area than before.

III : Among the members of Graminae, grasses seem to have received but little attention as far as micronutrient deficiencies. Until very recently, only scanty papers were published in this connection.

Experimental :-

Preparation of the Glasshouse :- The soil floor of the glasshouse, for two main reasons, was cultivated with grass.

1 To avoid contamination from dust as much as possible.

2 To maintain a humid atmosphere favoured by grasses.

The soil used in this experiment was from the top six inches of the Silwood Park Station fallow plots. It was air dried and sieved through a 2 m.m. sieve before use. The soil is described in Chapter VIII.

Purification of the silver sand used :- The sand was subjected to prolonged treatment to remove impurities and other nutrients particularly iron and manganese whose oxides are usually associated with sands of different sources. The technique used in this work was essentially that described by Hewitt (137). As modified by the author, the purification process was as follows :-

a Small portions of the sand were placed in polythene buckets (4 gallons capacity) and treated with 1% caustic soda solution, stirring at intervals. The sand was subsequently washed with tap water. The process was repeated with intermediate washing till all the clay and silt particles had disappeared.

b The sand was then transferred to large glazed containers (1½ cwt. capacity) with a tubulare near the bottom which was plugged with glass wool and connected to the drains. There the sand was soaked in 1 : 1 (v/v) hydrochloric acid for seven days with

occasional stirring. The acid was changed if it showed any colouration of iron oxides (reddish-brown).

c The sand was then leached continuously for another seven days with hydrochloric acid 1:1 (v/v) which is 2% with respect to oxalic acid. The leached sand was occasionally stirred to help in crushing the iron concretions through the abraising action of the sand particles. Stirring is very essential at this stage.

d Sand was then leached with distilled water till it was chloride free, dried in the glasshouse and then sieved through a 1 m.m. sieve.

POTS :- Plastic pots of 1000 gms. capacity were used. Each pot was fitted with a piece of acid-washed glass cloth at the bottom to hold the culture used in position without hindering the aeration of the roots. The glass cloth was boiled in concentrated hydrochloric acid for half an hour and rinsed thoroughly with distilled water till chloride free before use. Each pot stood in a plastic saucer to allow alternate watering (from bottom and top of the pot) in order to ensure even distribution of the nutrient in the pot.

Sowing :- Perennial rye grass (Lolium perenne) was sown at the rate of 0.5 gm. in each pot i.e. in each 1000 gms. of soil.

For analysis of the seeds see Table 71.

Nutrient solution used :- The nutrient solution used was essentially that adopted at the Long Ashton Research Station.

The following stock solutions were made :-

Salt.		Weight in gms. dissolved in 1 litre of distilled water.	
Potassium nitrate :	KNO_3	33.60	
Calcium "	(anhydrous) $Ca(NO_3)_2$	65.60	
Sodium hydrogen phosphate	$NaH_2PO_4.2H_2O$	20.80	
Magnesium sulphate	$MgSO_4.7H_2O$	36.90	or 34.8 gms. $MgSO_4$ anhyd. for preparation of "minus" Mg nutrient solution. or 49.9 gms. $MgCl_2.6H_2O$ to prepare the "minus" S solution.
Ferric citrate		2.45	
Manganese sulphate	$MnSO_4.4H_2O$	0.223	
Copper " "	$CuSO_4.5H_2O$	0.024	
Zinc " "	$ZnSO_4.7H_2O$	0.029	
Boric acid	H_3BO_3	0.186	
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0035	
Aluminium sulphate	$Al_2(SO_4)_3.12H_2O$	0.0196	
Cobalt " "	$CoSO_4.7H_2O$	0.0028	
Nickel " "	$NiSO_4.7H_2O$	0.0028	

All the salts used were A.R. grade.

The actual nutrient solutions were prepared immediately before application, in polythene containers (5 litres capacity). Six nutrient solutions were used :-

(1) Complete Solution :- This contains fifty ml. of each of the above stock solutions diluted to 5 litres with distilled water.

(2) "Minus Iron" Solution :- Fifty ml. of all the above stock solutions except ferric citrate, diluted to five litres with distilled water.

(3) "Minus Manganese" Solution :- Fifty ml. of each of the above stock solutions except manganese, diluted to five litres.

(4) "Minus Aluminium" Solution :- Fifty ml. of each of the above stock solutions except aluminium sulphate, and the final volume being to five litres.

(5) "Minus Magnesium" Solution :- Fifty ml. of each of the above stock solutions except magnesium sulphate. In order to supply the same amount of sulphate without magnesium, sodium sulphate anhydrous (34.8gms/l and 50 ml. were used for each 5 l. of nutrient solution) was used.

(6) "Minus Sulphate" Solution :- This was made by pipetting fifty ml. of each of the above stock solutions except $MgSO_4.7H_2O$ and diluting to 5 litres. The amount of

sulphate added with the different micronutrients was neglected, being very small.

In order to supply the same quantity of magnesium as in the other nutrient solutions, an equivalent amount of magnesium chloride ($MgCl_2.6H_2O$) was used to replace the sulphate.

After preparation of the diluted nutrient solutions (5 litres), the pH of each was adjusted to pH 5.5 - 6.0 using 0.08N-KOH solution. Each nutrient solution required 20 ml. of the potassium hydroxide for this purpose except the "minus sulphur" nutrient solution which required 30 ml.

The stock solutions were always prepared from the same batch of Analar Reagents throughout the whole experiment. Analyses of the dilute nutrient solutions applied to the pots is given in Table 72.

A :- Sand Culture Pots.
Technique of Pot Experiment :- 1000 gms. of the prepared sand were weighed into each pot. Six treatments, each of which was replicated four times were set up for studying the visual symptoms. These pots were randomised on the bench in the glasshouse. Another fallow pot experiment was set up similarly, but each treatment was replicated six times.

B Soil Sand Mixture Pots :- 100 gms. of Silwood soil were mixed with 900 gms. of the sand in a small polythene bag. Each lot was prepared separately to ensure thorough mixing. Each treatment was replicated 14 times. Fallow pots were prepared similarly.

To ensure equal distribution of heat and light and to eliminate the effect of other environmental factors in the glasshouse, the cropped pots were moved periodically from one place to another.

Sampling :- At the time of each cutting, duplicate fallow pots from each treatment were mixed thoroughly on a clean polythene sheet. A subsample was immediately taken for moisture determination, usually 10 gms. Another subsample (usually 90 gms.) was air dried in the constant temperature room and used for Morgan extracts. Throughout this investigation, the extracts were obtained by shaking one part of soil with two parts of Morgan's solution for one minute and filtering through No. 1 Whatman filter paper.

Soil Solution :- Soil solution was obtained by alcohol displacement as described by Parker(52). The soil was packed tightly into resin cylinders especially used for the purpose and 75 ml. of alcohol were carefully added to the top. From previous trials, it was found that 90 - 100 ml. of soil solution could be obtained safely without contamination. This was enough for the analysis and pH determination.

Plant Material :- The tops were cut with stainless steel scissors kept especially for the purpose. The tops from each pot were weighed separately, dried in an oven at 70 - 80°C overnight and weighed separately for the dry matter yield. Average of

fresh and dry matter yield obtained from each treatment are given. The tops from each individual treatment and substratum were mixed and crushed by hand ; a subsample was ground in an agate mortar and sieved through a 70 mesh sieve and kept in an air-tight container for analysis. At the time of each cutting, a block of the soil sand mixture cropped pots was always taken for analysis of the soil to keep a check on the application of nutrients. Meanwhile, the extracted roots were washed thoroughly and dried and their dry matter yield was recorded.

At the time of the third cutting, "minus magnesium" and "minus sulphur"and treated pots from the sand culture series were taken for analysis. At the end of the experiment, roots from the rest of the treatments in the sand culture pots were removed, washed, dried and weighed and analysed. The Morgan extract of the soil was also analysed.

The roots were extremely difficult to grind in the agate mortar and it was necessary to use the mechanical mill for their grinding.

Preliminary Experiment :-

To test the washed sand, its Morgan extract was analysed and no trace of the elements to be studied for their deficiency level (iron, manganese, sulphur and magnesium) was found. Moreover, four pots were sown at the rate to be used (0.5gm./pot), two with acid washed sand and the other two with soil sand mixture. One pot of each was given distilled water while the corresponding two received 50 ml. of complete nutrient solution

the plants cultivated in sand with nutrient solutions deficient in the element concerned.

The first signs of magnesium deficiency showed in the outer layers of leaves (immediately adjacent to the wall of the pot) and gradually spread into the whole plants growing in the centre of the pot. Coloured plate 5 shows magnesium deficiency in the rye grass (1st. stage).

A close examination of the leaf at this stage shows intravenal chloresis as the veins keep their green colour. Looking at the pots in Plate 5, apart from the yellowish green colour the magnesium deficient plants acquired, it was observed that their growth at this early stage of the experiment was vigorous and superior to that of the plants receiving the complete nutrient solution. This observation is confirmed by the dry matter yield percent of green weight of these plants being higher than those of plants given any of the rest of the treatments, Table 77. This is attributed to the beneficial effect of the extra sodium added to the nutrient solution on which these plants were fed, sodium sulphate was used to replace the magnesium sulphate to supply the sulphate. The dry matter yield from"minus magnesium" was 23.9% as compared with 21.7% for the plants receiving complete nutrient solution.

The sulphur defeicient plants grown in sand culture (coloured Plate 6) were markedly stunted in growth as compared with plants receiving complete nutrient solution. The tops of the sulphur-deficient grass showed a very characteristic

upright form of growth and almost formed a right angle with the surface of the sand partifularly towards the middle of the pot.

In the soil-sand pots, on the other hand, the magnesium deficiency showed similar symptoms as in the sand cultures but the colour of the tops was more greenish with only a slight yellow tinge. Sulphur deficiency in these pots showed in the stunted growth of plants which retained their green colour. Thus, it was decided not to take any coloured plates for these soil sand mixture pots till the symptoms became clearer. The delay in appearance of clear symptoms of either magnesium or sulphur deficiency is obviously due to extra reserve of nutrients in the soil mixed with the sand in these pots.

Second Cutting :- The second cutting was taken four weeks from the date of the first one (26.8.1960). The magnesium deficiency on the sand cultured plants became acute. The colour of the tops was straw-yellow but still the green colour could be seen in the veins as light green streaks. At the fourth week (three days before the 3rd. cutting) the tops in two pots showed very severe symptoms as white spots appeared on the basal portion of the leaf as well as the upper third Plate 10. These white spots were presumably due to severe shortage of magnesium which resulted in complete absence of chlorophyll in these white tissues. The spots seemed to extend upwards from the basal portion and downwards from the upper portion. The leaves suffering from magnesium deficiency were veryb smooth to the touch, very

fragile and the least breath of air would result in the lodging of tops (coloured Plate 8). Sulphur deficiency in the sand cultures resulted in very stunted growth of tops, leaves were extremely short and narrow and the plants were showing their upright growth very clearly. Moreover, the leaves developed chlorotic tips and chlorosis was progressing steadily towards the base of the leaves. In the soil-sand pots however, the magnesium-deficient plants did not show the white spots which appeared in the sand cultured pots. plants. The sulphur-deficient plants partly retained their green colour but still with chlorotic appearance being greenish-yellow in colour. In general, the plants growing on soil-sand mixture looked stronger and made comparatively better progress than those grown on sand culture. Comparison between coloured Plates 8 and 9 illustrates this.

Immediately after the second cutting, the cropped pots of the treatments "minus magnesium" and "minus sulphur" were divided into two halves, one was kept under deficient conditions as before and the other received the deficient element in the form of a complete nutrient nutrient solution for the four weeks between the 2nd. and 3rd. cuttings.

The pots which received the corresponding deficient element will be termed the treated pots.

Third Cutting :- The magnesium-deficient plants in the sand culture which were kept under deficient conditions collapsed and died. Sulphur-deficient plants on the other hand, became

severely stunted in growth and leaves became needle-like, being short and narrow with a pointed end. In the pots treated after the 2nd. cutting (with sulphate or magnesium), plants regained their green colour and in an interval of three weeks, all signs of chloresis disappeared completely and made remarkable progress in growth. The plants treated with sulphate, the tops made an extraordinary progress, and regained their deep green colour. Astonishingly, the sulphur-deficient plants when treated with sulphate (in the form of complete nutrient solution), despite their remarkable progress in growth which by far, exceeded that of plants given complete treatment, kept their upright form of growth. Plates 12, 13, 14 and 15. Iron and magnesium manganese deficiencies made their first appearance on the plants growing in the sand cultures at the interval between the second and third cuttings. Ten days after the second cutting, the newly formed tillers were chlorotic while the older leaves retained their green colour. At this early stage it was difficult to differentaiteb between iron and manganese deficiencies in the pots. It was observed however, that iron deficiency caused a remarkably stunted growth of the grass while manganese deficiency did not. After approximately two weeks, iron deficieney became preminent in the leaves which showed chlorotic streaks throughout the length of the leaves, this being more definite in the younger leaves than in the older ones. Coloured Plate 16 shows the iron deficiency immediately before the third cutting 24.9.1960. Manganese deficiency was not

very clear at that stage. In the plants growing in soil-sand mixture, on the other hand, no definite signs of either iron or manganese deficiencies apart from chlorotic tillers scattered in each pot. However, the dry matter yield of these plants was lower as compared with plants receiving complete nutrient solution.

4th. Cutting :- After the third cutting, half the remaining pots received a complete nutrient solution carrying the deficient elements, while the other half was kept under deficient conditions.

The iron deficiency in sand-cultured plants became very prominent and the plants showed remarkably stunted growth. The whole area of the leaf apart from the mid-rib which had a green tinge, developed a dull yellowish-green colouration. There was blotching condition varying in colour from light-green to yellowish-green. Treatment of the iron deficient pots with complete nutrient solution carrying ferric citrate (5.6 p.p.m.) removed the iron deficiency symptoms, the plants resumed their growth, regained their green healthy appearance and the characteristic blotchiness disappeared. Coloured Plate No. 20 shows the difference in growth and colour between iron-deficient grass and treated grass; the latter almost identical with that receiving complete nutrient solution.

Manganese deficiency, by contrast, did not appear to affect the growth of the grass but manganese-deficient plants

gave lower dry matter yield than those receiving the complete treatment. The tops of the manganese-deficient grass acquired a canary-yellow colouration except the mid-rib which partly retained its green colour. These plants on treatment with complete nutrient solution, recovered their green colour. Coloured Plate 21 shows manganese deficiency in the grass grown in sand-culture (-Mn pot) in contrast with pot receiving manganese after the 3rd. cutting. and that given the complete treatment throughout.

In the plants grown in the soil-sand mixture, iron and manganese deficiencies showed only in the occurence of chlorotic young tillers which were scattered among the plants kept deficient in these elements. Closer examination however, showed that the leaves were narrower and their green colour was not as deep as in the plants given complete nutrients. The deficiencies or more precisely, the inadequate supply of iron and manganese were shown in the analysis of the soil solution obtained by alcohol displacement, in Morgan extracts of the soil and in the analysis of plant material, particularly the tops which showed notable differences in the levels of these elements, especially the manganese when compared with the plants receiving complete nutrient solution.

It is evident from the foregoing that magnesium deficiency in perennial rye grass was shown initially by chlorotic intravenal tissues, the tissues becoming light-green. At a later stage, these chlorotic areas became whitish and white spots were formed at the base of the leaf as well as at its upper third. These spots grew upwards and downwards and resulted in the death of the plant.

Sulphur deficiency mainly resulted in very stunted growth, the tops developed chlorotic tips and upright form of growth.

Onn treatment with the deficient element in question, these plants made a very quick recovery and symptoms disappeared completely in a short time (10 - 12 days). This confirms the findings that both magnesium and sulphur translocation is easily effected within the plant. Moreover, it suggests that the limit between the deficiency level and sufficiency level is narrow and that observed deficiencies in the field, particularly that of magnesium, must be remedied quickly. Application of magnesium after the development of white patches on the leaves was not always effective One deficient pot was treated with $MgSO_4.7H_2O$ in a complete nutrient solution but did not recover and ultimately the plants died. Iron and magnesium deficiencies symptoms made their first appearance as chlorotic newly formed tillers. At this stage, it was extremely difficult or even impossible to differentiate between the two deficiencies. At a later stage iron deficiency could be differentiated from that of manganese in the perennial rye grass by :-

1st :- The iron deficient grass having a yellowish-green colouration with bletches differing in colour from yellowish green to greenish colouration apart from the mid-rib , while manganese deficiency showed a canary-yellow colouration of the tops.

2nd.:- The iron deficiency affected the yield more severely, particularly the tops while nom such effect occurred in

the manganese deficient plants. On treatment with solutions carrying the deficient element in question, it was observed that sulphur and magnesium moved more freely in plant tissues than did iron and manganese, particularly iron.

Studies on the Soil Solution obtained by alcohol displacement :-

Analysis of displaced solutions obtained from fallow pots of the sand and soil-sand mixture are shown in Tables ,4, 82, 90.

Discussion of Results :- In the solution from the sand, the micronutrients studied were always present in traces (i.e. less than 0.002 p.p.m. oven dry soil basis) except in one case namely "minus iron" in the 3rd. sampling; its concentration reached 0,002 p.p.m. The appearance of this relatively high iron concentration in the soil solution may be attributed to the possible contamination, some of the pots with iron containing dust or the fact that sometimes the sand grains become stained with a very thin film of iron oxides which extends in the grooves and cavities of these grains, as pointed out by Hewitt. In time, some of this iron might pass into the soil solution. However, the impurities did not affect the object of the experiment as the iron deficiencies symptoms appeared and became gradually more severe. The concentration of iron in the displaced solution from the other sand culture pots (given iron in their nutrient solution) ranged from 0.006 to 0.008 p.p.m./unit oven dry soil at the time of the first sampling, from 0.008 to 0.013 p.p.m. at the time of the

2nd. sampling and from 0.008 - 0.011 p.p.m. soil at the 3rd. sampling.

Manganese on the other hand, was not detected in the soil solution obtained from "minus manganese" treatment of the sand culture pots at any sampling. Its concentration ranged from 0.002 to 0.009 p.p.m. of soil (oven dry basis) at the first sampling, from 0.006 to 0.009 p.p.m. at the time of the 3rd. sampling, thus gradually increasing in other treatments which received manganese in their nutrient solution.

The concentrations of magnesium and sulphur in the displaced solution from sand cultures supplied with "minus Mg" and "minus S" nutrient solutions deficient in these elements were $\frac{1}{7}^{th}$ - $\frac{1}{10}^{th}$ the concentrations in the displaced solutions obtained from the other treatments supplied with these two elements. The concentration of magnesium in displaced solution from the "minus" "magnesium" treatment at the appearance of the first signs of visual symptoms was 0.019 p.p.m. as compared with 0.146 p.p.m. Mg (soil oven dry basis) in the complete treatment. Despite the fact the values obtained from the 2nd. and 3rd. samplings werehigher than the value of Mg in the first sampling magnesium deficient plants died except those which received magnesium after the second cutting.

Sulphur concentration in the displaced solution from sand cultures ranged from 0.32 to 0.46 p.p.m. (oven dry basis) where the concentration of S in "minus-sulphur" treatment was 0.1 p.p.m. At the time of the second sampling, the sulphur concentration

ranged from 0.6 to 0.89 p.p.m. where in "minus-sulphur" treatment it was 0.13 p.p.m. In the 3rd. sampling the values increased from 0.81 to 1.44 p.p.m. except in the "minus-sulphur" treatment it was 0.07 p.p.m. S. Thus there was a gradual increase in the sulphur concentration in the soil solutions from the different sulphur-containing and a slight decrease in those from the "minus -sulphur treatment.

Aluminium was examined mainly to investigate its effect if any, at such low concentrations on the uptake of phosphate. it is evident however, that such differences in concentration of aluminium applied to the pots could hardly be expected to have any effect. The nutrient solutions (Table 72) contained from 0.01 to 0.030 p.p.m. Al. The concentration of aluminium in the displaced solution obtained from the sand-culture-fallow pots at the time of the first sampling ranged from 0.003 - 0.011 p.p.m. while in the "minus-aluminium" treatment it was 0.004 p.p.m. At the time of the 2nd. sampling the concentration of aluminium had increased from 0.005 to 0.010 p.p.m. while with the "minus-aluminium" treatmentit was 0.003 p.p.m. At the time of the third sampling both values were unchanged.

The analysis of the phosphate in the displaced solution from the sand-culture showed that fluctuations in phosphate concentration were small and more or less uniform. Nearly always there was a slight increase in the phosphate concentrations in the solutions obtained from the "minus-iron; "minus- aluminium", "minus-magnesium" and to a certain extent, from the "minus-manganese

treatments. The concentration of phosphate at the time of the second sampling showed a slight decrease as compared with that from the first sampling. The range of values at the time of the first sampling was 0.23 - 0.51 p.p.m. P (oven dry basis), 0.20 - 0.31 p.p.m. in the second sampling and 0.1 - 0.5 p.p.m. P in the 3rd. sampling.

Potassium, on the contrary, showed a gradual increase in the values obtained from successive analyses of the displaced solutions from fallow pots of the sand-cultures and values for the "minus-iron" pots were slightly higher than from the rest of the treatments. This phenomenon could not be explained.

In the Soil Sand mixture pots, it was eveident that the displaced solution contains slightly higher quantities of iron, manganese and aluminium but lower quantities of sulphur, phosphorus, potassium and magnesium than that from the sand-culture pots; probably due to adsorption or fixation of the latter elements on the clay and organic matter complexes present. It is possible however, that such elementswere released in an available form under cropping conditions. Thus the first symptoms of magnesium and sulphur deficiencies which were prominent and clear in the sand-culture pots at the time of the first cutting were less obvious in the soil-sand mixture pots indicating that the level of the defisient nutrients studied in the latter was high enough to hinder the appearance of typical symptoms but not enough to supply adequate nutrients to maintain healthy plants. Moreover, the slightly higher iron and manganese values in the

soil solution obtained from the soil-sand pots explains the masking of the appearance of the visual symptoms in the plants growing in these pots even after four cuttings. The only visual symptoms recorded were scattered chlorotic tillers in each iron or manganese deficient pots. The pH of the soil solutions (Tables 73, 81 and 89) from the soil-sand mixture pots gradually decreased in the "minus-iron", "minus-manganese" and to a certain extent, "minus-aluminium" pots from the first to the third sampling e.g. with the "minus-iron" treatment the pH decreased from 6.43 to 5.44 in 12 weeks while with the "minus-manganese" the pH decreased from 5.94 to 5.50. The pH from the "minus-aluminium" treatmentwas 5.66, 5.74 and 5.46 in the successive samplings. The decrease in pH values following the "minus-iron and "minus-manganese" treatments possibly contributed to delaying the visual iron and manganese deficiency symptoms by releasing extra iron and manganese from the soil mixed with sand in these pots.

Analysis of Morgan Extracts :-

A Analysis of Morgan extracts from Fallow Pots of Sand Cultures:-

The Morgan extractable nutrients increased with the progress of the experiment, the only exception being the phosphates, the extractability of which decreased markedly from the first to the third sampling. With the "minus-sulphur" treatment however, the extractability of phosphates increased from the first to the third sampling.

When comparing the Morgan extractable values of phosphate

at one and the same time, it is evident that extractability of p phosphate was slightly higher from the "minus-iron", "minus-manganese" and to a certain extent "minus-aluminium" treatments than from the sand-cultures which received complete nutrients. At the time of the third sampling, the "minus-aluminium" treatment showed slightly higher Morgan extractable phosphate than the rest of the treatments with the exception of the "minus-sulphur" treatment, the extractable phosphate from which was considerably higher than from the "minus-aluminium" treatment.

Results of Morgan extractable nutrients are shown in Tables 75, 83, 91 and 98.

B Analysis of Morgan extracts from the Fallow Pots of the Soil-Sand mixture :-

In the pots receiving complete nutrient solution, the Morgan extractable iron, manganese, aluminium gradually increased from the first sampling to the second where it either remained approximately constant in the case of iron and manganese or slightly increased as in the case of aluminium.

The Morgan extractable magnesium increased from the first to the second sampling and slightly further in the third and fourth sampling.

Morgan extractable sulphates increased gradually but steadily from the first to the third sampling and more markedly in the fourth sampling. The Morgan values from the pots given the "minus-sulphur" treatment were comparatively lower and decreased

progressively with time.

Morgan extractable phosphates also showed a gradual increase with the progress of the experiment. At the time of the first sampling, the values ranged from 4.0 to 6.6 p.p.m. P, at the second sampling from 6.1 to 10.0 p.p.m. and at the third sampling from 8.9 to 19.6 p.p.m., the higher values being generally associated with "minus-iron", "minus-manganese", "minus-aluminium" and to some extent "minus-magnesium" treatments. Evidently, the Morgan extractability of phosphate increases with decrease of iron, manganese, aluminium or magnesium levels in the soil. This is- is also apparent from the data for the sand-culture experiment.

Potassium increasedv steadily at successive sampling. Analysis of Morgan extracts from the fallow pots of both the sand-culture and soil- sand mixture are given in Tables 75, 83, 91 and 98.

C Morgan Extracts of Residual Soils from the Cropped Pots :-

The main objects of the analysis of the residual soil from the cropped pots were :-

(1) To ascertain whether the rate of application of nutrients used was sufficient to give adequate supplies to the plants.

(2) To examine the rate at which the different nutrients were uptaken by the growing plant as shown by

Morgan tests and by plant analysis and to seek relationships between these two factors.

The analysis of the Morgan extracts of the residual soils from the cropped pots (Tables 76, 84, 92, and 99) show a gradual increase in the level of Morgan extractable elements studied from the first to the fourth samplingexcept when a particular element has been omitted from the nutrient solution in which case all elements except the deficient one increased as time advanced. Values for the element omitted showed a gradual decrease. The only exception to this statement was manganese values for which decreased considerably in all treatments from the first sampling, reached a minimum at the third sampling in the soil-sand mixture pots but in the 4th. sampling showed a slight increase over previous determinations. The concentration of manganese in the tops pf plants growing on soil-sand mixture and receiving complete nutrient decreased from 115 p.p.m. at the time of the first sampling to 52.5 p.p.m. at the time of the fourth cutting. In plants grown in sand-culture and also given complete nutrient solution the manganese concentration in the tops ranged from 90 p.p.m. in the first cuttings to 52.5 p.p.m. at the time of the fourth sampling. The manganese deficient plants grown in sand-culture showed clear visual symptoms of manganese deficiency when manganese concentration in the tissues decreased to 18 p.p.m. (dry matter).

In the soil-sand mixture, the Morgan extractable aluminium

from the cropped pots remained more or less constant in the first three samplings but showed a slight increase at the fourth sampling.

Magnesium showed more or less a constant level at the first and second samplings, but increased slightly at the third and fourth samplings.

The levels of sulphur, phosphorus and potassium showed a steady but a gradual increase in the Morgan extracts of these cropped pots from the first to the fourth sampling. The level of Morgan extractable potassium in the residual soil was the lowest from "minus-magnesium" treatments indicating the increased uptake of potassium by these magnesium-starved plants. This was clear in the first and second samplings but not so prominent in the third sampling, probably due to decreased top growth occurred by magnesium deficiency.

Yields and Mineral Composition as affected by mineral deficiencies

A :- Yield :-

I : Sand Culture Plants :- At the time of the first cutting, the yield of tops following the "minus-magnesium" treatment(fresh weight) were higher than those from the rest of the treatments including the complete nutrient. This is attributed mainly to the beneficial effects of the extra sodium added in the form of sodium sulphate to replace the magnesium sulphate usually used in supplying the magnesium and sulphur in the rest of the treatments. The dry matter yield percent of the tops from the "minus magnesium was 23.9 as compared with 21.7 for the complete nutrient

The dry matter yields were also highest from the "minus magnesium" treatment, those from the other treatments decreased in the order "complete treatment", "minus-manganese", "minus aluminium","minus iron", and"minus-sulphur".

At the second cutting, the "minus-magnesium manganese" and "minus-sulphur treatments gave the lowest fresh and dry matter yields. Yields from other treatments decreased in the order :- complete treatment, "minus-aluminium", "minus-manganese", "minus-iron), "minus-magnesium" and "minus-sulphur".

At the time of the third cutting, all the magnesium deficient plants collapsed and died ; no cuttings were taken. An attempt to save plants growing in one pot by adding complete nutrient solution containing $MgSO_4.7H_2O$ two weeks after the second cutting failed. The magnesium deficient plants which were treated with complete nutrient solution carrying magnesium sulphate immediately after the second cutting showed a remarked steady progress and recovered completely from visual symptoms (Plates 12, 13, 14 and 15). Yields from the plants were 6.267 gms/pot (fresh weight) against 6.304 gms/pot for those given complete treatment throughout (Table 94). The corresponding dry yield = 1.753 and 1.644gms/pot respectively.

The fresh weight of tops from the sulphur-deficient plants which received sulphate after the second cutting in the form of $MgSO_4.7H_2O$ at the same rate as was applied to the complete treatment increased tremendously being 10.212 gms/pot as compared

with 6.304 gms/pot from the complete treatment. Moreover, the dry matter yield in the sulphur-treated ~~pots~~ Plants exceeded that obtained from the complete treatmentbeing 1.719 gms/pot in the former against 1.644 gms/pot in the latter. However, the percentage of dry matter in the fresh cutting of the sulphur-treated plants decreased considerably being 16.83 when compared with complete treatment which reached 26.07%. The explanation of this phenomenon is that :-

1st : The sulphur-deficient plants subsequently treated with sulphur increased considerably in size and accordingly the rate of transpiration increased and with it the uptake of of water increased.

2nd : Another important factor is the presence of the chloride ion in the sulphur-deficient pots as magnesium was added in the form of chloride ($MgCl_2.6H_2O$) to supply an equal magnesium concentration in the "minus-sulphur" nutrient solution. Chlorides often increase succulence,. According to Black(138), truck-crop growers in the United States observed that tomatoes and other fruits are more firm when fertilised with potassium sulphate than when with potassium chloride. Also it was found that cabbage, lettuce are more succulent when treated with potassium chloride than when treated with potassium sulphate. It is suggested that this is the case with plants from sulphur-deficient pots which were supplied with a chloride ion and showed a low percentage of dry matter (i.e.high water

content) while in the "minus-magnesium", plants supplied with sodium sulphate, the percentage of dry matter was considerably higher (i.e. relatively lower water content).

The effect of Magnesium and Sulphur deficiencies on the growth and yield of the root system of Rye grass grown in Controlled Sand Culture :-

Both magnesium and sulphur deficiencies in the growing medium, particularly the latter exerted a considerable negative effect on the growth and development of the grass roots. Plate 18 shows roots of grass deficient in magnesium and sulphur compared with the treated roots (which received the deficient element after the 2nd. cutting). The roots suffering from magnesium deficiency are very thin, weak and fragile as compared with those which received magnesium. The sulphur-deficient roots were stunted in growth but in general, looked better than those of magnesium-deficient roots.

It is of interest to note how quickly these deficient roots respond to magnesium or sulphur when supplied to the starved root in a solution. The magnesium deficient roots increased in weight by 46.84% when treated with $MgSO_4.7H_2O$ solution for four weeks and the sulphur-deficient roots gained 31.25% in weight when treated with the same solution for the same time; The treated plants thus received a complete nutrient solution after the second cutting at the same rate as the original complete nutrient for the interval between the second and third

cuttings.

Iron and Manganese deficient plants :- gave slightly lower yields than those given the complete nutrient when one and the same cuttings are compared. This lasted for the first three cuttings. At first, the iron deficiency did not exert any effect on the weight of the plants as it happened at the time of the fourth cutting when the iron deficiency resulted in a very serious reduction in fresh and dry matter yields, particularly so in the plants grown in sand-culture. The weight of the fresh yield from "minus-iron" treatment was 2.980 gms/pot as compared with 7.418 gms/pot from the complete treatment. At this time, the visual symptoms were very clear.

Meanwhile, in manganese-deficient plants, visual symptoms were apparent as bright or canary-yellow colouration which spread all over the tops of the starved plants. It is interesting to note that the reduction in fresh or dry matter yields was not as great as in the case of iron deficiency and thus the effect of manganese differed from that of iron. The fresh weight yield at this time (4th. cutting, Table 100) was 6.416 gms/pot as compared with 7.419 gms/pot from the complete treatment. The corresponding dry matter yields were 1.172 and 1.609 respectively.

The effect of Iron and Manganese deficiencies on the root growth.

Although these deficiencies resulted in decreased root yields when compared with the complete treatments, there were

no serious effects on root growth or development unlike the effects of sulphur and magnesium. Plate 22 shows the roots from the "minus-iron","minus-manganese" and "minus-aluminium" treatments as compared with the complete treatments. Table 101 gives the weights of plants treated with Fe and Mn after the 3rd. cutting and those kept under deficient condition throughout. The treated roots received the deficient element in question as a complete nutrient solution for an interval of four weeks. During this period, roots of "minus-iron" plants gained 8.25% of their weight when treated with ferric citrate in a complete nutrient solution, roots of "minus-manganese plants gained 4.96% of their weight on treatment with $MnSO_4.4H_2O$ and roots from "minus-aluminium" treatment gained 3.30% of its weight on treatment.

Minus Aluminium Treatment :-

There was a slight decrease in fresh and dry matter yields from the "minus-aluminium" treatment. On treatment with a complete nutrient solution(containing aluminium sulphate) the dry matter yield increased from 1.614 gms/pot to 1.719 gms/pot as compared with 1.609 gms/pot for the complete treatment.

B : Soil Sand Mixture Plants :-

Yields from magnesium and sulphur-deficient plants showed reductions similar to those observed in the sand-cultures. The beneficial effect attributed to sodium was not observed except

at the time of the second cutting where the fresh and dry matter yields of magnesium-deficient plants exceeded those obtained from the complete nutrient treatments. On treatment of the deficient plants with the complete nutrient solution ~~of the deficient plants~~, carrying the missing nutrient, the fresh yield of "minus-magnesium" was increased from 2.768 gms/pot to 7.538 gms/pot and dry matter yield increased correspondingly from 0.914 gms/pot to 1.613 gms/pot. Again, the sulphur-deficient plants on treatment with sulphate showed a similar marked increase in fresh weight which increased from 2.216 gms/pot to 9.896 gms/pot and dry weight from 0.466 gms/pot to 1.868 gms/pot. The dry matter weight percent of the sulphur-treated plants also showed a remarkable decrease when compared with the rest of the treatments. This is attributed, as noted previously to the effect of the chloride which probably increased the succulence of the grass. The effect of sulphur and magnesium deficiencies on the root growth and development were the same as in the sand culture and after the addition of the deficient element, the roots in both cases gained weight; the "minus-magnesium" roots gained 22.5% of its weight on treatment with complete nutrient solution while the "minus-sulphur" gained 37.2% on treatment with the same solution.

Both iron and manganese-deficient plants showed a slight decrease in fresh and dry matter yields but the difference between these deficient plants and those grown with complete nutrient was not consistent as in the case of sand cultures. The effect of iron and manganese deficiencies on the roots of the

plants was not as serious as under sulphur and magnesium deficiency conditions. At the time of the fourth cutting, the root yield of "minus-iron" plantswas 5.896 gms/pot, of"minus-manganese" was 5.108 gms/pot and "minus-aluminium" was 6.439 gms/pot against 7.169 gms/pot from plants receiving complete nutrient solution. On treatment with the respective deficient element added in the form of complete nutrient solution at the same rate as the original complete nutrient solution for an interval of four weeks, there was an increase in weight of 8.13% in the treated iron roots and of 2.5% in the treated manganese roots and of 6.76% after the aluminium treatment (Table 101).

The effect of Magnesium, Sulphur, Iron and Manganese deficiencies on the Mineral Compodition of tops of perennial Rye Grass.

A : Effect of Magnesium deficiency :-

The most important characteristic of magnesium-starved plants is their high concentration of potassium. This was observed in the analysis of the tops of each cutting both from sand-cultures and soil-sand mixtures. In the first cutting, the potassium concentration in the tops of magnesium-deficient grass was 5850 p.p.m. K (dry matter basis) as compared with 3800 p.p.m. from the plants fed on complete nutrient solution.

The magnesium-deficient plants also had comparatively low nitrogen concentration e.g. in the first cutting the minus-magnesuim tops from the sand-culture contained 20750 p.p.m. N as

compared with 26875 p.p.m. N for those tops given the complete nutrient . This condition persisted throughout the whole experiment as shown by the folowing examples from the data of the analyses given in Tables 79, 87, 96 :- "minus-magnesium" plants from the soil-sand mixture at the time of the first cutting contained 15000 p.p.m. N dry matter basis as compared with 25688 p.p.m. for the complete treatment. At the time of the second cutting in the sand culture, the concentration of nitrogen in the magnesium-deficient plants was 20250 p.p.m. as compared with 23500 p.p.m. from the complete nutrient while in the soil-sand mixture, "minus-magnesium" plants gave 16125 p.p.m. in the dry matter and complete nutrient plants contained 18939 p.p.m. It is worth mentioning however, that the difference between concentration of nitrogen in the "minus-magnesium" treatment and complete treatment became smaller as the experiment progressed.

However, when comparing the uptake of the tops of "magnesium-deficient" plants which were kept as such and those which received magnesium in their nutrient solution, it is observed that the uptake of nutrients studied, increased considerably on treatment of the deficient plant with $MgSO_4.7H_2O$ in the form of a complete nutrient solution. The difference in the uptake of potassium between magnesium-deficient and "magnesium-treated" plants however, was very small viz, 9.414 mgm K in the tops of "minus-magnesium" plants and 9.758 mgm. K in "magnesium-treated" plants.

Taking into consideration the big difference in the dry matter yield between the two treatments, it is clear that the potassium concentration in "magnesium-deficient" plants is notably high. The detailed calculated uptake of other nutrients is in Tables 104 - 109.

B : Effect of Sulphur deficiency :-

Sulphur deficiency in most cases resulted in an increase in concentration in most of the elements studied obviously due to low yields obtained. However, it is seen that the concentration of magnesium is almost always lower in these plants than in those receiving other treatments, the only exception to this generalisation being magnesium concentration in tops of the sulphur-deficient plants at the time of the first cutting in the sand-culture pots.

The nitrogen concentration was considerably higher in the sulphur-deficient plants than in those given the other treatments. Again, this was not observed at the time of the first cutting but showed consistently in the second and third cuttings both in sand-cultures and soil-sand cultures.

On treatment of sulphur-deficient plants with sulphur (in the form of $MgSO_4.7H_2O$ added at the same rate as in the other treatments), the magnesium concentration increased considerably (from 4100 p.p.m. dry matter basis in the sulphur-deficient plants to 5400 p.p.m. in tops of treated plants from sand-culture and from 4200 p.p.m. Mg. to 5300 p.p.m. in the soil-sand mixture pots.

Nitrogen concentration on the other hand, decreased

markedly in the treated pots e.g. nitrogen concentration decreased from 32500 p.p.m. N dry matter in sulphur-deficient tops grown in sand-culture to 27500 p.p.m. on treatment with sulphate and from 31000 p.p.m. N (dry matter basis) in the soil-sand mixture to 28625 p.p.m. on treatment Table 96.

Considering the difference in uptake of the nutrients studied, on treatment of deficient plants the uptake increased greatly, obviously due to increased growth and considerable increase in dry matter yields. Table 106.

C : Effect of Iron deficiency on the Mineral Composition of Grass.

Iron deficiency affected the concentration of phosphorus in the starved plants. The phosphorus concentration is higher in the iron-deficient plants than in those given by the rest of treatments, the effect becoming clearer with the progress of the experiment. At the time of the first cutting, the concentration of iron in tops of grass grown in sand-culture and receiving complete nutrient solution was 450 p.p.m. and that of P was 2048 p.p.m. (dry matter basis) while in the "minus-iron" treatment, iron concentration was 80 p.p.m. and P was 2320 p.p.m. dry matter basis. At the time of the second cutting, the iron concentration in tops receiving complete nutrient was 170 p.p.m. and P was 2593 against 40 p.p.m. Fe and 2740 p.p.m. P (dry matter) for the deficient tops. The same picture was maintained in the 3rd. and 4th cuttings, Tables 96 and 103.

Calculating the ratio of phosphorus to iron (P/Fe ratio) in the tops of plants[(22)] receiving complete nutrient solution and those fed on nutrient solution deficient in iron, it was observed that P/Fe ratio is always considerably higher in iron-deficient plants (Table 110), Showing that these plants tend to take up more P than do plants receiving iron.

On treatment of iron-deficient plants with iron salts (ferric citrate applied at the same rate as the rest of treatments in the form of complete nutrient solution to the soil for four weeks), a reduction was observed in the P/Fe ratio of the treated plants which recovered from their chlorosis and regained their green healthy colouration in their tops. The P/Fe ratio in chlorotic plants when the visual symptoms were clear, was 14.7 as compared with 5.46 in tops receiving the complete nutrient. After treatment of the plants with ferric citrate, the ratio of P/Fe in chlorotic plants decreased from 14.7 to 11.6 at which the plants regained their healthy appearance again. The potassium concentration of iron-deficient grass in most cases showed a slight increase over that in plants receiving complete nutrient. This phenomenon was clear in plants whether grown in sand-culture or soil-sand mixture but was more obvious in the former. The only exception to this generalisation is that at the time of the first sampling of plants grown in the soil-sand mixture where the potassium concentration in the iron-deficient plants equalled that of the complete treatment. It was also observed that

the difference in potassium concentration between the iron-deficient plants and those supplied with iron becomes bigger in magnitude with increasing deficiency of iron i.e. the concentration of potassium in the iron-deficient tissue increased with the severity of the deficiency symptoms. For example, at the time of the first cutting, in the tops of the sand-cultured grass, the concentration of K in the "minus- iron" plants was 3900 p.p.m. K (dry matter) against 3800 p.p.m. K in those having the complete nutrient, at the second cutting, the corresponding concentrations of K were 4950 p.p.m. K and 4550 p.p.m. K and at the third sampling when the visual symptoms were prominent the values were 5850 p.p.m. and 5350 respectively.

Magnesium concentrations in the iron-deficient plants showed no regular trend probably due to a disturbance in the formation of chloroplasts. Possibly also the slight increase in the potassiumm concentration in the iron-deficient leaves was a contributory factor. This irregularity in magnesium concentration of iron-deficient plants was more marked in the plants growing in the soil-sand mixture than in those from sand-cultures. The ratios of magnesium concentration in the iron-deficient plants to that in those given the complete nutrient in sand-cultures were 0.68, 1.00, 1.29 and 1.25 from the first, second and third and fourth cuttings. The ratio decreased from 1.25 to 1.0 on addition of ferric citrate to the nutrient solution of the "minus-iron" plants after the third cutting. In the soil-sand mixture however, the ratio of magnesium in "iron-deficient" plants to

that in those given complete nutrient solution was irregular being 1.34, 0.8, 0.77, and 0.9 for the first, second, third and fourth cuttings respectively. On treatment of the "minus-iron" plants with iron the ratio increased from 0.9 to 1.03.

The interrelation of iron and manganese (total) is shown by the calculation of the Fe/Mn ratio (Table 111). The Fe/Mn ratio in the iron-deficient plants increased steadily in the sand-grown grass. In the soil-sand mixture the ratio Fe/Mn in the tops was irregular being 0.92, 1.06, 0.66 and 1.77 at the time of the first, second, third and fourth cuttings. After addition of ferric citrate, the Fe/Mn ratio rose from 1.77 to 2.66 in the soil-sand mixture series and from 7.22 to 9.22 in the sand-cultured plants.

D : Effect of Manganese Deficiency on the Mineral Compositio of the Grass :-

Manganese-deficient plants are characterised by an extremely low manganese concentration ; by contrast, chlorotic plants due to iron deficiency, contained as mudh as 320 p.p.m. Fe (dry matter basis) whereas at the time when visual symptoms were definite in the manganese-deficient plants grown in sand-culture, the concentration of manganese in the tops was 15 p.p.m. of the dry matter.

Manganese-starved plants showed a lower concentration of nitrogen. This statement is true for the first three cuttings of the sand-cultured plants but at the time of the fourth cutting

the reverse occurred. Plants grown on soil-sand mixture followed the same trend in the fourth as in the first three cuttings i.e. lower nitrogen concentration than in those receiving the complate nutrient.

Analysis of Roots :-

The roots of the magnesium-deficient plants grown in sand-cultures and soil-sand mixture were observed to have a lower potassium concentration than those receiving any other treatment, the exterme opposite of the dondition on the tops of these plants which possibly drained the potassium from the roots. This is particularly so in plants grown in sand-cultures.

The roots of plants deficient in sulphur, showed a higher concentration of almost all the nutrients studied. This is obviously due to the small root yields obtained under conditions of sulphur deficiency. However, on treatment of these plants with sulphur (as $MgSO_4 \cdot 7H_2O$), there was not only increased growth but also an increase in concentration of magnesium and potassium.

The roots of iron-deficient plants again contained as much and often more potassium than the plants receiving the other treatments e.g. in the roots from the soil-sand mixture at the time of the fourth cutting, those receiving complete nutrient contained 1300 p.p.m. K (dry basis) while the roots given the "minus-iron" nutrient contained 1550 p.p.m. K. On treatment of these plants with iron, the potassium concentration in the roots decreased to the level of those given complete nutrient

1300 p.p.m. K (dry basis) Table 103.

The P/Fe ratio in the roots (Table 110) was always higher in iron-deficient roots than in other treatments, a similar picture to that in the tops of these plants thus confirming the higher uptake of phosphates by the iron-deficient plants.

Discussion of Results :-

Minus-Magnesium Plants :-

It is evident from the data obtained in this experiment that a deficiency of one of the essential nutrients results almost always in characteristic changes in colour of tops, growth habit, rate of growth and yield to varying extents and also in changing the chemical composition of plants. Any unbalanced nutritional condition caused by the absence of any of these elements in the soil in an "available" form will invite the plant to increase its uptake of other elements. The uptake of potassium by the manganese-deficient plants is a good example of this. Lucas and Scarseth[139], studied the balance of potassium, calcium and magnesium and their reciprocal relationships and noted that if the uptake of one increased that of the other two will decrease. In the present work, the ratio K/Mg in grass given the complete nutrient was very consistent and ranged from 0.80 - 1.24 in those from the soil-sand mixture. The K/Mg ratio at the time of the first appearance of the magnesium-

deficiency symptoms ranged from 5.6 - 6.3 and increased to 19 - 20 when the deficiency was serious enough to cause the death of the tops.

Nitrogen is concerned with magnesium in the building of the chlorophyll molecule and under magnesium-deficient conditions it is obvious that little or even no chlorophyll may be formed and this explains the low nitrogen content of the magnesium-deficient tops. It is observed however, that the roots deficient in magnesium contain the highest nitrogen concentration.

It is well established fact that the nitrogen supply frequently controls the amount of protein formation. The dry matter yield of plants from the "minus-magnesium" treatment of the first cutting in the sand-culture pots and the second cutting in the soil-sand mixture series exceeded that of the complete-nutrient Plants and it is suggested that the increased weight could be attributed to the formation of proteins from sulphur and phosphorus. This suggestion receives some support from the data for the sand-cultured grass at the time of the first cutting (Table 104) when the uptake of sulphur from the complete nutrient = 5.708 mgm. S/pot and with the "minus-magnesium" treatment = 4.520 mgm/pot. Meanwhile, the the uptake of P by these magnesium-deficient plants exceeded that from the complete nutrient e.g. with the complete nutrient the uptake of the tops was 2.398 mgm P/pot and with the "minus-magnesium" treatment 2.893 mgm/pot.

Minus-Sulphur Plants :-

Apart from the serious effect on the yields of tops and roots caused by sulphur deficiency, the chemical composition of sulphur-deficient plants was observed to change as follows :-

1 : The most important characteristic of these plants is the greater concentration of nitrogen in their tissues compared with that in plants given complete nutrient. This observation was not apparent at the time of the first cutting but was definite in the second and third cuttings. This is probably due to the incomplete utilisation and assimilation of amino acids in the sulphur-deficient plants as indicated by the findings of Eaton(140).

2 : The concentration of P in the tops of the sulphur-deficient plants exceeded that in plants given other treatments. Comparing the P and K concentrations in the sand-culture pots, it is evident that there is extra accumulation of P. e.g., at the time of the first cutting in the complete treatment of the sand-culture pots the concentration of P was 2593 p.p.m. (dry matter) while that of potassium was 4550 p.p.m. as compared with 4309 p.p.m. P and 4550 p.p.m. K in the sulphur-deficient plants. This means that the potassium concentration was the same in both treatments (complete and "minus-sulphur") while the concentration of P was considerably higher in sulphur-deficient plants. In the soil-sand mixture, the concentration of P in the plants given the complete nutrient

was the same as in the corresponding plants in sand-culture (2593 p.p.m.) while the concentration of K was 4775 p.p.m. against 4203 p.p.m. P and 4950p.p.m. K in the sulphur-deficient plants. Generally speaking, it was observed that there was extra accumulation of P in sulphur-deficient plants, a phenomenom which was not observed except in the iron-deficient plants.

Iron and Manganese deficient plants :-

Iron-deficient plants were characterised by their high phosphate concentration. The phosphorus/iron ratio (P/Fe ratio) was consistently higher in the iron-deficient plants than in those receiving an adequate iron supply. The importance of this ratio lies in the fact that it increases considerably even if the visual symptoms of deficiency are not clear.

The iron content of deficient plants did not show a consistent relationship with the appearance of visual symptoms i.e. the iron content of deficient plants was not an effective index of the deficiency. In one case, tops of plants given the "minus iron" treatment contained as much as 320 p.p.m. Fe in the dry matter as compared with 550 p.p.m. for the plants given complete nutrient. It seems possible that plants can accumulate iron when growing in cultures containing even traces of iron. This phenomenon applied to the tops as well as the roots. In this respect, iron differed from the rest of the elements studied where the deficiency symptoms are associated with the low

concentration of the element concerned in the tissues. Manganese deficiency symptoms on the other hand, were observed when the manganese concentration in the dry matter of the tops was very low. In the sand-cultured grass, the first symptoms (chlorotic newly-developed tillers) coincided with a manganese concentration in the tops of 18 p.p.m. dry matter. This value decreased to 15 p.p.m. when the visual symptoms had spread all over the plant and caused then characteristic canary-yellow colouration of the tops as described previously.

Inter-relation between Soil Solution Contents, Morgan Extractable Nutrients and their uptake by Grass Tops.

From the curves relating these quantities (Fig. 49 - 56 for the sand-cultures receiving complete nutrients and Figs. 92 - 98 for the corresponding treatment of the soil-sand mixture), it is evident that the Morgan extractable nutrients and soil solution contents were good indices of the uptake of the nutrients studied by the tops. The only exception was the phosphate and to some extent, iron where the Morgan extractable and soil solution values in certain cases disagreed with the uptake in the sense that when there was considerable decrease in the values of phosphates or iron from one sampling to another, there was a continual increase in the phosphate uptake by the tops. This is particularly so in the sand cultures (Figs. 49 and 55). In the soil-sand mixture, however, it appears that the Morgan extractable phosphates furnished a better index of the phosphate

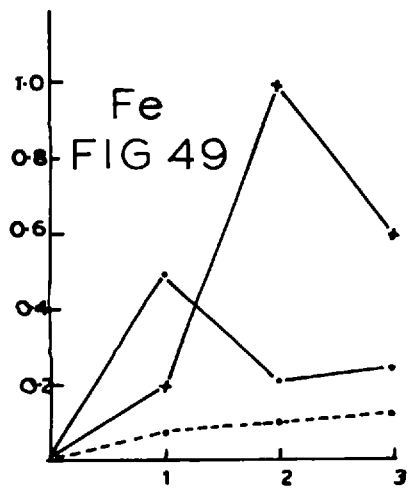
KEY
(for FIG 49-133)

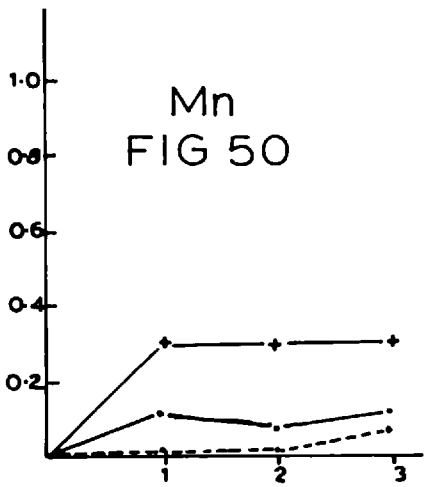
•·····•·····•·····• SOIL SOLUTION PPM SOIL oven dry basis
(X10 for Fe, Mn, Al, Mg & K)

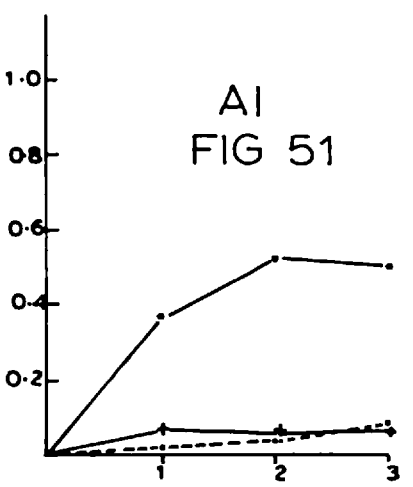
+——+——+——+ MORGAN EXTRACTABLE NUTRIENTS PPM
SOIL OVEN DRY BASIS.

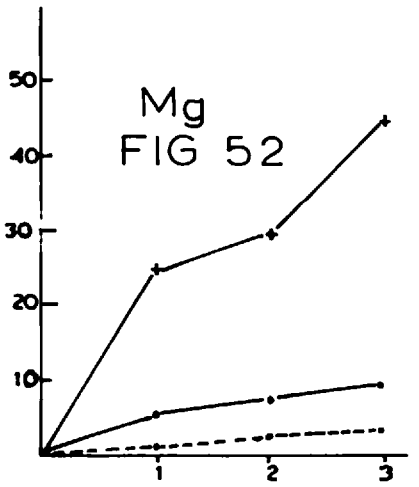
•—•—•—•—• PLANTS UPTAKE (TOPS ONLY) mgm/pot
(dry matter basis)

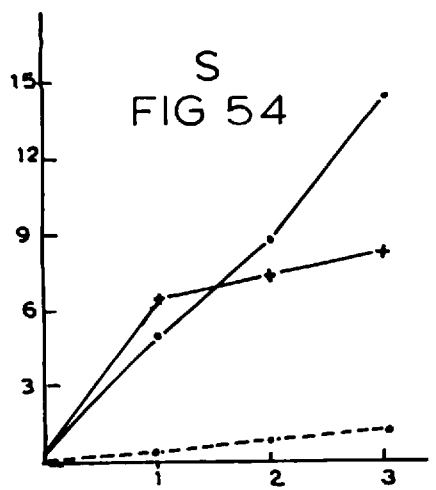
1,2,3, along the ordinate : Samplings or cuttings.

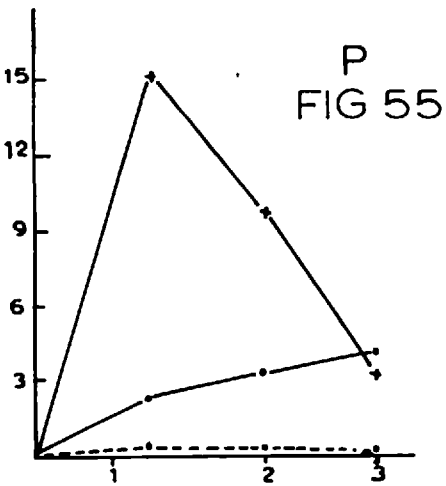


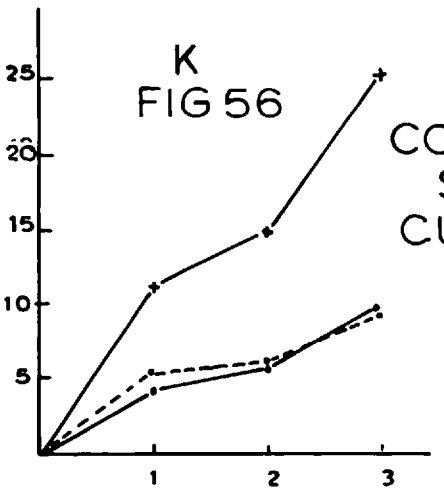




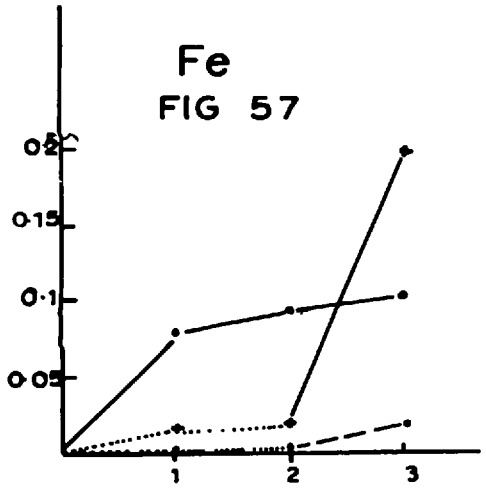


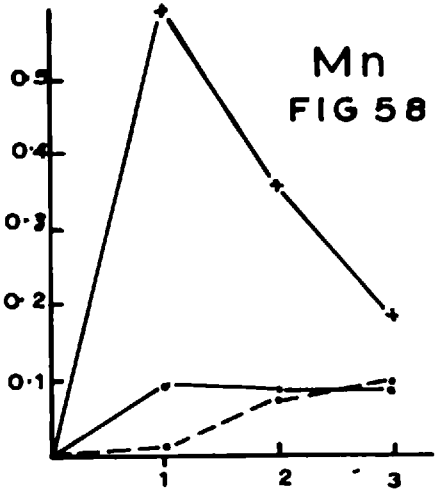


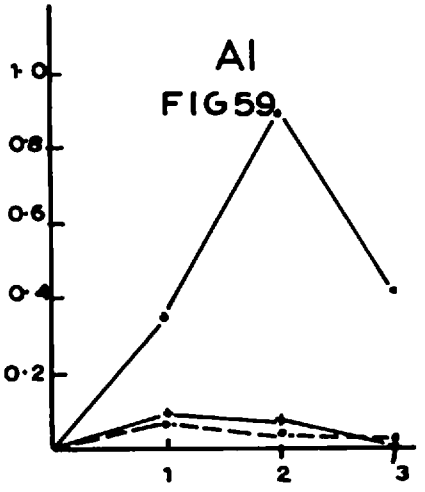


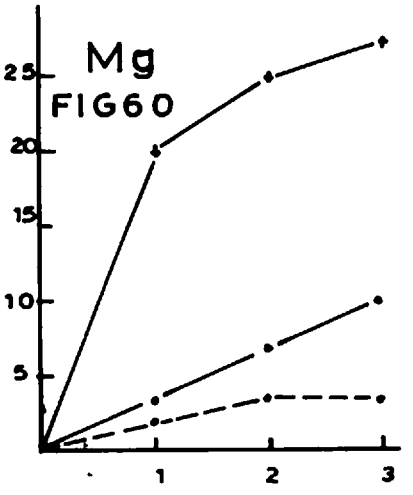


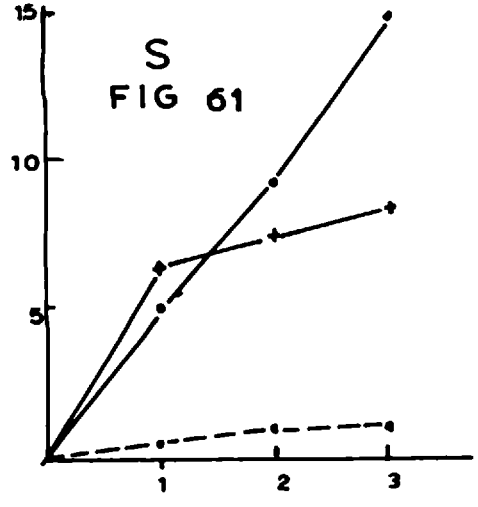
I
COMPLETE
SAND
CULTURE

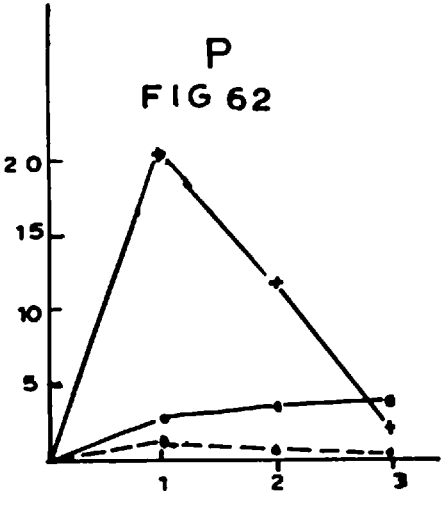


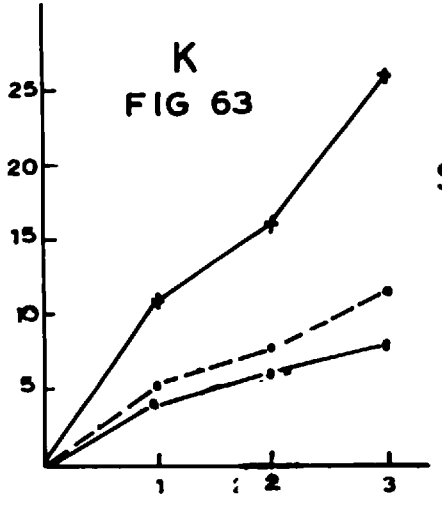




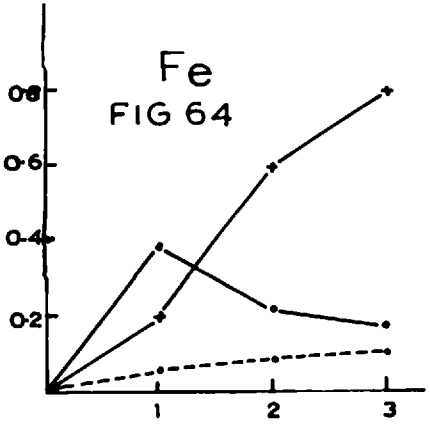


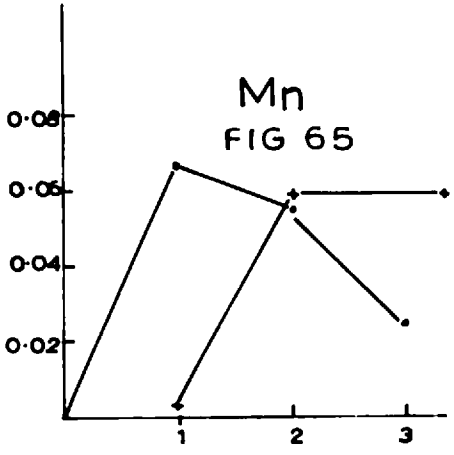


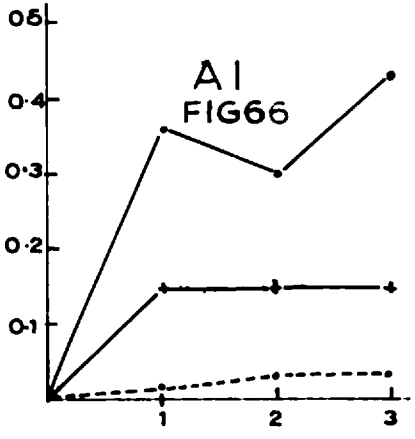


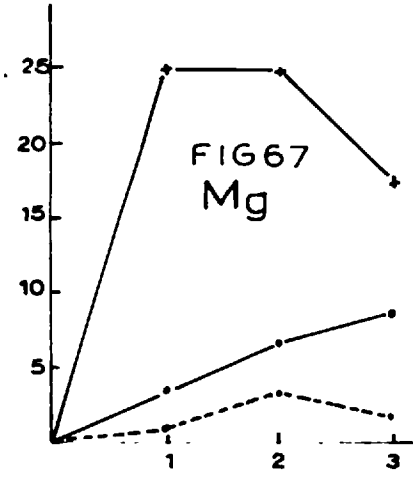


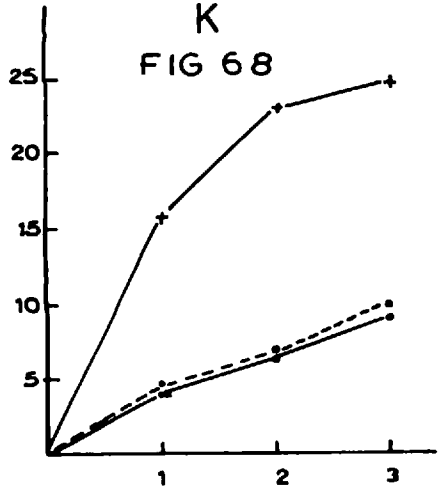
II MINUS IRON SAND CULTURE

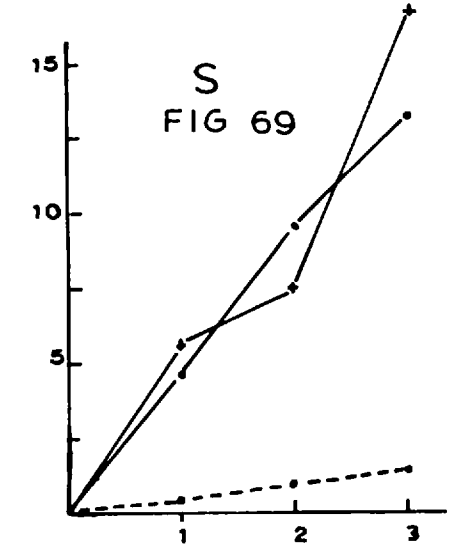


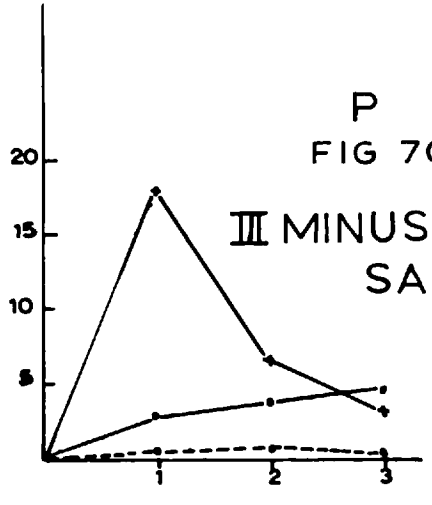




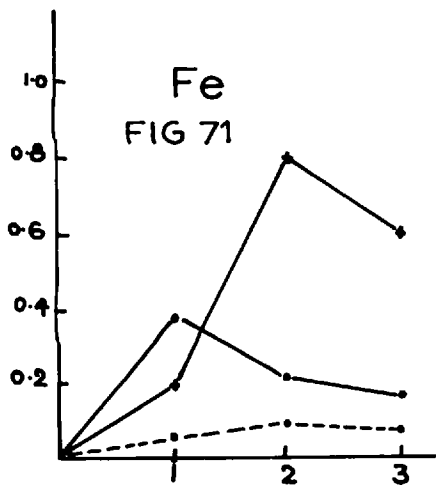


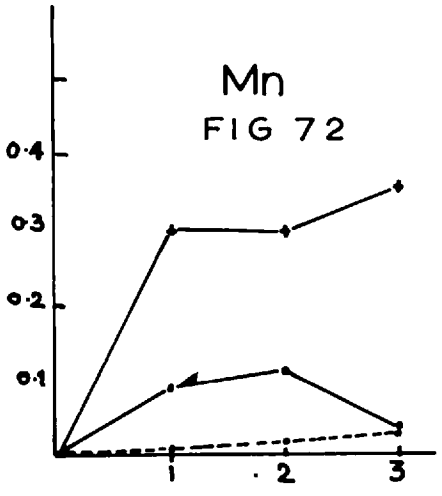


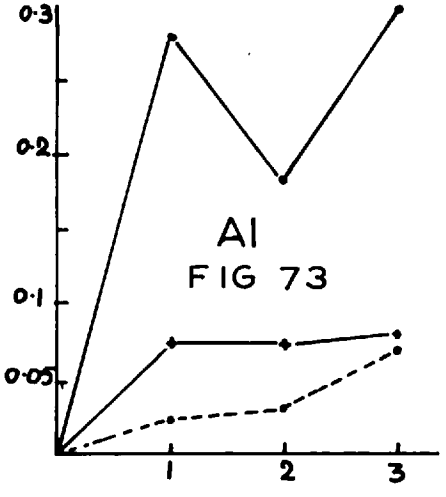


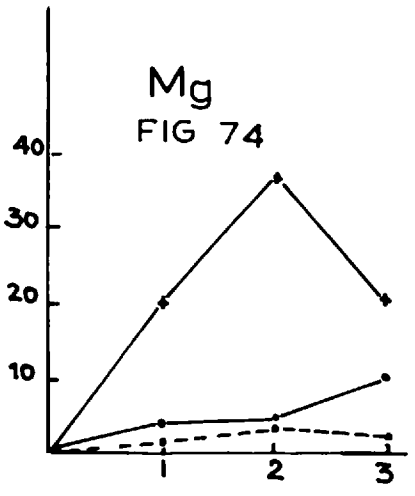


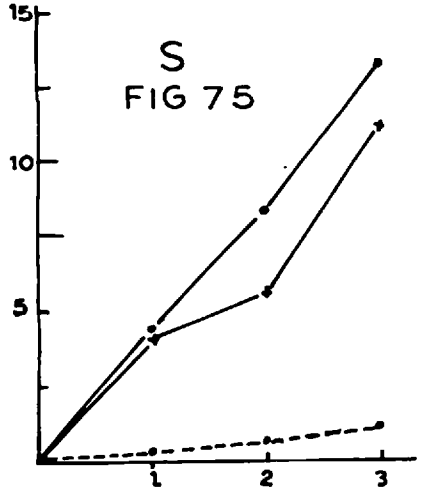
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SAND CULTURE

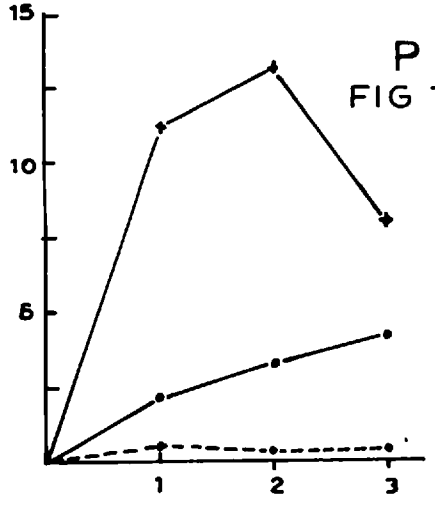


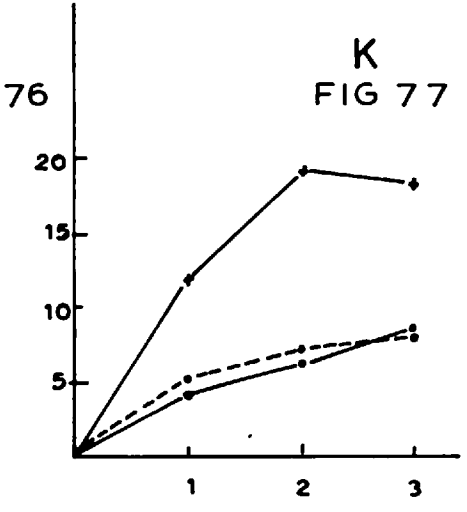




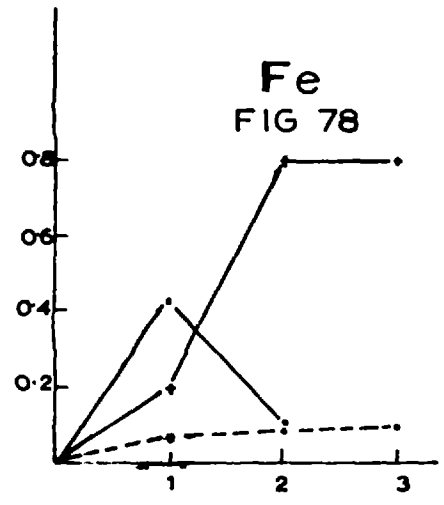


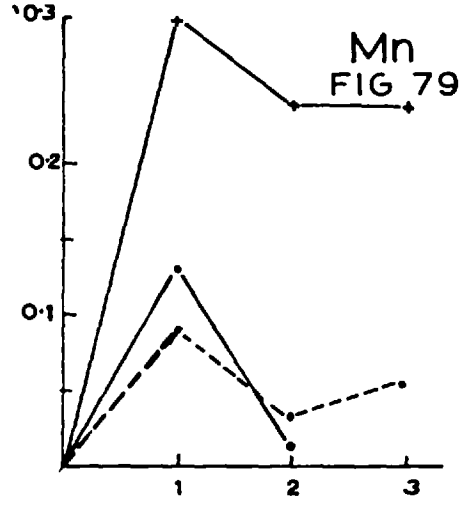


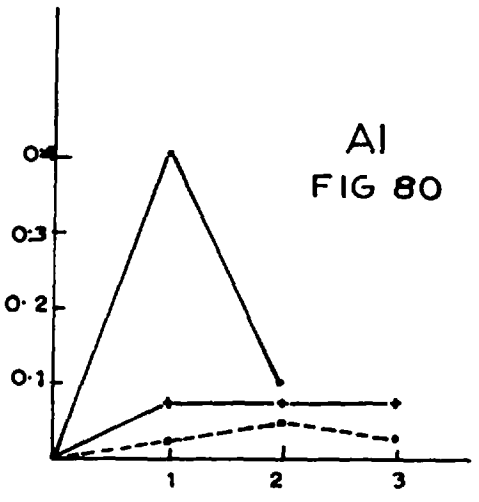


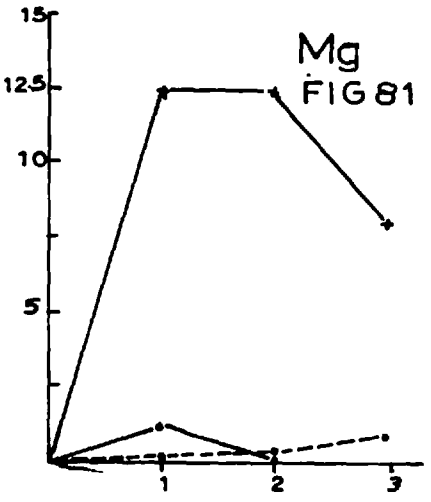


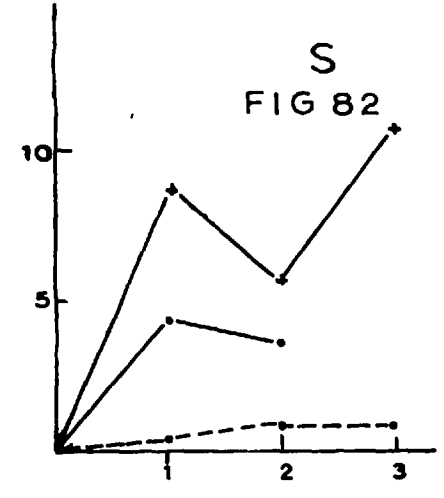
IV MINUS
AIUMINIUM
SAND CULTURE

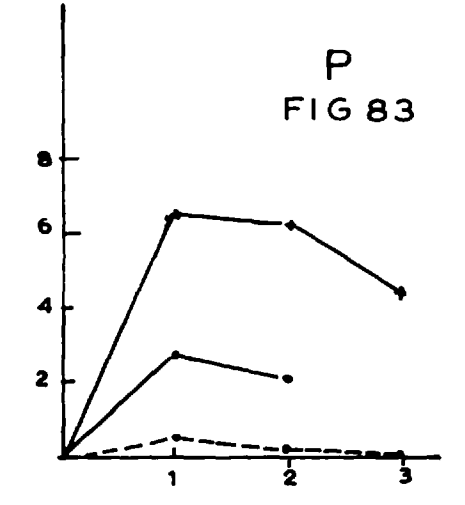


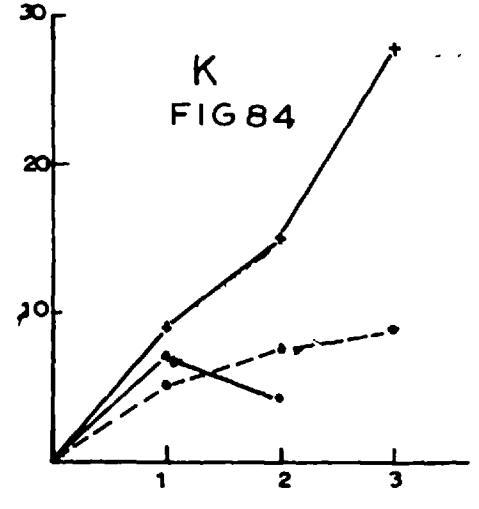




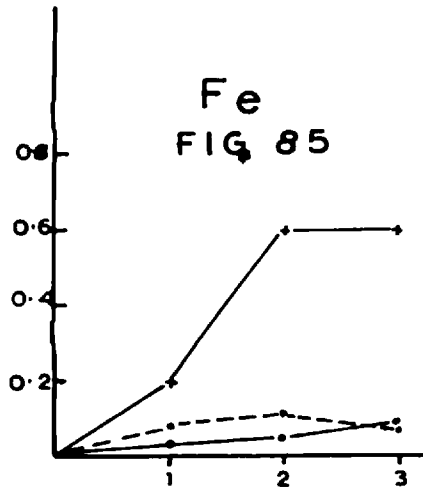


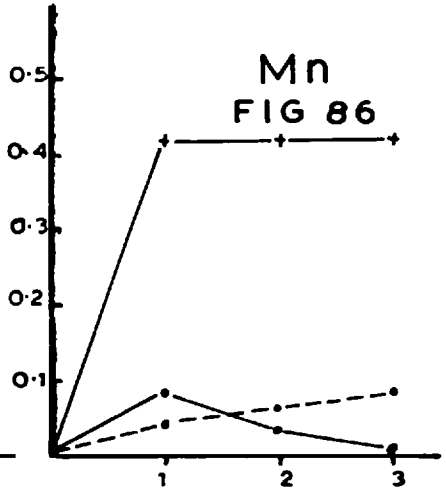


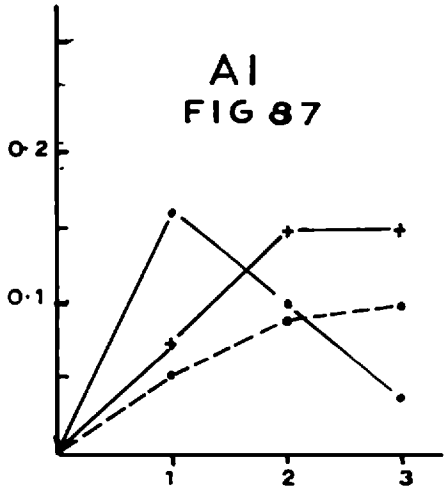


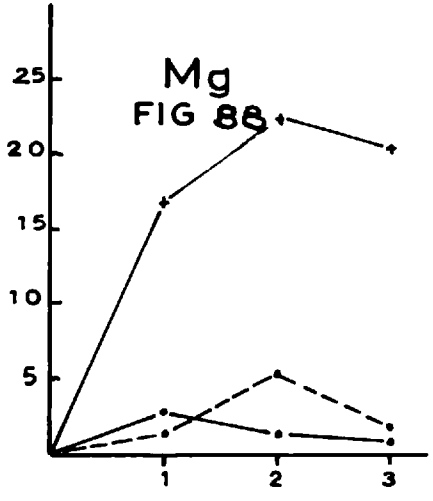


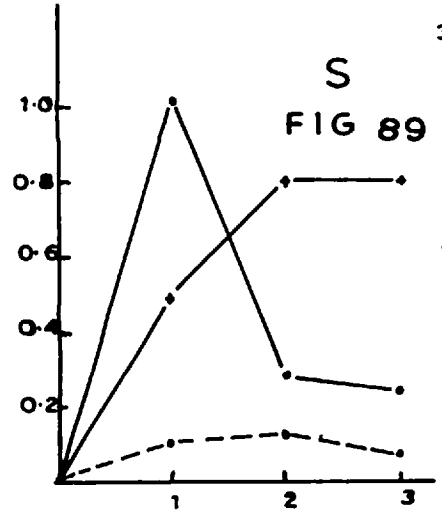
V MINUS
MAGNESIUM
SAND CULTURE

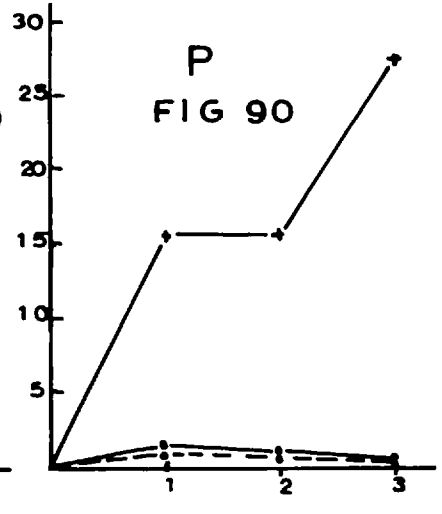


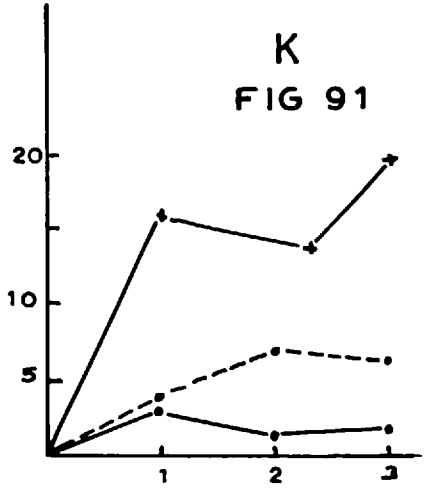




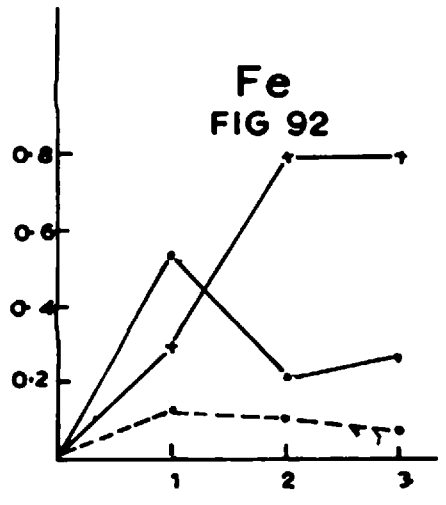


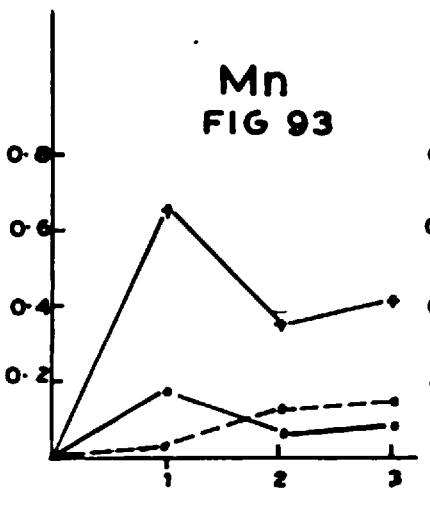


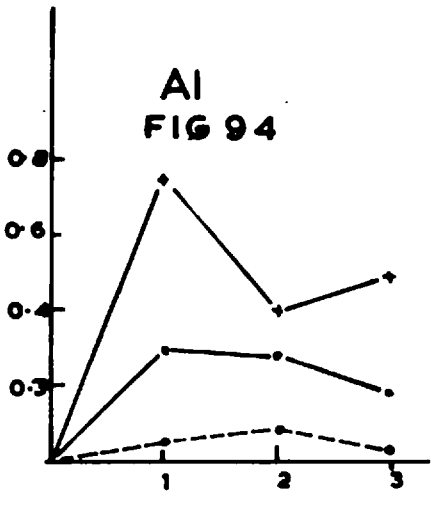


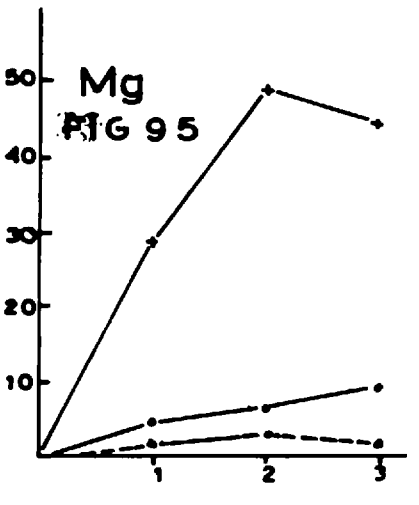


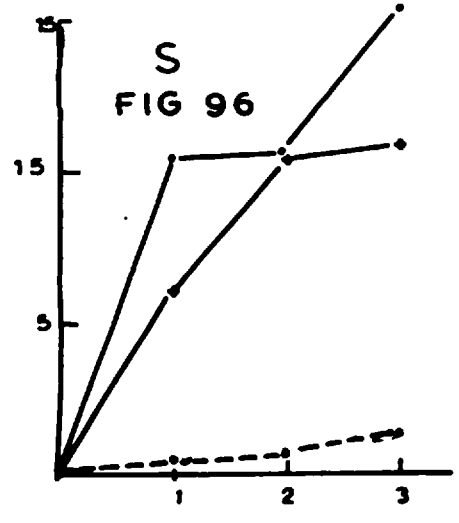
VI MINUS
SULPHUR
SAND CULTURE

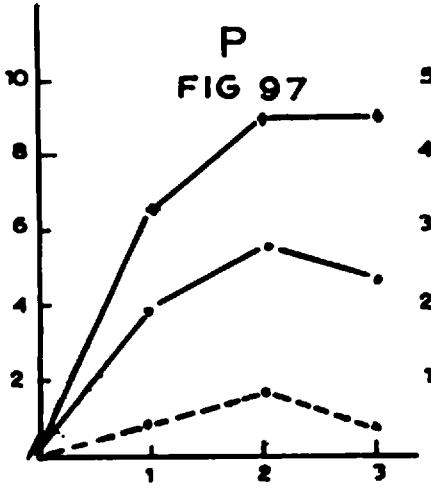


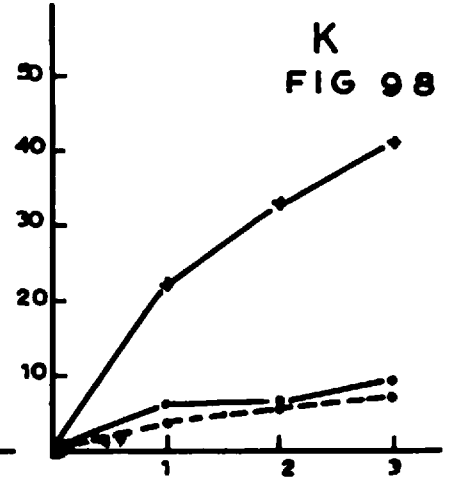




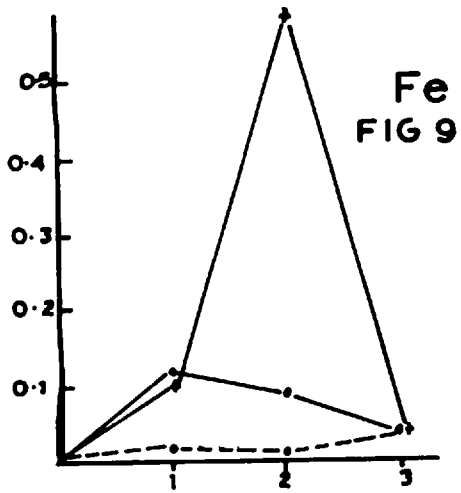


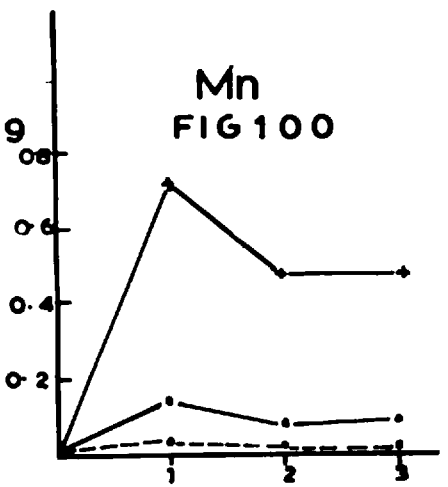


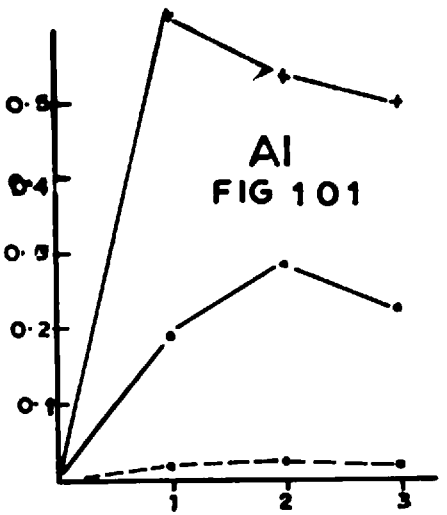


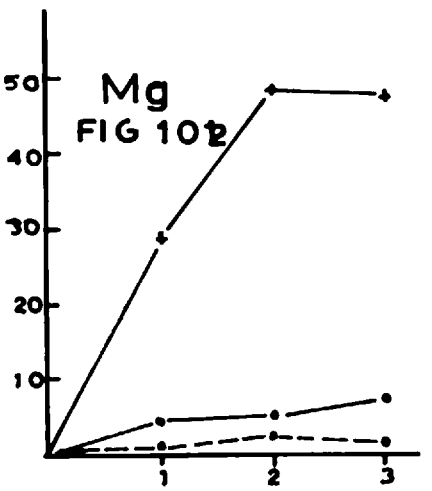


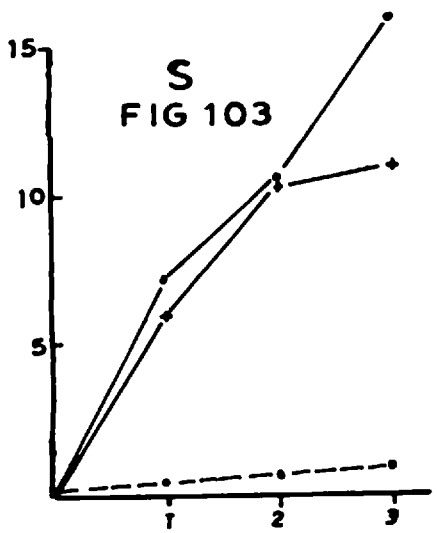
(1) COMPLETE SOIL SAND MIXTURE

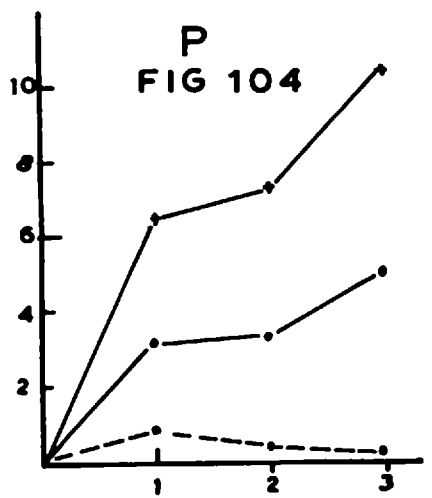


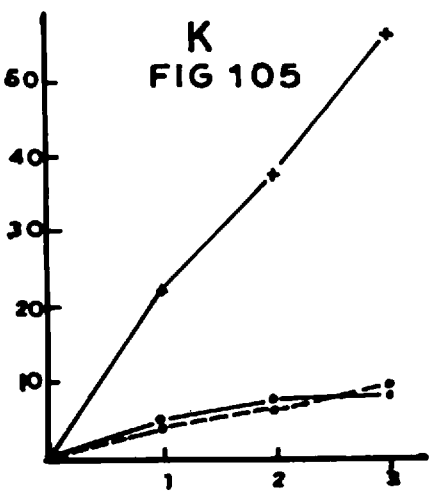




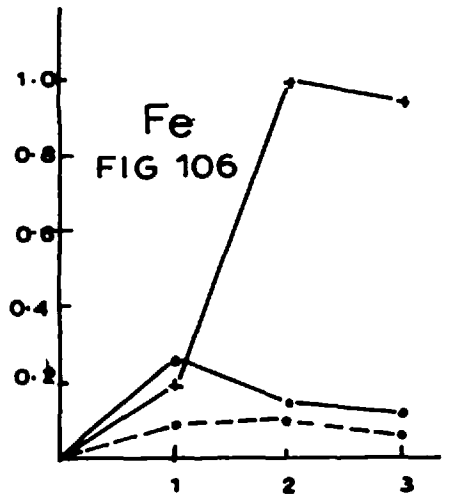


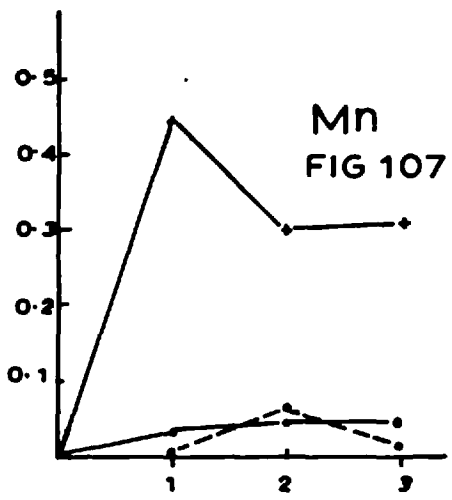


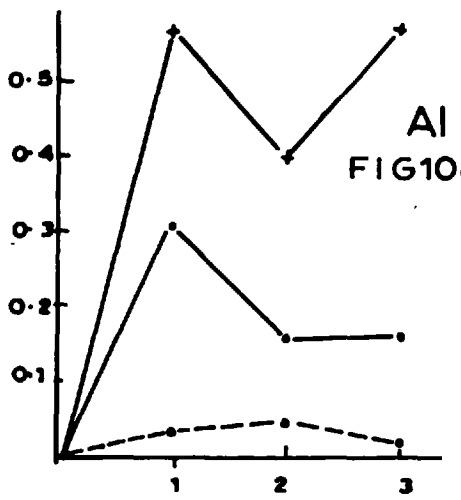


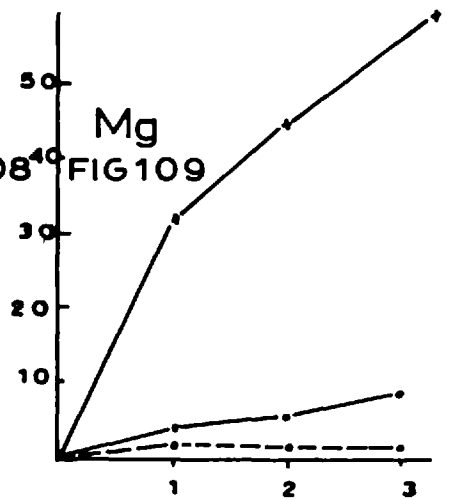


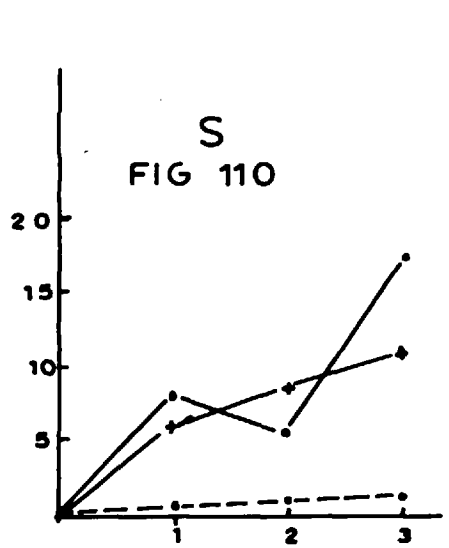
2) MINUS IRON SOIL SAND MIXTURE

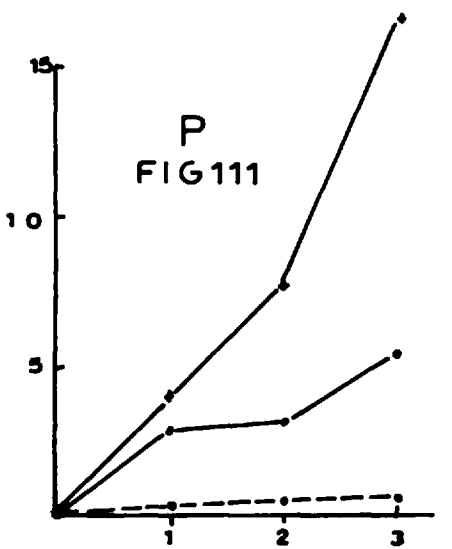


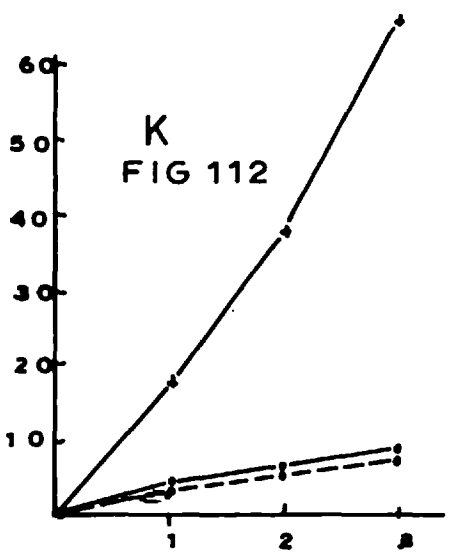




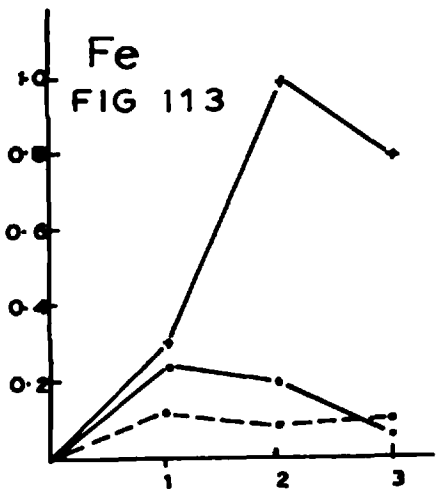


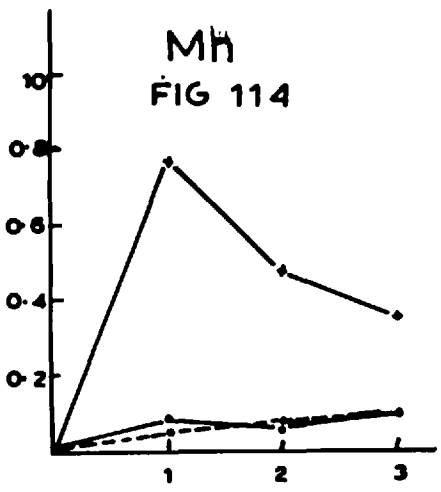


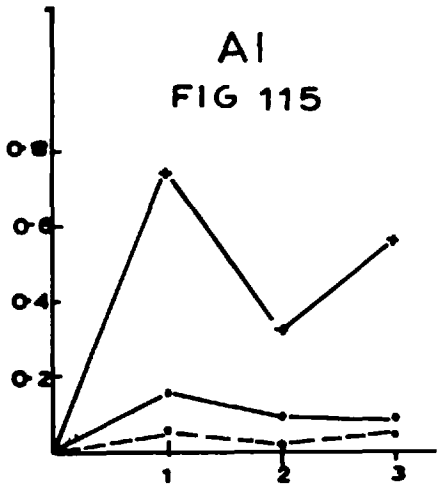


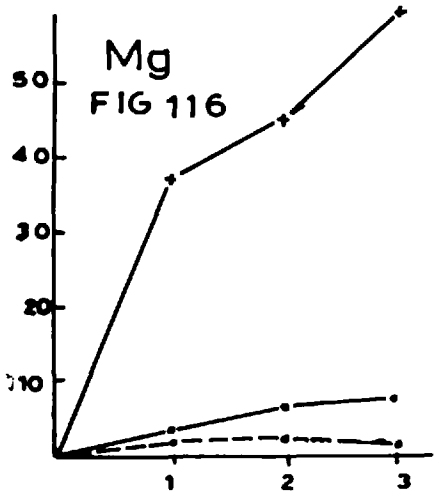


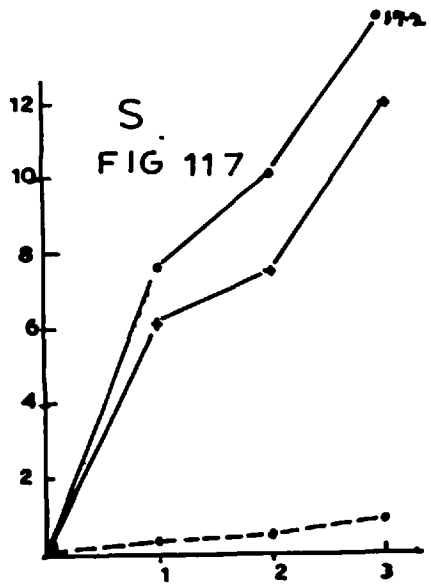
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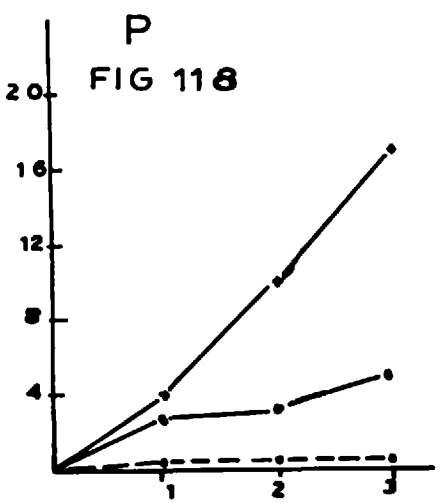


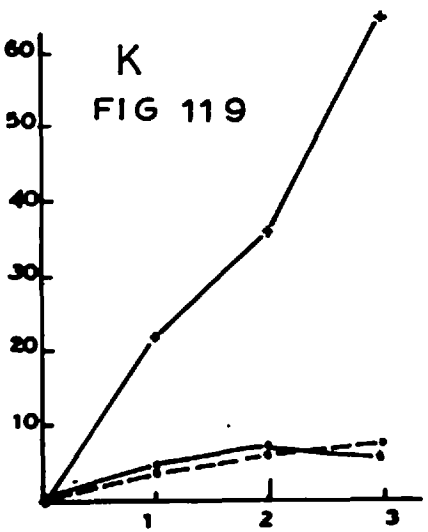




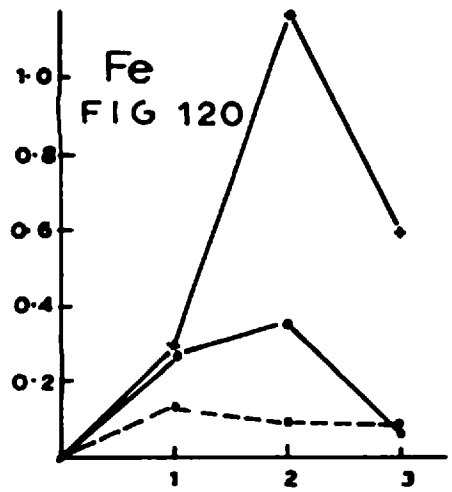


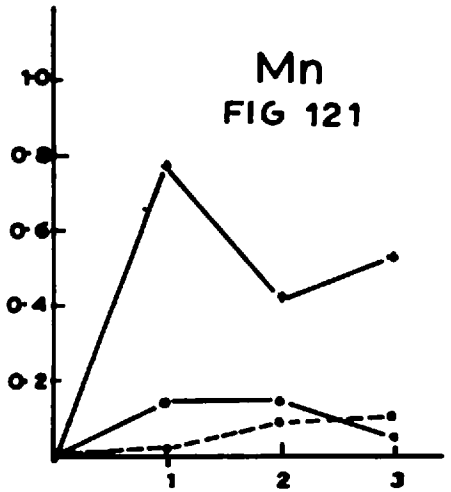


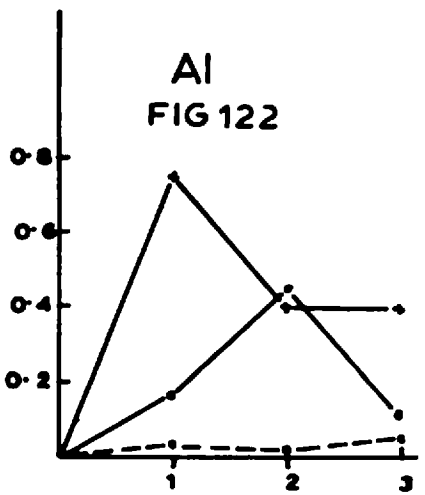


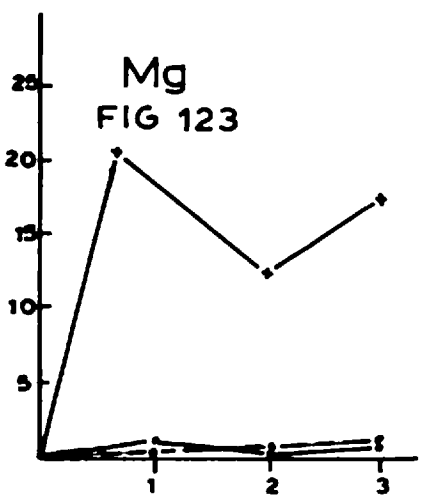


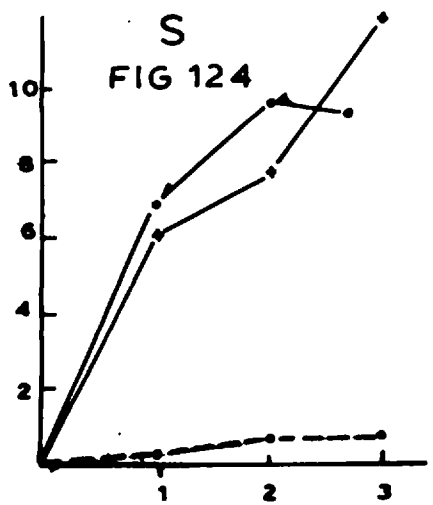
4) MINUS ALUMINIUM SOIL SAND MIXTURE

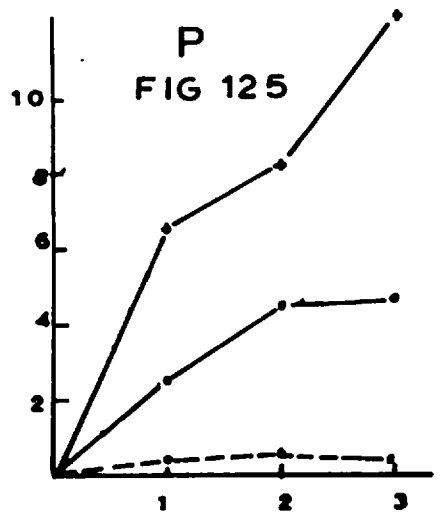


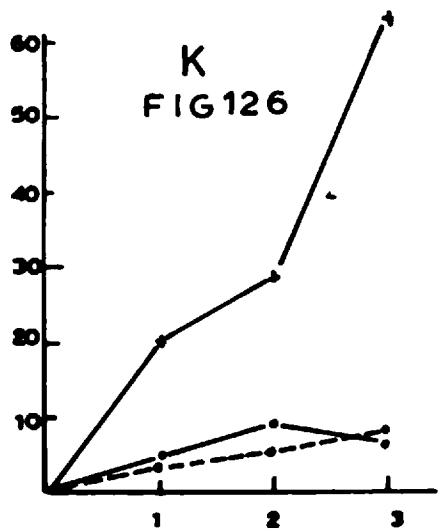




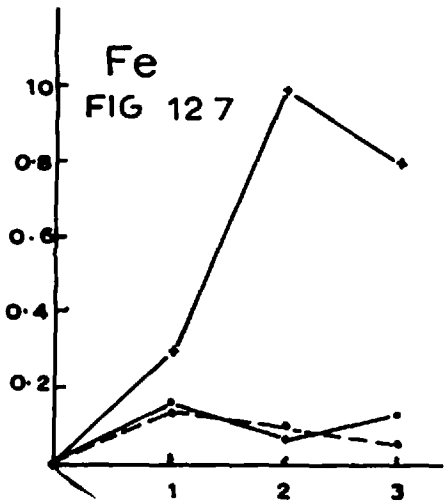


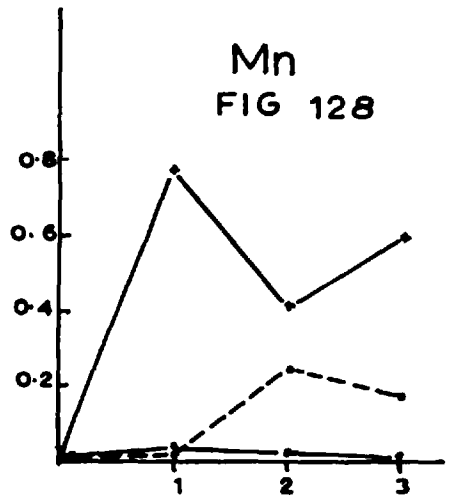


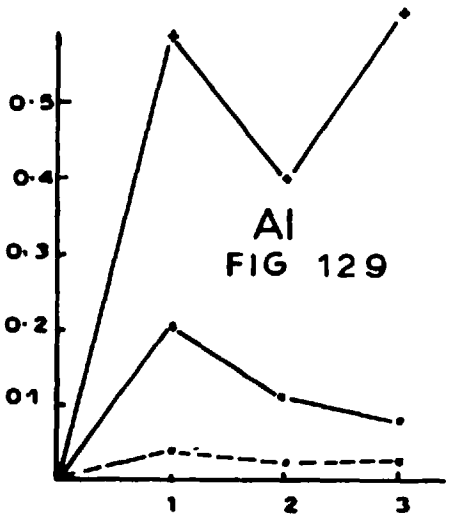


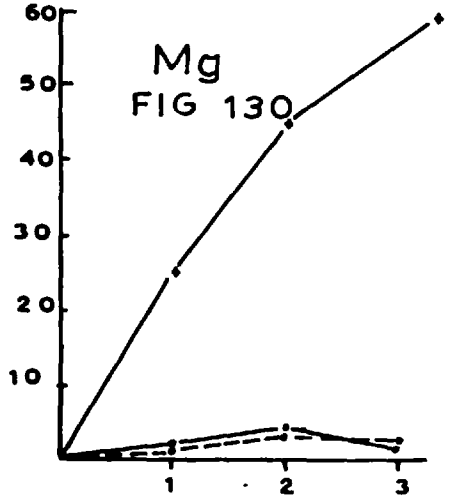


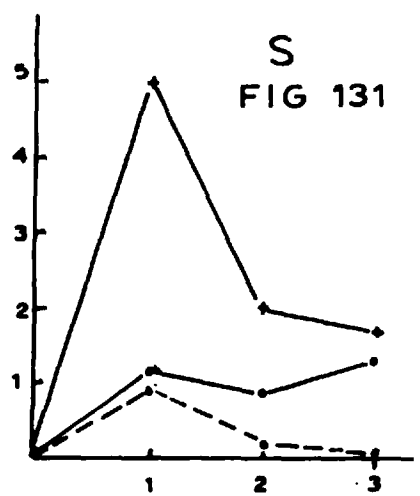
5) MINUS MAGNESIUM SOIL SAND MIXTURE

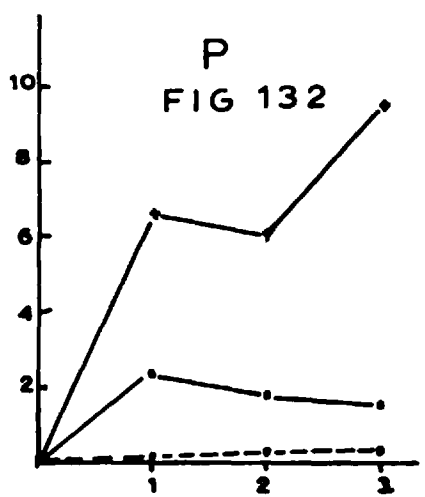


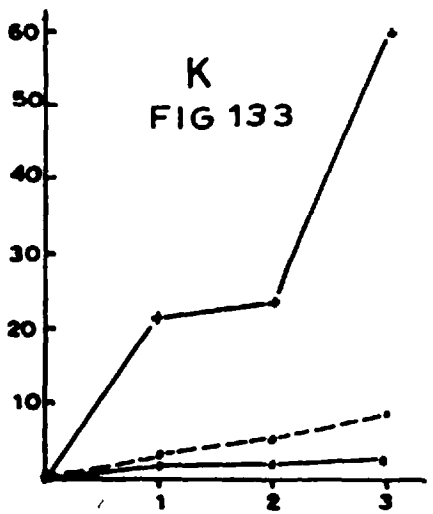












6) MINUS
SULPHUR
SOIL SAND
MIXTURE

uptake than in the sand-culture Fig. 97.

Iron :-

In the case of iron, the rye grass tended to absorb and accumulate iron from very small concentrations in the medium in which the plants were growing. For example, at the third cutting of the grass from the sand-cultures receiving the "minus-iron" treatment, the concentration of iron in the soil solution from fallow pots was 0.002 p.p.m. iron (oven dry soil), the Morgan-extractable iron was 0.2 p.p.m. and the concentration of iron in the tops from the corresponding cropped pots was 320 p.p.m. iron (dry basis). In the pots given the complete nutrient nutrient, iron concentration in the soil solution was 0.011 p.p.m., the Morgan-extractable iron was 0.6 p.p.m. and the concentration of iron in the tops from the corresponding cropped pots was 550 p.p.m. However, the total uptake of iron in the tops from the two treatments was 0.2466 mgm Fe/pot with the complete nutrient, iron concentration in the soil solution was 0.011 p.p.m. and 0.1055 mgm Fe with the "minus-iron" treatment. Thus, despite the fact that the plants showed a tendency tom absorb and, to some extent, accumulate iron in their tops from dilute solutions, the growth and total iron uptake was considerably smaller in the "minus-iron" plants than in those receiving adequate iron in the nutrient solution. The iron content of Morgan extracts of the soil as well as soil solution were two useful and successful indications of the iron uptake by the plants.

Magnesium, Sulphur and Manganese :-

Low concentration of these three elements in the soil solution and in Morgan extracts were without exception associated with a low uptake by the plant.

Aluminium :-

Differences in soil solution content and Morgan extractable aluminium between the complete nutrient and the "minus-aluminium" treatments were comparatively small. However, from the figures (No. 94, 115) relating soil solution content, Morgan-extractable aluminium and the aluminium uptake of the tops in pots receiving complete nutrient and "minus-aluminium" treatment in the soil-sand mixture, it is evident that both soil solution and Morgan extracts could serve as good indicators of the likely uptake of this element by the plant. It is observed however, at the time of the 3rd. cutting in the soil-sand mixture plants receiving complete nutrient, the uptake by the tops had increased while the aluminium in both Morgan-extracts and soil-solution decreased Fig. 94.

SUMMARY AND CONCLUSIONS :-

Magnesium deficiency showed as an intravenal chlorosis which increased in severity with the progress of time and resulted in the formation of white spots on the leaf surface almost always at the basal portion and at the upper third of the leaf. Ultimately, the plants collapsed. Treatment with magnesium at this stage did not bring recovery. Magnesium applied to the soil in a soluble form before this stage is reached produced rapid recovery.

Magnesium-deficient plants are characterised by high potassium, low nitrogen and comparatively high phosphorus and sulphur percentage in the dry matter of the tops.

Sulphur deficiency : showed itself in the stunted growth of the tops and greatly reduced leaf area. The tops acquired a characteristic upright habit of growth. Finally, the tops developed chlorosis at their tips which spread backwards and ultimately covered most of the leaf area.

Sulphur-deficient plants are characterised by high nitrogen and comparatively high phosphate content in the dry matter of the tops.

Sulphur and magnesium deficiencies had a marked adverse effect on the growth of the roots which on close examination showed a hairy appearance and were considerably weak and fragile.

3 Iron and Manganese deficiencies : showed a chlorosis in newly formed tillers as the first sign of these two deficiencies. Later, in both cases, leaves were chlorotic along the veins in the intravenal tissues. Up to this stage, it was extremely difficult to differentiate between the two deficiencies (iron and manganese). In a later stage however, the iron-deficient plants acquired a dirty yellowish-green colour but still the veins retained a green colour while the manganese-deficient grass developed a canary-yellow colouration of the tops.

Iron deficiency lowered the yield of the tops severely while the effect of manganese deficiency was much smaller.

The most marked change in the chemical composition of the iron-deficient grass was the considerable increase in phosphorus concentration in the tops which was always higher than in normal healthy plants. The P/Fe ratio was a consistent index of iron-deficiency, as it was being higherb in the tops of deficient than in those of normal plants (complete treatment.)

Manganese deficient plants, on the other hand, apart from the characteristic canary-yellow colouration are characterised by the increased nitrogen percentage in the dry matter of the tops as compared with those given complete nutrient.

Plate 2 : Difference in growth between grass grown in soil-sand mixture and acid-washed sand only. The two pots were sown on the same day and received distilled water only.

Plate 3 : Difference in growth between grass grown on sand culture supplied with complete nutrient solution (complete) and that supplied with distilled water only (S)



PLATE 2.



PLATE 3.

Plate 4 : Difference in growth between grass grown on soil-sand mixture supplied with complete nutrient solution.
(complete) and that given distilled water only (SSM).



PLATE 4.

Plate 5 : Magnesium deficiency in Perennial Rye Grass grown in acid-washed sand. (ist. cutting).
Note the yellowish-green colour of grass tops in the (minus-magnesium) pots as compared with the green colour of tops of grass receiving the complete nutrient solution.

Plate 6 : Sulphur deficiency in Perennial Rye Grass grown in acid-washed sand (ist. cutting).
Note the stunted growth of "minus-S" grass tops and their upright habit of growth as compared with the grass tops from the complete treatment.



PLATE 5.



PLATE 6.

Plate 7 : Root yield from soil-sand mixture pots (ist. cutting). Note the reduced yield from " minus-Mg" and "minus-S" plants as compared with the rest of the treatments.

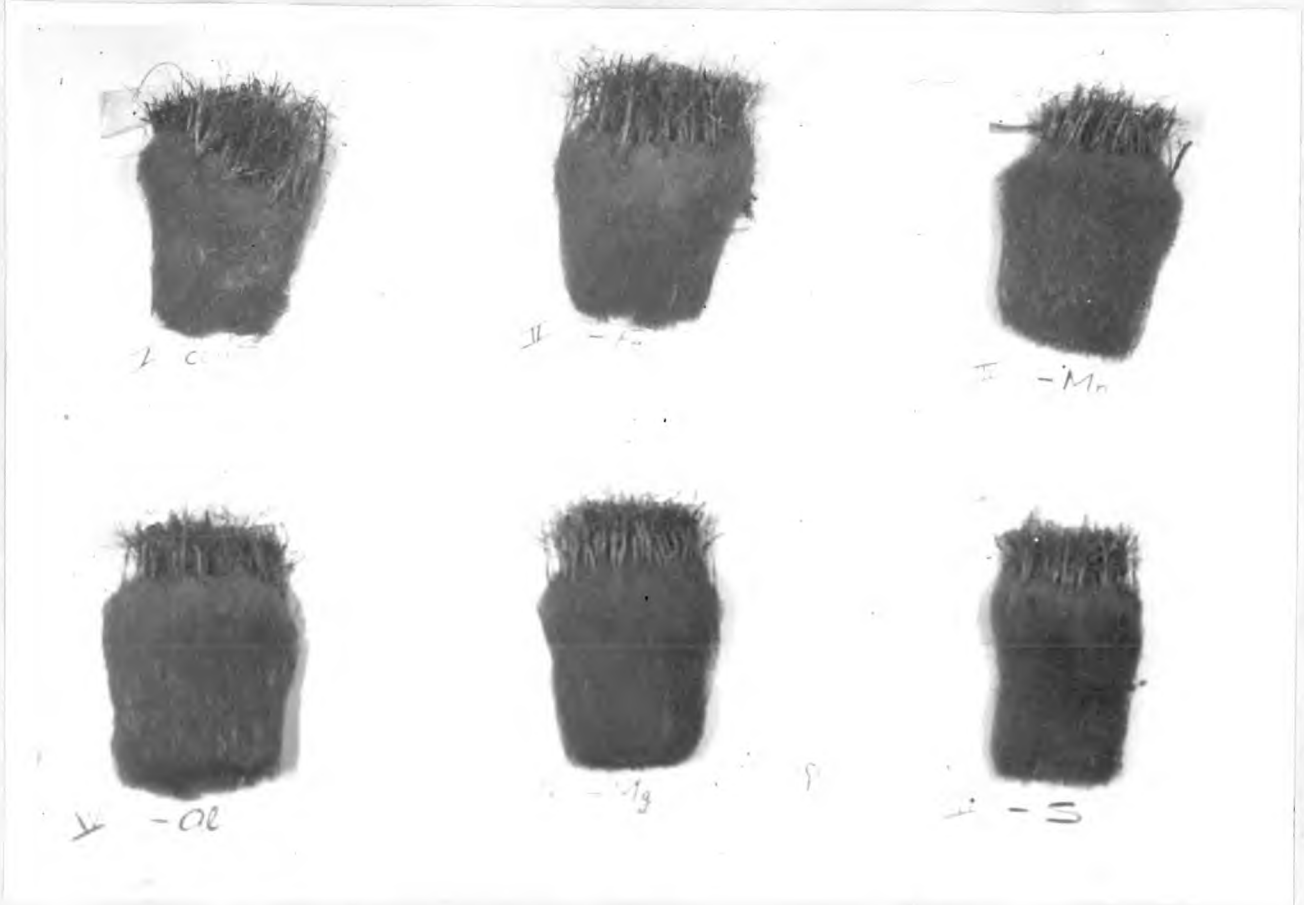


PLATE 7.

Plate 8 : Magnesium and Sulphur-deficient Rye Grass grown in sand-culture as compared with grass receiving complete nutrient solution.

"Minus-Mg" pots : note the lodging of grass tops as a result of the formation of white spots at the basal as well as the upper thi third of the leaves.

"Minus-S"pots :- note the stunted growth of the tops, the appearance of chlorotic tips which spread backwards towards the basal portion of these leaves. The upright form of, growth became very prominent.

Plate 9 : Magnesium and Sulphur deficient Rye Grass grown in soil-sand mixture as compared with grass receiving complete nutrient solution.

"Minus-Mg" pots : note the vigorous growth in these as compared with plants receiving complete nutrients.

"Minus-S" pots : are also stunted in growth and still acquire upright habit of growth.



PLATE 8.



PLATE 9.

Plate 10 : Magnesium deficiency in Perennial Rye Grass. White spots developed at the basal portion and the upper third of the leaf.

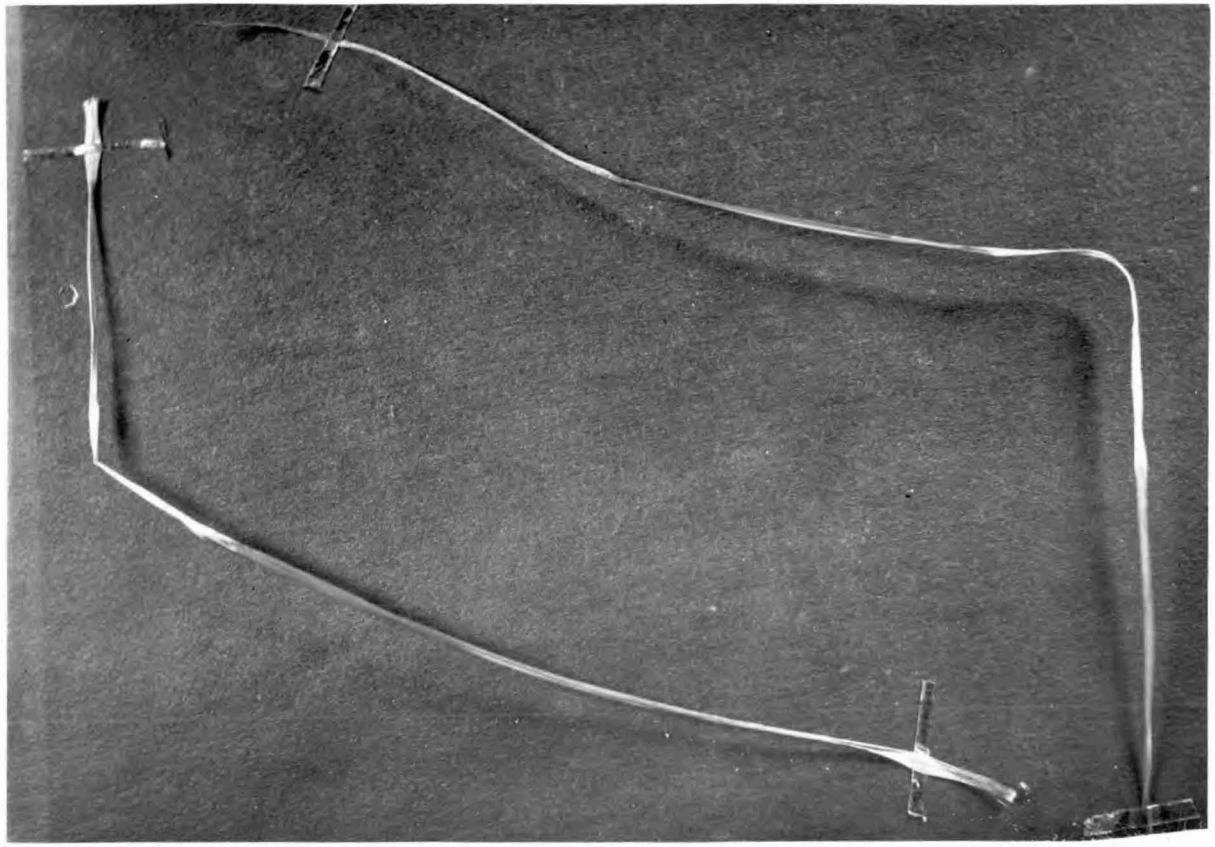


PLATE 10.

Plate 11 : Root yield of Rye Grass from soil-sand mixture pots. (2nd sampling).

Note the reduced root growth of "minus-sulphur" and "minus-Mg" plants as compared with the rest of the treatmemts.



PLATE 11.

Plate 12 : Sulphur-deficient and magnesium-deficient Rye Grass as compared with plants treated with S or Mg (in the form of $MgSO_4 \cdot 7H_2O$ solution)(after the 2nd. cutting) and those which received complete nutrient solution throughout. (soil-sand mixture pots, 3rd. cutting)
Note the disappearance of the visual symptoms as well as the vigorous growth in the plants which received treatment.

Plate 13 : Sulphur-deficient and magnesium-deficient Rye Grass as compared with plants treated with S or Mg (in the form of $MgSO_4 \cdot 7H_2O$ solution after the 2nd. cutting) and those which received complete nutrient solution, throughout. (Sand-culture pots - 3rd. cutting).
Note the vigorous growth of the plants which received sulphur after the 2nd. cutting and the complete disappearance of the chlorosism from the tops.
The "minus-magnesium" plants died at this stage.



PLATE 12.



PLATE 13.

Plate 14 : Shows Plate 13 in black and white.

Plate 15 : Shows Plate in black and white.



PLATE 14.



PLATE 15.

Plate 16 : Iron-deficiency in Perennial Rye Grass grown in sand-culture (3rd. cutting).
Note the stunted growth in "minus-iron" plants and the appearance of chlorosis on the tops of these plants.

Plate 17 : Shows Plate 16 in black and white.



PLATE 16.



PLATE 17.

Plate 18 : Effect of Magnesium and Sulphur deficiencies on root growth.
Magnesium-deficient and Sulphur-deficient roots as compared with roots from plants treated with Mg and S (as $MgSO_4.7H_2O$ after the 2nd. cutting).
Sand-culture plants, 3rd. cutting.



PLATE 18.

Plate 19 : Root yield from grass grown in Soil-Sand Mixture Pots. (3rd. cutting).

Note the stunted root growth of the "minus-magnesium" and "minus-sulphur" plants as compared with the rest of the treatments.

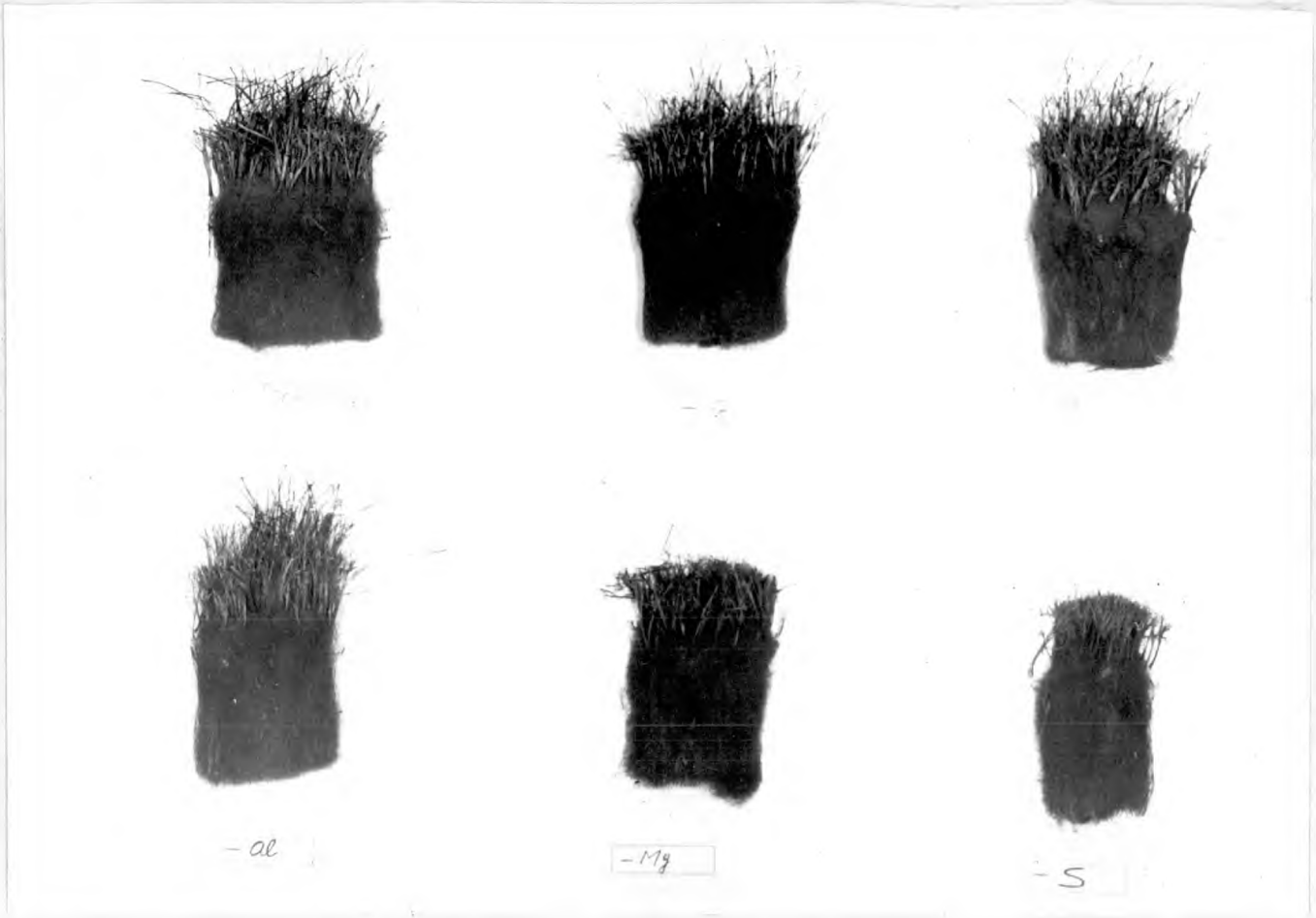


PLATE 19.

Plate 20 : Iron-deficient Rye Grass as compared with treated grass (which received ferric citrate after the 3rd. cutting) and those which received complete treatment throughout. (Sand-culture pots 4th. cutting).
Note the stunted growth and the chlorotic appearance of the "minus-iron plants.

Plate 21 : Manganese deficiency in Perennial Rye Grass grown in Sand-culture (4th. cutting).
Note chlorosis spreading in the tops of the "minus-manganese" plants.



PLATE 20.



PLATE 21.

Plate 22 : Root yield of grass grown in Sand-culture at the time of the 4th. sampling.

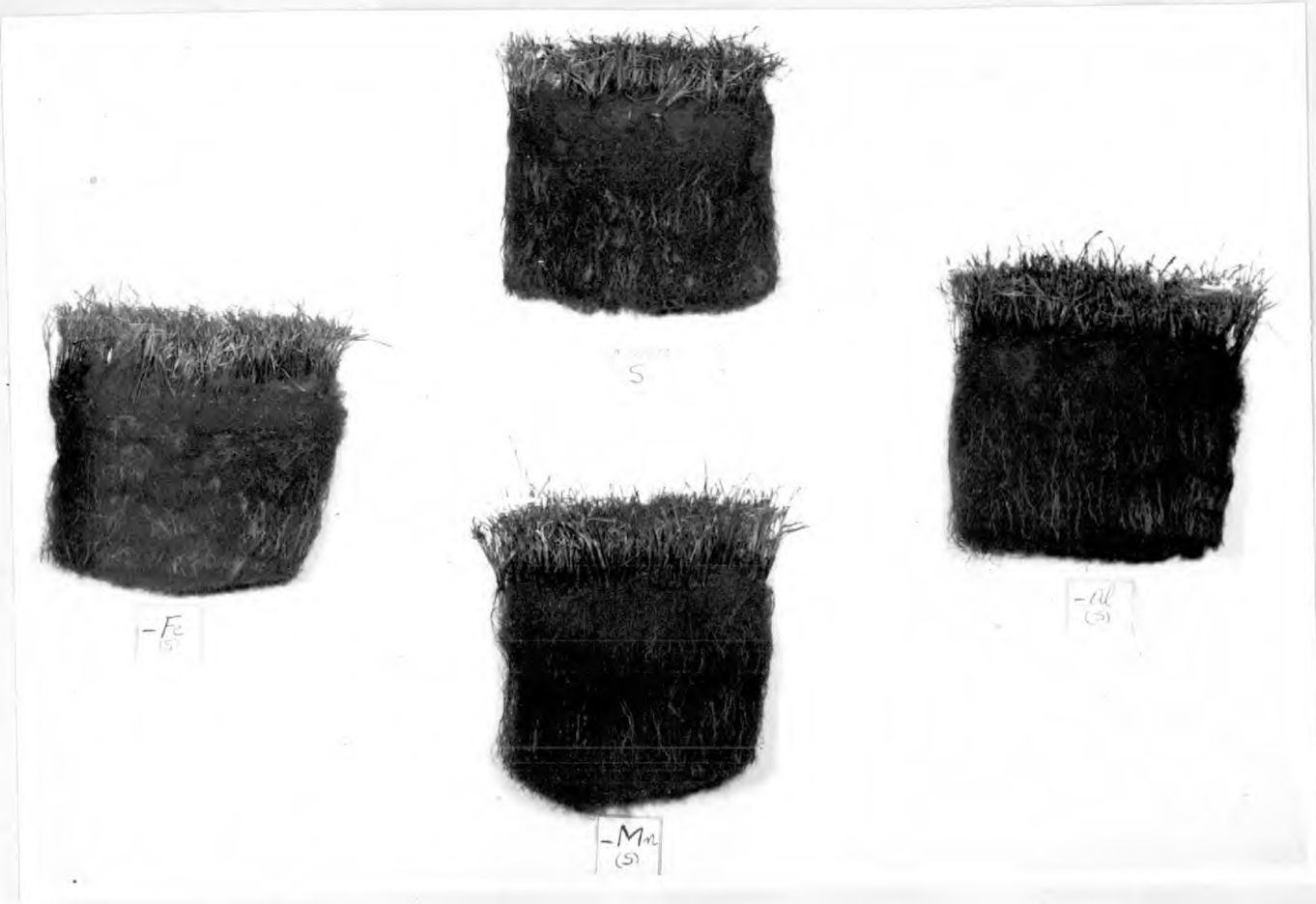


PLATE 22.

Plate 23 : Root yield of grass grown in Soil-sand Mixture at the time of the 4th. cutting.









PLATE 23.

Table 71.

Analysis of perennial Rye Grass seeds :- Results are expressed as p.p.m.

Fe	Mn	Al	Mg	S	N	P	K
350	60	150	250	773	15000	2252	1200

Table 72.

Analysis of Nutrient Solution used :- (mgm/l).

Solution	Fe	Mn	Al	Mg	S	P	K
Complete	5.5	0.57	0.02	41.3	78	40	147
minus Fe	Trace*	0.54	0.03	40.0	75	41	145
" Mn	5.3	0.01	0.03	41.3	80	41	142
" Al	5.4	0.56	0.01	41.3	76	40	145
" Mg	5.1	0.57	0.02	5.5	80	42	142
" S	5.3	0.58	0.02	42.5	4	44	144

Trace :- means less than 0.002.

Table 73.

pH of displaced soil solution and soil moisture % even dry basis of the fallow pots (ist. sampling) :-

	Sand Culture		Soil Sand Mixture	
Sample	pH	Moisture %	pH	Moisture %
Complete	6.50	12.9	6.27	12.5
minus Fe	6.66	13.6	6.43	13.1
" Mn	6.43	11.9	5.94	14.0
" Al	6.25	14.0	5.66	12.3
" Mg	5.52	13.3	6.22	12.2
" S	5.44	12.5	5.66	14.0

Table 74.

Analysis of Soil Solution from Fallow Pots :- (1st. Sampling).

Analytical data re-calculated as p.p.m. of oven dry soil :-

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Culture :-							
Complete	0.008	0.002	0.011	1.46	0.46	0.34	0.60
minus Fe	Trace	0.002	0.008	1.92	0.42	0.45	0.60
" Mn	0.006	Trace	0.003	1.05	0.32	0.23	0.50
" Al	0.006	0.002	0.004	1.62	0.32	0.44	0.50
" Mg	0.007	0.009	0.004	0.19	0.46	0.51	0.50
" S	0.009	0.005	0.005	1.40	0.10	0.34	0.40
Soil Sand Mixture :-							
Complete	0.013	0.008	0.046	0.93	0.41	0.09	0.40
minus Fe	Trace	0.004	0.037	0.905	0.32	0.08	0.40
" Mn	0.010	Trace	0.040	0.66	0.26	0.05	0.35
" Al	0.012	0.005	0.050	1.08	0.22	0.06	0.40
" Mg	0.014	0.003	0.034	0.08	0.21	0.05	0.40
" S	0.014	0.005	0.040	1.01	0.09	0.14	0.40

* Trace = Less than 0.002

Table 75.

Analysis of Morgan Extracts from Fallow Pots :- (1st. Sampling).

p.p.m. oven dry basis (mean of duplicates)

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Culture :-							
Complete	0.20	0.30	0.08	25	10.9	15.2	12.0
minus Fe	Trace	0.60	0.08	20	8.4	20.7	11.0
" Mn	0.20	Trace	0.05	25	11.2	17.9	13.0
" Al	0.20	0.30	0.08	20	11.7	11.1	12.0
" Mg	0.20	0.30	0.08	12.5	8.9	6.6	9'0
" S	0.20	0.40	0.08	17.0	5.0	15.2	16.0
Soil Sand Mixture :-							
Complete	0.30	0.66	0.80	29.0	6.1	6.6	22.0
minus Fe	0.10	0.72	0.70	29.0	6.1	6.6	19.0
" Mn	0.20	0.26	0.60	32.5	6.1	4.0	18.0
" Al	0.30	0.78	0.80	35.5	6.1	4.0	22.0
" Mg	0.30	0.78	0.80	22.5	6.1	6.6	20.0
" S	0.30	0.78	0,65	25.5	3.5	6.6	22.0

* Trace = less than 0.002

Table 76.

Analysis of Morgan Extracts from Cropped Pots :-(Soil Sand Mixture
1st Sampling.

p.p.m. oven dry basis (mean of duplicates

Treatments	Fe	Mn	Al	Mg	S	P	K
Complete	1.2	6.0	1.5	32.5	2.1	5.0	10.0
minus Fe	0.8	5.6	1.4	32.5	2.9	5.2	9.0
" Mn	1.3	6.1	1.6	35.5	2.9	4.0	10.0
" Al	1.1	6.3	1.4	37.5	2.9	4.9	9.0
" Mg	1.1	5.6	1.6	22.5	2.9	5.0	8.0
" S	0.9	3.6	1.6	32.5	1.6	5.2	9.0

Table 78.

Root Yield if the First Cutting :- (Soil Sand Mixture)

Treatment		Weight of oven dry roots gm/pot (average of two pots)
Complete		4.872
minus	Fe	3.998
"	Mn	3.596
"	Al	4.188
"	Mg	3.660
"	S	2.060

Table 77.

Yield ofm the First Cutting :- (Tops).

Treatment	Green Yield (gms)	Dry Matter Yield (gms)	Dry Matter Yield (%)
Sand Culture :-(average of 4 pots)			
Complete	5.374	1.171	21.7
minus Fe	5.034	1.089	21.7
" Mn	4.958	1.125	22.8
" Al	4.877	1.111	22.7
" Mg	5.214	1.247	23.9
" S	3.034	0.654	21.5
Soil Sand Mixture :- (average of 12 pots)			
Complete	5.691	1.532	26.8
minus Fe	4.018	1.121	27.9
" Mn	4.770	1.250	26.2
" Al	4.350	1.120	25.7
" Mg	4.430	1.120	25.3
" S	2.910	0.709	24.0

Table 79.

Analysis of Tops from First Cutting :-

p.p.m. dry matter , mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Sand Culture :-								
Complete	450	90	325	4400	4875	26875	2047	3800
minus Fe	80	90	325	3000	4750	26500	2320	3900
" Mn	350	60	325	3900	4125	23750	2220	3900
" Al	350	90	250	4000	4000	19750	1902	3650
" Mg	350	105	325	900	3625	26750	2320	5850
" S	350	135	250	4600	1563	24500	2593	4150
Soil Sand Mixture :-								
Complete	355	115	200	3050	6719	25688	2593	3775
" Fe	125	135	175	3760	6520	25000	2798	3775
" Mn	215	60	250	3370	6406	21436	2247	3650
" Al	215	80	150	3200	6344	18750	2457	3575
" Mg	260	128	150	750	6250	15000	2320	4225
" S	205	113	282	2600	1750	16565	3339	3775

Table 80.

Analysis of Roots :- (Soil Sand Mixture) 1st. Cutting.

p.p.m. dry matter, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Complete	385	105	325	250	1438	6125	819	550
minus Fe	320	165	1075	450	1813	5250	1172	550
" Mn	353	150	1000	150	1543	5250	819	550
" Al	385	195	1325	1850	1563	5260	819	500
" Mg	600	170	825	1050	1438	6625	919	450
" S	400	225	1500	750	1189	5875	1172	450

Table 81.

pH of Soil Solution and Soil Moisture % oven dry basis of sampled Fallow Pots :- 2nd. Sampling.

Treatment	Sand Culture		Soil Sand Mixture	
	pH	Moisture %	pH	Moisture %
Complete	6.22	20.0	5.62	18.0
minus Fe	6.28	17.9	6.02	16.8
" Mn	6.30	19.5	5.52	19.2
" Al	6.36	20.0	5.74	17.4
" Mg	6.38	16.9	5.51	18.6
" S	5.80	20.2	5.24	16.6

Table 82.

Analysis of Soil Solution from Fallow Pots :- 2nd. Sampling

Analytical Data re-calculated fpe as p.p.m. even dry soil.

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Culture :-							
Complete	0.010	0.008	0.006	0.30	0.74	0.22	0.70
minus Fe	Trace	0.008	0.004	0.35	0.60	0.28	0.80
" Mn	0.009	Trace	0.007	0.34	0.89	0.31	0.70
" Al	0.012	0.003	0.008	0.34	0.97	0.27	0.70
" Mg	0.008	0.008	0.005	0.04	0.89	0.20	0.80
" S	0.012	0.006	0.009	0.55	0.13	0.22	0.75
Soil Sand Mixture :-							
Complete	0.015	0.013	0.009	0.26	0.47	0.17	0.60
minus Fe	Trace	0.021	0.005	0.29	0.51	0.22	0.70
" Mn	0.010	0.005	0.005	0.24	0.40	0.19	0.50
" Al	0.008	0.007	0.002	0.24	0.39	0.19	0.60
" Mg	0.011	0.013	0.002	0.08	0.65	0.13	0.60
" S	0.010	0.025	0.002	0.35	0.02	0.09	0.50

Table 83.

Analysis of Morgan Extracts from Fallow Pots :- 2nd. Sampling.

p.p.m. oven dry soil, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Culture :-							
Complete	1.00	0.30	0.08	30.0	17.5	9.8	15.0
minus Fe	0.02	0.36	0.08	25.0	17.5	12.0	16.0
" Mn	0.60	0.06	Trace	25.0	15.6	6.5	16.0
" Al	0.80	0.30	"	37.5	15.6	13.1	15.0
" Mg	0.80	0.24	0.08	12.5	15.6	16.3	15.0
" S	0.60	0.42	0.15	22.5	0.8	15.2	14.0
Soil Sand Mixture :-							
Complete	0.80	0.36	0.40	49.0	10.3	9.0	33.0
minus Fe	0.60	0.48	0.58	49.0	10.3	7.2	38.0
" Mn	1.00	0.30	0.40	45.0	8.9	7.6	38.0
" Al	1.00	0.48	0.32	45.0	7.5	10.0	36.0
" Mg	1.20	0.42	0.40	12.5	7.9	8.3	28.0
" S	1.00	0.42	0.40	45.0	2.0	6.1	24.0

Table 84.

Analysis of Morgan Extracts of Cropped Pots :- 2nd. Sampling.

P.p.m. oven dry soil, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	P	K
Complete	0.8	2.4	1.3	32.5	3.3	7.9	12.0
minus Fe	0.8	2.2	1.4	40.0	3.3	7.2	13.0
" Mn	0.8	1.8	1.4	32.5	2.0	7.2	12.0
" Al	0.8	2.0	1.1	37.5	3.3	5.9	10.0
" Mg	1.0	2.3	1.2	25.0	3.3	7.2	9.0
" S	1.2	1.9	1.4	40.0	1.4	7.9	16.0

Table 85.

Yield of the Second Cuttings :- (TOPS).

Treatment	Green weight of yield (gms)	dry matter yield (gms).	percent dry matter yield.
Sand Culture(average of 4 pots):-			
Complete	5.272	1.284	24.35
minus Fe	5.188	1.219	21.56
" Mn	5.337	1.222	22.95
" Al	5.299	1.239	23.38
" Mg	1.317	0.406	30.38
" S	1.363	0320	23.65
Soil Sand Mixture :- (average of 10 pots)			
Complete	4.627	1.178	25.45
minus Fe	4.683	1.189	25.38
" Mn	4.437	1.116	25.15
" Al	4.793	1.207	25.18
" Mg	5.510	1.388	25.19
" S	2.022	0.443	21.90

Table 86.

Root yield of the 2nd. Cutting :- Soil Sand Mixture.

Treatment	Dry matter yield in gms. (average of two determinations)
Complete	5.450
minus Fe	4.160
" Mn	3.940
" Al	4.860
" Mg	3.945
" S	2.615

Table 89.

Analysis of Tops from 2nd. Cutting :-

p.p.m. dry matter, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Sand Culture :-								
Complete	170	60	325	5700	6875	23500	2593	4550
minus Fe	80	75	250	5700	7750	19750	2740	4950
" Mn	405	45	250	5700	7500	20250	2740	5450
" Al	170	90	150	3750	6687	20250	2740	4850
" Mg	260	45	250	500	9188	20250	5458	10500
" S	170	105	325	4600	930	28000	4309	4550
Soil Sand Mixture :-								
Complete	220	60	250	5825	8688	18939	2593	4775
minus Fe	80	75	250	4670	8813	18688	2729	4875
" Mn	130	90	150	5350	5094	18812	2646	4650
" Al	170	60	75	5450	8313	18188	2456	4825
" Mg	260	105	325	350	6938	16125	3275	6925
" S	170	150	250	4500	2000	22437	4203	4950

Table 88.

Analysis of Roots from 2nd. Cutting :- (Soil Sand Mixture).

p.p.m. dry matter, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Complete	660	105	325	600	2138	4875	1173	650
minus Fe	420	135	250	450	2563	4250	1023	650
" Mn	620	210	250	250	2000	3124	1023	650
" Al	490	105	150	205	4750	4250	1023	350
" Mg	400	105	250	690	4875	4875	1160	650
" S	560	150	325	750	1438 5250	5250	1160	750

Table 89.

pH of displaced Soil Solution and Soil Moisture % from Fallow Pots :- 3rd. Sampling.

	Sand Culture		Soil Sand Mixture	
Treatment	pH	Moisture %	pH	Moisture %
Complete	6.82	19.4	5.55	20.0
minus Fe	5.92	16.9	5.44	18.9
" Mn	6.04	18.8	5.50	19.0
" Al	5.80	16.5	5.46	18.6
" Mg	6.90	19.5	5.50	20.1
" S	6.48	18.5	5.57	19.6

Table 90.

Analysis of Soil Solution from Fallow Pots :- 3rd. Sampling.

Analytical Data re-calculated as p.p.m. soil oven dry basis:-

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Culture :-							
Complete	0.011	0.009	0.009	0.32	0.88	0.10	0.90
minus Fe	0.002	0.006	0.006	0.27	0.92	0.22	1.17
" Mn	0.010	Trace	0.006	0.19	1.44	0.18	1.00
" Al	0.008	0.007	0.003	0.22	1.12	0.30	0.90
" Mg	0.009	0.006	0.005	0.09	0.81	0.30	0.90
" S	0.008	0.009	0.010	0.20	0.07	0.50	0.62
Soil Sand Mixture :-							
Complete	0.008	0.015	0.004	0.14	1.35	0.07	0.64
minus Fe	0.004	0.020	0.006	0.17	0.85	0.13	0.93
" Mn	0.007	0.003	0.005	0.25	1.16	0.17	0.80
" Al	0.011	0.008	0.003	0.18	0.92	0.12	0.70
" Mg	0.009	0.013	0.005	0.08	0.70	0.12	0.82
" S	0.005	0.017	0.003	0.26	0.05	0.15	0.92

Table 91.

Analysis of Morgan Extract from Fallow Pots :- 3rd. Sampling.

p.p.m. Soil oven dry basis, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Cultire :-							
Complete	0.6	0.30	0.08	45	16.6	3.3	26
minus Fe	0.2	0.18	0.08	27.5	16.6	2.1	26
" Mn	0.8	0.06	0.07	17.5	17.5	3.3	25
" Al	0.6	0.36	Trace	20.5	14.1	8.1	18
" Mg	0.8	0.24	0.08	8.0	16.9	4.4	23
" S	0.6	0.42	0.15	20.5	1.8	27.8	20
Soil Sand Mixture :-							
Complete	0.8	0.42	0.50	45.0	10.9	8.9	41
minus Fe	Trace	0.48	0.50	48.5	10.9	10.4	56
" Mn	0.95	0.32	0.56	69.0	12.0	16.5	66
" Al	0,80	0.36	0.56	60.0	12.0	17.0	65
" Mg	0.60	0.54	0.40	17.5	12.0	11.7	64
" S	0.80	0.60	0.70	69.0	1.7	19.6	66

Table 92.

Analysis of Morgan Extract from Cropped Pots :- Soil Sand Mixture, 3rd. Sampling.

p.p.m. soil oven dry basis, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	P	K
Complete	1.2	0.90	1.60	45	4.1	14.6	13
minus Fe	1.0	0.78	1.40	45	4.1	11.7	11
" Mn	1.0	0.18	1.10	37.5	3.3	9,6	10
" Al	1.6	1.02	1.50	52.5	4.1	16.2	16
" Mg	1.0	1.62	1.70	28.0	5.5	12.4	14
" S	1.2	1.74	2.1	65.0	1.7	20.5	35

Table 93.

Analysis of Morgan Extract of Treated Pots :- Fallow and Cropped.

p.p.m. oven dry basis, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Culture :-							
minus Mg untreated cropped.	0.4	0.30	0.33	12.5	9.7	15.8	18
minus Mg treated cropped	1.0	0.18	0.50	17.5	10.3	10.0	9
minus S untreated cropped	1.0	0.36	0.75	32.5	3.3	20.7	30
minus S treated cropped	0.8	0.42	0.65	45.0	5.5	17.9	16
Soil Sand Mixture :-							
minus Mg treated cropped	1.4	1.14	1.83	37.5	6.6	17.2	13
minus Mg treated fallow	1.0	2.82	0.90	52.5	5.5	38.8	87
Minus S treated cropped	1.8	1.26	1.35	48.5	5.5	12.9	15
minus S treated fallow	1.6	2.82	1.08	187.5	6.6	37.1	110

Table 94.

Yield of Grass, 3rd. Cutting (Tops).

Treatment	Weight of green grass (gms/pot)	Dry matter yield (gms/pot)	Dry matter %
Sand Culture :-			
Complete	6.304	1.644	26.07
minus Fe	5.266	1.319	25.04
" Mn	6.351	1.415	22.20
" Al	6.595	1.569	23.78
" S	1.175	0.269	23.10
Treated Mg*	6.267	1.753	25.07
" S	10.212	1.719	16.83
Soil Sand Mixture :-			
Complete	6.703	1.612	24.04
minus Fe	6.984	1.653	23.88
" Mn	6.845	1.668	24.40
" Al	7.199	1.650	22.91
" Mg	2.768	0.914	33.02
" S	2.216	0.466	21.02
Treated Mg	7.538	1.613	21.39
" S	9.896	1.868	18.87

*minus Mg plants grown on sand culture died at this stage and no cuttings were taken.

Table 95.

Root yield of the 3rd. Cutting :-

		Weight in gms/pot (mean of duplicates)	Increase % gained by the roots on treatment
Sand Culture :-			
minus	Mg	3.337	
treated	Mg*	5.837	46.34
minus	S	2.310	
treated	S**	3.360	31.25
Soil Sand Culture :-			
Complete		6.855	
minus	Fe	4.894	
"	Mn	4.477	
"	Al	5.880	
"	Mg	3.490	
treated	Mg*	4.275	22.5
minus	S	2.862	
treated	S**	3.936	37.2

* Treated Mg :- received magnesium immediately after 2nd.cutting

** " S " " " " " "

Table 96.

Analysis of Tops 3rd. Cutting :-

p.p.m. dry matter, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	N	K	P
Sand Culture :-								
Complete	550	65	313	6000	8937	33750	2593	5350
minus Fe	320	68	313	7750	11312	33750	2740	5850
" Mn	440	18	313	6300	9427	31250	3275	6650
" Al	80	45	200	6500	8427	28750	2729	5350
treated Mg	550	60	163	2500	9437	20500	2740	5350
minus S	260	30	163	4100	960	32500	3411	7500
treated S	170	60	125	5400	6500	27500	2729	6900
Soil Sand Mixture :-								
Complete	175	60	125	6025	9624	28375	3007	5650
minus Fe	40	60	144	4675	9906	26062	3075	5275
" Mn	80	60	101	5925	10312	27625	3275	5400
" Al	30	60	55	4750	9500	26625	3007	5400
" Mg	80	50	120	525	10224	22812	5186	10300
" S	290	75	180	4200	3054	31000	3480	6525
Treated Mg	170	75	100	3550	10469	26875	3411	6050
" S	215	85	125	5300	7565	28625	2740	7750

Table 97.

Analysis of the Roots from the 3rd. Cutting :[2]

p.p.m. dry matter, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Sand Culture :-								
minus Mg	600	120	163	600	4750	10875	1965	1300
treated Mg	555	53	163	1250	3875	9000	1742	1550
minus S	458	45	125	2200	1563	17500	1902	1550
treated S	363	45	125	3150	2313	18625	1742	1800
Soil Sand Mixture :-								
Complete	313	60	125	1400	2313	13750	1365	1050
minus Fe	235	45	75	750	2438	12625	1419	1050
" Mn	225	30	75	1550	2500	14375	1527	1450
" Al	220	45	125	1250	2438	11875	1365	1200
" Mg	665	75	200	1100	2613	7875	1419	600
" S	535	90	75	1400	1438	12500	1910	950
treated Mg	305	45	250	1250	2438	9625	1419	1050
" S	635	120	163	1750	1938	10375	1527	1050

Table 98.

Analysis of Morgan Extract from Fallow Pots, Soil Sand Mixture:-
4th. Sampling.

p.p.m. oven dry soil, average of duplicates.

Treatment		Fe	Mn	Al	Mg	S	P	K
Complete		1.00	0.60	0.58	40	19.5	12.0	47
minus	Fe	0.08	0.70	0.58	45	18.9	18.0	51
"	Mn	0.80	0.50	0.70	49	19.5	18.0	55
"	Al	0.80	0.60	0.40	40	16.6	17.4	53
treated	Fe	0.40	0.60	0.60	45	15.5	14.0	55
"	Mn	0.80	0.70	0.90	49	17.5	20.1	68
"	Al	1.0	0.70	0.80	49	15.5	14.0	53

Table 99.

Analysis of Morgan Extracts from Cropped Pots :- 4th. Sampling.

p.p.m. oven dry soil, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	S	P	K
Sand Culture :-							
Complete	0.8	0.5	1.5	15	9.5	19.0	12
minus Fe	Trace	0.5	1.3	17.5	9.5	18.0	19
" Mn	0.6	0.2	1.3	12.5	7.5	19.0	14
" Al	0.6	0.5	1.3	17.5	7.5	14.0	11
treated Fe	0.2	0.5	1.0	15.0	6.6	15.8	12
" Mn	1.0	0.4	1.4	12.5	6.6	18.0	10
" Al	0.6	0.5	1.4	12.5	6.6	15.8	11
Soil Sand Mixture :-							
Complete	1.4	1.2	2.5	37.5	8.9	18.0	18
minus Fe	0.06	1.0	2.1	32.5	7.5	14.0	15
" Mn	1.4	1.0	1.8	22.5	8.9	12.4	14
" Al	1.4	1.6	2.2	37.5	7.5	14.8	15
treated Fe	0.15	1.5	2.0	37.5	8.9	18.0	15
" Mn	1.0	1.4	1.9	22.5	7.5	14.0	15
" Al	1.4	1.8	2.0	37.5	7.5	16.3	15

Table 100.

Yield of the 4th. Cutting (TOPS).

Treatment	Weight of green yield (gms/pot)	Dry matter yield (gms/pot)	Percentage dry matter yield.
Sand Culture :- (average of two pots)			
Complete	7.419	1.609	21.68
minus Fe	2.860	0,676	23.63
" " Mn	6.416	1.172	18.27
" " Al	7.872	1.614	20.50
treated Fe	5.991	1.202	20.06
" " Mn	7.877	1.603	20.35
" " Al	6.760	1.719	25.43
Soil Sand Mixture :- (average of 4 pots)			
Complete	9.772	2.354	24.08
minus Fe	9.566	2.169	22.67
" " Mn	9.629	2.175	22.57
" " Al	9.424	2.078	22.04
treated Fe	10.177	2.303	22.63
" " Mn	10.312	2.381	23.09
" " Al	10.473	2.279	21.76

Table 101.

Root yield of the 4th. Cutting :-

Treatment		Weight of dry matter yield gm/pot (mean of duplicates)		
			Treated pots*	Difference % gained on treatment.
Sand Culture :-				
Complete		7.510		
minus	Fe	5.149	5.573	8.25
"	Mn	4.956	5.202	4.96
"	Al	5.910	6.105	3.30
Soil Sand Mixture :-				
Complete		7.169		
minus	Fe	5.896	6.327	8.13
"	Mn	5.108	5.246	2.50
"	Al	6.439	6.875	6.76

* These treated pots received the deficient element in a soluble form immediately after the 3rd. cutting:-

minus iron pots :- received ferric citrate
" Mn " " manganese sulphate
" Al " " aluminium " "

} all applied at the same rate as in a complete nutrient solution.

Table 102.

Analysis of the Tops from the 4th. Cutting :-

p.p.m. dry matter, average of duplicates.

Treatment		Fe	Mn	Al	Mg	N	P	K
Sand Culture :-								
Complete		650	52.5	325	10000	26875	3542	7200
minus	Fe	325	45.0	125	12500	32500	4776	8050
"	Mn	80	15.0	75	8000	33000	4367	8950
"	Al	80	45.0	163	7400	32500	3547	7750
treated	Fe	325	35.0	163	10000	32500	3777	8050
"	Mn	170	45.0	250	8000	31500	4367	7900
"	Al	170	35.0	375	7700	32000	3412	7500
Soil Sand Mixture :-								
Complete		170	52.5	200	6000	28500	3542	6650
minus	Fe	80	45.0	125	5400	26250	3411	6400
"	Mn	170	35.0	200	6000	26125	2740	6400
"	Al	50	45.0	163	5250	24250	3411	6400
treated	Fe	120	45.0	75	6200	26250	3411	6400
"	Mn	80	45.0	163	5700	24250	2740	6100
"	Al	80	15.0	163	5700	23875	3411	6400

Table 102.

Analysis of the Roots of the 4th. Cutting :-

p.p.m. dry matter, mean of duplicates.

Treatment	Fe	Mn	Al	Mg	N	P	K
Sand Culture :-							
Complete	465	30	250	2050	10500	1527	1450
minus Fe	258	45	250	2500	10500	1201	1300
" Mn	395	30	200	2200	13375	1742	1300
" Al	443	45	125	2700	10250	1419	1300
Treated Fe	703	53	250	2200	17375	1902	1300
" Mn	258	45	125	2800	19750	1638	1450
" Al	258	45	225	2200	21250	1364	1300
Soil Sand Mixtrue :-							
Complete	715	45	163	950	9375	983	1300
minus Fe	515	45	125	600	8375	893	1550
" Mn	765	45	163	1250	11875	1092	1300
" Al	605	53	125	600	8625	1201	1550
treated Fe	635	53	163	950	6875	983	1300
" Mn	355	53	163	950	10875	1201	1450
" Al	385	30	125	600	9375	1201	1450

Table 104.

Uptake of Tops :- 1st. Cutting, mgm/pot.

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Sand Culture :-								
Complete	0.53	0.11	0.38	5.15	5.70	31.47	2.40	4.45
minus Fe	0.09	0.10	0.35	3.27	5.17	28.86	2.52	4.14
" Mn	0.39	0.07	0.37	3.71	4.64	27.71	2.61	4.39
" Al	0.39	0.10	0.28	4.44	4.44	21.94	2.11	4.06
" Mg	0.44	0.13	0.40	1.12	4.52	25.38	2.90	7.30
" S	0.02	0.09	0.16	2.90	1.02	16.62	1.69	2.71
Soil Sand Mixture :-								
Complete	0.54	0.18	0.31	4.67	10.29	39.35	3.97	5.78
minus Fe	0.14	0.14	0.20	4.22	7.32	28.03	3.14	4.23
" Mn	0.27	0.08	0.31	4.31	8.00	26.75	2.81	4.56
" Al	0.24	0.09	0.17	3.59	7.67	21.00	2.75	4.00
" Mg	0.29	0.14	0.17	0.84	7.00	16.80	2.60	4.73
" S	0.15	0.08	0.20	1.84	1.19	11.75	2.37	2.68

Table 105.

Uptake of Tops :- 2nd. Cutting (mgm/pot).

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Sand Culture :-								
Complete	0.22	0.08	0.54	7.32	8.83	26.32	3.33	5.84
minus Fe	0.10	0.09	0.91	6.95	9.45	24.08	3.24	6.03
" Mn	0.22	0.05	0.31	6.97	9.17	24.75	3.35	6.66
" Al	0.21	0.11	0.19	4.35	8.29	25.09	339	6.00
" Mg	0.11	0.02	0.10	0.02	3.73	5.44	2.12	4.26
" S	0.05	0.03	0.16	1.47	0.30	8.96	1.38	1.46
Soil Sand Mixture :-								
Complete	0.21	0.07	0.29	6.86	10.23	23.31	5.63	5.62
minus Fe	0.10	0.09	0.30	5.55	10.48	22.22	3.24	5.80
" Mn	0.15	0.10	0.17	5.97	5.68	20.99	2.95	5.17
" Al	0.21	0.07	0.09	6.58	10.03	21.95	2.95	5.82
" Mg	0.36	0.15	0.45	0.49	9.63	22.38	4.55	9.61
" S	0.08	0.07	0.11	1.99	0.89	9.84	1.86	2.19

Table 106.

Uptake of Tops :- 3rd. Cutting (mgm/pot).

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Sand Culture :-								
Complete	0.25	0.11	0.51	9.86	14.69	55.49	4.26	8.80
minus Fe	0.11	0.09	0.41	10.22	14.92	44.52	3.61	7.71
" Mn	0.20	0.03	0.44	8.91	13.35	44.22	4.63	9.41
" Al	0.19	0.07	0.31	10.20	13.24	45.11	4.28	8.39
treated Mg	0.26	0.11	0.28	4.38	14.44	35.94	4.80	9.29
minus S	0.07	0.01	0.04	1.10	0.26	8.75	0.92	2.02
treated S	0.29	0.10	0.43	9.28	11.17	47.27	4.69	11.86
Soil Sand Mixture :-								
Complete	0.28	0.10	0.20	9.71	15.51	45.74	4.85	9.11
minus Fe	0.07	0.10	0.24	7.73	16.37	43.08	5.08	8.71
" Mn	0.13	0.10	0.17	9.88	17.20	46.08	5.46	9.00
" Al	0.05	0.10	0.09	7.84	17.23	43.83	4.98	8.91
" Mg	0.07	0.05	0.11	4.80	9.34	20.85	4.74	9.41
" S	0.14	0.03	0.08	1.96	1.42	14.45	0.16	3.04
treated Mg	0.27	0.12	0.16	5.72	16.89	43.35	5.85	9.76
" S	0.40	0.16	0.23	9.90	14.13	53.47	5.12	14.48

Table 107.

Uptake of Tops :- 4th. Cutting (mgm/pot).

Treatment	Fe	Mn	Al	Mg	N	P	K
Sand Culture :-							
Complete	0.24	0.08	0.52	16.09	43.24	3.71	11.58
minus Fe	0.05	0.03	0.08	8.45	20.97	3.19	5.44
" Mn	0.09	0.02	0.03	9.38	38.68	5.12	10.49
" Al	0.20	0.07	0.26	11.94	52.46	3.79	12.51
treated Fe	0.15	0.04	1.95	12.02	39.07	4.84	9.67
" Mn	0.27	0.07	0.40	12.82	50.49	7.00	12.66
" Al	0.29	0.06	0.06	16.54	55.00	3.72	12.89
Soil Sand Mixture :-							
Complete	0.40	0.12	0.47	20.12	67.09	8.35	15.65
minus Fe	0.17	0.09	0.27	11.71	56.94	7.40	13.88
" Mn	0.37	0.07	0.44	13.05	60.82	5.96	13.92
" Al	0.10	0.09	0.34	10.91	50.39	7.09	13.30
treated Fe	0.28	0.10	0.17	14.28	60.45	7.86	14.74
" Mn	0.19	0.11	0.39	13.57	57.73	6.52	14.52
" Al	0.18	0.03	0.37	12.99	56.11	7.77	14.59

Table 108.

Roots uptake :- 3rd. Cutting (mgm/pot)

Treatment	Fe	Mn	Al	Mg	S	N	P	K
Sand Culture :-								
Complete								
minus Mg	2.00	0.40	0.54	2.00	15.85	36.29	6.56	4.34
treated Mg	3.24	0.30	0.95	7.30	22.72	52.53	10.17	9.05
minus S	1.06	0.10	0.29	5.08	3.61	40.43	4.39	3.58
treated S	1.22	0.25	0.42	10.58	7.77	62.58	5.85	6.05
Soil Sand Mixture :-								
minus Mg	2.32	0.26	0.70	3.84	9.12	27.48	4.95	2.09
treated Mg	1.30	0.19	1.07	5.34	10.42	41.14	6.07	4.49
minus S	1.53	0.26	0.22	4.02	4.12	35.25	5.48	2.72
treated S	2.50	0.47	0.64	6.89	7.63	40.84	6.01	4.13

Table 109.

Roots uptake :- 4th. Cutting (mgm/pot)

Treatment	Fe	Mn	Al	Mg	N	P	K
Sand Culture :-							
Complete	3.49	0.23	1.48	15.40	78.86	11.47	10.88
minus Fe	2.36	0.23	1.29	12.87	54.06	9.27	6.69
" Mn	1.96	0.15	0.62	10.90	66.29	8.63	6.44
" Al	2.62	0.27	1.18	15.96	60.58	8.39	7.68
treated Fe	3.92	0.29	1.39	12.26	96.83	10.50	7.24
" Mn	1.34	0.23	0.65	14.57	70.47	8.52	7.54
" Al	1.57	0.27	0.46	13.43	129.73	8.33	8.94
Soil Sand Mixture :-							
Complete	5.13	0.32	1.16	6.81	67.21	7.04	9.32
minus Fe	4.22	0.27	0.74	3.54	49.38	5.79	9.14
" Mn	3.91	0.23	0.83	6.39	60.66	5.58	6.64
" Al	3-90	0.34	0.80	3.86	55.53	7.73	9.98
treated Fe	4.02	0.33	1.03	6.01	43.50	6.22	8.23
" Mn	1.86	0.28	0.85	4.98	57.05	6.30	7.61
" Al	2.65	0.21	0.86	4.13	67.89	8.26	9.97

Table 110.

Phosphorus :- Iron ratio P/Fe ratio

	Sand Culture		Soil Sand Mixture	
	Complete	Minus Fe	Complete	Minus Fe
TOPS :-				
1st. Cutting	4.55	29	7.3	22.38
2nd. " "	15.25	34.25	11.78	34.1
3rd. " "	4.71	8.56	17.1	76.87
4th. " "	5.45	14.69	20.87	42.63
	Treated Fe 11.62		28.42	
ROOTS :-				
1st. Cutting	2.12	3.66		
2nd. " "	1.77	2.43		
3rd. " "	4.35	6.03		
4th. " "	1.24	1.73	3.28	6.95
	Treated Fe 1.54		2.1	

Table 111.

Fe/Mn ratio in Tops and Roots of Grass.

	Sand Culture Plants.			Soil Sand Mixture Plants.		
Cutting	Complete treatment	Minus Fe	Minus Mn	Complete treatment	Minus Fe	Minus Mn
TOPS :-						
1st.Cutting	5.0	0.89	5.83	3.52	0.92	3.59
2nd. "	2.83	1.06	9.00	3.66	1.06	2.16
3rd. "	8.46	4.70	24.40	2.91	0.66	1.33
4th. "	12.38	7.22	5.33	3.23	1.77	4.85
Treated Plants :-	Treated Fe 9.22	Treated Mn 3.77		Treated Fe 2.66	Treated Mn 1.77	
ROOTS :-						
1st Cutting				3.66	1.94	2.35
2nd Cutting	--	--	--	6.28	3.10	2.95
3rd "	--	--	--	5.20	5.22	7.5
4th. "	--	--	--	15.88	11.40	17.0
Treated Plants		13.38	5.72		12.09	17.0

CHAPTER X

General Summary and Conclusions.

1. ANALYTICAL METHODS :-

The methods were developed :-

i) To avoid or eliminate the interference from Morgan's extractant and

ii) To be suitable for field use with the minimum of apparatus.

A A colorimetric technique for the determination of sulphates in Morgan extract of soils was introduced. The reaction between solid barium chloranilate and soluble sulphate in 50% alcoholic solution at pH 3 to 3.5 liberates the highly coloured chloranilic acid. The purple colour is stable for at least fifteen hours.

The cations which might interefre if present in appreciable quantities in soil extracts (by precipitating the liberated chloranilic acid) are removed prior to the determination by treatment of the soil extracts with an ion-exchange resin (Zeo-carb 225,H-form)

The method as suggested is sensitive to 5 - 80 p.p.m. sulphate and in the presence of up to 100 p.p.m. of the other anions which are usually present in soil extracts (Cl', NO_3', HCO_3', CO_3'', and PO_4''') no interference occurred.

For field work, a lightly constructed shaker and centrifuge were designed. A coloured standard disc was made for the purpose.

B <u>The determination of Manganese in Morgan Soil extracts using leucomalachite green.</u>

The presence of very small quantities of manganese in soil solutions and Morgan extracts made it necessary to adopt an extremely

sensitive method for these studies. A colorimetric method using p:p' tetramethyldiaminotriphenyl methane covering the range from 0.03 to 0.3 µgm. Mn was sought. The manganous ions are oxidised to permanganic acid using potassium periodate in acetate-acetic buffer solution at pH 3.35. Permanganic acid oxidises the leuco-malachite green to malachite green, the colour of which is proportional to the amount of manganese present.

The sensitivity of the method as well as the interference of Morgan's solution made it necessary to take 0.1 ml. of the soil extract for analysis. This made the method almost free from interferences. The colour produced is stable for two hours at a temperature ranging from 2o - 30°C. Temperatures above 25°C seemed to accelerate the reaction rather than interfere with the colour.

A coloured disc was made covering the range from 0.03 to 0.3 µgm. manganese for field work.

C (1) Thiocyanate method originally developed for determining iron using an organic extractant was standardised for the examination of Morgan extract of soils. It was found that :-

a Morgan's solution in reasonable proportions had no effect on the thiocyanate complex formed.

b Increasing the volume of hydrochloric acid added increased the toleranceof the method to presence of phosphates.

c Increasing the volume of $KMnO_4$ added to a limited extent, did not affectb the colour of the iron thiocyanate complex but increased its stability and guaranteed the complete oxidation

of any ferrous iron present.

d The extracted thiocyanate complex is stable for one hour in the shade and half an hour in the direct sunlight.

(2) The thiocyanate method for determination of iron using a stabiliser. The method was further modified and simplified by stabilising the thiocyanate complex in situ by adding a ethyl-methyl ketone/acetone mixture. Again the complex formed was stable for one hour in the shade and half an hour in the direct sunlight.

For field work, in both cases, permanent standards were made covering the range 2 - 20 µgm. Fe (i.e. 0.4 - 4 p.p.m. Fe if 5 ml. of the extract were used.)

(3) Ophenanthroline Method :- The test was developed for field work by :-

i Increasing the volume of the reagent used, thus furnishing a better differentiation between the different levels of

ii iron to be tested.

ii The 10% hydroxylamine hydrochloride solution could be safely substituted for the solid reagent without interference with the test. The colour formed was stable for at least fifteen hours. Permanent standards were made covering the range from 10 - 140 µgm. Fe.

D The titan yellow method was standardised to suit analysis of Morgan extracts of soils. It was found necessary to neutralise the test solution before the determination in order to obtain accurate results.

E The test for potassium using cobaltinitrite turbidimetrically

was modified to suit the field work as follows :-

The cobaltinitrite reagent was dissolved in 2% ethylene glycol and mixed with iso-propyl and ethyl alcohols.

Both solid sodium cobaltinitrite and sodium nitrite may be prepared in the tablet form to avoid weighing chemicals in the field.

The test is sensitive enough to differentiate between 10 p.p.m. (Low), 25 p.p.m. (Medium), and 50 p.p.m. K (High) with respect to soil.

III STUDIES on the EFFECT of DIFFERENT MOISTURE LEVELS and AIR DRYING of SOILS on the AVAILABILITY of NUTRIENTS as ASSESSED by MORGANS' TECHNIQUE :-

An attempt was made to study the fluctuations in the availability of iron, manganese, aluminium, magnesium, sulphates, phosphates and potassium under different soil moisture levels in soils varying in texture, pH and organic matter content.

The soils were incubated at 28°C at three moisture levels (25%, 50% and 75% of the corresponding moisture holding capacity). The fluctuations in Morgan-extractable (or available) elements were studied by analysing three-weekly samples. At the end of the incubation period, soils were air-dried and Morgan extracts of the air-dried soils were analysed. The changes in extractability of these elements as a result of air drying were studied and compared with a corresponding analysis of the Morgan extracts from the soils maintained at the same moisture level throughout.

The general increase in the iron, manganese and to a certain extent, aluminium availabilities with increasing acidity of the soil was accentuated at the higher moisture levels.

Magnesium availability increased progressively with rising moisture level. Potassium also showed a steady increase in Morgan-extractable values with increasing moisture level, the effect being more marked in acid soils than in neutral or alkaline soils.

Sulphates : With a few exceptions, the Morgan-extractable sulphate increased with increase of soil moisture level. An increase in sulphates was almost always accompanied with an increase of the Morgan-extractable elements studied, particularly aluminium and potassium.

Phosphates :- Generally, the Morgan-extractable phosphates increased the higher the moisture level. Mobilisation of phosphates in acid conditions was greater than under alkaline or neutral conditions probably due to solubility of iron and manganese phosphates under such acid conditions. It is probable, however, that transformation of organic phosphate to the available inorganic form is accelerated in alkaline soils considerably rich in organic matter.

EFFECT of AIR DRYING on the EXTRACTABILITY of the NUTRIENTS STUDIED :-

(1) Iron, Manganese and Aluminium :- The extractability of iron, particularly of manganese and to a certain extent, aluminium, increased on air drying acid soils but decreased in the case of neutral or alkaline soils.

(2) Magnesium :- Of the plant nutrients studied, magnesium showed the least changes in extractability on air drying the soil irrespective of its reaction. Air drying the soils from 25% and 50% moisture holding capacity did not result in any appreciable change Air drying the soil from 75% moisture holding capacity caused certain fluctuations in the extractability which in no case exceeded ± 25% of the value the corresponding air-dried sample.

(3) Sulphates :- Air drying almost always resulted in a decrease in Morgan-extractable sulphates. The only exception to this generalisation was in the case of a very sandy soil rich in organic matter

(4) Phosphates :- Air drying of acid soils resulted in increased values for Morgan-extractable phosphates while in neutral or alkaline soils a negative effect was obtained.

(5) Potassium :- A similar effect to that of phosphate was obtaind Air drying acid soils increased the Morgan-extractable potassium which in the case of neutral or alkaline soils, there was always a decrease.

It is suggested that Morgan extracts should be made with made with soils while fresh (field moisture) instead of air-drying as a preliminary precaution.

III STUDIES on the VISUAL SYMPTOMS of IRON, MANGANESE, MAGNESIUM and SULPHUR DEFICIENCIES in PERENNIAL RYE GRASS and the EFFECT OF THESE DEFICIENCIES on its MINERAL COMPOSITION:-

An approach to the study of the visual symptoms caused by iron, manganese, magnesium or sulphur deficiencies in rye grass

grown in pot culture (sand-culture and soil-sand mixture) was made with parallel analyses of Morgan extracts, soil solution and plant material.

A ! STUDIES on the VISUAL SYMPTOMS :-

(1) Effect of Iron deficiency :- appeared at first as newly developed chlorotic tillers. In a later stage leaves acquired an intravenal chlorosis along the midrib which retained its green colour. Finally, the tops acquired a dirty yellowish-green colouration. Iron deficiency resulted in a severe reduction in growth of tops but affected the root growth only slightly.

(2) Manganese deficiency :- The symptoms were very similar to those of iron deficiency when they made their first appearance as chlorotic newly produced tillers. In a later stage, the tops had a characteristic canary-yellow colour as a result of the spreading of intravenal chlorosis. Manganese deficiency decreased the yield from tops and roots but to a much smaller extent than did the rest of the deficiencies studied.

(3) Magnesium deficiency :- The first signs of deficiency showed as a lighter green colour of the tops as a result of intravenal chlorosis while midribs retained their greenish colour. The plants were fragile, twisted and lodged very easily. Later, white spots appeared on the leaves, almost always at the basal portion and the upper third. These spread upwards and downwards and ultimately the plants died. Both tops and root growth were severely reduced as a result of magnesium deficiency.

(4) Sulphur deficiency :- Tops were severely stunted in growth and, as a result, acquireda very upright habit of growth. In a later stage, leaves showed a characteristic chlorosis starting at the tip and spreading towards the base of the leaf. Sulphur deficiency restricted the growth of the plants (tops and roots) very severely.

On treatment with the corresponding deficient nutrient, plants made a quick recovery in the case of sulphur and magnesium deficiencies while the iron and manganese-deficient plants took a longer time to recover, obviously due to the ease with which the former two nutrients are translocated in plant tissues.

Iron, manganese, magnesium and sulphur deficiency symptoms were very definitely shown in the plants grown in sand-cultures and fed on a nutrient solution deficient in the respective element while in those grown in the soil-sand mixture, only sulphur and magnesium deficiency symptoms were clearly defined. In the latter however, iron and manganese contents in Morgan extracts and soil solution were markedly lower than the corresponding values in soils receiving complete nutrients. The iron and manganese deficiency symptoms were manifested only in the appearance of scattered chlorotic newly-formed tillers. In this case, soil and plant analyses were the only possible techniques for detecting theb deficiencies present.

B : CHANGES in MINERAL COMPOSITION ACCOMPANYING DEFICIENCIES of IRON, MANGANESE, MAGNESIUM and SULPHUR in RYE GRASS:-

Iron deficiency resulted consistently in an increased

uptake of phosphate by plants. The phosphorus/iron ratio was found to be a satisfactory index of iron deficiency being higher in chlorotic plants (even when no visual symptoms are clear) than in plants receiving complete nutrient solution. Moreover, these plants showed a tendency to absorb and accumulate iron from very dilute solutions, an observation which was not recorded in any of the other nutrients studied.

Manganese-deficient plantsb apart from their considerably low manganese cöntent, are characterised by their high nitrogen percentage in the dry matter.

Magnesium-deficient plants showed a very high potassium, low nitrogen and comparatively high percentage of phosphorus and sulphur in the tops (dry matter). Sulphur deficient plants are characterised by high nitrogen and comparatively high phosphate percentage in the dry matter.

C INTERRELATION between MORGAN EXTRACTABLE, SOIL SOLUTION CONTENT and UPTAKE of TOPS :-

A correlation was established between these three values. It is concluded that Morgan-extractable nutrients and soil solution content are good indices of plant uptake.

Rye grass is capable of absorbing iron from extremely dilute solutions and accumulating it in the tops.

The absorption of nutrients from the soil solution is affected by the absence or inadequacy of any which leads to an unbalanced condition which manifest itself in visual symptoms orn disturbed mineral composition, of the plant.

It is suggested that in the field, such deficiencies could be detectedv by analysis of Morgan extracts of the soil and data obtained are confirmed by plant analysis, due regard being paid to the reaction of the soil particularly in the case of iron and manganese.

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