INVESTIGATIONS ON THE RÔLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part VIII. Influence of Fermentable Organic Matter on the Transformations of Iron in the Swamp Soil.

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The discovery of the importance of minerals in plant nutrition and the realization of increased yields on application of mineral fertilisers led to considerable amount of interest in the related chemical and biological transformations in the soil. Particular attention was paid to the changes in essential fertilising ingredients such as potash and phosphoric acid and their availabilities under diverse soil conditions and to different types of crops. Comparatively little is known, however, regarding similar changes relating to certain other minerals such as iron, aluminium and silicon which, though not so important, are nevertheless present in very much larger quantities and do, under certain conditions, profoundly influence plant growth and metabolism. It is thus well known that although most soils contain sufficient amounts of iron to meet the normal requirements of crops, there are yet some which are deficient in that element—at any rate, in readily available form. There are also certain conditions when abnormally large quantities of that metal pass into solution and adversely affect plant growth by either direct action or by precipitating other elements such as phosphorus and thus reducing their availability in the soil. Very little information is available however regarding the cycle of changes undergone by those minerals in the soil and their relation to the decomposition of organic matter, especially under the conditions of the swamp soil.

There is considerable amount of literature relating to the chemistry and bacteriology of iron in geological formations, purification of water and disposal of sewage. They have been summarised in the monographs of Molisch (1910), Ellis (1919) and Cholodny (1926). The transformations of iron relating to pan formation in the soil have been studied by Morison and Sothers (1914). The physico-chemical and the biological considerations relating to the solution and precipitation of iron have been investigated by Halvorson and Starkey (1927, 1931). The latter authors have drawn attention to the importance

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of H-ion concentration, oxygen pressure and carbon dioxide tension in the associated changes. They have also shown that, in addition to the auto-trophic iron bacteria, several of the commoner saphrophytes can bring about transformations of iron. Precipitation of iron may be due to a variety of causes some of which are purely chemical. Their researches have revealed a number of new facts and have emphasised the need for further systematic work on the subject.

In the course of an enquiry on the chemical and biological changes attendant on the decomposition of cane molasses in the swamp soil (Bhaskaran, Narasimhamurthy, Subrahmanyan and Sundara Iyengar, 1934), it was observed that considerable amounts of ferrous iron were first brought into solution and after a certain period were either oxidised to insoluble ferric forms or otherwise reprecipitated into the soil system. The period of maxi-mum dissolution of iron corresponds to the early stages of puddling. At that stage the soil is known to contain toxic principles which kill out the existing vegetation and are inimical to any crop which may be planted. After some time, corresponding to the stage when ferrous iron is removed from the soil system, the toxicity also wears off. Since even minute quantities of ferrous iron are toxic to plant growth, the foregoing observations are highly significant. The changes that follow the oxidation and reprecipitation of iron are also of much interest because they represent conditions that are highly favourable to the crop and are responsible for the phenomenal increase in yields reported from different parts of the World (*vide* Subrahmanyan, 1933). Since molasses contains very little of nitrogen or minerals (except potash) and has otherwise no direct fertilising value (the sugars disappearing within the first few days), it may be reasonably expected that its beneficial effects are largely associated with the increased availability of minerals which follows its application (Bhaskaran *et al.*, *loc. cit.*). The precipitated iron is in a finely divided condi-tion and may be eventually expected to turn into ferric oxide. Since the previous work of Harihara Iyer, Rajagopalan and Subrahmanyan (1934) has shown that such oxides act as fertilisers by hastening the rate of decomposi-tion of organic matter in the soil and thus providing increased supply of carbon dioxide and other forms of plant food, it may be reasonably expected that the changes that follow the precipitation of iron are also of considerable practical interest. In view of the importance of the above and the need for further knowledge regarding the transformations of iron in the swamp soil, the present study was undertaken.

Experimental.

Materials.—Four types of soils, representing different parts of India and varying widely in their reactions were used. They were, (1) peaty (*Kari*)

soil from Central Travancore, (2) black cotton soil from Nagpur, (3) alkali (*Kalar*) soil from Sindh, and (4) laterite type of soil from Bangalore. Their total iron contents (A.O.A.C., 1930) on the air-dry basis and H-ion concentrations (Colorimetric, using a Hellige comparator) were as follows (Table I).

TABLE I.

Item	Peaty soil— Travancore	Black cotton soil— Nagpur	Alkali soil—Sindh	Laterite soil— Bangalore
Total iron (as Fe) per cent.	3·5	6·6	3·6	3·9
P$_H$	2·8	8·0	8·0	6·6

It may be seen from the above that, with the exception of the black cotton soil, all of them contained nearly the same amounts of iron. The reaction showed considerable variation. The peaty soil contained free sulphuric acid (Pillai and Subrahmanyan, 1931) which was probably mainly responsible for its high acidity. The laterite soil was free from carbonate. The Sindh soil contained alkali carbonate and the black cotton soil, largely calcium carbonate.

Molasses was not used in the present study because it contained (1) a number of other organic substances—though in small quantities—in addition to the sugars and (2) small amounts of nitrogen and minerals which might interfere with the transformations of iron in the soil. It was also found that it was difficult to obtain accurate estimates of iron (ferrous as well as ferric) in presence of molasses and its products of decomposition. For the above reasons, commercial glucose was applied as the fermentable organic matter. In view of the simplicity of composition and comparative freedom of that product from impurities, it was hoped that it would be possible not only to follow the changes in iron but also to correlate the observations with the attendant transformations of carbon.

METHODS.—*Estimation of iron.*—The usual methods adopted for the estimation of iron in solution cannot be applied to the system under study because of the presence of the organic matter of the soil and the various products of fermentation. It was chiefly on account of this difficulty that Morison and Doyne (1914) could not draw any conclusion regarding the ferrous iron of the different soils which they studied. The introduction of diphenylamine as an internal indicator (Knop, 1924) and the use of phosphoric acid to sharpen the end-point greatly facilitated the study. Kolthoff and Sarver (1930) studied the properties of diphenylamine and showed that it is a true oxidation—reduction indicator developing a characteristic blue colour at a P.D. of 0. 51 V. Schollenberger (1931) further investigated

the conditions relating to the development of blue colour and showed that the ferric iron formed during the titration interfered with the accuracy and led to the development of the blue colour even before all the ferrous iron was oxidised. He found that either hydrofluoric acid or alkali fluoride helped to convert ferric iron into poorly dissociated salts and thus sharpened the end-point. In spite of these useful advances, it was found that the procedure was largely empirical: that the end-point varied with the concentration of mineral acid (sulphuric) in the medium, the quantity of fluoride and the volume of indicator solution, none of which, beyond a certain minimum, should normally have any influence on the accuracy of estimation. The procedure had therefore to be standardised under the conditions of the present research and corrections applied for the different forms of organic matter present in the medium.

In one set of experiments, the same volume of a solution of ferrous-ammonium sulphate was titrated against standard dichromate, the concentration of sulphuric acid being varied while the total volume of the mixture, the quantity of sodium fluoride and the volume of indicator solution were maintained constant. In another, the quantity of fluoride was varied, while, in a third, the volume of indicator was altered, the other items being maintained the same. The details of the experiments, as also the results obtained, have been given in Table II.

The indicator solution used in the above experiment was prepared by dissolving 0·5 g. of diphenylamine in 100 c.c. of concentrated sulphuric acid and then diluting with 20 c.c. of distilled water.

It may be seen from the above results that a fairly high concentration of acid, a useful quantity of sodium fluoride and a very small volume of indicator are required to obtain the correct results. More concentrated acid and larger quantity of fluoride are unnecessary, while a still smaller volume of the indicator does not develop sufficient colour to obtain a sharp definition of the end-point.

Correction for the organic matter of the soil and the reducing action of the products of fermentation.—To 5 c.c. lots of the solution of ferrous ammonium sulphate used in the previous experiment, known amounts of ferric sulphate (in solution) were added and the mixtures titrated against dichromate in presence of either internal or external indicator. Another set of samples was titrated as such against standard permanganate. The trials were next repeated after reduction with zinc and sulphuric acid to include ferric iron. The same experiments were next carried out after adding to the iron solution a mixture containing small quantities of each of the following—glucose,

TABLE II.

Effect of varying the quantity of fluoride, the concentration of acid and the volume of diphenylamine indicator on the accuracy of the titre value.

Composition of titration mixture	Dichromate (0·02N) required (in c.c.)	Remarks
I. Fe. Am. sulphate (5 c.c.)+ sulphuric acid (1 : 3, 30 c.c.) + Indicator (0·2 c.c.)	4·2	End-point not sharp.
Do. + NaF (1 g.)	6·0	Do.
Do. + ,, (2 g.)	6·1	
Do. + ,, (3 g.)	6·2	} End-point sharp. Duplicates agree closely.
Do. + ,, (4 g.)	6·2	
II. Fe. Am. sulphate (5 c.c.) + NaF (3 g.) + Indicator (0·2 c.c.) + sulphuric acid (30 c.c., 1 : 17)	?	A dirty, pale-brown colour develops during titration ; no end-point.
Do. + sulphuric acid (30 c.c., 1 : 8)	5·9	Colour change not pronounced.
Do. + sulphuric acid (30 c.c., 1 : 4)	6·1	
Do. + sulphuric acid (30 c.c., 1 : 3)	6·2	} Sharp end-point. Duplicates agree closely.
Do. + sulphuric acid (30 c.c., 1 : 2)	6·2	
III. Fe. Am. sulphate (5 c.c.) + NaF (3 g.)+ sulphuric acid (1 : 3, 30 c.c.) +Indicator (0·5 c.c.)	6·9	} The oxidation product of the indicator interferes with the sharpness of the end-point.
Do. + Indicator (0·3 c.c.)	6·7	
Do. + ,, (0·2 c.c.)	6·2	} End-point quite sharp. Duplicates agree.
Do. + ,, (0·1 c.c.)	6·2	

5 c.c. of Fe. Am. sulphate=6·2 c. c of $K_2Cr_2O_7$ (0·02N)

lactic acid, acetaldehyde (in traces), acetic acid, propionic acid and butyric acid, the organic acids being present in quantities corresponding to those formed during decomposition of cane molasses in the swamp soil (Bhaskaran *et al., loc. cit.*). In another set of experiments known quantities of ferrous iron were added to the soil, which was subsequently extracted with excess of 1 : 3 acid and the extract titrated both before and after reduction. In

addition to the above, acid extracts of dry soils were also obtained and titrated against permanganate and dichromate respectively. The results have been given in Table III.

TABLE III.

Correction for Organic Matter.

Composition of titrating mixture	Titre values as c.c. of 0·02 KMnO₄ or K₂Cr₂O₇			
	Expected	Obtained by titration against KMnO₄	Obtained by dichromate titration with external indicator	Obtained by dichromate titration with internal indicator
Fe. Am. sulphate (5 c.c.) + $Fe_2(SO_4)_3$ ..	6·2	6·2	6·2	6·2
Do. after reduction ..	6·8	6·8	6·8	6·8
Do. + mixed products of fermentation (5 c.c.)	6·2	6·9	6·8	6·4
Do. + Do. after reduction ..	6·8	7·3	6·6	6·7
Supernatant from swamp soil (5 c.c.) (contains ferrous iron) ..	2·4	4·5	2·6	2·4
Do. + Fe. Am. sulphate (5 c.c.) ..	8·6	11·2	9·0	8·7
Do. + Do. after reduction ..	9·2	11·6	..	9·0
Acid extract of alkali soil (10 c.c.)	Nil	0·4	Nil	Nil
,, black cotton (10 c.c.)	Nil	0·2	Nil	Nil
,, laterite soil (,,)	0·9	1·5	1·2	0·9
,, peaty soil (,,)	4·3	5·4	4·5	4·3

It would be seen from the above that although the oxidising agent reacted preferentially with ferrous iron, there was always some correction to be applied for the organic matter present in the medium. Direct titration against permanganate led to erroneous results because there was no sharp end-point. The organic matter reacted steadily, though somewhat slowly, with the permanganate so that the colour continued to disappear even after all the ferrous iron was used up. Titration with external indicator was an improvement, but the results were still inaccurate: the procedure was also slow and somewhat tedious. Titration with internal (diphenylamine) indicator yielded the best results though correction for organic matter could not be entirely avoided.

The results obtained with the mixed products of fermentation, as also with the extracts from swamp soil, both before and after reduction, would suggest that they contained certain products which were either flocculated or otherwise rendered ineffective on treatment with zinc and sulphuric acid. The extracts from all the soils contained mostly ferric iron, but those from the laterite and the peaty soils also contained some ferrous iron. The quantities present in the peaty soil were rather large and may be accounted for by the presence of ferrous-aluminium alum referred to in an earlier communication (Pillai and Subrahmanyan, *loc. cit.*).

Influence of the nature of the soil on the dissolution of iron.—Representative specimens of the four soils mentioned above were air-dried and ground to pass the 80-mesh sieve. They were then weighed out in 100 g. lots into a number of conical flasks (cap., 250 c.c.). Glucose was added as concentrated solution in quantities corresponding to 1·8 g. per flask. It was expected that the sugar thus applied would correspond to about 30 tons per acre at which rate molasses is being applied in Hawaii and elsewhere. Water was then added to the contents of the flasks so as to make the total volume 200 c.c. in each case. The flasks were then plugged with cotton wool and incubated at 30°. At weekly intervals, representative specimens were taken out and examined as follows : (1) An aliquot of the supernatant was pipetted out and its P_H determined colorimetrically. (2) Another sample of the supernatant was filtered out and its iron content determined. Ferrous iron was estimated in the manner described already. Total iron was estimated by the same method after reduction with zinc and sulphuric acid. Ferric iron present in solution at each stage was calculated from the difference between, the two foregoing estimates. (3) The supernatant was decanted out as far as possible and the sediment (including a small portion of the supernatant) was then extracted with 1 : 3 sulphuric acid. Aliquots of the extract were titrated and their ferrous iron contents determined. From the figures thus obtained the ferrous iron of the residual supernatant was subtracted. The difference represented the total insoluble ferrous iron of the soil at each stage. Samples were thus examined at seven-day intervals over a period of five weeks. The results have been presented in Figs. 1 and 2.

Except in the case of the peaty soil, the total soluble iron contents of the different soils were very nearly the same as the ferrous iron in solution. This would show that iron brought into solution was almost exclusively in the ferrous condition and continued to remain so as long as it was present in the supernatant. In the peaty soil, which was exceptional in several respects, small quantities of ferric iron were present in solution throughout the period of observation, so that the total iron was invariably higher than
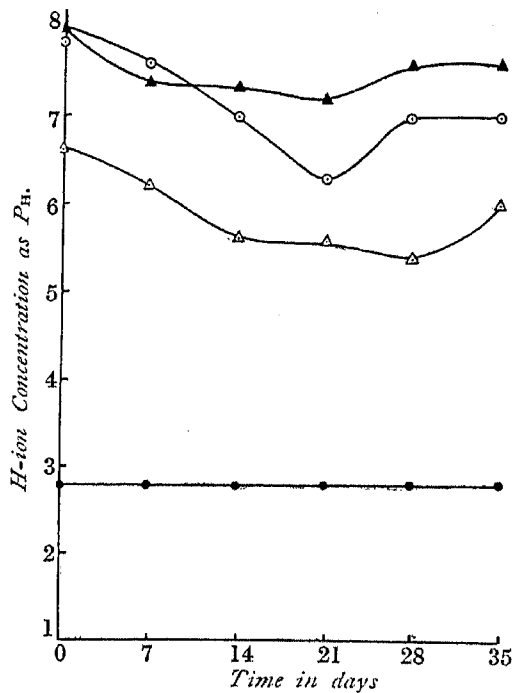
FIG. 1. Changes in soil reaction during fermentation
●—● Peaty (*Kari*) Soil
△—△ Laterite Soil
⊙—⊙ Black Cotton Soil
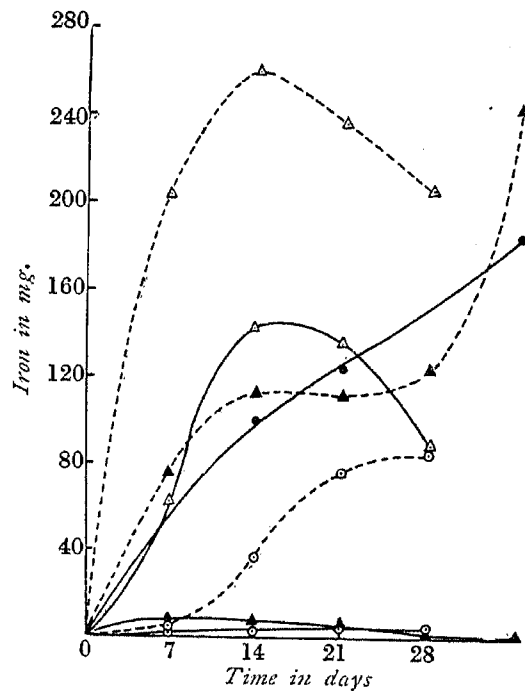▲—▲ Alkali (*Kalar*) Soil

FIG. 2. Distribution of ferrous iron in four types of soils
●—● Soluble ferrous iron in Peaty Soil
△—△   ,,   ,,   Laterite Soil
⊙—⊙   ,,   ,,   Black Cotton Soil
▲—▲   ,,   ,,   Alkali Soil
△--△ Insoluble ferrous iron in Laterite Soil
⊙--⊙   ,,   ,,   Black Cotton Soil
▲--▲   ,,   ,,   Alkali Soil

that in the ferrous state. The total soluble iron contents, together with the quantities of insoluble ferric iron left at each stage, have been presented in Table IV.

TABLE IV.

Total soluble and insoluble ferric iron at different stages.

Soil used	Iron in mg. at the end of									
	1st week		2nd week		3rd week		4th week		5th week	
	Total sol.	Insol. ferric	Total sol.	Insol. ferric	Total sol.	Insol. ferric	Total sol.	Insol. ferric	Total sol.	Insol. ferric
Alkali soil ..	10·9	3510·2	10·2	3482·8	5·4	3479·2	1·5	3452·1	1·5	3321·0
Peaty soil ..	84·5	..	100·8	..	133·7	..	147·7	..	211·3	..
Laterite soil ..	64·2	3630·3	141·4	3496·7	132·2	3531·3	93·9	3594·9	..	..
Black cotton soil ..	1·5	6591·1	2·4	6561·0	3·2	6522·2	3·2	6506·6	..	..

In all the soils the fermentation set in vigorously after the first seven days. The maximum frothing was noticed in the case of the black cotton and the alkali soils. As these soils originally contained carbonates (black cotton soil, 0·41 per cent. ; alkali soil, 0·66 per cent.), it may be assumed that the additional gas production was due to the reaction of the carbonates with the organic acids formed during the fermentation. Iron was dissolved to varying extents from the different soils. The largest quantities were dissolved from the peaty and the laterite soils, while only traces passed into solution from the other two. This may first appear inexplicable especially when considering the total iron contents ; but a comparison with the figures for H-ion concentration would show that the conditions were favourable to dissolution from two of the soils but rather unfavourable in the other two. It may thus be observed that the peaty soil remained highly acid (P_H 2·8) throughout the period of observation. The laterite soil became acid (P_H 6·6—5·4) with the progress of the fermentation. On the other hand the black cotton soil remained more or less neutral and the *Kalar* soil faintly alkaline throughout the period of study.

There was steady increase in the quantity of ferrous iron dissolved from the peaty soil. This was largely due to the presence of mineral acid which protected the ferrous iron from precipitation. On the other hand there was marked decrease in the case of the laterite soil. This was probably due to the steady reaction in the case of the former soil whereas, in the latter, there was a tendency to return to the neutral condition.

It is difficult to state whether the occurrence of ferric iron in the supernatant above the peaty soil was abnormal. It may be pointed out however that such a high acidity, as also the presence of free mineral acid, are not encountered in the commoner types of soils.

Quite considerable amounts of ferrous iron were present in the insoluble condition in all the three soils for which data are available. In the case of the laterite soil, over 200 mg. were present in that form even at the end of one week. There was not very much increase after that period and indeed there was a small decline at the end of four weeks. The other two soils—especially the alkali one—showed steady increase throughout the period of observation. This was rather significant when considering that the above three soils contained only small quantities of ferrous iron to begin with. The production of increased amounts of the insoluble form should therefore be attributed to the fermentation consequent on the addition of sugar. There is not sufficient material to show whether the corresponding quantities of iron were brought into solution prior to precipitation or whether the reduction proceeded exclusively in the insoluble condition. The general chemical considerations would

suggest however that the iron was first brought into solution, but was precipitated immediately afterwards—especially in the black cotton and the alkali soils—so that only a small part could be found in the supernatant.

The forms in which ferrous iron is brought into solution and subsequently precipitated will be considered in a later section of the paper.

The quantities of iron affected by the fermentation were fairly large when compared with the total amounts present in the different soils. In the case of the laterite soil about one-tenth of the total iron was either brought into solution or reduced to insoluble, ferrous condition. Much smaller quantities were involved in the black cotton and the alkali soils.

Effect of concentration of sugar on the dissolution and precipitation of iron.— These experiments were carried out with only the laterite soil from Bangalore because it responded best to the treatment and did not contain either mineral acid or carbonate that would affect the normal course of fermentation and the attendant changes. The samples (100 g.) were weighed out and treated with sugar solution in quantities corresponding to 0·3, 0·6, 1·2 and 1·8 g. respectively, which, in turn, would approximate to 5, 10, 20 and 30 tons per acre. The volumes were made up to 200 c.c. in all the cases and the flasks incubated at 30°. At weekly intervals, samples were taken out and examined in the manner outlined already.

As may be naturally expected, the fermentation was more vigorous at the higher concentrations of the sugar than at the lower ones. The corresponding changes in H-ion concentration have been presented in Table V.

TABLE V.

Changes in H-ion concentration with different concentrations of sugar.

Sugar applied at	H-ion concentration (as P_H) at the end of				
	1st week	2nd week	3rd week	4th week	5th week
5 tons	6·0	6·0	6·4	6·4	6·8
10 ,,	6·2	5·8	6·0	6·4	6·4
20 ,,	6·3	5·6	5·4	5·7	5·8
30 ,,	6·2	5·6	5·6	5·4	6·0

P_H at the commencement, 6·6.

It may be observed that with 5 and 10 tons the suspension became slightly acid at the end of two weeks but recovered rapidly after that period. At the higher concentrations not only did the medium become more acid

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but it also continued to be so over a period of three weeks. There was a tendency to recover at the end of the fifth week. Since acidity is favourable to the dissolution of iron, it may be expected that larger quantities of that metal would be present in solution at the higher concentrations than at the lower ones. This is supported by the results which have been presented in Table VI.

TABLE VI.

Influence of concentration of sugar on the transformations of iron.

Sugar applied at	Iron in mg. at the end of									
	1st week		2nd week		3rd week		4th week		5th week	
	A	B	A	B	A	B	A	B	A	B
5 tons	1·5	10·0	1·5	15·9	·7·0	137·9	4·1	187·1	2·3	292·4
10 ,,	25·8	82·1	27·3	149·8	23·3	210·1	29·7	377·5	28·2	319·3
20 ,,	69·6	261·1	96·2	287·7	..	..	73·4	318·4	71·9	311·2
30 ,,	64·2	269·7	141·4	403·3	132·2	368·7	93·9	305·1	..	..

There is no direct proportion however between the quantities of iron brought into solution and the corresponding concentrations of sugar. A similar observation will also apply to the total quantities of iron affected by the fermentation. These figures which represent the sum of total iron in solution and ferrous iron in the insoluble condition are also highly suggestive. They show that in the earlier stages of the fermentation only a small proportion of the total iron was affected when the concentration of sugar was small. With increasing concentration, very much larger quantities were either dissolved or reduced to insoluble ferrous condition. At 30 tons, the maximum quantity of iron was thus transformed in the course of two weeks. At lower concentrations the reaction changed more slowly, but nevertheless steadily, so that the quantities of iron affected by the fermentation increased continuously throughout the period of observation. The quantities of dissolved iron in such samples were always low, but there was steady increase in insoluble ferrous iron as may be seen from Fig. 3.

The available material is not sufficient to explain the nature of the agencies concerned in the production and distribution of ferrous iron at different concentrations. It is of much interest however to note that even in presence of very small quantities of fermentable organic matter, the reduc-
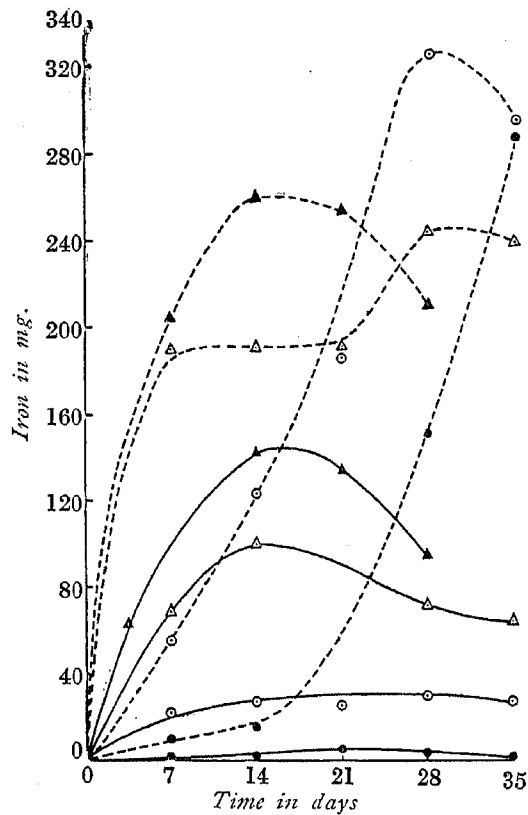
FIG. 3. Distribution of ferrous iron at different concentrations of sugar

●—●	Ferrous iron in solution when sugar is applied at 5 tons
○—○	,, ,, ,, ,, 10 tons
△—△	,, ,, ,, ,, 20 tons
▲—▲	,, ,, ,, ,, 30 tons
●--●	Insoluble ferrous iron when sugar is applied at 5 tons
○--○	,, ,, ,, ,, 10 tons
△--△	,, ,, ,, ,, 20 tons
▲--▲	,, ,, ,, ,, 30 tons

tion of iron continued over a fairly long period. Since the entire quantity of sugar was used up in the course of the first few days, it may be assumed that the production of insoluble ferrous iron was the result of interaction between some of the products of fermentation and the insoluble ferric iron of the soil. The related changes would appear to continue over a period of several weeks. It is difficult to explain, however, as to why similar changes did not proceed at the higher concentrations, say, when the sugar was applied at 30 tons : as to how a maximum of soluble as well as insoluble ferrous iron was attained in two weeks, after which there was only steady decrease in both of these forms. Further research is needed to elucidate the significance of these observations.

The percentages of total iron affected by the fermentation were not proportional to the quantities of sugar applied. It would, indeed, appear from

Table VII that, after the first four weeks, the percentages were about the same in all the cases.

TABLE VII.

Percentages of total iron affected by the fermentation at different concentrations of sugar.

Sugar applied at	Percentages affected at the end of				
	1st week	2nd week	3rd week	4th week	5th week
5 tons	0·3	0·4	3·5	4·8	7·5
10 ,,	2·1	3·8	5·4	9·7	8·2
20 ,,	6·7	7·4	..	8·2	8·0
30 ,,	6·9	10·3	9·5	7·8	..

The practical significance of the foregoing observation requires further elucidation. Although it is known that soluble ferrous iron is toxic to plant growth, the influence of insoluble ferrous iron is still obscure. It would no doubt be less toxic than the soluble form, but as to whether it is entirely harmless, especially when present in fairly large quantities, is yet to be determined.

Influence of the proportion of soil to water on the dissolution of iron.—Under similar soil conditions, the proportion of soil to water determines, to a large extent, the extent of submergence, available air-supply and other related changes. Since in normal field practice, the extent of submergence is variable and is often determined by conditions over which the farmer has no control, it was considered desirable to ascertain the possible influence of such conditions on the dissolution of iron. Some experiments were carried out, therefore, using the laterite soil (in 100 g. lots) for the study. The same quantity of glucose (1·8 g.) was applied in all the cases and water added so as to make up the total volumes to 100, 200 and 400 c.c. respectively. The flasks were then incubated as usual and samples examined at weekly intervals.

It was observed that the changes in H-ion concentration were of the same order in all the cases. The figures were the same as those recorded for 30 tons in Table V and have not therefore been repeated. There were slight variations in the quantities of ferrous iron in solution (Table VIII), but they appear to bear no relation to the degree of submergence. The results would indeed suggest there is some inconsistency even with regard to the samples treated in the same manner. It would thus appear that the degree

TABLE VIII.

Influence of the degree of submergence on the quantities of ferrous iron in solution.

Proportion of soil to water by weight	Ferrous iron (in mg.) in solution at the end of				
	1st week	2nd week	3rd week	4th week	5th week
1 : 1	138·4	122·0	110·3	72·7	111·2
1 : 2	64·2	143·1	135·3	97·0	..
1 : 4	95·4	114·1	147·0	131·4	109·4

of submergence is only secondary to certain other factors which determine the quantities of ferrous iron in solution.

The quantities of total iron in solution were more or less identical with those of ferrous iron cited above. It may be inferred, therefore that, at any rate in the case of the soil under study, the degree of submergence had no influence on the form in which iron was brought into solution.

Effect of temperature on the dissolution and precipitation of iron.—The range of temperature in the tropics, at any rate in the equatorial regions, is comparatively small. Since the present study would largely relate to such conditions, the following experiments were carried out at temperatures which may be attained during different seasons of the year. The soil (laterite type) was weighed out in 100 g. lots and treated with glucose (1·8 g.) and water (200 c.c.) in the usual way. They were then divided into three batches and incubated at 30°, 37° and 45° respectively. Samples were taken out at weekly intervals and examined in the usual way.

The changes in H-ion concentration which have been presented in Fig. 4 show that, with the rise in temperature, the soil became quickly acid thereby indicating that the fermentation changes proceeded more rapidly. The recovery was also fairly rapid. Thus at 45°, the reaction changed from P_H 5·6 to 6·0 in the course of two weeks, whereas a similar change occupied four weeks at 30°. These observations would suggest that the corresponding changes in iron would also proceed rapidly at the higher temperatures. Such was indeed the case as may be seen from Fig. 5. At 45°, there was rapid dissolution of iron in the course of the first two weeks after which the quantities were more or less stationary for about one week and then dropped suddenly to less than a third of the original value. The corresponding formation of insoluble ferrous iron also proceeded very fast at the higher temperature and
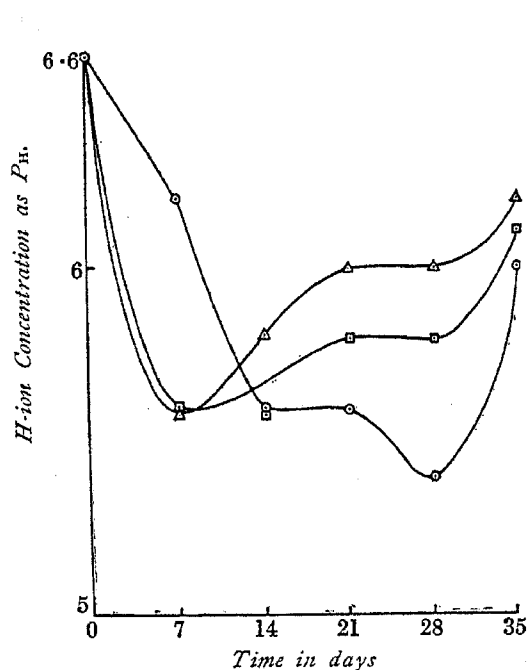
FIG. 4. Effect of temperature on soil reaction during fermentation

O—O Changes in H-ion concentration at 30°C
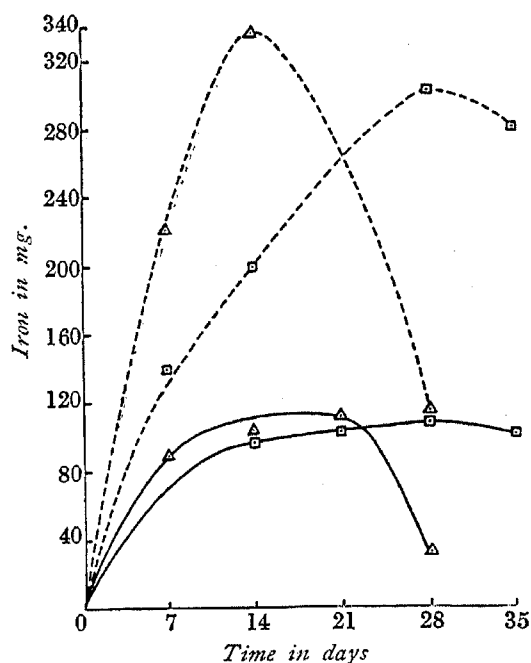□—□ ,, ,, 37°C
△—△ ,, ,, 45°C

FIG. 5. Total soluble and insoluble ferrous iron at different temperatures.

□—□ Total soluble iron at 37°C.
△—△ ,, ,, 45°C.
□--□ Insoluble ferrous iron at 37°C.
△--△ ,, ,, at 45°C.

then dropped suddenly at the end of four weeks. The parallel changes at 37° proceeded comparatively more slowly.

The results obtained at 30° were the same as those shown in Fig. 2. The quantities of ferrous iron present in solution at different stages were very nearly the same as those of total soluble iron. Those figures have not therefore been recorded.

Fermentation in shallow, open dishes.—The foregoing experiments were carried out in narrow-necked conical flasks, which did not permit of free access to air. The air supply was further restricted by the presence of cotton wool which was intended to keep out dust and other foreign bodies. Since, under field conditions, the surface involved is very large and the soil is directly exposed to the atmospheric conditions, it may be reasonably expected that the air supply will be more favourable. With a view to determining the influence of such conditions on the distribution of iron, some experiments were carried out in open, shallow glass dishes. The soil, the treatment and the conditions of incubation were the same as in the previous experiments. The results have been given in Table IX.

TABLE IX.

Fermentation in open, shallow dishes.

Observations at the end of	P_H	Iron in mg.		
		Total soluble	Soluble ferrous	Insoluble ferrous
1st week	6·3	82·1	82·1	264·7
2nd ,,	5·8	45·4	45·4	245·0
3rd ,,	6·0	29·0	29·0	208·4
4th ,,	6·0	2·4	2·4	14·1

Although the soil turned faintly acid, the P_H did not become so low as in some of the previous experiments. Furthermore, the change was only momentary and there was a tendency to return to normal conditions at the end of about three weeks. This is reflected in the dissolved iron contents which attained a fairly high value at the end of the first week but decreased rapidly in the later stages. Insoluble ferrous iron also followed a similar course. The largest quantities were present at the end of the first week. This was followed by slow decrease during the two subsequent weeks and then a sudden fall at the end of the fourth week.

The precise nature of the changes which attend the precipitation of the dissolved iron or the disappearance of insoluble ferrous iron is still obscure. Judging from purely chemical considerations, however, it may be inferred that those forms of iron passed into the insoluble, ferric condition. The nature of the products thus obtained will be considered in a later section of the paper.

Effect of sulphur or lime on the solution and precipitation of iron.—Attention has already been drawn to the fact that in two of the soils, which contained useful quantities of carbonates, the quantities of iron passing into solution were comparatively small, whereas in another, which remained steadily acid, very large quantities were dissolved. These observations suggested that the reaction of the soil is an important factor determining the solution of iron; that the mineral constituents of the soil have probably no influence on the related changes. With a view to determining whether by artificially rendering conditions favourable to increased acidity or alkalinity the extent of dissolution of iron can be altered, the following experiments were carried out. Suspensions of the Bangalore (laterite) soil containing glucose were prepared in the usual way and divided into two batches. To one set of flasks, burnt

lime was added at 0·3 g. each (corresponding to 3 tons per acre), while, to the other flowers of sulphur were added at 0·1 g. per flask (corresponding to 1 ton per acre). It was hoped that the lime would help to maintain a neutral or faintly alkaline reaction while sulphur would turn the medium increasingly acid and thus introduced a condition similar to that obtained in the peaty soil. The flasks were incubated at 30° and representative samples examined at weekly intervals. The results have been presented in Table X.

TABLE X.

Effect of sulphur or lime on the transformations of iron.

Observations at the end of	Treated with sulphur				Treated with lime			
	P_H	Iron in mg.			P_H	Iron in mg.		
		Total in solution	Soluble ferrous	Insoluble ferrous		Total in solution	Soluble ferrous	Insoluble ferrous
1st week	5·8	108·6	108·6	245·0	6·8	13·3	13·3	107·0
2nd ,,	6·0	135·3	135·3	267·5	6·4	84·5	84·5	261·9
3rd ,,	6·0	138·9	138·9	342·7	6·6	90·7	90·7	..
4th ,.	6·0	104·8	104·8	371·7	6·6	39·8	39·8	309·8
5th ,,	6·0	24·2	24·2	357·6	6·6	..	..	354·8

A comparison with the figures for the untreated soil (Fig. 2 and Table IV) would show that the addition of sulphur did not produce the desired effect. The medium was in fact less acid than the control. On the other hand, lime was helpful in checking the acidity. The medium was nearly neutral throughout the period of observation. The corresponding distributions of iron would show that whereas sulphur was not effective in enhancing the dissolution of iron, lime was fairly useful in depressing it, at any rate in the early stages. The production of ferrous iron was also retarded by the presence of lime. The effect was however only temporary, so that, at the end of two weeks, the quantities present in the two sets were nearly the same.

Although the samples treated with lime maintained nearly the same P_H as those of the black cotton soil, they were still not so effective in preventing the solution of iron. It would appear, therefore, that in addition to reaction there are certain other factors which determine the quantities of iron passing into solution.

Effect of flooding on the distribution of iron.—In field practice, the land is first puddled for a few weeks after which fresh quantities of water are let

in and allowed to flow over the surface. The transformations studied in the earlier part of the enquiry would correspond to the period of puddling. It was considered desirable therefore to extend the observations to the later stage and to study the attendant changes, especially of ferrous iron. After standing for seven weeks, the samples were treated with fresh quantities of distilled water in such a manner that while the supernatant was diluted, the soil sediment was left unaffected. The top layer was then decanted out and fresh water added upto the neck of the flask. After a few minutes, the supernatant was again decanted out followed by refilling. This operation was repeated several times for each sample. The flasks were then incubated and representative specimens examined at weekly intervals. The results have been given in Table XI.

TABLE XI.

Effect of flooding on the soluble as well as insoluble ferrous iron in the soil system.

Soil and treatment before flooding	Iron in mg. at the end of					
	8 weeks		9 weeks		10 weeks	
	Sol. ferrous	Insol. ferrous	Sol. ferrous	Insol. ferrous	Sol. ferrous	Insol. ferrous
Laterite soil (100 g.) + sugar (1·8 g.)	34·7	385·3	1·4	398·1	Nil	388·8
Do. + Do. + lime (0·3 g.)	2·4	508·3	Nil	497·2	Nil	502·7
Do. + Do. + sulphur (0·1 g.)	Nil	391·2	Nil	394.0	Nil	385·6
Black cotton soil (100 g.) + sugar (1·8 g.)	Nil	113·9	Nil	111·1	Nil	..
Alkali soil (100 g.) + sugar (1·8 g.)	Nil	283·4	Nil	272·2	Nil	..

It may be seen from the above that flooding removed almost the entire quantity of ferrous iron in a very short time. In the case of the laterite soil treated with sugar alone, it took about a week to eliminate the major part of the ferrous iron whereas, in others, it followed immediately after the flooding. Since ferrous iron forms the bulk of that element in the supernatant, the above observations will also apply to the total iron in solution.

The quantities of insoluble ferrous iron present in the different samples remained more or less the same throughout the period of flooding. This would show that the insoluble ferrous compounds formed in the soil system were fairly stable and were not affected by the dissolved oxygen of the irrigation water.

Forms in which iron is brought into solution.—A study of the anions with which ferrous iron is associated is of much scientific interest as it would help to throw light on the mechanism of dissolution. It is also of some practical importance as it would determine the stability of the related compounds and their physiological effects on crops. Robinson (1930) suggested that the ferrous iron in submerged soils is associated with carbon dioxide and is largely present as ferrous bicarbonate. He did not, however, consider the possibility of the iron being associated with the organic acids produced during fermentation being probably unacquainted with the slightly previous work of Subrahmanyan (1929). The chemical nature of those acids would indeed suggest that they would combine with the ferrous iron even after displacing any carbon dioxide that may remain in combination. The resulting compounds may also be expected to be comparatively stable. It is also probable that a part of the ferrous iron may exist as the sulphate or chloride as the result of some double decomposition changes in the soil. The available information being inadequate, the following experiments were carried out. In the case of the supernatant from the Bangalore soil, there was neither chloride nor sulphate in solution. On heating samples taken at different stages of fermentation, there was rapid change in colour to the characteristic yellowish brown of the ferric condition. After cooling, there was the slow separation of a reddish brown precipitate which was mostly ferric oxide. Continued bubbling of air through the liquid also yielded similar results. The formation of ferric oxide as one of the products of oxidation is further supported by the fact that the scum formed at the sides, especially in the later stages of fermentation, consisted exclusively of that oxide.

Even prolonged bubbling of carbon dioxide, nitrogen or hydrogen through the liquid did not produce any appreciable change in its composition. When the gases were admixed with traces of oxygen, however, there was steady change in colour accompanied by diminution in ferrous iron in solution. There was also the slow separation of ferric oxide.

When evacuated either by itself or over alkaline pyrogallol, there was apparently no change in the composition of the supernatant. Quantitative analyses of samples taken at short intervals showed that the ferrous iron contents remained the same for a fortnight.

On heating a dilute, aqueous solution of lactic acid with ferric oxide, it was found that small quantities of ferric as well as ferrous iron passed into solution. Similar effects were also observed on prolonged standing. Experiments with the volatile fatty acids (acetic, propionic and butyric) also showed that small quantities of ferric, though not ferrous, iron could thus be brought into solution. On the other hand even prolonged bubbling of carbon

dioxide through a suspension of ferric oxide in water could not bring more than traces of iron into solution.

The foregoing evidence, though not conclusive, is yet fairly suggestive of the manner in which ferrous iron is present in solution. If it is present as a bicarbonate or otherwise held in solution by the dissolved carbon dioxide of the medium, there should occur the steady separation of the insoluble ferrous carbonate either on evacuation or on bubbling an inert gas such as nitrogen through the medium. On the other hand, the iron in solution remained comparatively stable and was unaffected by any treatment except heating or bubbling of air. These observations would suggest that the ferrous iron was either in combination with, or otherwise held in solution by, the organic acids produced during the fermentation.

Forms in which iron is precipitated.—Attention has already been drawn to the formation of the scum on top and ring at the sides which consists almost exclusively of ferric oxide. These would form only a small percentage of the total precipitated iron which, as has already been observed, was mostly in the ferrous condition.

Precipitation of ferrous iron under nearly similar or allied conditions has been studied by some previous workers (Van Hise and Leith, 1911 ; Tache, 1923 ; Starkey and Halvorson, *loc. cit.* ; Robinson, *loc. cit.* ; Halvorson, *loc. cit.*). Evidence was adduced to show that the sulphide and the carbonate are two of the forms in which ferrous iron is present under such conditions. In the present study it was found that the white precipitate formed at the surface of the soil was a carbonate and contained ferrous iron. Ferrous sulphide was also present as a fluffy, dark-brown mass covering portions of the white precipitate. Indeed the entire mass of soil sediment yielded positive tests for sulphide after the fermentation had progressed for some time. The presence of sulphide was most pronounced in the case of samples receiving small quantities of sulphur. This observation would partly account for the unexpectedly poor dissolution of iron in presence of sulphur. The added sulphur was reduced to hydrogen sulphide which, instead of dissolving more iron, actually precipitated a part of what was already present in solution. The white precipitate did not yield positive tests for the presence of phosphate but this was probably due to the deficiency of phosphorus in the soil itself.

It is not clear as to what extent the ferrous iron first brought into solution is chemically precipitated and how far it is adsorbed by the mineral and the organic colloids of the soil. In view of the wide variation observed in regard to the retention of iron by different soils examined in the present study and the difference which they exhibit in their adsorptive capacities in other

directions (Sreenivasan, 1935), it is probable that similar phenomena may partly be responsible for the removal of iron from solution.

Discussion.

The present study has brought to light certain important aspects of the transformations of iron in the swamp soil. It has also indicated certain promising lines of future research.

Although the findings under discussion relate to the changes attendant on the decomposition of a readily fermentable form of organic matter, they are nevertheless of considerable practical interest. The commoner organic manures and, particularly green manures which are so effective in swamp soils, either contain small quantities of fermentable sugars or produce some, through hydrolysis, in the early stages of puddling. The fermentation which attends the application of such manures would therefore largely relate to transformations of the type described in the present study.

Attention has already been drawn (Bhaskaran et al., loc. cit.) to the biochemical significance of resting soil for at least a few weeks after the application of a manure. The presence of toxic concentrations of certain minerals—particularly ferrous iron—in the supernatant and the need for rapidly removing them were indicated. It was not then realized, however, that all soils may not require the rest at any rate, to the same extent. Even under the rather exaggerated conditions of the present study and the large quantities of sugar that were added, only minute quantities of ferrous iron were brought into solution from the black cotton soil. It may be confidently expected therefore that the quantities dissolving, under normal field conditions, from such soils, will be negligible and will not have any adverse effect on plant-growth. On the other hand, fairly large quantities may be dissolved from other soils such as that of the laterite type examined in the present study. Even under favourable conditions, small quantities of ferrous iron may be expected to persist in the soil for at least a few weeks, so that the soil would require some rest before planting. These observations show that the different soils vary in their need for rest, so that it would be useful to devise some simple method of determining when the toxic concentrations are reduced so that the planting operations may be begun. Premature planting may be inimical to the crop or, at any rate depress the yield, while late planting may lead to unnecessary wastage of fertilizing ingredients, particularly organic matter. Test papers dipped in ferricyanide or other suitable reagent would provide a useful indication which may be applied in field practice. Experiments designed to bring out the efficacy of such tests are in progress and will be described in a later communication.

The possible significance of the formation of insoluble ferrous iron has already been discussed. Such forms, though not so toxic as those in solution, may yet have some effect on plant growth. Although they are fairly stable in presence of water, it would yet appear that they get oxidised at some later stage. The average dry soil contains practically no ferrous iron. It would be of interest to follow the mechanism of the related changes.

The influence of the dissolution of iron on the availabilities of other minerals—particularly phosphoric acid—is of much practical importance. It has already been shown (Bhaskaran *et al.*, *loc. cit.*) that when increasing quantities of iron and aluminium are dissolved, there is actually a lowering in the quantities of phosphorus in solution. Since any ferrous salt would precipitate phosphates from solution, it would appear that the conditions favouring increased dissolution of iron would reduce the availability of phosphorus. As has already been shown, fermentation changes of the type described in the present paper could attend—though to varying extents—the decomposition of other forms of organic matter. It may be expected therefore that, under such conditions, there may actually be a lowering in the availability of phosphorus. This conclusion is apparently the reverse of that drawn by Sivan (1925) in regard to the availability of rock phosphate in presence of green manures. It may be noted, on the other hand, that the quantities of phosphate used in that study were quite large so that the reverse of that reported in the present study may be expected under such conditions. The small quantities of ferrous iron passing into solution would be rapidly precipitated by the larger quantities of phosphate brought into solution by the decomposition of the green manure. It may be further observed that the mineral phosphate used by Sivan contained a useful percentage of calcium carbonate which, as has been already observed, would discourage the dissolution of iron. It would appear therefore that the availability of phosphorus under such conditions would depend on the extent to which the dissolution of iron is favoured. Since, in any case, there would be mutual precipitation of the two elements, it would appear to be desirable to either adjust the conditions so as to discourage the dissolution of iron or to apply the phosphate, especially when it is present in some readily available form such as superphosphate, sometime after the initial fermentation has subsided. The relative merits of the two methods, as applied to different organic manures and various types of soils, would require careful study.

There are two other important aspects of the transformations of iron which emerge out of the present enquiry. One is the significance of the precipitation of iron in the soil and the attendant chemical and biological changes. An extension of that aspect will be the study of the effect of applying

different forms of ferrous and ferric iron with and without organic manures and their bearing on crop yield. The other line of interest will be the influence of different manurial treatments, both individually and in combination, on the dissolution and precipitation of iron. A systematic study of the first aspect would show whether the precipitated iron plays any useful rôle in the subsequent transformations of minerals and organic matter. In view of the previous findings of Harihara Iyer *et al.* (*loc. cit.*), it may be reasonably expected that the oxidation of organic matter may be hastened in presence of the precipitated iron. If this leads to some useful increase in yield, the precipitated iron would actually become a fertiliser. The results may even justify the application of ferrous or ferric salts in field practice.

The other aspect would, in one sense, be an extension of the present enquiry. In addition to the different organic manures, some trials may also be carried out with mixtures of known C–N ratios so as to determine the influence of the inter-relation between those two elements on the transformations of iron. The study of the effects of applying different mineral fertilisers, both by themselves and in combination with organic manures, would also lead to findings of much practical interest.

Some of the foregoing and allied problems are under investigation and will form the subjects of later communications.

Summary.

1. On addition of fermentable organic matter to swamp soils of four different types, it was found that fairly large quantities of ferrous iron were brought into solution from two of them (peaty and laterite soils) whereas only minute quantities were present in the supernatants from the other two (black cotton and alkali types). In the case of the peaty soil, small quantities of ferric iron were also present in solution, whereas, in the others, the dissolved iron was almost exclusively in the ferrous condition. In all the soils, there was steady increase in the quantities of insoluble ferrous iron present in the soil sediment. The corresponding changes in H-ion concentration showed that the peaty soil remained strongly acid(P_H 2·8) ; the laterite soil changed from P_H 6·6 to 5·4 and then tended to revert to neutral condition ; but the other two remained either neutral or faintly alkaline throughout the period of observation.

2. Increasing concentration of organic matter led to larger quantities of ferrous iron being brought into solution. The quantities of insoluble ferrous iron found at the end of five weeks were, however, more or less the same in all cases.

3. The extent of submergence did not have any appreciable influence either on the total quantities or the mode of distribution of iron in solution.

4. Rise in temperatures upto 45° hastened the dissolution and subsequent precipitation of iron. The total quantities were not, however, appreciably affected.

5. Fermentation in shallow, open vessels led to rapid precipitation of the iron from solution. Insoluble ferrous iron was also mostly oxidised to the ferric condition after the third week.

6. Addition of lime checked the dissolution of iron, at any rate in the early stages. On the other hand, sulphur did not lead to any increase in dissolution. In presence of organic matter, it was reduced to hydrogen sulphide and was eventually precipitated as ferrous sulphide.

7. Flooding the soil system accompanied by proper drainage of the supernatant removed all the ferrous iron in solution in the course of one week. The insoluble forms were left unaffected.

8. Evidence has been adduced to show that the ferrous iron in solution was largely present in association with the organic acids produced during the fermentation. The insoluble ferrous iron was present partly as the carbonate and partly as the sulphide. There are indications to show that a part of the ferrous iron first brought into solution was adsorbed by the soil colloids.

9. The significance of the foregoing and other observations in relation to field practice has been discussed. Although the dissolved ferrous iron may be toxic to plant life and may reduce the availability of essential fertilising ingredients such as phosphates, the precipitated forms, especially after oxidation to the ferric condition, may prove beneficial. The need for standardising the conditions so as to reduce the quantities in solution has been shown. Some useful lines of future research have been indicated.

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