

Microbiological Reduction for the Estimation of Free Iron Oxides in Soils

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(Received March, 1969)

INTRODUCTION

A considerable proportion of the total iron in most (dry) soils exists in the ferric state although some of it may be in the ferrous form as in residual fragments of hornblende and other ferromagnesium silicates containing this element. Under the conditions of abundant precipitation and high temperatures that prevail in the humid tropics iron in soils is readily converted into hydrated oxides, some of which may subsequently become dehydrated. Appreciable amounts of iron compounds coat clay, silt and sand particles in many soils to give them their characteristic colours.

Chemical methods for removing free iron oxides from soils have been described by several investigators (1, 5, 6, 7, 9, 10, 14, 15). Most of these methods are based on the relative ease with which naturally occurring iron oxides may be brought into solution through reduction to the ferrous state. In contrast to these is the biological method employed by Allison and Scarseth (2) where a readily available source of energy (sucrose) is mixed with soil which is covered with distilled water and incubated under anaerobic conditions. It appears probable that chemical methods of extracting free iron oxides from soils would bring into solution appreciable amounts of iron from attack on minerals other than the oxides. Thus Williams (15) obtained evidence to indicate that biotite, ilmenite, hydrobiotite and nontronite were susceptible to attack according to the method of Jeffries (10), and that nontronite was attacked by the reagents of the methods of Haldane (9).

A knowledge of the occurrence and distribution of free iron oxides is valuable in the study of soils, especially that of rice soils. The experiments described herein were to study the feasibility of microbiological reduction for routine determination of free iron oxides in soils.

MATERIALS AND METHODS

Experiment 1

A preliminary experiment was conducted to compare the biological reduction method of Allison and Scarseth (2) with the chemical method of Deb (5) for removing free iron oxides from soils. The effect of particle size of soil on the removal of free iron oxides was studied by grinding the soil to two sizes. Portions of the soil were ground to pass through 2 mm. and 0.5 mm. sieves respectively. The procedures were as described by the investigators (2, 5). Iron removed from soil was determined by the method of Fortune and Mellon (8).

Experiment 2

The effect of pH on the efficiency of the microbiological reduction was studied by using the soil of experiment 1 at its natural pH (5.1) and at pH 7.0, so adjusted by mixing the calculated amount of slacked lime with the soil. The soil samples were incubated for a period of 7 days with a solution of sucrose supplying the equivalent of 5 per cent. of the weight of soil. The incubation was carried out under anaerobic conditions as described by Allison and Scarseth (2), and maintained at room temperature (27° C). The solubilized iron was washed off the flocculated soil with portions of a 5 per cent. NaCl solution acidified to pH 3.0 with HCl. This was repeated until the washings indicated no evidence of iron when tested with H_2O_2 and dilute alcoholic KCNS solution. The extract was made up to 250 ml. before use. Iron was estimated by the method of Fortune and Mellon (8).

Experiment 3

Three soils, varying in texture and iron content, were used to study the effect of pH, concentration of sucrose and time of incubation on the biological reduction of free iron oxides. Some characteristics of the experimental soils are presented in Table I.

TABLE I—SOME CHARACTERISTICS OF THE SOILS

<i>Place of Collection</i>	<i>Texture</i>	<i>pH</i>	<i>Total Fe percent.</i>
Ambalantota	.. clay	.. 5.7	.. 1.16
Weweldeniya	.. loam	.. 4.5	.. 7.80
Dankotuwa	.. sand	.. 5.0	.. 0.64

The reaction of the soils were maintained at their natural value and at pH 7.0. The adjustment of the soil reaction was accomplished by mixing the required amount of slaked lime with the soil as in experiment 2.

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Two concentrations of sucrose were used to supply 1 and 5 per cent. of the weight of the soil. Ten gram soil samples were incubated under anaerobic conditions in large boiling tubes, fitted with one hole stoppers, containing 100 ml. of sucrose solution. A piece of glass tube was fitted to each stopper, and the outer end of the tube was fitted with a rubber valve which allowed gases to escape from within but prevented their entry to the system from outside. The samples were incubated for 5, 10, 15 and 30 days. At the end of the incubation period the supernatant liquid was filtered off and the soluble iron remaining in the soil was washed out with a 10 per cent. solution of $(\text{NH}_4)_2 \text{SO}_4$ acidified to pH 3.0 with H_2SO_4 and combined with that previously removed. The washings were repeated if they indicated any evidence of iron when tested with $\text{H}_2 \text{O}_2$ and dilute alcoholic KCNS solution. The extracted iron was estimated by the method described earlier (8).

RESULTS AND DISCUSSION

Experiment 1

The amounts of iron released from a soil by the biological reduction method (2) and the (Deb's) chemical method (5) at two particle sizes are compared in table II where the indications are that the former method brings more of the element into solution from easily reducible sources than the latter. This is in accordance with findings of other investigators (1) who reported that removal of free iron oxides from latosols was extremely slow according to the procedure proposed by Deb.

TABLE II.—COMPARISON OF THE BIOLOGICAL AND CHEMICAL METHODS OF
REDUCTION OF FREE IRON OXIDES IN A SANDY LOAN SOIL AT
TWO PARTICLE SIZES

(ppm. Fe in Extract)					
Particle Size mm.		Biological Method		Chemical Method	Per cent. Increase in Biological Method
2.0	..	40.8	..	35.0	.. 16.8
0.5	..	45.5	..	41.2	.. 10.4

Grinding the soil to pass a 0.5 mm. sieve gives higher values of soluble iron for both methods of extraction the difference being approximately the same for both methods. This may most probably be due to increase of surface area. Because the biological method of

Allison and Scarseth consistently yielded higher amounts of iron from the 2 mm. and 0.5 mm. soil this method was used in subsequent experiments.

Experiment 2

The effect of reaction of the medium on the microbiological reduction of iron shows that elevation of pH to neutrality considerably reduces the content of solubilized iron. (Table III). The concentration of iron at pH 5.1 is almost 200 per cent. more than that at pH 7.0. A low pH is known to favour the accumulation of reduced iron in soils.

TABLE III—EFFECT OF pH OF MEDIUM ON BIOLOGICAL REDUCTION OF IRON IN SOIL
(ppm. Fe in Extract)

pH of Soil	Conc. of Iron ppm.		Relative Conc.
5.1	..	45.5	.. 100
7.0	..	16.8	.. 36.9

Experiment 3

The influence of reaction of the medium (pH), concentration of fermentable substrate and period of incubation on the quantities of iron solubilized from the three soils are shown graphically in figure 1. The relatively large amounts of iron in the extracts from Weweldeniya soil is related to the amount of total iron in the soil (Table I). It is seen in the Ambalantota and Dankotuwa soils that the concentration of iron increases rapidly within the first five days. In the Ambalantota soil the concentration of iron gradually increases after five days and continues to increase even up to thirty days. In the Dankotuwa soil the indications are that no appreciable increases would have taken place after five days of incubation. In the Weweldeniya soil the initial increase in concentration of iron continues until approximately the tenth day after which the increase is gradual up to thirty days. The contents of iron solubilized from the three soils after incubation for thirty days as affected by pH and concentration of sugar are presented in Table IV.

TABLE IV—IRON CONCENTRATION IN SOIL EXTRACTS AFTER THIRTY DAY INCUBATION AS AFFECTED BY REACTION AND CONCENTRATION OF SUCROSE
(ppm. Fe in Extract)

pH of Medium	Unamended		7.0	
	1	5	1	5
Sugar Conc. Percentage				
Soil				
Ambalantota (pH 5.7)	.. 50	.. 47	.. 36	.. 49
Weweldeniya (pH 4.5)	.. 620	.. 730	.. 520	.. 700
Dankotuwa (pH 5.0)	.. 36	.. 34	.. 41	.. 41

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There were no marked effects of pH or sugar concentration on the reduction of free iron oxides in the soils from Ambalantota or Dankotuwa. This probably is because these soils are relatively low in their contents of free iron oxides, and the quantity of free iron oxides may be proportional to the amounts of total iron in the soils, (Table I). In the Weweldeniya soil which contained approximately 7 times more total iron than any of the other two soils, the differences were more marked where the 5 per cent. sucrose solution brought out more iron than the 1 per cent. solution at both pH values, confirming the microbiological nature of the reduction process. At pH 7.0 however the effect of sugar concentration was more marked, there being an increase of more than 30 per cent. with the 5 per cent. solution over that of the 1 per cent. solution. The inhibitory effect of high pH on the release of free iron oxides from this soil is evident from the data where higher concentrations of iron were recorded at pH 4.5 than at pH 7.0.

Iron transformations in soil are due to activities of micro-organisms, and bacterial action is reported to be effective in reducing iron in rock and soil (3, 4). Micro-organisms decrease the oxidation-reduction potential of their surroundings which lead to the formation of soluble ferrous iron. Some bacteria and fungi produce acidic end-products such as carbonic, nitric, sulphuric and organic acids which help to bring iron into solution. In addition other complex organic products of microbial metabolism help to maintain iron in soluble complex forms. The quantity of iron, or the maximum activity of iron that can be present in solution is a function of the oxidation-reduction potential (13) and reaction (11, 12) of the system.

The rates of release of iron from these three soils at their natural unamended pH values with 5 per cent. sucrose solution were calculated from the data in figure 1, and are presented in figure 2. It is observed that in all three soils the rate of release of iron tends to diminish from the first day of incubation. The very negligible rate of release of iron from the Dankotuwa soil after ten days of incubation is due most probably to its low content of iron.

The picture is therefore clear that generally the microbiological reduction of free iron oxides in soils is rather rapid. The concentration of iron in extracts of soils with unamended pH and incubated

with 5 per cent. their weight of sucrose, as affected by time are presented in Table V.

TABLE V—IRON CONCENTRATION IN SOIL EXTRACT AS AFFECTED BY TIME OF INCUBATION*

Days of Incubation	Ambalantota				Weweldeniya				Dankotuwa			
	Fe in Extract		Per cent. of Total Solubi- lized		Fe in Extract		Per cent. Solubi- lized		Fe in Extract		Per cent. of Total Solubi- lized	
5	..	26	..	55	..	285	..	39	..	26	..	76
10	..	31	..	66	..	470	..	64	..	34	..	100
15	..	34	..	72	..	588	..	81	..	34	..	100
30	..	47	..	100	..	730	..	100	..	34	..	100

*pH unamended, 5 per cent. Sucrose.

Within the first five days of incubation much of the activity is over in soils containing relatively low amounts of iron as in the case of the Dankotuwa soil where 76 per cent. of the iron in the extract was released. Further incubation of this soil made no more contribution to the total amount of iron solubilized. In the case of the Ambalantota soil approximately 55 per cent. of the total iron solubilized was released in five days while 72 per cent. was released by fifteen days. The percentage of iron initially released was relatively less in the Weweldeniya soil that contained the highest amount of total iron. However, by the fifteenth day of incubation approximately 80 per cent. of the total solubilized iron was in the extract. These results indicate that fifteen days of incubation may be sufficient for comparative studies.

The maximum amount of iron liberated per 100 gms. of each soil is shown in Table VI. Comparison of this with the data in Table I shows a direct relationship between total iron and easily reducible iron oxides in these soils.

TABLE VI—MAXIMUM CONTENT OF IRON SOLUBILIZED AND ITS RELATION TO THE TOTAL CONTENT OF THE ELEMENT

Soil	Treatment	Fe in Extract per 100 gms. soil	Per cent. of Total Fe- liberated
Ambalantota	.. pH=5.7, 1% Sucrose	.. 0.05	.. 4.39
Weweldeniya	.. pH=4.5, 5% Sucrose	.. 0.73	.. 9.36
Dankotuwa	.. pH=7.0 (unamended) 1 and 5% Sucrose	.. 0.04	.. 6.25

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CONCLUSIONS

Microbiological reduction affords a convenient method for routine estimation of free iron oxides in soils. The results of the experiments indicate that this method gives higher values than the chemical method of Deb (5), from soils ground to 2 mm. or 0.5 mm. To obtain maximum solubilization it is feasible to use sucrose solution equivalent to 5 per cent. of the weight of soil as the fermentable carbohydrate. The soils should be incubated unamended at their natural pH values. A disadvantage of this method is the relatively long time required for incubation.

ACKNOWLEDGEMENTS

Thanks are due to Mr. D. M. Rodrigo, Research Officer in Soil Science, Department of Agriculture, for his valuable suggestions during the preparation of the manuscript and Messrs. M. M. M. Jauffer and A. Ratnayake for assistance in the preparation of the script and figures respectively.

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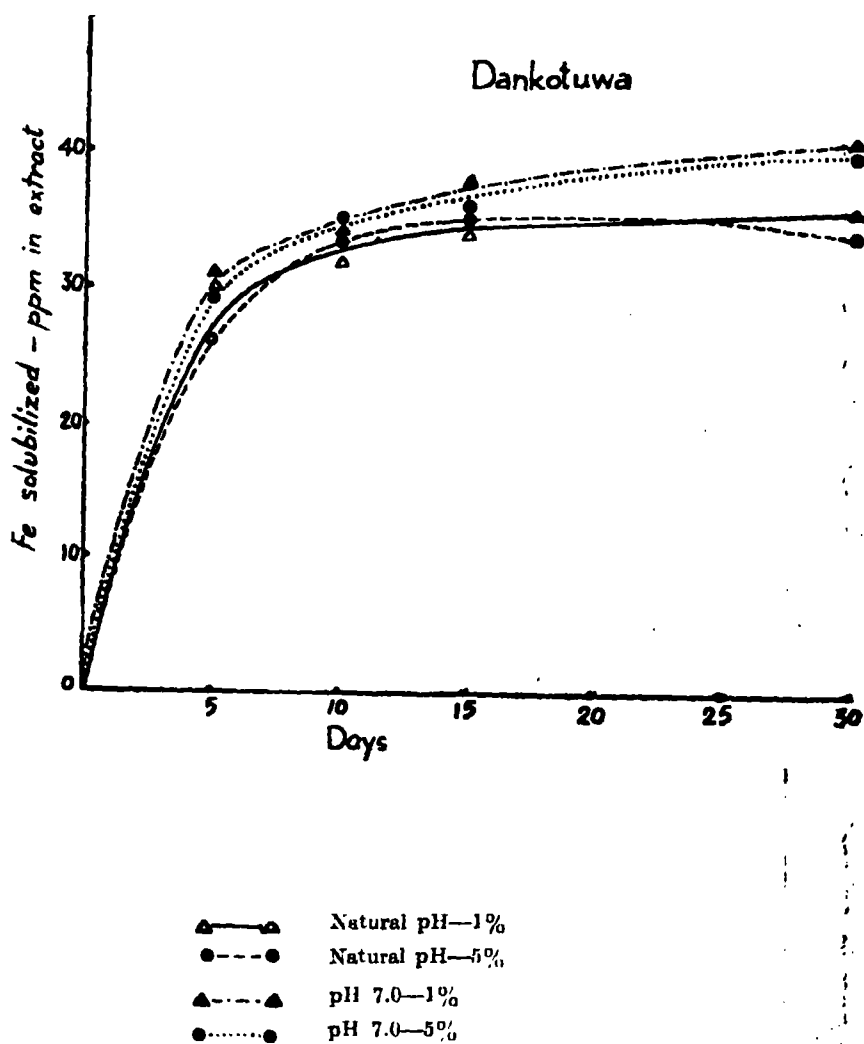


Fig. 1. Iron solubilization curves for the soils as affected by pH and sucrose concentration. (Continued on page 84)

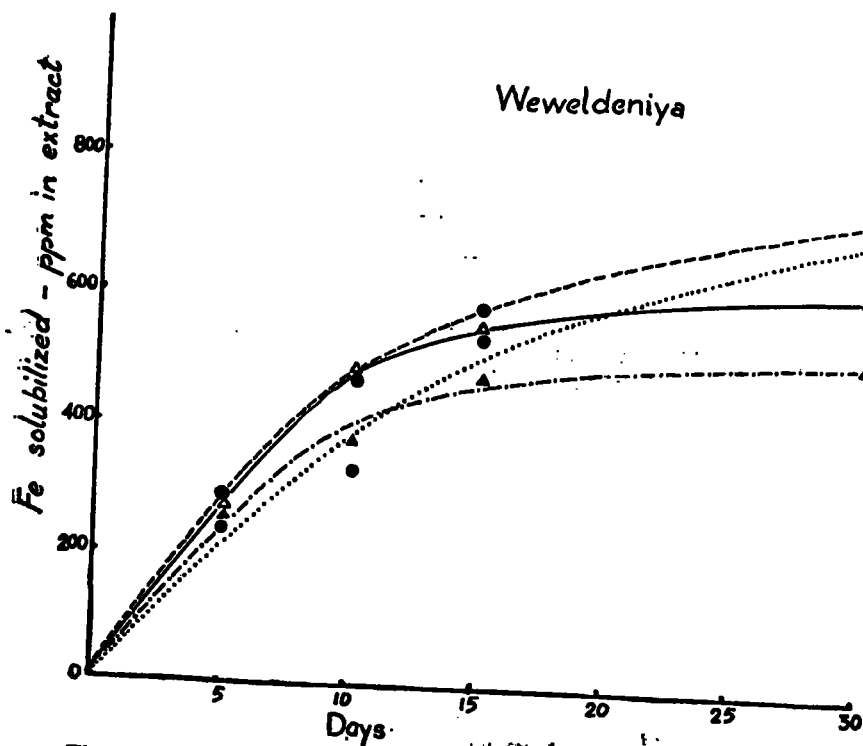
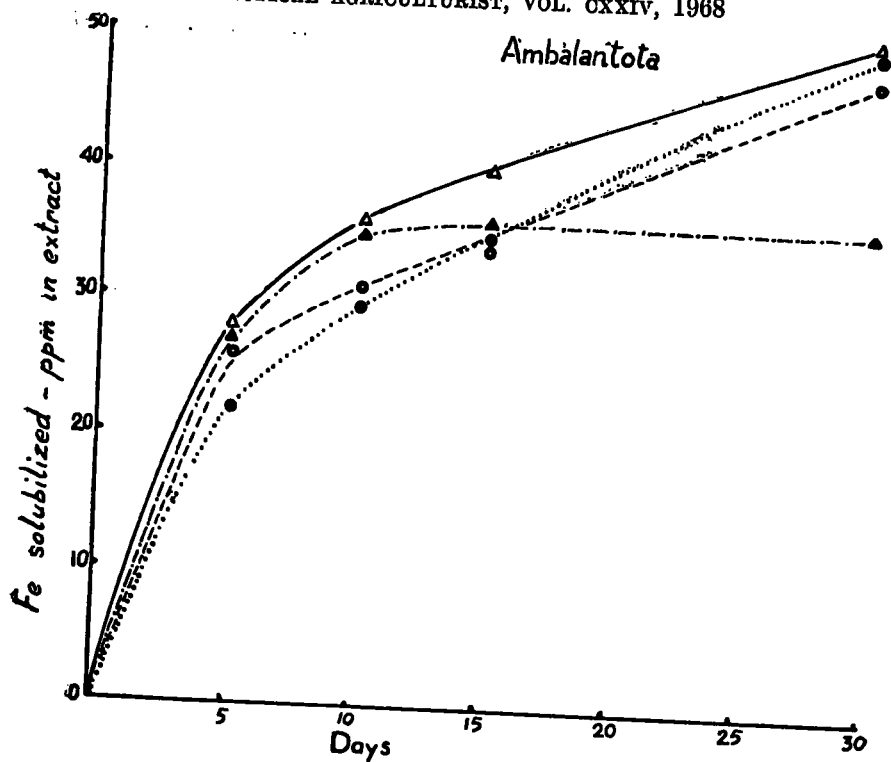


Fig. 1. Continued from page 83

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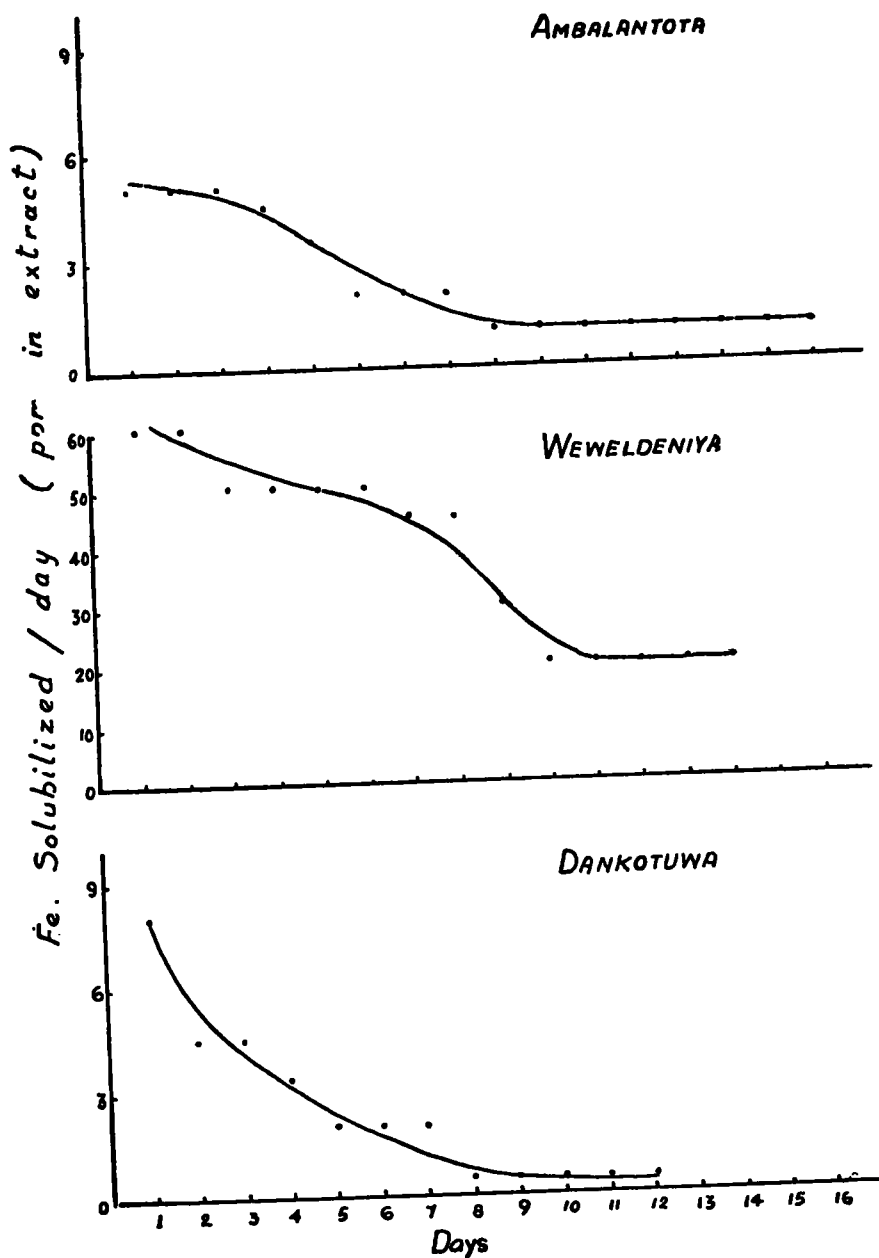


Fig. 2. Rate curves of iron solubilized form soils at the unamended pH values with 5 per cent sucrose.