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Waite Agricultural Research Institute,
Adelaide, Australia.

AVAILABILITY OF PHOSPHORUS AND COPPER IN SOME AUSTRALIAN SOILS, WITH SPECIAL REFERENCE TO THE SEDDON SERIES OF KANGAROO ISLAND.

A Thesis submitted
by
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in

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PART I. PHOSPHORUS

I. INTRODUCTION

There are few inorganic compounds of agricultural importance, which have been so extensively studied and so little understood, as phosphates. As Burd (1948) has aptly remarked "the interpretations of phosphate behaviour in soil involve some of the most interesting and baffling problems in the domain of Soil Science". This is largely owing to the diversity of types of complexes in which the phosphate ion occurs, and the variety of conditions which affect the solubility of the phosphate carrier.

The role of phosphorus in plant nutrition hardly needs any emphasis. Its deficiency is very wide-spread in the World and in many countries such as Australia and South Africa crop production is limited over enormous areas by phosphate supply (Russell, 1952). Like any other nutrient problem, the phosphate problem is also that of deficiency and availability and perhaps the latter more than the former, in some cases. The absolute deficiency of a nutrient is easy to correct by the use of fertilisers, provided there are no further complications, but in the case of phosphate similar fertiliser treatments on different soils have produced different results because of the phosphate and soil interactions being so complex as to defy any universal solution. Unlimited dressings of superphosphate - a time honoured remedy for phosphate deficiency - are neither economical nor feasible, for after all the rich deposits of phosphate and sulphur are limited.

Despite the voluminous literature that has grown around the two aspects of the phosphate problem - fixation and availability - it is very disappointing that the problem is still as elusive as ever. These terms have become commonplace but difficult to define. The available phosphate as generally understood depends as much on the plant as on the soil and perhaps more on the latter than on the former.

The need, from the point of view of land settlement, to study the fertility status of the Seddon Series of Kangaroo Island presented an opportunity for studying the availability of phosphate in these soils. For the proper study of the available phosphate in these soils it is important:

(a) To assess the existing phosphate status of the soil.

(b) To ascertain the future of the applied phosphate to the soil.

(c) To measure the actual response of plants in the field over a period of years.

The Seddon Series, which occur extensively on Kangaroo Island (Northcote and Tucker, 1948), have developed on pre-Cambrian shales and are confined to an undulating plateau top or eroded plateau remnants. These soils have been classified (Northcote et al., ibid) as podsols,containing laterite or ironstone gravel, which occurs in the profile as an outstanding feature. They are the residuals on truncated laterite profile and are highly weathered. These series possess a distinctive overall yellow brown colour throughout the profile, although the intensity of the colour varies and the subsoil, particularly the deep subsoil, is mottled.

The general level of fertility of these soils is low and the phosphate is the initial limiting factor to pasture development. The mean P_2O_5 content in the surface soils of the Seddon series is 0.011% (Northcote et al., 1948) which is a sufficient proof of their absolute deficiency in the nutrient. Their low phosphate content, as compared to the Urrbrae Fine Sandy Loam and other red brown earths which are also derived from the pre-Cambrian shales and have a model value of P_2O_5 0.04 to 0.05% (Piper, 1938), is also suggestive of the very severe weathering of the parent material of these soils. As suggested by Piper and Prescott (1949) and Prescott (1952) it seems quite likely that factors other than the normal weathering processes may also have been instrumental in producing such low resultant figures.

Besides the basic phosphate deficiency, these soils also have an availability problem. As in field experiments it has been found (Report 1941-44,45) that at least 10 cwt. of superphosphate per acre is required to get an optimum yield of the pasture. The principal object of the investigations outlined in this part of the thesis is to study the changes in the availability of phosphate, when it is applied as a fertiliser, to correct the deficiency of the nutrient in the soil.

Soils

The Seddon Gravelly Sandy Loam, virgin soil as a representative of the Seddon Series, was chosen for the study because of its extensive occurrence and because some fertility problems of this soil are being examined on an experimental block. From the uncleared country, soil samples representative of the Seddon Gravelly Sandy Loam (0-4") were collected by A.G. Tyson of this laboratory during 1951, and these samples have been used for all the investigations reported in this thesis. For comparison, a Sample of Urrbrae fine Sandy Loam, virgin, (0-4") soil from the Arboretum of the Waite Agricultural Research Institute, was also collected. Laboratory data of these samples and of others used for these investigations, along with the abbreviations used to designate these soils, are given in Table 1.

TABLE 1

Analytical data relating to soils used
(on air dry basis)

Soil	Urrbrae fine sandy loam (0-4") U287	Urrbrae fine sandy loam virgin (0-4")	Seddon gravelly sandy loam virgin (0-4")	Seddon gravelly sandy loam virgin (4-8")	Seddon gravelly sandy loam experimental block (0-4")
Abbreviated name	U287	Urrbrae.V. (0-4")	Seddon.V. (0-4")	Seddon.V. (4-8")	Seddon (E.B.)
pH	6.51	5.91	6.19	6.02	6.05
* **Analysis of fine earth < 2mm.**	%	%	%	%	%
Mechanical analysis					
Coarse sand	$\frac{3}{4}$	4	$16\frac{3}{4}$	$18\frac{1}{8}$	$22\frac{3}{4}$
Fine sand	$42\frac{1}{2}$	$46\frac{3}{4}$	$58\frac{3}{4}$	$58\frac{3}{4}$	$55\frac{1}{8}$
Silt	$32\frac{1}{4}$	$11\frac{1}{8}$	11	$8\frac{1}{8}$	$8\frac{1}{4}$
Clay	$20\frac{3}{4}$	16	9	12	9
Loss on ignition	4.9	4.9	7.2	4.1	8.6
Organic carbon	1.22 C/N 10	1.33 C/N 9	1.60 C/N 20	0.85 C/N 16	2.40 C/N 12
Nitrogen	0.125	0.147	0.081	0.054	0.202
P_2O_5	0.050	0.049	0.011	0.009	0.033
Total soluble salts	0.020	0.015	0.019	0.013	0.035
Chlorides as NaCl	0.003	0.002	0.004	-	0.010
Exchange capacity at pH7.0	-	9.9m.e.%	10.2m.e.%	6.6m.e.%	11.4m.e.%
Moisture in air dry soil	2.0%	1.6%	3.6%	1.5%	2.6%
X-ray diffraction analysis of clay fraction < 2μ		**	*** approximate %	*** approximate %	
Quartz		< 10	5	10	
Kaolinite		10 - 20	55	45	
Illite		23 - 47	-	-	
Chlorite		10 - 20	-	-	
Goethite		-	5	2	
Gibbsite		-	10	5	
Unidentified minerals (possibly chlorite or vermiculite)		-	25	35	

* By Chemistry Section, Division of Soils (C.S.I.R.O.)

** As interpreted by E.W. Radoslovich (1951), Division of Soils (C.S.I.R.O.) (Soil colloids of several Adelaide red brown earths. Report 1951)

*** As interpreted by K. Norrish (1953), Division of Soils (C.S.I.R.O.)

II. PHOSPHATE RETENTION

In order to assess the phosphate supplying power of the Seddon V. soil, an attempt was made to obtain some information on phosphate retention capacity and the relative importance of the different factors affecting the retention and release of the phosphate ions.

It is most unfortunate that different workers have used the same term phosphate fixation to imply different meanings. It has been used to denote retention or sorption of phosphate from the solution, adsorption of phosphate precipitation and also its becoming unavailable to plants. It is in fact the latter sense in which the term phosphate fixation can be most appropriately used, while in the ordinary sense, for denoting the loss of PO_4 ions from the solution to the soil,[x] the more comprehensive term retention or sorption is more suitable. Recently Wild (1950), and Owen (1947) have also used the term retention or sorption in this sense.

(i) Phosphate retention capacity of soils

The amount of phosphate retained by soils, when treated with phosphate solutions, depends not only on the mechanical and chemical composition of the soil but also on the concentration of phosphate solution (Russell and Prescott, 1916; Hibbard, 1935; Davis, 1935; Owen, 1947), the ratio of soil to solution, the pH, time of contact and temperature (Black, 1942; Perkins, 1945; Coleman, 1944). Consequently the methods used for the determination of phosphate retention capacity of soils are mainly empirical. Two techniques have been used by most of the workers:-

(a) Shaking the soil with phosphate solutions of known concentration and pH, for a standard time, followed by centrifuging or filtration. Phosphate not sorbed is estimated and the phosphate retained by the soil determined by difference. Being an indirect estimation it has the drawback that very often the change in concentration is small and consequently the experimental error can be considerable.

(b) Leaching the soil with phosphate solutions and then replacing the phosphate retained by the soil by NaOH or KOH solutions (Stout, 1939;

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[x] Way (1850) called it absorption of phosphoric acid.

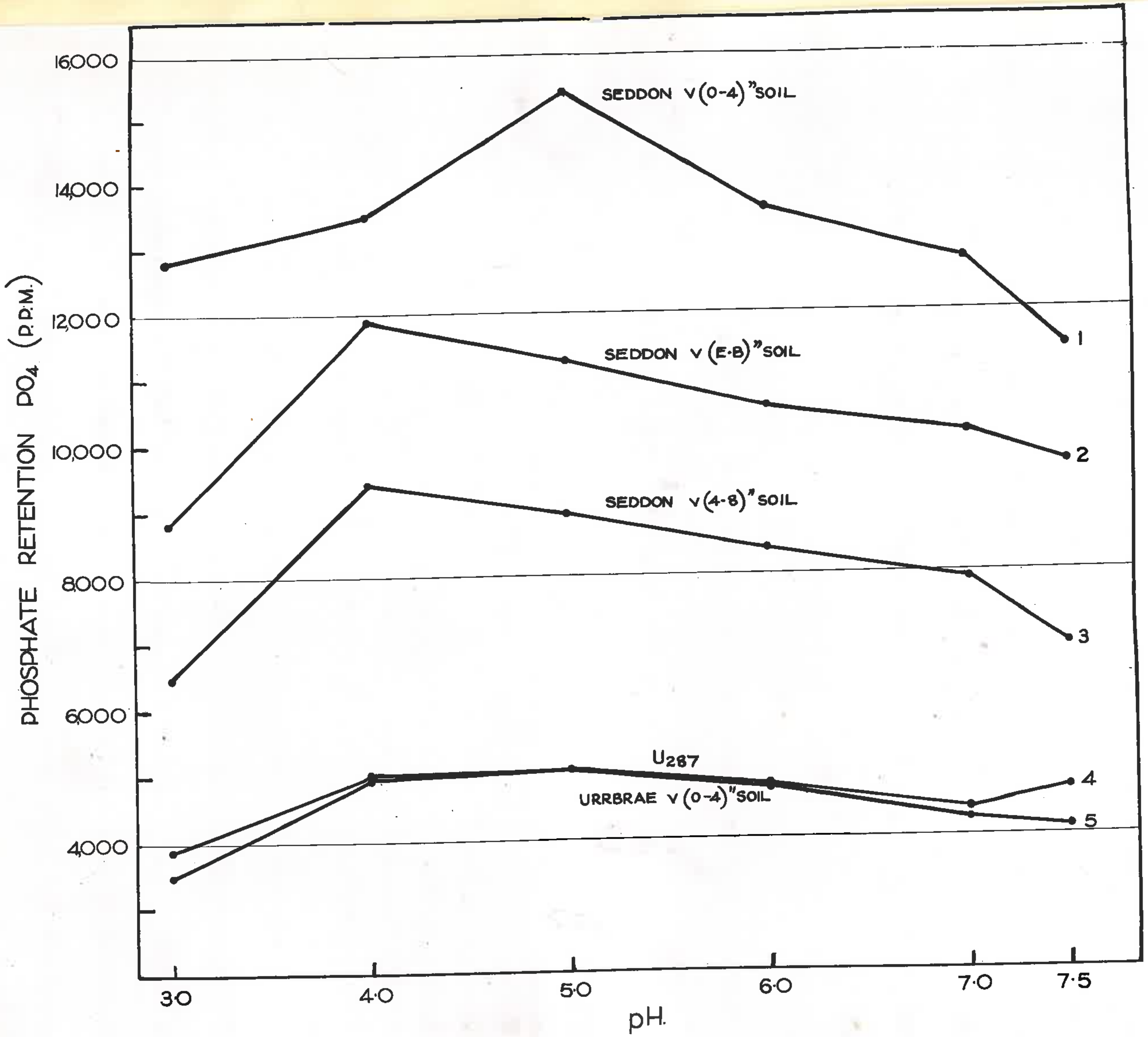


FIGURE I.

Showing phosphate retention capacity of soils at different pH.

Hosking (Piper, 1950), Rubins and Dean, 1947). These methods are analogous to the methods followed for cation exchange capacity. In these methods also, the choice of phosphate buffer, pH of the buffer, ratio of solution to soil, temperature and time of reaction are the important factors, but these methods are more direct than the former and for the studies mentioned here, Hosking's method for determination of anion exchange capacity as outlined by Piper (1950), but with slight modifications, was used. Normal solutions of N. ammonium phosphate buffer of pH 3.0, 4.0, 5.0, 6.0, 7.0 and 7.5 were used. 2.5g. soil or soil separate (1 g. in case of clay) in duplicate were heated for one hour on a water bath at 70°C. and for the estimation of phosphate, Williams' (1950a) technique for the determination of inorganic phosphate in the extract obtained with 0.1N.NaOH, was employed.

TABLE 2

Phosphate retention capacity of soils at varying pH

Soil	Phosphate retained PO_4 (p.p.m.) pH					
	3.0	4.0	5.0	6.0	7.0	7.5
1. U287	3880	5000	5024	4800	4396	4740
2. Urrbrae V.(0-4")	3500	4950	5024	4780	4240	4112
3. Seddon V.(0-4")	12800	13500	15390	13600	12800	11440
4. Seddon V.(4-8")	6504	9400	8960	8040	7920	6912
5. Seddon (E.B.)	8816	11900	11260	10560	10160	9648

A reference to table 2 and Fig.I. will show that within the range of these studies (pH3 to 7.5) the Seddon Soils retained more phosphate than the Urrbrae soils. The phosphate retention capacity of the Seddon V. (0-4") soil is three times as high as that of the Urrbrae V. (0-4") and twice that of the Seddon V. (4-8") soil. These differences are not explainable on the basis of pH, total clay, Kaolin and organic carbon content of these soils (See Table 1), but the role of comparatively high humus content as indicated by the N. content in imparting low phosphate retention capacity to the Urrbrae soils can not be ruled out. This high phosphate retaining power of the Seddon V. (0-4") soil may be partially responsible for the high applications of phosphatic fertilisers required for getting optimum yields on this soil.

It is also interesting that the maximum sorption of phosphates in all the soils was at reactions between pH4.0 to 5.0. It is strongly suggestive of the formation of some compounds with aluminium and iron and not with Kaolin like clay minerals, because many workers (Murphy, 1939; Stout, 1939; Black, 1942; Perkins, 1945; Chatterjee and Datta, 1951) have observed that highest phosphate retention by Kaolin lies in the highly acid range. On the other hand, minimum solubility of phosphates adsorbed by aluminium lies between pH4-6, (Teakle, 1928; Gaarder, 1930; Rathje, 1942; Swenson et al., 1949; Cole & Jackson, 1950; Perkins, 1948), and of those by iron between pH 2 to 4 (Schoen, 1953).

(ii) Phosphate retention by soil separates

To assess the relative role of mechanical separates in phosphate retention by these soils, coarse sand (2-0.2mm.), fine sand (0.2 to 0.02 mm.), silt (0.02 to 0.002mm.) and clay (< 0.002mm.) were separated by dispersing the soil with the ammonium carbonate method of Puri (1935), without removal of organic matter. Phosphate retention capacity was determined only at pH5.0, employing the same procedure as mentioned earlier.

TABLE 3

Phosphate retention capacity of soil separates at pH5.0

	Soil	Phosphate retained PO_4 (p.p.m.)			
		Coarse Sand	Fine Sand	Silt	Clay
		PO_4(p.p.m.)	PO_4(p.p.m.)	PO_4(p.p.m.)	PO_4(p.p.m.)
1.	U287	-	1632	4000	17440
2.	Urrbrae V.(0-4")	2640	1308	4000	19600
3.	Seddon V.(0-4")	6920	8200	31840	43000
4.	Seddon V.(4-8")	3136	2200	18000	34600
5.	Seddon E.B. (0-4")	4040	4200	27520	38250

From Table 3 it may be seen that in all soils phosphate retention increased as the particle size decreased, which is in conformity with the conclusions of many investigators such as Hibbard, (1935); Perkins et al., (1942); Beater (1937); Owen, (1947); Neller and Comar, (1947); Aderekhin et al., (1952). The clay fraction of the Seddon V. (0-4") soil retained twice, while the silt fraction about eight times as much phosphate as their counterparts in the Urrbrae soils. The phosphate retention by the coarse sand fraction of the Seddon V. (0-4") soil was much higher than even the silt fraction of the Urrbrae V. (0-4") soil. Since the Seddon soils have about 90% of the particles bigger than two microns (see Table 1), it means that most of their phosphate retention resides in their coarse fractions and not in the clay, whereas the reverse is the case with the Urrbrae soils. The contribution made by the clay fraction of the Seddon V.(0-4") soil, towards its total phosphate retention capacity is only 20.4% against 62.4% by the corresponding fraction in the Urrbrae V. (0-4") soil.

The higher phosphate retention by the coarse fractions of the Seddon soils may be due to the

(a) Presence of Kaolinite

(b) High amounts of hydrated oxides of iron and aluminium such as goethite and gibbsite.

Hosking (1953) has emphasised that clay minerals like Kaolin are not confined to soil fractions smaller than 2 microns only; they occur in coarser fractions as well. It is probable that in the Seddon soils some Kaolin may be present in these separates also, but it may not be contributing much to their phosphate retention capacity, as coarse Kaolin retains very little phosphate (Midgley, 1940; Raychaudhuri and Mukherjee, 1941; Coleman, 1944; Cooke, 1951).

Graham (1953) reported that 2-20 μ fraction of a lateritic soil (No.10143) from Kangaroo Island, contains a very high amount of gibbsite and since the Seddon soils also belong to the same group of soils they are likely to have a fair amount of gibbsite and possibly goethite.

The importance of free (active) sesquioxides, particularly that of gibbsite in comparison with Kaolinite as a factor for phosphate retention has been emphasised by many investigators such as Midgley, 1940; Coleman, 1944; Sieling, 1946; Mehlich, 1950; Chu and Sherman, 1952. Hence it was thought desirable to investigate the role of free sesquioxides in phosphate retention by the separates of these soils as well as the whole soils.

(iii) Effect of the free (active) oxides of iron and aluminium on the retention of phosphate by soils

The effect of the active iron and aluminium of the soil on phosphate sorption has been studied by most of the investigators by any of the following five approaches:

(i) Correlations have been established between the amount of phosphate retained by the soils and the amount of iron and aluminium dissolved therefrom by acids (concentrated or dilute), (Coleman, 1944; Fraps, 1922; Vries et al., 1938; Raychaudhuri et al., 1941; Metzger, 1941).

(ii) Iron and aluminium compounds have been added to soils and soil colloids and their effect on phosphate retention studied (Doughty, 1930; Scarseth, 1935; Davis, 1943).

(iii) Compounds formed during phosphate retention have been identified by comparing the effect of pH on phosphate retention with the known effect of pH on the solubility of iron and aluminium phosphates (Teakle, 1928; Doughty, 1935; Rathje, 1942; Stelly and Pierre, 1942).

(iv) Phosphate retention by the soils or soil colloids before and after removal of free sesquioxides has been studied (Toth, 1937; Kelly and Midgley, 1943; Black, 1942; Chandler, 1941; Metzger, 1941; Coleman, 1942, 1944).

(v) Sesquioxides have been rendered ineffective by chelation with suitable complexing agents such as oxine (Ghani, 1943b), citrate anions (Struther and Sieling, 1950), seleneous acid (Cooke, 1951), and the phosphate retention studied.

All of these methods are open to criticism, but the last two approaches, in spite of their shortcomings, have found more favour with a large number of workers. The serious drawback of the commonly used methods of removing free ironoxides like the acid oxalate method of Tamm (1922), the H_2S method of Trueg et al., (1936), is their attack on clay minerals. The microbiological reduction method of Allison and Scarseth (1942) and the modified hydrosulphite method by Deb (1950), although seemingly promising, have not been well established for critical work. Jeffries' method (1946) has been found, by Williams (1950) in Australia, Rice and Levick (1953) in Canada, to dissolve very small amounts of silica from soils, and this may be taken as an indication of its less vigorous attack on clay minerals. Deb (1950) has also pointed out that the stability of clay minerals towards reducing agents commonly used for removing free iron oxides, is in the decreasing order Kaolinite, Illite and Montmorillonite, respectively. Since the Seddon soils are in the present state of knowledge presumed to be kaolinitic and the Urrbrae soils Illitic-kaolinitic (Table 1), the effect on clay minerals is not likely to vitiate the results. Considering these points, the method of Jeffries, as slightly modified by Williams (1950) eliminating the pretreatment with H_2O_2, was used. Iron in the extract was determined by the dichromate method as adapted by Haldane (1953) and aluminium by grovimetric method (Piper, 1950).

(a) **Phosphate retention by the soils after the removal of free oxides of iron and aluminium**

A reference to Table 4 shows that:

(i) All the soils suffered a substantial decrease (about 80%) in phosphate retention after the treatment with the method of Jeffries, though the decrease was much greater in the case of the Seddon soils than in the Urrbrae soils.

(ii) The decrease also depended on the pH of the phosphate solution. Thus in all cases the maximum decrease occurred at pH5.0, though the phosphate retention was still higher at this pH than at pH3.0 or 7.0.

(iii) When the reduction in phosphate retention capacity is considered in relation to the amount of iron and aluminium oxides removed by the method of Jeffries, and the molecular ratio of PO_4/R_2O_3 is calculated as in Table 4 the distinction between these two series of soils becomes quite apparent.

(iv) Within the series and at the same pH these ratios in different soils are very close to each other. Thus at pH3.0, 5.0 and 7.0 the average PO_4/R_2O_3 ratio in the Urrbrae soils is 0.142, 0.175 and 0.142 respectively, whereas in the Seddon soils it is 0.232, 0.303 and 0.276 respectively.

These results show that the phosphate retention in these soils is dependent not only on the amounts but also on the reactivity of the sesquioxides present therein. Thus the Seddon soils have nearly twice as reactive sesquioxides as the Urrbrae soils. This may have some practical implications on the availability of phosphates in these soils. Tyson (1953) in pot experiments observed that from an application of monocalcium phosphate equivalent to 2 cwt. super. per acre, in the case of Seddon V. (0-4") soil, subterranean clover, utilised only 20.8% of the applied phosphate, whereas from the Urrbrae V. (0-4") soil under the same conditions 38.7% was available. Likewise after incubating these soils for 13 weeks with $Ca[H_2PO_4]_2 H_2O$ equivalent to 12 cwt. super per acre, the amount of phosphate extractable with the borate buffer + versenate, was found to be 122 p.p.m. (see Table 26 , page 43) PO_4 in the case of Seddon V. (0-4") soil against 220 p.p.m. from the Urrbrae V. (0-4") soil. These results confirm that in the Seddon V. (0-4") soil, phosphate fixation is twice as

TABLE 4

Effect of removal of sesquioxides by the method of Jeffries on the retention of phosphate by soils

Soils	Phosphate retention after removal of free sesquioxides by the method of Jeffries at pH			Reduction in phosphate retention due to removal of free sesquioxides at pH			Amount of sesquioxides dissolved in Jeffries' extractant (Moles %)			Molecular ratio of PO_4/R_2O_3: Reduction in the amount of phosphate retained (moles PO_4 per 100 g. of soil) / Moles % of sesquioxides dissolved by the extractant		
	3.0	5.0	7.0	3.0	5.0	7.0	Fe_2O_3% Moles %	Al_2O_3% Moles %	Total R_2O_3% Moles %	at pH3.0	at pH5.0	at pH7.0
	PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)						
1. U287	970	1220	1170	2910	3804	3226	1.487	0.850	2.337	0.130	0.172	0.145
2. Urrbrae V.(0-4")	950	1138	1190	3350	3886	3050	1.450	0.842	2.292	0.153	0.178	0.140
3. Seddon V.(0-4")	3080	3280	2512	9000	12110	10228	1.312	2.86	4.172	0.228	0.305	0.259
4. Seddon V.(4-8")	1300	1880	1256	5204	7080	6644	1.212	1.23	2.442	0.224	0.305	0.287
5. Seddon (E.B.)	1600	2420	1816	7216	8840	8344	1.225	1.87	3.095	0.245	0.300	0.283

active as in the Urrbrae V. (0-4") soil. The higher PO_4/R_2O_3 ratio at pH5.0 than at pH3.0 and 7.0 is also indicative of the formation of less soluble compounds of phosphate at pH5.0 than at pH7.0, which can be expected as aluminium and iron phosphates are least soluble at this pH (Stelly and Pierre, 1942).

(b) <u>Phosphate retention by the soils after saturation with 8 hydroxy-quinoline at pH5.0</u>

To study the role of sesquioxides in the retention of phosphate, 2.5g. of soils were mixed with 0.2 - 0.4g. of 8 hydroxy-quinoline (oxine) before adding N. ammonium phosphate buffer (pH5.0), previously saturated with oxine.

<u>TABLE 5</u>

Phosphate retention after treatment with Jeffries' method and with 8 hydroxy-quinoline

Soils	Phosphate retention after treatments by		Mean	Mean for the series
	8 hydroxy-quinoline	Jeffries' method		
	A. PO_4 (p.p.m.)	B. PO_4 (p.p.m.)	PO_4 (p.p.m.) ± 60.8	
	± 85.98			
U.287	1050	1220	1135	1094.5
Urrbrae V.(0-4")	970	1138	1054	
Seddon V.(0-4")	3640	3220	3430	2626.7 significant at 0.1%
Seddon V.(4-8")	1960	1880	1920	
Seddon (E.B.)	2640	2420	2530	
	± 38.45			
	A.	B.		
Mean	2052.0 N.S.	1975.6		

L.S.D.	5%	1%	0.1%
Body of the Table	280.4	407.9	613.0
Vertical Margins	198.3	288.5	433.4
Horizontal Margins	125.4	182.4	274.1

<u>NOTE:</u> (i) Differences between methods A and B are non significant

(ii) Differences between both the series of soils are highly significant.

TABLE 6

Reduction in phosphate retention at pH5.0 after the treatment with 8 Hydroxy-quinoline or the removal of free sesquioxides

Soils	Phosphate retention at pH5.0 PO_4 (p.p.m.)			Decrease in phosphate retention due to chelating effect of 8 Hydroxy-quinoline	Decrease in phosphate retention due to removal of free sesquioxides	Free sesquioxides removed by Jeffries' treatment (Moles R_2O_3%)	Ratios PO_4/R_2O_3	
	Before removing free oxides	A. After saturation with 8 Hydroxy-quinoline	B. After removal of free oxides by Jeffries' method				By method A.	By method B.
	PO_4 (p.p.m.)	PO_4(p.p.m.)	PO_4(p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)	(moles %)	(Moles PO_4/R_2O_3)	
U.287	5024	1050	1220	3974	3804	2.337	0.179	0.172
Urrbrae V.(0-4")	5024	970	1138	4054	3886	2.292	0.186	0.178
Seddon V.(0-4")	15390	3640	3220	11750	12170	4.172	0.297	0.305
Seddon V.(4-8")	8960	1960	1880	7000	7080	2.442	0.301	0.305
Seddon (E.B.)	11260	2640	2420	8620	8840	3.095	0.293	0.300

TABLE 7

Effect of removal of sesquioxides by the method of Jeffries on the retention of phosphate by the soil separates

Soil separates	Phosphate retention capacity at pH5.0 before removal of sesquioxides	Phosphate retention capacity after removal of free sesquioxides	Reduction in the retention of phosphate due to removal of free oxides	F_2O_3 dissolved by the extractant Fe_2O_3%	Al_2O_3 dissolved by the extractant Al_2O_3%	Total Al_2O_3 and Fe_2O_3 dissolved by the extractant expressed as R_2O_3 moles %	Ratio PO_4/R_2O_3 $\frac{PO_4 \text{ moles } \%}{R_2O_3 \text{ moles } \%}$
	PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)	Fe_2O_3%	Al_2O_3%	R_2O_3 moles %	
U.287							
Coarse sand	-	-	-	-	-	-	-
Fine sand	1632	360	1272	0.96	0.64	1.227	0.109
Silt	4000	700	3300	1.98	1.32	2.532	0.137
Clay	17440	3200	14240	7.00	2.23	6.552	0.228
Urrbrae V. (0-4")							
Coarse sand	2640	2320	320	2.37	0.42	1.894	0.018
Fine sand	1308	300	1008	0.98	0.52	1.122	0.095
Silt	4000	760	3240	2.16	0.91	2.242	0.152
Clay	19600	3200	16400	7.00	2.22	6.553	0.263
Sedden V. (0-4")							
Coarse sand	6920	5170	1750	1.67	0.86	1.887	0.098
Fine sand	8200	3200	5000	1.40	2.76	3.581	0.147
Silt	31840	7500	24340	5.16	6.52	9.617	0.266
Clay	43000	8240	34760	5.65	4.15	7.601	0.482
Sedden V. (4-8")							
Coarse sand	3136	1688	1448	1.62	0.43	1.421	0.107
Fine sand	2200	550	1650	1.86	1.27	1.906	0.091
Silt	18000	3280	14772	4.04	3.45	5.907	0.263
Clay	34600	8080	26520	5.00	2.29	5.370	0.520
Sedden (E.B.)							
Coarse sand	4040	2360	1680	1.62	1.08	2.076	0.085
Fine sand	4200	1100	3100	1.28	1.73	2.495	0.131
Silt	27520	8080	18440	4.22	4.48	7.028	0.276
Clay	38250	4960	33290	4.50	3.66	6.402	0.548

The amount of phosphate retained (Table 5) after saturation with oxine shows that this treatment reduced the phosphate retention to the same extent as did the removal of the iron and aluminium oxides. Assuming that 8 Hydroxy-quinoline complexed the same amount of Fe and Al as was removed by the other treatment the calculated ratios $^{PO_4}/_{R_2O_3}$ (Table 6) in both cases are very close to each other. This is strong corroborative evidence of the fact that it is the active aluminium and iron which are mainly responsible for the retention of phosphate. Secondly, the differences between the two series of soils - Urrbrae and Seddon - as indicated by the $^{PO_4}/_{R_2O_3}$ ratios obtained with this method also substantiate the evidence of the differences in the reactivities of the sesquioxides in both these Series of soils.

(iv) Phosphate retention by the soil separates after removal of free oxides of iron and aluminium by the method of Jeffries.

The reduction in phosphate retention brought about by the removal of free sesquioxides from the different mechanical separates of these soils (Table 7) also confirms that phosphate retention was mainly due to sesquioxides, though this treatment did not reduce the phosphate retention to the same extent in different separates. The reactivity of the sesquioxides ($^{PO_4}/_{R_2O_3}$) as measured by the decrease in phosphate retention due to the amount of iron and aluminium dissolved by the method of Jeffries, increased with the decrease in particle size, which seems to be quite logical. Thus the sesquioxides removed from the clay fractions were nearly twice as reactive as those from the silt fractions. The reactivity of sesquioxides present in silt and clay fractions of the Seddon soils is also nearly twice, as compared to their counterparts in the Urrbrae soils, as is borne out by the molecular ratios of $^{PO_4}/_{R_2O_3}$ in both these series (Tables 7 and 8).

TABLE 8

Showing average ratio of $^{PO_4}/_{R_2O_3}$

Soil series	Molecular Ratio of $^{PO_4}/_{R_2O_3}$			
	Coarse sand	Fine Sand	Silt	Clay
Urrbrae	0.018	0.101	0.144	0.245
Seddon	0.096	0.123	0.268	0.517

The lower $^{PO_4}/_{R_2O_3}$ ratio in the case of Urrbrae soils as compared with the Seddon soils may be due to the differences in the:

(a) Nature of sesquioxide minerals (iron and aluminium oxides) - their relative proportion and degree of hydration.

Haseman et al. (1950) observed that gibbsite has greater phosphate retention capacity than goethite and far greater than the clay minerals. Likewise Mehlich (1952) reported that anion exchange capacity (PO_4 retention capacity) of aluminium and iron oxide minerals was, in the decreasing order, gibbsite, goethite, bauxite and haematite. As the Seddon soils, in comparison with the Urrbrae soils, have predominance of alumina over iron oxides (Tables 4 and 7) they are expected to retain more phosphate than the latter. Moreover, since the retention of phosphate by these hydrated oxides involves an exchange of hydroxylions, and the extent of this exchange depends on their degree of hydration (Ford, 1933; Weiser, 1933; Heck, 1934; Dean, 1934; Kelly and Midgley, 1943; Perkins and King, 1944) which may be another contributing factor to this difference

(b) Amount of iron and aluminium in complex forms with the organic matter.

The higher nitrogen and organic phosphorus content and the darker coloured extracts obtained with sodium versenate, sodium pyrophosphate and sodium hydroxide, suggest that the Urrbrae soils have much higher amount of humified organic matter than the Seddon soils. Since iron and even aluminium are known to exist in complex forms with humus (Aarnio, 1913; Udulf, 1924; Waksman, 1938; Winters, 1940; Bremner et al., 1946; Deb, 1949; Bloomfield, 1953) it is very likely that the Urrbrae soils have higher amounts of iron and aluminium existing in complex forms with the humus than the Seddon soils. As iron and aluminium present in such organic complexes are not altogether available for reactions with the phosphate (Doughty, 1930, 1935; Tyulin et al., 1952; Dalton et al., 1952) the amount of Fe_2O_3 and Al_2O_3 dissolved by the method of Jeffries does not necessarily represent the amount of reactive R_2O_3, and consequently the PO_4/R_2O_3 ratio in these soils may be low.

(c) Degree of crystallinity

It is also probable that in the oxides of aluminium and iron present in these two series of soils there is some difference in their degree of crystallinity which is such an important a factor in the fixation and release of phosphate (Fujiwara, 1950).

There is another important aspect which deserves some consideration. Even after treatment with the method of Jeffries, the soils as well as the soil separates still retained appreciable amount of phosphate. As this method is not designed to remove free alumina (Williams, 1950) and the amount of aluminium dissolved is only incidental, and some iron is also left undissolved (see Table 9).

TABLE 9

Total and free iron oxide in soils and clay fractions

	U287		Urrbrae V. (0-4")		Seddon V. (0-4")		Seddon V. (4-8")		Seddon (E.B.)	
	soil	clay	soil	clay	soil	clay	soil	clay	soil	clay
Total Fe_2O_3% *	3.70	10.1	3.68	10.70	4.50	7.95	4.88	6.20	5.00	-
Free Fe_2O_3% (extractable) (by Jeffries') (treatment)	2.38	7.0	2.32	7.0	2.10	5.65	1.94	5.0	1.96	4.50
Remaining Fe_2O_3%	1.32	3.1	1.36	3.7	2.40	2.30	2.94	1.20	3.04	-

* Total Fe_2O_3 in the soil = Fe_2O_3 dissolved in boiling HCl after 4 hours' digestion.

Total Fe_2O_3 in clay represents the Fe_2O_3 determined after fusion of clay with Na_2CO_3

Moreover in the retention of phosphate the role of clay minerals also can not be ignored. All these factors can account for the residual retention of phosphate. But if we attribute the whole of the remaining retention of phosphate to the residual Fe_2O_3 only, a lower ratio of PO_4/R_2O_3 is obtained. It signifies that all the iron not dissolved by the method of Jeffries is less reactive. Likewise 8 hydroxy-quinoline appears to have complexed only the most reactive iron and aluminium.

(v) Mechanism of retention of phosphate by iron and aluminium in the soil.

Wild (1950) discussing the mechanism of retention of phosphate by the iron and aluminium of the soils, concludes that it is difficult to resolve whether the retention is due to precipitation (Fisher, 1922; Comber, 1925) or chemical adsorption (Mattson, 1931; Ford, 1933; Heck, 1934; Pugh and duToit, 1936; Murphy, 1939; Kelly and Midgley, 1943), which entails exchange of hydroxyl ions from the surface of the hydrated oxides. The distinction between precipitation and adsorption (chemo-sorption) is rather a fine one, because according to Beer (1950) the various forces that hold a visiting molecule for a shorter or longer time at a surface, are exactly the same as those that form molecules from atoms or crystals from molecules. Swensen Cole and Sieling (1949) have adduced some evidence that in the presence of excess of phosphate, iron and aluminium hydroxides form basic phosphates of the type $R(H_2O)_3\ (OH)_2\ H_2\ PO_4$. It is very likely that in the Seddon and Urrbrae soils also, iron and aluminium are forming similar compounds and the reaction can be represented by the general equation:-

$$\left[R(H_2O)_3(OH)_3\right]_n + H_2PO_4 \rightleftharpoons \left[R(H_2O)_3\ (OH)_3\right]_{n-1} \cdot R(H_2O)_3(OH)_2H_2PO_4 + \overline{OH}$$

$$R = Al \text{ or } Fe$$

As an average at pH5.0 n = 4 in the case of clay fraction of Seddon soils and 8 in the corresponding fraction of Urrbrae soils, as the molecular ratios of PO_4/R_2O_3 are 1:2 and 1:4 respectively in both cases. The same mechanism can be extended to explain the reaction of 8 Hydroxy-quinoline in reducing phosphate retention, as 8 Hydroxy-quinoline also replaces the reactive hydroxyl groups.

III. AVAILABLE PHOSPHATE IN SOILS

1. A Review

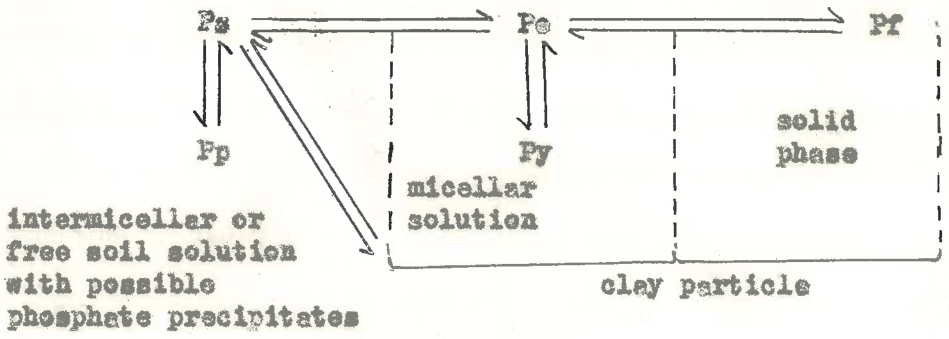
The efforts to determine some fraction of the total phosphate of the soil which may serve as an index of phosphate available to the crops have been made over a century. Since 1845 when Daubeny proposed extraction of available phosphate with CO_2 and Dyer (1894) suggested the use of citric acid, hundreds of extractants have been proposed, but not one of them is universally applicable.

After Behrens (1937) reviewed these laboratory methods, further reviews have appeared in Soils and Men (1938), Soils and Fertilisers (1950) and recently Williams (1952) has critically reviewed the whole situation.

In laboratory methods of extracting the available phosphate, an attempt is made to simulate the action of plants and most of these results are correlated with pot cultures or field trials. In spite of their shortcomings, they still remain the most popular and convenient tools in the hands of soil chemists and agronomists who are called upon to do advisory work.

The common laboratory methods are biological - Mitscherlich, Neubauer, and *Aspergillus niger* techniques - and chemical extractants - water, acids, alkalis, salts and buffer solutions. In recent years considerable attention has also been focussed on the methods of fractionation of phosphates, evaluation of anion and cation saturation capacity (Dean and Rubins, 1947; Birch, 1950, 1952) and radio active isotope technique (Fried and Dean, 1952). All of these chemical extractants are empirical and suitable only under certain limited conditions (Fraps and Fudge, 1945; Weeks and Karraker, 1941, Rubins and Dean, 1946). There another common failing is that the phosphate extracted is not the phosphate actually dissolved by the extractants, but the amount left in the solution after the adsorption of the dissolved phosphate. Thus the extractable phosphate depends not on the nature of the phosphate minerals but on the phosphate fixing factors. To overcome this defect in most of these methods a short time of extraction is recommended. Beater (1949) in Natal used 30 seconds' extraction with $^{N}/_{21}$ H_2SO_4. Such short time extractions besides being difficult to handle give results of low reproducibility.

The distribution of phosphate in the soil can be considered according to the schematic representation of Wiklander (1950)



Ps = phosphate in the free soil solution

Pe = exchangeable phosphate adsorbed on the particle surface

Pf = phosphorus fixed in the surface layer of the particles

Py = phosphate present as free salt in the micellar solution

Pp = phosphate precipitated as difficultly soluble compounds of Fe, Al and Ca, etc.

Ps, Pe and Py are the readily available forms at any time. From this it will also be appreciated that as the system is in dynamic equilibrium and a slight change in any portion will change the whole balance. Thus the phosphate from the soil available to the plants may be determined by the three factors:

(a) The intensity factor - the sum of Ps, Pe and Py.

(b) The ease of defixation factor - rate of conversion of $Pf \longrightarrow Pe$ and of $Pp \longrightarrow Ps$. It depends on soil, plant micro-organisms and climatic factors.

(c) The capacity factor - amount of Pf and Pp. The general equation for the available phosphate can be written as $-\frac{dP}{dt} = Kfi\ fe\ Cp$ (fi = intensity factor; fe = ease of defixation; Cp = capacity factor). The availability of phosphate in a soil could be determined by calculating all these factors. At the present state of knowledge, about the interaction of soil, plant, micro-organisms and climatic factors on this equilibrium, the solution of this equation is not possible.

The best that can be done, is to approximate to the readily soluble phosphate by determining the amount of Ps + Pe + Py, using suitable extractants. Since the amount of Ps + Pe + Py also depends on Pf and Pp, hence it is necessary to get a fair estimate of these slowly soluble forms of phosphate as well. This is only possible if some method of fractionation of phosphate is used so as to separate Ps + Pe + Py from the slowly soluble and extremely slowly soluble forms. Fisher and Thomas (1935) by extracting separate samples of soils with acetic acid - sodium acetate solution of pH5.0 for different periods and also with sulphuric acid solution of pH2.0, divided the phosphates of the soil into three groups. The main defect in the method is that there is no safeguard against the readsorption of the phosphates from solution. Secondly certain arbitrary factors are used for the calculation of the above-mentioned three forms of phosphate. R. Williams (1937) differentiated between fractions of phosphate soluble and insoluble in sodium hydroxide. This method besides other defects, had the disadvantage that the fraction of phosphate soluble in the alkali would depend on calcium content of the soil, because of the precipitation of calcium phosphate under alkaline conditions. Dean (1938) overcame this defect by extracting with sodium acetate solution prior to extraction with the alkali. He also suggested another extraction with sulphuric acid, after the extraction with the alkali. Ghani (1943a) modified Dean's method by extracting first with acetic acid, followed by sodium hydroxide and finally with sulphuric acid. The main disadvantage of this modification was that it removed interference due to calcium but introduced error due to adsorption of phosphates from the solution by the sesquioxides, as was originally noted by Russell and Prescott (1916). However, to overcome this shortcoming, Ghani (1943b) proposed the use of 8 hydroxy-quinoline as a chelating agent for sesquioxides. C.H. Williams (1950a, b, c) in Australia, simplified Ghani's technique of fractionating phosphate and used it successfully in some Australian soils, especially red brown earths. He differentiates between three categories of phosphate:

(a) soluble in 2.5% acetic acid + 1% 8 hydroxy quinoline

(b) soluble in 0.1N sodium hydroxide solution. This fraction is further subdivided into inorganic and organic phosphorus

(c) phosphate not extractable by either of the two extractants.

Bray (1943) and Bray and Kurtz (1945) extracted adsorbed phosphorus with 0.03N NH_4 F + 0.025N HCl and acid soluble + adsorbed phosphorus with 0.03N NH_4 F + 0.1N HCl. Arnold (1947) incorporated with these a third test using 0.25N HCl + 0.24N. ammonium acetate, for the extraction of "flush" phosphorus. Besides other considerations, the interference of fluoride with the molybdenum blue reaction in the estimation of phosphate, limits the use of this method.

From the foregoing it can be inferred that out of the methods of fractionating the phosphates, Ghani's method as simplified by Williams (1950a) seems to be promising, but it requires critical tests with a variety of soils before its utility for extracting available phosphate can be established. It may also be emphasised that it is doubtful that any laboratory method can determine the absolute amounts of phosphate available to particular plants grown under particular conditions. They can determine only the amounts of compounds of phosphorus readily soluble under the conditions of the test.

ii. Laboratory studies of the availability of phosphate - Changes in the forms of phosphate as affected by time.

The experiment reported here is a laboratory attempt to study the changes in the forms of phosphate as affected by time, in the Seddon V. (0-4") and the Urrbrae V. (0-4") soils, after the application of different phosphatic fertilisers. Williams' method (1950a) was used to determine the different forms in which the applied phosphate distributed itself in these soils.

Monocalcium phosphate $[Ca(H_2PO_4)_2\ H_2O]$ (A.R.) silico phosphate (Fertiliser $<$ 0.2mm) and rock phosphate (Fertiliser $<$ 0.2mm.) at rates equivalent to 0, 4, 12 and 30 cwt. super per acre (4" layer), were added to 100 g. of air dry soils ($<$ 1mm.) contained in small bottles. Six samples were prepared for each treatment and for each soil. Water to 50% of the water-holding capacity of the soil was added to 4 samples. Two of the moistened samples were stored for 6 weeks at 20°C. and another two for 13 weeks and the moisture lost restored occasionally. The remaining two were not moistened and were analysed just after thorough mixing. All samples were air dried and crushed to mix thoroughly and sieved through 0.5mm. sieve before use.

From Tables 10, 11 and Figs. II, III, IV, it will be seen that when the extraction of phosphate was done, just after mixing the soils and fertilisers, the acetic acid + 8 Hydroxy-quinoline solution removed only about 25-50% of the phosphate that was applied to the Seddon soil, while over 70% was recovered from the Urrbrae soil. When the fraction of inorganic phosphate soluble in 0.1N NaOH from both these soils is compared the position becomes reverse (Tables 10 and 11; Figs. V, VI, VII). As these

TABLE 10

Effect of incubation on the phosphates applied to the Seddon V. (0-4") soil

Period of incubation	Control (no phosphate applied)	Phosphate applied = 4 cwt. super/acre as 132p.p.m.			Phosphate applied = 12 cwt. super/acre as 396p.p.m.			Phosphate applied = 30 cwt. super/acre as 990p.p.m.			Remarks
		mono-calcium phosphate	silico phosphate	rock phosphate	mono-calcium phosphate	silico phosphate	rock phosphate	mono-calcium phosphate	silico phosphate	rock phosphate	
First fraction soluble in acetic acid + 8 hydroxy-quinoline (PO_4 p.p.m.) *											* fraction soluble in acetic acid + 8 hydroxy-quinoline is also the fraction of phosphate recovered in this case as the value of control is nil.
Initial (0 weeks)	Nil	35	31	30	123	120	126	400	535	505	
6 weeks	Nil	5	8	14	33	61	102	161	253	408	
13 weeks	Nil	8	6	14	31	36	92	142	207	418	
Second fraction soluble in 0.1N. NaOH (PO_4 p.p.m.) (inorganic)											** phosphate recovered means the value left after subtracting the value of control
initial (0 weeks)	19	82	79	76	197	196	174	344	352	312	
6 weeks	14	82	98	91	248	256	211	568	539	381	
13 weeks	14	82	94	95	247	262	215	607	592	384	
Organic phosphorus soluble in 0.1N. NaOH (PO_4 p.p.m.)											*** total inorganic phosphate recovered is the total recovery in the first and second fractions
initial (0 weeks)	21	26	11	22	23	29	28	28	29	28	
6 weeks	16	25	36	25	27	29	23	26	26	35	
13 weeks	28	29	30	26	32	21	29	33	30	32	
Recovery of inorganic fraction soluble in 0.1N. NaOH (PO_4 p.p.m.) **											
initial (0 weeks)	-	63	60	57	178	177	155	325	333	293	
6 weeks	-	68	83	77	234	242	197	554	525	367	
13 weeks	-	68	80	81	233	248	201	593	578	370	
Total inorganic phosphate recovered (PO_4 p.p.m.) ***											
initial (0 weeks)	-	98	91	87	301	297	281	725	868	798	
6 weeks	-	73	91	91	267	303	299	715	778	775	
13 weeks	-	76	86	95	264	284	293	735	785	788	

TABLE 11

Effect of incubation on the phosphate applied to the Urrbrae V. (0-4") soil

Period of incubation	Control (no phosphate applied)	Phosphate applied ≡ 4 cwt.super /acre (132 p.p.m.) as			Phosphate applied ≡ 12 cwt.super /acre (396 p.p.m.) as			Phosphate applied ≡ 30 cwt.super /acre (990 p.p.m.) as		
		monocalcium phosphate	silico phosphate	rock phosphate	monocalcium phosphate	silico phosphate	rock phosphate	monocalcium phosphate	silico phosphate	rock phosphate
First fraction soluble in acetic acid + 8 hydroxy-quinoline (PO$_4$ p.p.m.)										
initial (0 weeks)	15	105	120	100	320	343	312	775	789	771
6 weeks	15	62	76	92	211	248	289	516	574	717
13 weeks	16	72	77	98	188	228	273	505	579	708
Second fraction soluble in 0.1N. NaOH (inorganic phosphate PO$_4$ p.p.m.)										
initial (0 weeks)	67	95	95	97	132	111	103	183	162	140
6 weeks	66	111	118	98	213	189	148	390	368	185
13 weeks	69	120	120	98	208	214	146	374	354	206
Organic phosphorus (PO$_4$ p.p.m.) soluble in 0.1N. NaOH										
6 weeks	248	286	253	282	249	242	244	241	247	255
13 weeks	258	252	264	246	252	242	263	268	254	256
First fraction soluble in acetic acid + 8 hydroxy-quinoline (PO$_4$ p.p.m.) Recovery *										
initial (0 weeks)		90	105	85	305	328	297	760	774	756
6 weeks		47	61	77	196	233	274	501	559	702
13 weeks		56	61	82	172	212	257	489	563	692
Second fraction soluble in 0.1N. NaOH (inorganic phosphate PO$_4$ p.p.m.) Recovery *										
initial (0 weeks)		28	28	30	65	44	36	116	95	73
6 weeks		45	52	32	147	123	82	324	302	119
13 weeks		51	51	29	139	145	77	305	285	137
Total inorganic phosphate (first + second fractions) (PO$_4$ p.p.m.) Recovery *										
initial (0 weeks)		118	133	115	370	372	333	876	869	829
6 weeks		92	112	109	343	356	356	825	861	821
13 weeks		107	112	111	311	357	334	794	848	829

* Recovery means the increase over the control

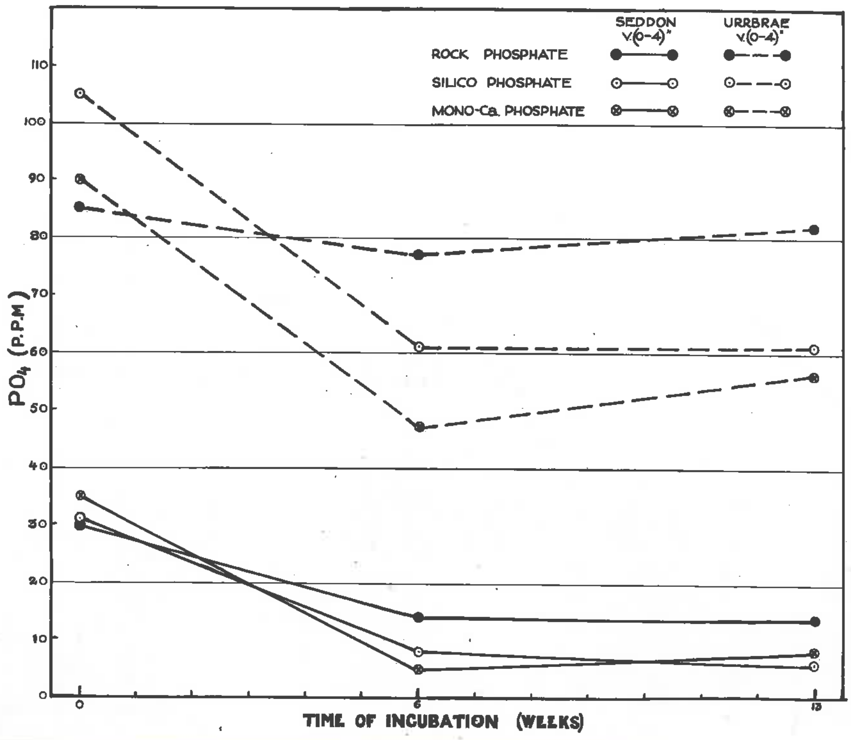


FIGURE II.
Illustrating changes in the amounts of phosphates recovered with acetic acid + 8 hydroxy-quinoline from soils incubated with different fertilisers equivalent to 4 cwt. super per acre.

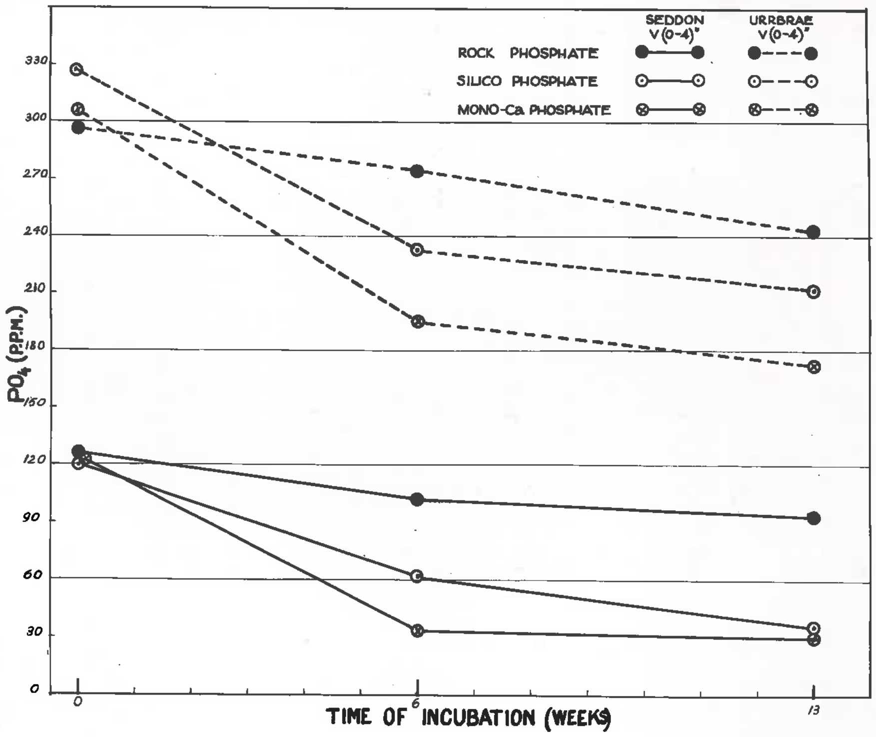


FIGURE III.
Illustrating changes in the amounts of phosphates recovered with acetic acid + 8 hydroxy-quinoline from soils incubated with different fertilisers equivalent to 12 cwt. super per acre.

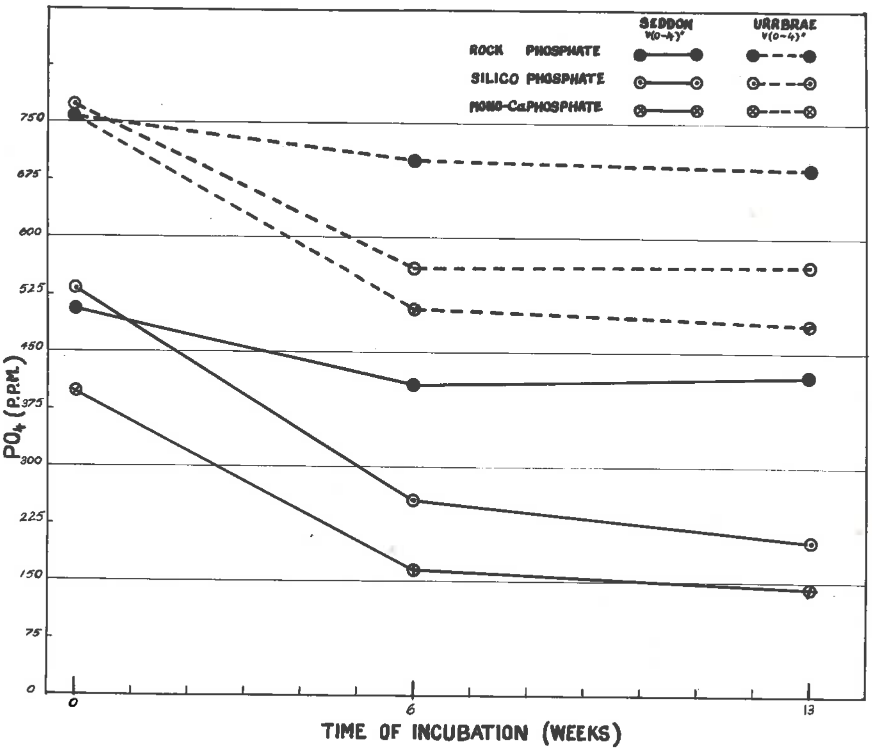


FIGURE IV.

Illustrating changes in the amounts of phosphates recovered with acetic acid + 8 hydroxy-quinoline from soils incubated with different fertilisers equivalent to 30 cwt. super per acre.

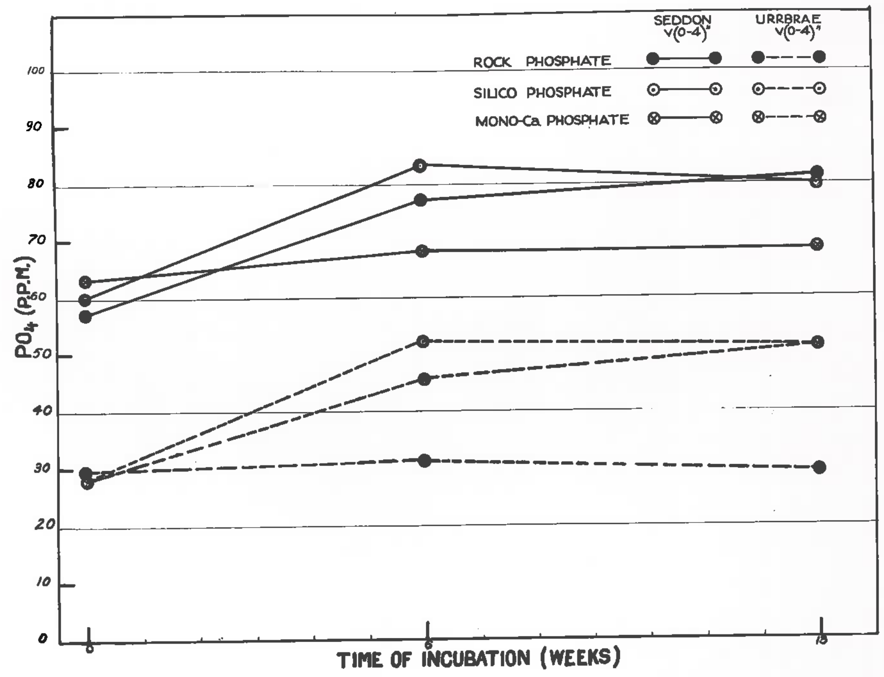


FIGURE V.

Illustrating changes in the amounts of inorganic phosphate recovered in the second fraction soluble in 0.1N NaOH, from soils incubated with different fertilisers equivalent to 4 cwt. super per acre.

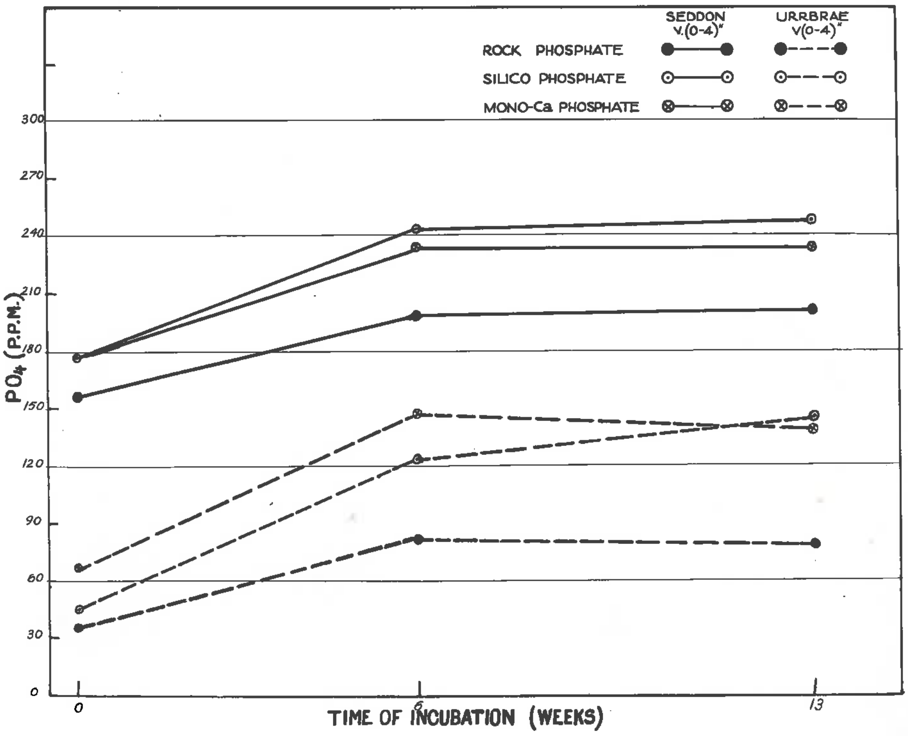


FIGURE VI.

Illustrating changes in the amounts of inorganic phosphate recovered in the second fraction soluble in 0.1N NaOH, from soils incubated with different fertilisers equivalent to 12 cwt. super per acre.

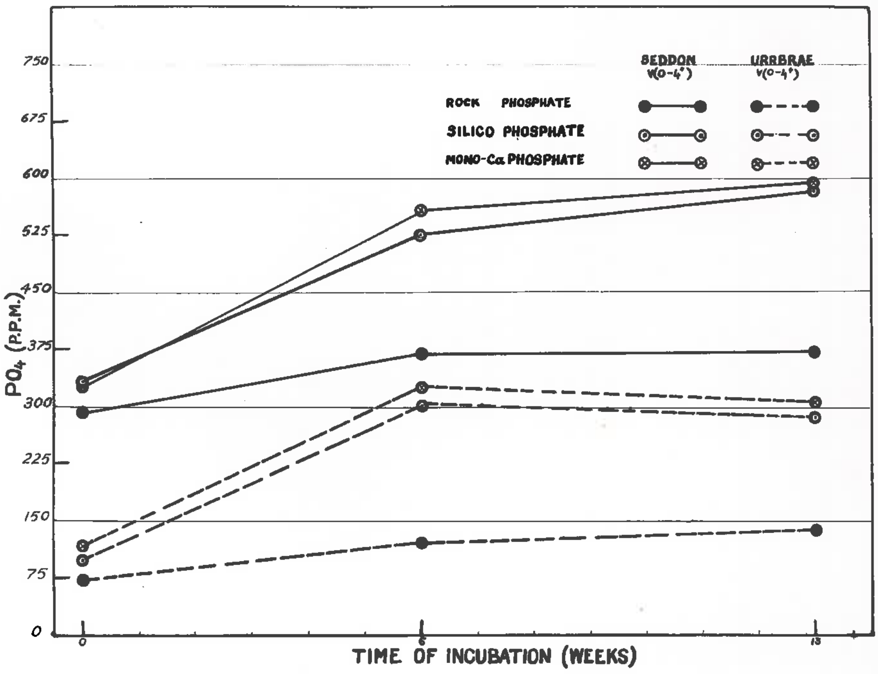


FIGURE VII.

Illustrating changes in the amounts of inorganic phosphate recovered in the second fraction soluble in 0.1N NaOH, from soils incubated with different fertilisers equivalent to 30 cwt. super per acre.

fertilisers are readily soluble in acetic acid + 8 hydroxy-quinoline [Williams, (1950a) and Table 12] the whole of the applied phosphate should have been removed in the first fraction and none in the second.

TABLE 12
Solubility of different phosphatic fertilisers in acetic acid + 8 hydroxy-quinoline and 0.1N NaOH.

Form of phosphate	Weight taken	Total phosphate	Phosphate soluble in acetic acid + 8 hydroxy quinoline during 17 hours shaking	Phosphate soluble in 0.1N. NaOH in 17 hours shaking and after the extraction with acetic acid + oxine	Total phosphate dissolved by both the extractants
	mg.	mg.	(% dissolved)	(% dissolved)	(% dissolved)
Monocalcium					
phosphate	13.2	9.90	91.9	0.2	92.1
$Ca(H_2PO_4)_2H_2O$	26.3	19.80	96.0	0.2	96.2
(A . R)	46.0	34.65	93.1	0.1	93.2
Silico					
phosphate	22.4	9.90	93.5	0.5	94.0
fertiliser	44.9	19.80	95.0	0.6	95.6
(< 0.2mm.)	78.5	34.65	92.9	0.3	93.2
Rock					
phosphate	18.4	9.90	82.5	0.1	82.6
fertiliser	36.9	19.80	68.2	0.9	69.1
(< 0.2mm.)	64.4	34.65	53.4	0.8	54.2

It seems that the added phosphate was mostly dissolved by the acetic acid + 8 hydroxy-quinoline, but during prolonged shaking some of it has been adsorbed by the soil. The adsorption was much more in the Seddon V. soil than in the Urrbrae V. soil. It may be due to incomplete chelation of aluminium and iron of the soil by 8 hydroxy-quinoline, as it is not an ideal blocking agent particularly for aluminium, at pH3.9 (Mellan, 1941). Welcher, (1947) has given the limit of precipitation of Al with 8 hydroxy-quinoline within the pH range of 4.2 to 9.8. Cooke (1951) observed that even in the presence of one gram of oxine in 0.5N. acetic acid Fe_2O_3, TiO_2 and Al_2O_3 retained from 47 to 74% of the added phosphate. Ghani (1943b) also reported that oxine did not prevent adsorption of phosphate completely, though the efficiency was higher at pH5.0 than below it. Williams (1950a) noted that cupferron [$C_6H_5N(NO)O\ NH_4$] was much better in preventing adsorption of phosphate, by minerals as well as by soils than oxine. In view of the above evidence it seems reasonable to suppose that in the case of Seddon V. soil, because of the predominance of gibbsite among the sesquioxides (Tables 1, 4 and 7) 8 hydroxy-quinoline, failed to prevent the adsorption of phosphate dissolved by the acetic acid. Consequently the amount of phosphate in the first fraction is lower and in the second higher than it should have been.

During 6 weeks incubation there appeared a sharp decrease in the fraction of phosphate soluble in acetic acid + 8 hydroxy-quinoline (Figs.II - IV) and a corresponding increase in the amount of inorganic phosphate extractable with 0.1N NaOH (Figs.V-VII). After 6 weeks' incubation equilibrium seems to have been reached and except for slight redistribution during the interval from 6 to 13 weeks, there is no major change in the two inorganic fractions of phosphate. If some suitable method of extracting available phosphate were employed, this equilibrium value will be a better index of the usefulness of a given fertiliser than the initial value.

From these data, it can also be inferred that during this incubation the organic phosphorus extractable with NaOH (Total phosphate soluble in NaOH - Inorganic phosphate soluble in NaOH) remained practically unchanged and it was also little affected with the nature of fertilisers or their amounts. The slight differences however are conceivable because of this fraction being an indirect estimation thus reflecting the experimental error of two independent determinations. It is also worth mentioning that the total recovery of the applied inorganic phosphate from the Seddon V. soil is less than from the Urrbrae V. soil. It may possibly be due to the greater phosphate retaining power of the former than that of the latter soil.

The change over of phosphate from the fraction soluble in acetic acid + 8 hydroxy-quinoline to the inorganic fraction soluble in NaOH was most marked in the treatments with monocalcium phosphate, followed by silico phosphate and least in rock phosphate, irrespective of the soil (Figs.II - VII). It seems that the rock phosphate being least soluble of the three fertilisers did not change from the apatite form and thus released very little phosphate for adsorption. On the other hand, monocalcium phosphate and silico phosphate being more soluble rapidly passed into the forms soluble in the alkali.

It may however be mentioned in the case of Seddon V. soil that none of these fractions of phosphate, as they stand (Table 10) represent true values because of the adsorption during extraction. Some recalculation is necessary to get a fair estimate of their real value. Assuming that while extracting with NaOH alone there is not much interference due to calcium, then at least from the treatment with rock phosphate the same amount of phosphate should be extractable as after pre-treatment with acetic acid + 8 hydroxy-quinoline. But, as will be evident from Table 13, this is not the case, for on an average there is an adsorption of 312 p.p.m. of PO_4 from 30 cwt. treatment series in the Seddon V. (0-4") soil and only 38.5 p.p.m. in the Urrbrae V. (0-4") soil.

TABLE 13.

Recovery of inorganic phosphate with 0.1N NaOH from soils treated with rock phosphate equivalent to 30 cwt. super/acre.

Description of samples	Amount of phosphate dissolved by 0.1N NaOH in 17 hours		Difference (A-B)	
	A. After 17 hours extraction with acetic acid + 8 hydroxy-quinoline	B. No pre-treatment with acetic acid + 8 hydroxy-quinoline		
	PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)	
Seddon V. (0-4") soil treated with rock phosphate = 30 cwt. super/acre				
after 6 weeks	381	62	+ 319	mean 312
after 13 weeks	384	79	+ 305	
Urrbrae V. (0-4") soil treated with rock phosphate = 30 cwt. super/acre				
after 6 weeks	185	150	+ 35	mean 38.5
after 13 weeks	206	164	+ 42	

When the phosphate was fractionated just after applying rock phosphate equivalent to 30 cwt. super per acre, 312 p.p.m. of PO_4 appeared in the second fraction soluble in NaOH (see Table 10), which closely agrees with the above calculated difference. Thus it seems that in 30 cwt. treatments from the Seddon soil, the amount of phosphate soluble in acetic acid + 8 hydroxy-quinoline is lower by 312 p.p.m. and the fraction soluble in the alkali is higher by the same amount. If the amounts dissolved by these extractants from the Seddon V. (0-4") soil treated with different fertilisers equivalent to 30 cwt. super per acre are recalculated by adding this constant factor (312 p.p.m.), the new recalculated values appear as under:

TABLE 14

Showing recalculated values of different fractions of phosphate in the Seddon V. (0.-4") soil.

Soil	Treatment	Phosphate soluble in acetic acid + 8 hydroxy-quinoline (Recalculated value)	Phosphate soluble in 0.1N. NaOH inorganic fraction (Recalculated value)
		PO_4 (p.p.m.)	PO_4 (p.p.m.)
Seddon V. (0-4")	Monocalcium phosphate ≡ 30 cwt. super /acre		
	Initially	712	32
	after 6 weeks incubation	473	256
	after 13 weeks incubation	454	295
	Silico phosphate ≡ 30 cwt. super /acre		
	Initially	847	40
	after 6 weeks incubation	565	227
	after 13 weeks incubation	519	280
	Rock phosphate ≡ 30 cwt. super /acre		
	Initially	817	nil
	after 6 weeks incubation	720	69
	after 13 weeks incubation	730	72

It may however be mentioned that this correction factor may not be exactly applicable to monocalcium phosphate and silico phosphate, especially in 6 and 13 weeks' incubation series, because during this interval these comparatively more soluble phosphates may even have changed over to the fractions soluble in the alkali. However, there is no escaping the fact that these values in the case of Seddon V. (0-4") soil without some recalculation are not satisfactory.

It may also be pointed out that whether we accept the recalculated values or the original values the fraction of phosphate soluble in acetic acid + 8 hydroxy-quinoline (Figs.II - IV; Tables 10, 11) indicates higher availability of phosphate from the treatments with rock phosphate, which seems to be quite improbable. Even in the case of Urrbrae V. (0-4") soil, the situation is similar. It shows that the use of acetic acid + 8 hydroxy-quinoline as a solvent for the so-called available phosphate requires reconsideration.

iii. Availability of phosphate as indicated by the crop (Subterranean Clover)

Having studied the changes in the forms of phosphate after the application of different fertilisers to the Seddon V. soil the next point to verify was the usefulness of such fractionation as a means of predicting available phosphate in the soil. In 1951 A.G. Tyson of this laboratory conducted some pot experiments on a sample of the Seddon V. (0-4") soil. He used subterranean clover as the indicator plant and tried six fertilisers, three rates of application equivalent to 2, 4 and 6 cwt. super per acre and three replicates of each. The fertilisers tried were Monocalcium phosphate $[Ca(H_2PO_4)_2 H_2O]$, Basic slag, Calcium metaphosphate, Silico phosphate, Fused Tricalcium phosphate and Defluorinated phosphate. After the crop had been harvested the top 4" layer of soil from all the pots was removed. The soil was air dried and sieved and the fine earth ($<$ 2mm.) was further subsampled and suitable aliquots were ground finer than 0.5mm., and used for analysis reported here.

A reference to table 15 shows that the highest phosphate uptake was from silico phosphate, closely followed by monocalcium phosphate and basic slag and least from fused tricalcium phosphate. Statistical analysis (Table 16) reveals that the silico phosphate is significantly more available and the fused tricalcium phosphate less available than the monocalcium phosphate and the other phosphatic fertilisers. Moreover, there is no significant difference between the rest of the treatments. The fraction of phosphate soluble in acetic acid + 8 hydroxy-quinoline (Table 17) shows that fused tricalcium phosphate, basic slag and defluorinated phosphate treatments have significantly more phosphate in this fraction than monocalcium phosphate. If it is assumed that the phosphate extracted by acetic acid + 8 hydroxy-quinoline is the most available fraction this would mean that these three fertilisers were more available than monocalcium phosphate, silico phosphate and metaphosphate, which is quite contrary to the actual availability as revealed by the crop (Tables 15, 16). This suggests that the phosphate soluble in acetic acid + 8 hydroxy-quinoline does not represent the available phosphate.

TABLE 15

Showing percentage of applied phosphate taken up by the crop [x]
(Subterranean clover)

Rate of application equivalent to	Mono-calcium phosphate	basic slag	calcium meta-phosphate	Silico phosphate	Fused tri-calcium phosphate	Defluorinated phosphate
	Percentage of the applied phosphorus taken up by the crop					
2 cwt. super/acre	11.3	12.2	10.9	14.5	6.8	10.9
4 cwt. super/acre	12.2	12.0	10.6	14.5	7.2	10.6
6 cwt. super/acre	12.4	12.8	13.4	15.4	7.5	12.7

TABLE 16

Phosphate uptake by subterranean clover [x]
(Milligrams phosphate taken up per pot.)

Type of phosphate	Rate of phosphate equivalent to 2 cwt. super/acre	4 cwt. super/acre	6 cwt. super/acre	Mean
	Mean of three pots			
		± 4.66		± 2.7
1. Monocalcium phosphate	33.0	72.7	110.2	72.0
2. Basic slag	35.7	70.9	114.2	73.6
3. Calcium meta-phosphate	32.6	63.3	119.5	71.8
4. Silico phosphate	42.8	85.6	136.5 **	88.3***
5. Fused tri-calcium phosphate	20.5	44.2**	66.5***	43.7***
6. Defluorinated phosphate	31.7	62.4	111.9	68.7
		± 1.9		
Mean	32.7	66.5	109.8	

Least significant differences

	5%	1%	0.1%
1. Body of the table	13.5	18.1	24.0
2. Vertical margins	7.7	10.4	13.7
3. Horizontal margins	5.5	7.3	9.6

* Significant at 5%
** Significant at 1%
*** Significant at 0.1%

[x] Data collected by A.G. Tyson

TABLE 17

Phosphate soluble in acetic acid + 1% 8 hydroxy-quinoline (first fraction) x

Form of phosphate or fertiliser applied	Rates of phosphate equivalent to 2 cwt. super/acre	4 cwt. super/acre	6 cwt. super/acre	Mean
		PO_4 (p.p.m.)		
		± 1.45		± 0.83
1. Monocalcium phosphate	1.3	7.1	9.5	6.0
2. Basic slag	4.2	7.2	21.8^{xxx}	11.0^{xxx}
3. Calcium meta-phosphate	3.4	6.6	8.7	6.2
4. Silico phosphate	0.9	4.3	8.9	4.7
5. Fused tri-calcium phosphate	5.0	6.3	21.7^{xxx}	11.0^{xxx}
6. Defluorinated phosphate	4.0	11.0	15.8^{xx}	10.3^{xx}
		± 0.59		
Mean	5.1	7.1	14.4	

Least Significant Differences

	5%	1%	0.1%
Body of the Table	4.2	4.6	7.4
Vertical margin	2.4	3.2	4.3
Horizontal margin	1.7	2.3	3.0

x Data by the author.

TABLE 18

Fraction of inorganic phosphate soluble in 0.1N NaOH (second fraction)
PO_4 (p.p.m.)

Form of phosphate or fertiliser applied	Rates of phosphate equivalent to 2 cwt. super/acre	4 cwt. super/acre	6 cwt. super/acre	Mean
		± 3.85		± 2.2
1. Monocalcium phosphate	50.9	94.4	135.3	93.6
2. Basic slag	52.4	96.3	140.3	96.3
3. Calcium meta-phosphate	52.3	85.3	117.5^{xx}	85.02^{x}
4. Silico phosphate	46.4	95.3	127.2	89.6
5. Fused tri-calcium phosphate	45.3	78.5^{xx}	127.1	83.6^{xx}
6. Defluorinated phosphate	55.5	98.9^{x}	124.8	93.0
		± 1.6		
Mean	50.5	91.5^{xxx}	128.7^{xxx}	

Least Significant Differences

	5%	1%	0.1%
Body of the Table	11.0	14.8	19.5
Vertical margins	6.3	8.5	11.2
Horizontal margins	4.6	6.2	8.1

A comparison of the inorganic phosphate extracted by N/10 NaOH (Table 18) shows that tricalcium phosphate and calcium metaphosphate treatments give significantly lower results than the monocalcium phosphate and the rest. At first sight this appears to correlate with the uptake by the plant, but as has been shown previously this fraction is virtually high due to failure of 8 hydroxy-quinoline to prevent adsorption of phosphate during the first extraction.

The total inorganic phosphate extractable by these two extractants shows (Table 19) that in these fertilisers there does not exist any significant difference. Even basic slag, which shows a higher and just significant value is also a borderline case. Thus the total inorganic phosphate (fraction soluble in acetic acid - oxine + the fraction soluble in 0.1N. NaOH) also fails to show the differences in the availability of these fertilisers. Likewise, the organic phosphorus figures (Table 20) fail to differentiate between these treatments.

TABLE 19

Total inorganic phosphate extractable by Williams' method (first fraction + second fraction)

Type of phosphate	Rate of application of phosphate equivalent to 2 cwt. super /acre	4 cwt. super /acre	6 cwt. super /acre	Mean
	PO_4 (p.p.m.)	± 4.2		± 2.4
1. Monocalcium phosphate	52.3	101.5	144.8	99.5
2. Basic slag	56.6	103.5	162.1 ^{xx}	107.4 ^{x}
3. Calcium metaphosphate	55.6	91.9	126.2 ^{xx}	91.3
4. Silica phosphate	47.3	99.6	136.1	94.3
5. Fused tricalcium phosphate	50.3	84.8	148.7 ^{xx}	94.6
6. Defluorinated phosphate	59.5	109.9	140.6	103.3
		± 1.7		
Mean	53.6	98.5 ^{xxx}	143.1 ^{xxx}	

Least significant differences

	5%	1%	0.1%
1. Body of the Table	12.0	16.2	21.3
2. Vertical margins	6.9	9.2	12.2
3. Horizontal margins	4.9	6.5	8.6

TABLE 20

Organic phosphorus extractable with 0.1N. NaOH

Type of phosphate applied	Rates of phosphate equivalent to 2 cwt. super/acre	4 cwt. super/acre	6 cwt. super/acre	Mean
	PO_4 (p.p.m.)	± 3.7		± 2.1
1. Monocalcium phosphate	27.4	22.9	23.3	24.5
2. Basic slag	22.4	25.1	29.7	25.7
3. Calcium meta-phosphate	34.1	38.0^{**}	45.9^{***}	39.3^{***}
4. Silico phosphate	27.6	29.2	27.2	28.0
5. Fused tri-calcium phosphate	33.5	41.1^{**}	27.9	34.2^{**}
6. Defluorinated phosphate	27.1	24.7	24.5	25.4
Mean	28.7	± 1.5 $30.2^{N.S.}$	$29.7^{N.S.}$	

Least Significant Differences

	5%	1%	0.1%
Body of the Table	10.6	14.2	18.7
Vertical margins	6.1	8.2	10.8
Horizontal margins	4.3	5.8	7.7

These results illustrated that in the Seddon V. (0-4") soil the utility of this method both as a fractionating method for phosphate as well as a fertility index is somewhat questionable.

iv. A critical examination of the method of C.H. Williams (1950a, c) for fractionation of phosphates as an index of available phosphate in the soil.

From the results discussed above and in the previous section it becomes evident that there are some serious drawbacks in the method of fractionation of phosphate proposed by C.H. Williams (1950a).

1. In the first instance, the acetic acid + 8 hydroxy-quinoline extractant is unsuitable because at pH3.9 (pH of the extractant) in 17 hours' shaking it dissolves most of the tricalcium phosphates and other apatites which are of recognised low value to plants except under certain acid conditions. A perusal of the solubility data (Table 12) shows as the amount of rock phosphate in the extractant decreases the percentage solubility increases. Williams (ibid) observed similar increase in solubility of apatites with the increase in the degree of fineness of the particles. Thus from soils, the extractant dissolves most of the apatites

depending upon their degree of fineness, degree of crystallinity, chemical nature and amount in the soil. Consequently the extractant assigns as high a value to rock phosphate as to monocalcium phosphate or other more soluble phosphates. Tyson (1953) in a comparison of various forms of phosphate, as a source of phosphorus to Subterranean Clover, in the Seddon V. (0-4") soil observed that the crop did not remove more than 0.5% of the phosphate from the applications of rock phosphate equivalent to 2 to 20 cwt. super/acre. Similar conclusions have been reached by officers of the Department of Agriculture in South Australia and Tasmania. Some of these results will be discussed later. The data presented in Tables (15 to 17) also confirm that fused tricalcium phosphate which was half as available to subterranean clover as monocalcium phosphate shows an even higher amount of phosphate in the fraction soluble in acetic acid + 8 hydroxy-quinoline than the latter.

2. 8 hydroxy-quinoline is evidently not a suitable complexing agent, especially in soils having a measure of free alumina. The failure of the reagent to prevent adsorption of the phosphate dissolved by acetic acid from the Seddon V. (0-4") soil has already been discussed in the previous section, and recently Skene (1953) expressed doubt about the suitability of the acetic acid + 8 hydroxy-quinoline soluble phosphorus as fertility index in red loams.

3. Williams (1950c) working with Urrbrae soils noted that phosphorus in the plants was better correlated with the fraction of the soil phosphate soluble in 0.1N. NaOH than with the one soluble in acetic acid + 8 hydroxy-quinoline. The same author concluded that for neutral to slightly acid red brown earth soils either the amount extracted by acetic acid + 8 hydroxy-quinoline or inorganic fraction soluble in NaOH or their sum, may be used as an index of available phosphate, but for alkaline soils the inorganic fraction soluble in NaOH seems to be preferable. Skene in his critical examination of the method has remarked that inorganic phosphorus soluble in the alkali appears to give a better correlation with the yield of wheat grown on alkaline soils than the fraction soluble in acetic acid + 8 hydroxy-quinoline. Recently Woodroffe and Williams (1953), in working out the association of the wheat yields with available phosphate in the soil, have used the total inorganic phosphate (sum of the phosphate soluble in

acetic acid + 8 hydroxy-quinoline and the inorganic phosphate soluble in the alkali), and not the fraction extractable with acetic acid + 8 hydroxy-quinoline.

All this evidence confirms that the acetic acid + 8 hydroxy-quinoline method of fractionation of phosphate, as a guide to available phosphate status of the soil, is not altogether suitable especially for

(1) Soils having fairly high apatite and tricalcium phosphates

(2) For alkaline soils

(3) For acid soils with high free sesquioxide content such as red loams or krasnozems.

Even for laterites and podsolic soils derived from lateritic material such as the Seddon Soils, the applicability of the method is questionable.

v. Development of an alternative method for extracting available phosphate.

(a) The use of sodium versenate (disodium salt of ethylene diamine tetra-acetic acid).

The main defects in Williams' method (1950a) are the high attack on apatites due to high buffer acidity (pH3.9) and the unsuitability of 8 hydroxy-quinoline as a chelating agent. On the other hand, an extractant of pH5-6 will dissolve least amounts of less available sources of phosphate such as apatite, variscite, wavellite, dufrenite and vivianite (Stelly and Pierre, 1942).

Moreover, in critical comparison of methods, Fraps and Fudge (1945), Weeks and Karrakar (1941), Rubins and Dean (1946), Iowa Station (1946), and Brown (1940) have concluded that Morgan's acetate buffer pH5.0 is not so drastic a solvent as most of the acid extractants are. If the features of Morgan's method, i.e. a buffer of pH5.0 and short time of extraction, and those of Williams' method, i.e. presence of a suitable complexing agent, to prevent readsorption of phosphate are combined in one method, a better separation of easily soluble phosphates can be affected. Williams (1950a) observed that cupferron is a better chelating agent than oxine, and recently for complexing divalent and trivalent metals Schwarzenbach et al. (1948) have suggested the use of ethylene diamine tetra-acetic acid disodium salt (sodium versenate).

The data reported in Table 21 illustrates the effect of such combinations in extracting easily soluble phosphates. Though a combination of acetate buffer pH5.0 and cupferron, is better than any other combination, but in 30 minutes' extraction all the water soluble phosphate (396 p.p.m.) was not extractable. It would have required longer time of extraction, which has two defects:

(i) Much higher amount of rock phosphate is dissolved than its availability justifies.

(ii) Cupferron decomposes and thus its effectiveness as a chelating agent is affected.

On the other hand, sodium versenate at pH5.0 did not prevent the readsorption of phosphate, because it complexes with Fe successfully only below pH3.0 (Cheng, Bray and Krutz, 1953). Thus for the extraction of readily soluble forms of phosphate, none of these alterations appeared to be

TABLE 21.

Comparison of some methods of extracting phosphates from the Seddon V. (0-4") Soil incubated with different phosphatic fertilisers equivalent to 12 cwt. Super/acre.

Method of Extraction	Phosphate extracted PO_4 (p.p.m.) MonoCa.Phos. after 0 wks.	Rock Phos. after 0 wks.	Rock Phos. after 6 wks.	Remarks.
1. Water alone. 30 minutes shaking.	149	21	15	(extract filtered (through no.44 filter paper and (phosphate determined directly.
2. Water + 1.5g sodium versenate (30 minutes shaking).	26	16	5	(
3. Water + 1.5g sodium versenate (17 hours).	26	30	-	(extract centrifuged filtered & (phosphate determined directly.
4. Water + 0.2g sodium versenate + 1.0g cupferron (30 minutes extraction).	226	52	16	(extract filtered (ignited & phosphate determined (as usual.
5. Water + 1.0g cupferron (30 minutes extraction).	227	14	1	"
6. Ammonium Acetate buffer pH 5.0 + 0.2g sodium versenate + 1.0g cupferron (30 minutes extraction)	228	51	22	"
7. Ammonium acetate buffer pH 5.0 + 1.0g cupferron (30 minutes extraction).	240	37	11	"
8. Ammonium acetate buffer pH 5.0 + 0.2g sodium versenate (30 minutes extraction).	148	45	49	"

Note: Soil to extractant ratio was 2.5: 100 in all cases.

promising.

(b) Use of sodium versenate in alkaline solutions:- As mentioned earlier, that from the point of view of availability of phosphates to plants, an alkaline extractant would be more suitable than an acid one. Recently Rubins (1953) using radio active isotope technique, observed that Crops utilise more phosphorus from the fraction soluble in 0.1N NaOH, than from the fraction soluble in 0.002 N H_2SO_4.

Secondly if the contention of Overstreet and Dean (1951) Burstrom (1951) Overstreet and Jacobson (1952), that plants absorb anions by exchanging $\overline{OH}$ and $\overline{H}CO_3$ ions from the roots, be accepted, it would mean that the extraction of soil phosphate with alkaline solutions is more akin to plant roots' action, than the extraction with acids. Moreover, hydroxyl ions exchange phosphate ions better than $\overset{+}{H}$ ions. All these considerations show that for extracting available phosphate an alkaline extractant is preferable. But in alkaline solutions calcium interferes, because of the formation of insoluble calcium phosphates, as was observed by Teakle (1928) and Dean (1938). The former investigator used ammonium oxalate to prevent precipitation of the extracted phosphates as $Ca_3(PO_4)_2$ and the latter resorted to pretreatment with sodium acetate, to remove the exchangeable and water soluble calcium. Sodium versenate is reported to be very effective in complexing with Ca and Mg in alkaline medium (Schwarzenbach et al. 1946, Diehl 1950) It should be preferable to oxalate, because of the higher stability of its complex with Ca, than that of the calcium oxalate. Secondly the complex is water soluble, but does not ionise to liberate $\overset{++}{Ca}$ ions, and thus it is less likely to adsorb any phosphate from solutions as the precipitates of oxalates will do.

TABLE 22.

Solubility of Minerals in 0.1N NaOH with and without sodium versenate.

Mineral	Weight taken Mgs	PO_4 equivalent Mgs	Percentage Phosphate extractable in 17 hours with 0.1N NaOH	
			+ versenate %	- versenate %
Monocalcium Phosphate (Reagent A.R.)	46.00	34.65	93.0	59.0
Tricalcium Phosphate (Reagent A.R.)	56.6	do.	85.7	20.5
Silico Phosphate (Fertiliser < 0.2mm)	78.5	do.	72.5	0.7
Rock Phosphate (A) (Fertiliser < 0.2mm)	64.4	do	19.3	0.4
Rock Phosphate (B) (Fertiliser < 0.2mm)	64.4	do.		
1st extraction			20.5	0.4
2nd extraction			27.4	0.4

TABLE 23.

Effect of Sodium Versenate on the recovery of phosphate with 0.1N NaOH

Soils	Treatment	(Method A.) Phosphate extracted with 100 ml 0.1N NaOH containing 0.2g sodium versenate (+ versenate)	(Method B.) Phosphate extracted with 100 ml 0.1N NaOH alone (− versenate)
		(PO_4 p.p.m.)	(PO_4 p.p.m.)
Seddon V.(0–4")	Soil alone no treatment	16	16
	Soil treated with Monocalcium Phosphate ≡ 30 cwt. Super/acre		
	after 6 weeks incubation	728	728
	after 13 " "	768	704
	Soil treated with Silico Phosphate ≡ 30 cwt. Super/acre		
	after 6 weeks incubation	675	516
	after 13 " "	736	640
	Soil treated with Rock Phosphate ≡ 30 cwt. Super/acre		
	after 6 weeks incubation	156	62
	after 13 " "	163	79
Urrbrae V.(0–4")	Soil alone (no treatment)	76	55
	Soil treated with MonoCal.Phos.≡ 30 cwt. Super/acre		
	after 6 weeks incubation	876	836
	after 13 " "	872.	800
	Soil treated with Silico Phos.≡ 30 cwt.Super/acre		
	after 13 weeks incubation	868	788
	Soil treated with Rock Phos. ≡ 30 cwt.Super/acre	268	164
	after 13 weeks		

Summary of Results.

Method	Mean ± 9.50 p.p.m. PO_4	
A	516.8	
B	448.7	
Difference	68.1	Significant at 0.1% level.

L.S.D.

5%	29.6
1%	41.7
0.1%	59.6

The data given in Tables 22, 23, show that in preventing the precipitation of $Ca_3(PO_4)_2$ from soils as well as from minerals, 0.2% solution of sodium versenate in 0.1N NaOH, proved quite effective. Secondly from the soils treated with monocalcium phosphate it recovered nearly as much inorganic phosphorus as the two extractions of Williams' method (compare with Table 10), but rock phosphate was not appreciably dissolved. In some preliminary experiments it was also seen, that in the presence of over 0.2% sodium versenate in 0.1N NaOH, an addition of calcium as calcium acetate up to 4%, had no adverse effect on the recovery of phosphate from the soil.

These experiments illustrate the effectiveness of sodium versenate in removing interference due to calcium, but the phosphate soluble in 0.1N NaOH, is not necessarily available, because comparatively less available aluminium and iron phosphate minerals and other adsorbed phosphates are also hydrolysed. On these considerations and also keeping in view that aluminium phosphate begins to hydrolyse after pH 9.0 (Britton 1932) borate buffer pH 9.0, in conjunction with sodium versenate was tried. The extractant is aimed to dissolve:-

i. water soluble phosphates.

ii. mono, di and tri calcium phosphates but very little apatites.

iii. magnesium and manganese phosphates.

iv. exchangeable phosphorus.

v. limited amounts of aluminium and iron phosphates, as will be extractable under the same conditions, in which almost whole of the more soluble forms are dissolved.

Sodium versenate prevents the precipitation of these soluble phosphates with calcium. The solubility of some of these compounds in the extractant is given in Table 24.

TABLE 24.

Solubility of Phosphate Minerals in 100 ml borate buffer + 0.5g sodium versenate.

	Compound	Weight taken	PO_4 equivalent	% PO_4 extracted
1.	Monocalcium Phosphate $Ca(H_2 PO_4)_2 \quad H_2O$ (A.R.)	10.0 mg	7.53 mg	99.0
2.	Dicalcium Phosphate $Ca_2(H PO_4)_2$ (A.R.)	10.0 mg	5.510 mg	97.5
3.	Tricalcium Phosphate $Ca_3 (PO_4)_2$ (A.R.)	10.0 mg	6.120 mg	94.0
4.	Ferric Phosphate (L.R.)	10.0 mg	4.675 mg	21.4
5.	Aluminium Phosphate (L.R.)	10.0 mg	4.30 mg	62.8

(c) Comparison of 2 hours' extractions, with acetate buffer pH 5.0 and with borate buffer pH 9.0.

Duplicate samples of 2.5g Urrbrae V. Soil which had been previously treated with monocalcium phosphate and rock phosphate equivalent to 12 cwt Super/acre (396 p.p.m. PO_4), were extracted separately for 2 hours with

(A) 100 ml of acetate buffer pH 5.0 + 0.2g sodium versenate + 1.0g cupferron.

(B) 100 ml of borate buffer pH 9.0 + 0.2g sodium versenate.

(C) 100 ml of borate buffer pH 9.0 + 0.5g sodium versenate.

The results (Table 25) show, that there is no significant difference in the methods B and C, but extractant A, dissolved significantly higher amounts of phosphates particularly from soils treated with rock phosphate, than the B and C extractants. When just after the application of monocalcium phosphate the extraction was done, all the three extractants removed almost whole of the phosphate that was added. But after 6 and 13 weeks' moist incubation of the same treatment, the amounts extractable with the acetate buffer were higher than with the borate buffer. In 2 hours' extraction from treatments with rock phosphate, the high solubility of phosphate with acetate buffer, shows the unsuitability of this extractant, for readily available phosphates, because rock phosphate is not so available, as its solubility indicates.

(d) Effect of time of extraction on the recovery of phosphate with borate buffer and versenate.

The amounts of phosphate extractable with 100 ml of borate buffer + 0.2g or 0.5g sodium versenate, in 2 and 16 hours from the Seddon V. (0-4") and Urrbrae V. (0-4") soils, treated with phosphates, show (Table 26) that

TABLE 25.

Comparison of 2 hours' extractions of phosphate with borate buffer versenate, and acetate buffer versenate - cupferron methods.

Soil	Treatment	A. Phosphate Sol- in acetate buffer pH 5.0 + 0.2% sodium versenate + 1.0% cupferron solution	B. Phosphate Soluble in borate buffer pH 9.0 + 0.2% sodium versenate solution	C. Phosphate Soluble in borate buffer pH 9.0 + 0.5% versenate solution
		PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)
Urrbrae V.(0-4")	Incubated with monocalcium phosphate ≡ 12 cwt.Super/acre			
	For 0 weeks	398	373	380
	For 6 weeks	303	230	231
	For 13 weeks	306	233	220
	Incubated with Rock Phosphate ≡ 12 cwt. Super/acre			
	For 0 weeks	162	28	46
	For 6 weeks	160	44	45
	For 13 weeks	155	64	59
		± 9.58		
Means		247.3 xxx	162.0 NS	163.5 NS

L.S.D. @ 0.1% = 62.15

xxx Significant at 0.1%

Remarks: Phosphate extracted was determined after ignition in method A and directly in the methods B and C, using Williams' technique for the estimation of inorganic phosphate in alkali extracts.

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there is no difference in 2 hours' extractions in the presence of 0.2g or 0.5g sodium versenate, but 16 hours' extraction is significantly more drastic, particularly from the samples treated with the rock phosphate. These results clearly indicate that 2 hours' extraction is adequate to remove most of the so-called available phosphates, such as $Ca(H_2PO_4)_2$ and longer extraction dissolves comparatively less available forms as well. Thus in view of these data, and also some preliminary observations it seems more appropriate to use 2 hours' extractions with borate buffer containing 0.5g versenate.

TABLE 26.

Comparison of the effect of the time of extraction with borate buffer and versenate on the recovery of phosphate (PO_4 p.p.m.)

			A. 2 hours extraction with borate buffer + 0.2% versenate	B. 16 hours extraction with borate buffer + 0.2% versenate	C. 2 hours extraction with borate buffer + 0.5% versenate
			PO_4 (p.p.m.)	PO_4 (p.p.m.)	PO_4 (p.p.m.)
Seddon V.(0-4") Soil	Monocalcium Phos. ≡ 12 cwt. Super/acre (incubated)				
		initially	292	304	300
		after 6 weeks	128	160	130
		after 13 weeks	111	168	122
	Rock Phosphate ≡ 12 cwt Super/acre (incubated)				
		initially	14	68	24
		after 6 weeks	8	64	21
		after 13 weeks	16	66	24
Urrbrae V.(0-4") Soil	Monocalcium Phos. ≡ 12 cwt.Super/acre (incubated)				
		initially	373	368	380
		after 6 weeks	230	231	231
		after 13 weeks	233	220	220
	Rock Phosphate ≡ 12 cwt. Super/acre (incubated)				
		initially	28	92	46
		after 6 weeks	44	112	45
		after 13 weeks	60	120	59

	A	B	C
	± 4.76		
Means	128.1	164.4	133.5
Differences from C	- 5.4 (non significant)	+ 30.9 (significant) (@ 1%)	-
L.S.D.			
@ 5%	13.96		
1%	19.00		
0.1%	25.35		

(e) Procedure for the extraction of available phosphate.

2.5g sample of soil is shaken for 2 hours at a constant temperature (20°C), with 100 ml borate buffer (pH 9.0), containing sodium versenate. The buffer is prepared by mixing 850 ml of borax-versenate solution, containing 32.47 gram borax ($Na_2 B_4 O_7 . 10H_2O$) and 5.0g disodium salt of ethylenediamine tetraacetic acid, with 150 ml 0.2 N HCl. The suspension is centrifuged for 15 minutes and in 25 ml of the extract, phosphate is determined by the technique of Williams' (1950a) for the determination of inorganic phosphate, in the alkali extract of the soil. It is essential to prepare a standard curve with the extractant solution in the same way.

The remaining liquid is completely poured off and the residue is shaken with 100 ml of 0.1N NaOH for 17 hours at 20°C and centrifuged as usual. Inorganic phosphate is determined in 25 ml of the aliquot by the same technique as mentioned above.

The inorganic phosphate soluble in the borate buffer and 0.5% versenate may be considered as readily soluble or readily available, while in the second fraction i.e. 0.1N NaOH, as slowly soluble or available. The sum of these two fractions may be termed as total soluble or available inorganic phosphate.

(f) A Comparison of some Methods of fractionation of phosphate.

The soils which had been treated with different fertilisers at rates equivalent to 12 cwt. Super/acre (396 p.p.m. PO_4) for incubation studies (Tables 10 and 11) were extracted with ((A) Williams' method (B) Acetate buffer pH 5.0 + 0.2% versenate (C) Borate buffer pH 9.0 + 0.5% versenate. 2.5g of the treated soils were extracted with 100 ml of each extractant for 17 hours in method (A), 30 minutes in method (B) and for 2 hours in method (C). The second fraction in all cases was 17 hours' extraction with 0.1N NaOH.

A comparison of the first fraction extracted by these three methods (Table 27), shows that in both the soils treated with monocalcium phosphate, the borate buffer method extracted more phosphate than any of the other methods, which was to be expected, because of the high adsorption from the solution in the other two methods. Thus in the extraction of phosphate just after adding monocalcium phosphate (396 p.p.m. PO_4) to the Seddon V. (0-4") soil, 300 p.p.m. of PO_4 were extractable, with borate buffer + versenate, whereas acetic acid +8 hydroxyquinoline, gave a figure of 123 and acetate buffer 148. From the soils treated with rock phosphate, the borate buffer + versenate, as com-

TABLE 27.

Comparison of some methods of fractionation of phosphate.

Soil	Treatment	Williams' Method			Acetate buffer (ph5.0) Method			Borate buffer (pH 9.0) Method		
		Phosphate Soluble in Acetic acid + 8 Hydroxy-Quinoline in 17 hours.	Phosphate Soluble in NaOH (0.1N) (inorg. PO_4) in 17 hours	Total inorganic phosphate extracted	Phosphate soluble in amm.acetate buffer + 0.2% versenate in 30 minutes	Phosphate soluble in 0.1N NaOH (inorg. PO_4) in 17 hours	Total inorganic phosphate extractable	Phosphate soluble in borate buffer + .05% versenate in 2 hours	Phosphate soluble in 0.1N NaOH (inorganic PO_4)	Total inorganic phosphate extractable
					PO_4 (p.p.m.)					
	Samples incubated with different phosphates equiv. to 12 cwt. Super/acre.									
Seddon V. (0-4")	Monocalcium phosphate									
	initially	123	197	320	148	168	316	300	66	366
	after 6 weeks	33	248	281	20	212	232	130	140	270
	after 13 weeks	31	247	278	16	212	228	122	141	263
	Rock Phosphate									
	initially	126	174	300	45	-	-	24	-	-
	after 6 weeks	103	211	314	49	80	129	21	45	66
	after 13 weeks	92	215	307	52	80	132	24	48	72
	Silico Phosphate									
	initially	120	196	316	128	111	239	156	42	198
	after 6 weeks	61	256	317	59	224	283	154	128	283
	after 13 weeks	36	262	298	48	214	262	103	113	216
Urrbrae V.(0-4")	Monocalcium Phosphate after 13 weeks	188	208	396	106	248	354	220	164	384
	Rock Phosphate "	273	146	419	102	128	230	59	86	145
	Silico Phosphate "	228	214	442	115	264	379	170	184	354

Note:- 2.5g Soil was extracted with 100 ml of the extractant in each case and phosphate extracted determined by Williams' technique (1950a) pared with other methods, extracted very little phosphate, which shows that this treatment had produced very little of phosphates of a type soluble in the borate buffer + versenate. The higher recovery from all the fertiliser treatments in the second fraction soluble in 0.1N NaOH by Williams' method and the acetate method, as compared to the corresponding fraction of the borate buffer-versenate method confirms that in all these cases some phosphate dissolved in the first fraction was readsorbed by the soil, otherwise the second fraction should have been the same in all the three methods. The total inorganic phosphate extractable from the treatment with monocalcium phosphate in all the three methods is virtually the same, but from the soils treated with silicophosphate and rock phosphate, Williams' method recovered most of the added phosphate, but not the borate buffer versenate method. It is because of the comparatively less solubility of the apatites in the borate buffer + versenate.

From the above, it can be inferred that when compared with Williams' method, the borate buffer-versenate method has two advantages:

(i) It does not dissolve the slowly available phosphates like apatites and rock phosphate to any great extent.

(ii) The phosphate dissolved by the borate buffer + versenate does not get adsorbed from the solutions, because of the high pH, and the presence of versenate.

It may however be mentioned that if the soils containing more than 2% soluble calcium are to be extracted, a higher amount of versenate will be required. Secondly since the apatites are not without any availability, a third extraction with dilute solutions of organic acids such as citric acid to dissolve such compounds, after the first two extractions with borate buffer + versenate and 0.1N NaOH, may be desirable.

vi. The comparison of phosphate extracted by the borate buffer versenate method with the plant response.

(a) Pot Cultures.

Samples of soils from the pot culture experiments of Tyson (see Tables

15 - 20) have been fractionated by the borate buffer versenate method. The results (Tables 28,29) show that from the treatments with fused tricalcium phosphate, the borate buffer + versenate, as well as 0.1N NaOH extracted, significantly lower amounts of phosphate than from the treatments with mono-calcium phosphate and the others. The amount of phosphate taken up by the crop (Tables 15,16) compares fairly well with these results.

TABLE 29.

Phosphate extractable from Tyson's pot experiment (1951) by borate buffer versenate method.

Seddon V. (0-4") Soil.

	Phosphate added equiv. to	Form of Phosphate added	Phosphate soluble in borate buffer +0.5% versenate PO_4 (p.p.m.)	Phosphate (inorganic) soluble in 0.1N NaOH PO_4(p.p.m.)	Total inorganic phosphate extractabl PO_4 (p.p.m.)
			Mean of 3 pots. ± 1.41	± 1.95	± 2.92
72.7	4 cwt. Super/acre	Monocalcium phosphate	34.86	54.36	89.22
70.9		Basic Slag	31.93	50.70	82.6
63.[illegible]		Calcium Meta phosphate	28.53 xx	49.00	77.5 x
55.6		Silico Phosphate	33.40	54.03	87.4
40.[illegible]		Fused Tricalcium phosphate	23.86 xxx	38.40 xxx	62.3 xxx
62.4		Defluorinated phosphate	31.86	50.70	82.6
		L.S.D. @ 5%	4.32	6.0	9.01
		@ 1%	6.06	8.40	12.63
		@ 0.1%	8.59	11.88	17.83

xxx Significant at 0.1%

xx Significant at 1%

x Significant at 5%

TABLE 28.

Phosphate extractable from Tyson's Pot Experiments (1951) by borate buffer versenate method.

Pot No.	Phosphate added equivalent to	Form of Phosphate added	Phosphate extractable with borate buffer versenate method		
			1st fraction soluble in borate buffer + 0.5% versenate	2nd fraction soluble in 0.1N NaOH	Total inorganic Phosphate extractable
				PO_4 (p.p.m.)	
350	4 cwt. Super/acre	Monocalcium Phosphate	32.6	52.0	84.6
351	"	"	36.0	57.3	93.3
352	"	"	36.0	53.8	89.8
359	"	Basic Slag	30.6	48.0	78.6
360	"	"	32.2	55.8	88.0
361	"	"	33.0	48.3	81.3
368	"	Calcium meta Phosphate	28.6	49.5	78.1
369	"	"	28.0	48.3	76.3
370	"	"	29.0	49.2	78.2
377	"	Silico Phosphate	37.6	56.5	94.1
378	"	"	32.6	53.8	86.4
379	"	"	30.0	51.8	81.8
386	"	Fused Tricalcium Phosphate	24.6	40.0	64.6
387	"		23.0	38.4	61.4
388	"	"	24.0	36.8	60.8
395	"	Defluorinated Phosphate	28.6	44.5	73.1
396	"	"	31.0	55.8	86.8
397	"	"	36.0	51.8	87.8
353	6 cwt. Super/acre	Monocalcium Phosphate	51.6	85.0	136.6
354	"	"	56.0	87.0	143.0
355	"	"	51.6	87.0	138.6
389	"	Fused Tricalcium Phosphate	36.4	57.5	93.9
390	"		35.1	61.5	96.5
391	"	"	31.1	59.0	90.1

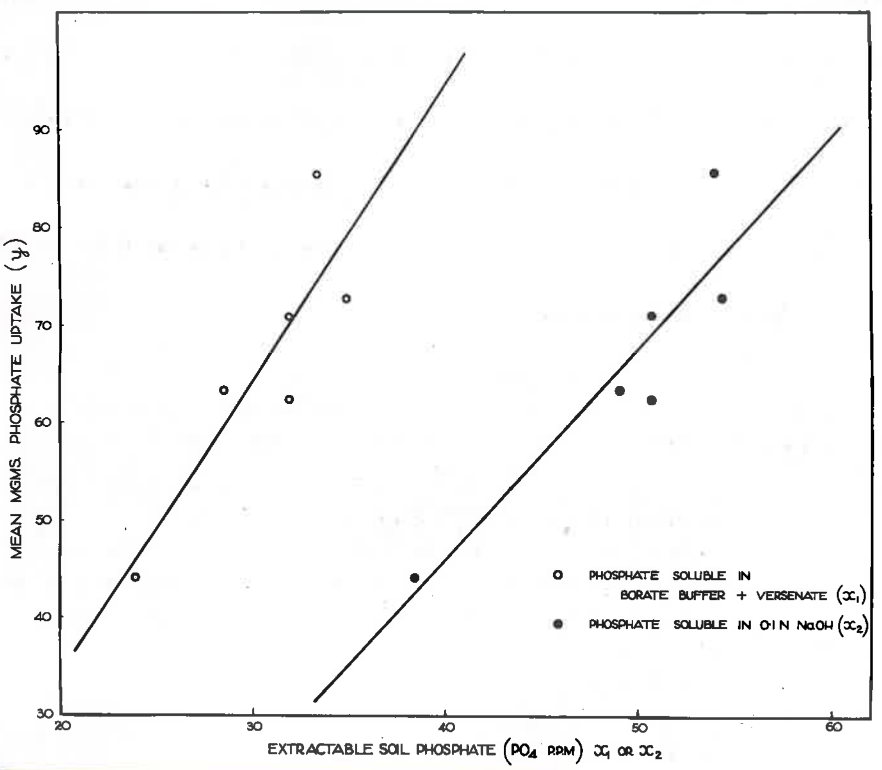


FIGURE VIII.

Showing relationship between milligrams PO_4 uptake by Subterranean Clover from each pot and the inorganic soil phosphate (PO_4 p.p.m.) extractable with: (a) Borate buffer + versenate
(b) 0.1N NaOH.

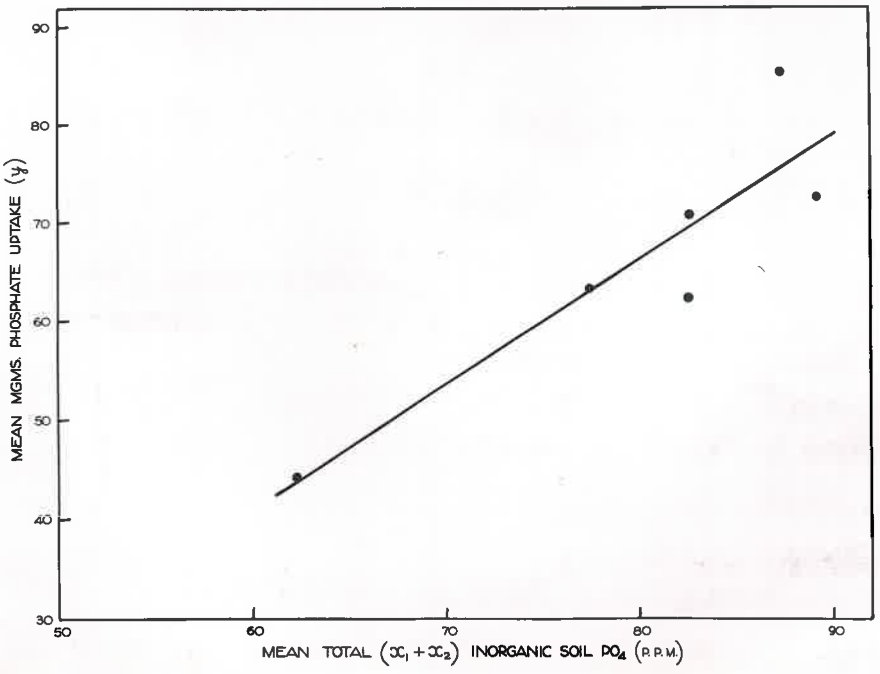


FIGURE IX

Showing relationship between milligrams PO_4 uptake by Subterranean Clover from each pot and the total extractable inorganic soil phosphate (PO_4 p.p.m.) by the borate buffer versenate method of fractionation.

It has also been possible to calculate significant regression equations relating phosphate uptake by the Subterranean Clover and the amount of phosphate extractable by this method in the first and second fractions or their sum (Table 30, Figs. VIII, IX). From Table 30 it may also be seen that in the case of Williams' method there is no significant regression on any one of these fractions or their sum, though a partial regression on both the first and second fractions exists.

TABLE 30

Showing regression of phosphate taken up by Subterranean Clover on the phosphate extractable from the soil by different methods.

Regression of Y on	Significance of the regression lines fitted to the means of PO_4 extracted from the different fertiliser treatments.		Equations relating milligrams of phosphate taken up by the crop from each pot to p.p.m PO_4 extractable from the treated soils.
	Williams' method	Borate buffer versenate method	
X_1	Non signif.	(1) Signif. at 5%	(1) $Y = - 26.01 + 3.01 X_1$
X_2	Non signif.	(2) Signif. at 5%	(2) $Y = - 39.98 + 2.15 X_2$
$X_1 + X_2$	Non signif.	(3) Signif. at 5%	(3) $Y = - 35.43 + 1.27 (X_1 + X_2)$
X_1 and X_2 both (Partial regression on both the fractions X_1 and X_2)	(4) Signif. at 5%	Non signif.	(4) $Y = - 60.83 - 4.36 X_1 + 1.73 X_2$

Y = Milligrams phosphate taken up by Subterranean Clover from each pot.

X_1 = PO_4 (p.p.m.) extracted by acetic acid + 8 hydroxy-quinoline or borate buffer + versenate.

X_2 = Inorganic PO_4 (p.p.m.) extracted by 0.1N NaOH in both the methods.

$X_1 + X_2$ = Total inorganic PO_4 (p.p.m.) extractable in both the methods.

(b) Field Experiments

Williams (1950b, c) had collected a number of soil samples from the red brown earths at the Waite Institute and some alkaline soils of known history of phosphate treatments. Some of these soils were extracted by the borate buffer versenate method and the results obtained are set against Williams' data in Table 31. From these slightly acid red brown earth soils from the farm of the Waite Institute, both the methods extracted virtually the same amount of phosphate (Table 31) and as a fertility index, one is as good as the other, which was expected because:-

(i) In these soils the phosphate dissolved by acetic acid + 8 hydroxyquinoline, suffers very little adsorption (see page 25)

TABLE 31.

The Comparison of Phosphate extractable (p.p.m.) by Williams and borate buffer versenate methods from soils of varying fertility.

Soil No.	pH	Treatment	Phosphate extractable with borate buffer-versenate method: Soluble in borate buffer + versenate	Soluble in 0.1N NaOH	Total extractable inorganic phosphate	Phosphate extractable with Williams' method [x]: 1st fraction soluble in acetic acid + 8 Hydroxyquinoline	2nd fraction soluble in 0.1N NaOH	Total extractable inorganic phosphate	Remarks
					PO_4 (p.p.m.)				
					Waite Institute Soils Red Brown Earths				
U454	6.31	2 cwt. Super/acre	45	118	163	42	112	156 (a)	(a) Phosphate uptake by wheat = 6 lb./acre (Williams 1948 p.73)
U442	6.39	23.5 cwt. Super/acre	118	230	348	150	212	362 (b)	(b) Phosphate uptake by wheat 23.4 lb/acre (Williams ibid)
U188	6.37	Nil	30	62	92	20	52	72 (c)	(c) Phosphorus uptake by wheat, oats, barley & peas was 3.3, 6.9, 7.4 & 8.9 lb P_2O_5/acre, whereas in the case of U185, it was 15.2, 12.7, n.d. & 27.0 lb respectively (Williams 1950c).
U185	6.34	60 lb P_2O_5/acre applied as superphosphate	82	140	222	72	126	198	
					Some alkaline Soils				
11034 Appila	8.88	Nil	80	33	113	60	45	105	
11024 "	8.76	30 cwt. Super/acre	298	77	375	352	121	473	
10774 Pallamana	8.17	Nil	32	43	75	28	43	71	
11010 Wokurna	8.81	Nil	77	31	108	77	25	102	
11036 Wepowie	8.51	Nil	185	91	276	255	129	384	

[x] Williams data.

(ii) These soils were treated with superphosphate, which being one of the highly soluble forms of phosphate, was equally well extractable by both the methods.

The differences become more apparent only where apatites or forms like the rock phosphate are present.

The data pertaining to some alkaline soils (Table 31), also show the same trend, in both the methods, except in soil No. 11024 and 11036. It seems that from both of these soils, acetic acid + 8 hydroxyquinoline, dissolved a fair amount of apatites, which may be either originally present in the soil, or may have been formed due to the reversion of superphosphate at such a high pH, particularly in soil No. 11024 (pH 8.76). Dean (1938) and Nagelschmidt and Nixon (1944) also observed that in an alkaline soil at Rothamsted, superphosphate had been retained by the soil as tricalcium phosphate or apatite.

TABLE 32.

The Comparison of phosphate extractable by Williams' and borate buffer-versenate methods from samples of soils from the permanent pasture experiment (W.A.R.I.)

Soil No.	Treatment	pH	Phosphate extracted by Williams' method*		
			1st fraction soluble in acetic acid + 8 Hydroxy-quinoline	2nd fraction soluble in 0.1N NaOH (inorganic) PO_4	Total extractable inorganic phosphate
			(PO_4 p.p.m.)		
U622	Nil	6.21	19	40	59
U618	Super	6.20	254	274	528
U624	Rock Phos.	6.36	608	139	747
U626	Basic Slag	6.64	282	215	497

Soil No.	Treatment	pH	Phosphate extractable by borate buffer versenate method		
			1st fraction soluble in borate buffer + versenate	2nd fraction soluble in 0.1N NaOH (inorganic) PO_4	Total extractable inorganic phosphate
			(PO_4 p.p.m.)		
U622	Nil	6.21	13	43	56
U618	Super	6.20	190	363	553
U624	Rock Phos.	6.36	36	152	188
U626	Basic Slag	6.64	169	276	445

* Williams' data.

In Table 32 are shown the results of phosphate extracted from the soils under natural pasture experiment, at the Waite Institute. The experiment was started in 1925, and the main treatments are:-

(1) no fertilizer

(2) superphosphate

[x] (3) superphosphate + nitrate of soda

(4) rock phosphate

(5) basic slag

The rate of application is 40 lb. P_2O_5 / acre per annum. The comparison of phosphate soluble in borate buffer + versenate (36 p.p.m. PO_4) with that soluble in acetic acid + 8 Hydroxyquinoline (608 p.p.m. PO_4) (Williams' data 1950b) shows that though in 22 years[xx] rock phosphate increased the more soluble forms such as $Ca(H_2PO_4)_2$ and $Ca_2(HPO_4)_2$ to a certain extent, but most of it remained in apatite form. Secondly there occurred an appreciable increase in the forms of inorganic phosphate soluble in NaOH; from 43 p.p.m. in the control plots to 152 p.p.m. in the plots treated with the rock phosphat This shows that this build up of inorganic phosphate soluble in the alkali has been due to the conversion of the first fraction soluble in borate buffer + versenate, to iron, aluminium and other adsorbed phosphates. The yields of herbage (Waite Report 1943 - 47) show that since 1930 the plots treated with rock phosphate, are yielding as high or even higher, than those treated with superphosphate or basic slag. It is difficult to offer any satisfactory explanation, but the following seems to be a plausible one.

Rock phosphate slowly dissolved, giving rise to $H\overset{=}{P}O_4$ and $H_2\overset{-}{P}O_4$ ions, which are partially depleted by the growing crop, as the plants take up their phosphorus mainly as $H_2\overset{\prime}{P}O_4$ ions (Russell 1952 p.38), and partially adsorbed by the sesquioxides. Thus at the prevailing pH of the soil, from an apatite form, the equilibrium is shifting to comparatively more soluble phosphates and adsorbed phosphates, and both these forms have been a source of phosphorus to plants./ The

---

[xx] Sampled in 1947.

[x] Sample not available.

evidence for this comes from the fact that in the first 5 years of the dressings with rock phosphate, the yield of the herbage was not increased appreciably, which indicates that some changes in rock phosphates were prerequisite, before it could be as good a source of phosphorus as superphosphate and basic slag. The more soluble phosphates thus formed being subjected to two way continuous removal could not build up large reserve. The following equation can illustrate the reactions involved.

$$\begin{tikzcd}
\text{Rock phosphate} \arrow[r, shift left] & H\,PO_4^{=} \arrow[l, shift left] \arrow[r, shift left] & H_2\,PO_4^{-} \arrow[l, shift left] \arrow[r, shift left] \arrow[d] & \text{adsorbed phosphates} \arrow[l, shift left] \\
 & & \text{available phosphate utilised by the crop.} &
\end{tikzcd}$$

(c) <u>Phosphate trials on Potatoes and Pastures.</u>

During 1951 and 1952 officers of the Department of Agriculture in South Australia and Tasmania conducted some field trials with various phosphatic fertilisers on a variety of soils and crops. Some of the representative soil samples of the top 4" soil from these experiments were available and it was thus possible to compare the responses obtained in the field, with the phosphate extractable by the borate buffer-versenate method.

TABLE 33.

The Comparison of phosphate extractable by borate buffer versenate method with the yield of potatoes.[x] (Field Expts. with Potatoes).

			Phosphate (p.p.m.) extractable by borate buffer versenate method			
			1st Fraction soluble in borate buffer + versenate	2nd Fraction soluble in 0.1N NaOH (inorganic) PO4	Total extractable inorganic phosphate	Yield of Potatoes Tubers (tons/ac.
Elliot	E_4 (pH 5.2)	Nil	58	494	552	3.5
	E_5	Fine rock phos. 10 cwt./acre	67	539	606	5.7
(Tasmania)	E_6	Superphos. 5 cwt./acre	125	574	699	7.4
North Morton	NMI(pH 5.44)	Nil	210	1600	1810	8.1
(Tasmania)	NM_2	Fine rock phos. 10 cw./acre	253	1848	2101	8.7
	NM_3	Superphos. 5 cwt./acre	280	1680	1960	10.3

[x] Experiments were conducted by the Department of Agriculture, Tasmania, during 1951 - 52, and samples analysed by the author during 1953.

From the data given in Table 33, it is apparent that on these soils the phosphate soluble in the borate buffer + versenate can fairly well be used as an index of readily available phosphate to potatoes. Secondly it also shows that these soils have very high amounts of inorganic phosphates soluble in the alkali, but the potatoes still respond to superphosphate. It indicates that they have high phosphate fixation capacity, which is probably tied up with high amounts of free sesquioxides in these soils. The unavailability of rock phosphate to potatoes, is also apparent.

Likewise broadly speaking, the data presented in Table 34 also show the value of borate buffer-versenate method in predicting the amount of available phosphate for pastures in different soils, but a high correlation is not possible because of the inadequacy of samples and yield records.

TABLE 34.

The Comparison of phosphate extractable by borate buffer versenate method with the yield of Pasture (Sub.Clover, White Clover, Perennial Rye grass) (Field Expt. with Pasture[x])

Locality	Sample No.	Treatment	Phosphate (p.p.m.) extractable by the borate buffer versenate method			Yield of Pasture	Remarks
			1st Fraction soluble in borate buffer + versenate	2nd Fraction soluble in 0.1N NaOH (inorganic PO_4)	Total extractable inorganic phosphate	Cwt./acre.	
Gressy	Cressy 1 (pH 5.62)	Nil	24	21	45	37.5	
	Cressy 2	Super 1 cwt./acre	32	53	85	63.0	
(Tasmania)	" 3	" 2 "	47	58	105	70.5	
	" 4	" 4 "	122	98	220	74.0	
	" 6	Rock Phosphate (Finely ground) 4 cwt./acre	73	124	197	76.5	
Elliot	Elliot 1 (pH 5.90)	Nil	42	452	494	Poor	No quantitative records available
	Elliot 2	Super 1 cwt./acre	45	490	535	Poor	
(Tasmania)	Elliot 3	" 2 " "	37	442	479	Fair	
	Elliot 4	" 4 " "	52	454	506	Fair good	
	Elliot 7	Rock Phos. (Finely ground) 4 Cwt./acre	44	522	566	v. Poor	
Xybybolite	A1 (pH 5.48)	Nil	24	28	52	36.6	
	A2	Superphos. 1 cwt./acre	24	32	56	59.5	Statistical Analysis of the yield shows:-
	A3	" 2 " "	33	37	70	65.8	
S. Australia	A5	Rock Phos. 4 " "	23	37	60	41.7	1. A2, A3, A7, A9, A10 significantly better than the control.
	A7	Very Fine Rock Phos. 4 cwt./acre	27	44	71	54.4	2. Treatments A3, A10 gave significantly greater yields than A5.
	A9	Sub.acid Phosphate 2 cwt./acre	26	37	63	57.5	3. No significant difference between A1 and A5.
	A10	Silico Phosphate + Gypsum 2 cwt./acre	74	55	129	65.3	

[x] Experiments conducted by the Department of Agriculture Tasmania and S.Australia.

PART II. COPPER

I. INTRODUCTION.

The necessity for copper in the nutrition of plants did not emerge as an isolated and clear cut discovery. Raulin's discoveries (1863-69), that zinc and probably manganese and silicon were also essential for the growth of *Aspergillus niger*, stimulated the search for other micro-nutrients. It stood by itself until early in the Twentieth Century.

At first, all increases in the yield of crops by copper were ascribed to chemical stimulation (Konda, 1904; Felix, 1927; Allison, Bryan and Hunter, 1927). The basis of the recommendations of Lutman (1911) and Floyd (1913, 1917) for copper sprays was the fallacy that it kills some fungi causing lower yields of potatoes and die back in citrus. Hudig et al. (1926) observed its beneficial effect on moor-lands in Holland, in overcoming the reclamation disease of cereals. In 1927 Bortels demonstrated the necessity of copper for producing black spores in *Aspergillus niger*, but credit goes to Sommer (1931) to establish its essentiality for sunflower, tomatoes and flax. In 1942, Piper in Australia, with critical water culture experiments, firmly established the indispensability of copper as a plant nutrient. Now it is well-known that in minute traces, copper is biologically essential for the growth and reproduction of green plants. Its deficiency is identified in cereals with reclamation disease, and in citrus with exanthema.

The importance of copper in the nutrition of animals can hardly be over-emphasised, in view of the deficiency diseases from which cattle and sheep are known to suffer when grazing in pastures with suboptimal copper content in the herbage, particularly in Australia, New Zealand, United States of America, United Kingdom and the Continent of Europe. The problem is not easily solved by applying copper to the soil, partly because it is liable to become toxic to plants and animals if present beyond certain limits, and partly because it is easily fixed, consequently becoming unavailable to plants. However, from the point of view of the soil, it is mainly a problem of amount, fixation and availability of the nutrient. This thesis is primarily concerned with these aspects of copper in the virgin soil of the Seddon Gravelly Sandy Loam from Kangaroo Island.

II. REVIEW OF LITERATURE.

1. Total Copper in the Soil.

Although considerable interest has been evinced in the copper content of the plants and some authenticated data are available, yet the information on the total copper content of the soil is very scanty. It may be partly due to the technical difficulties involved in the estimation of small quantities of copper in the soil, and partly due to the doubt in the reliability of such an analysis as an index of the availability of the nutrient to plants and animals.

McMurtrey and Robinson (1938), have found that the total copper content of most of the agricultural soils of United States of America ranges from 1 to 50 p.p.m., while Holmes (1943), from an extensive study, reported this range to be 2 to 67 p.p.m. Sedletski and Ivanov (1941) observed that in U.S.S.R., red earths, chernozems, peaty podsolized soils and highly podsolized sands have 140.0, 60.8, 32.0 and 3.0 parts of copper per million parts of soil respectively. In a later paper, Ivanov (1950) reported that the average copper content of the soils, he examined, was between 10 to 30 p.p.m., and primitive soils, formed under lichens, were found to contain many times more copper than the parent-rocks. Malyguga (1944), another Russian worker, reports that the chernozems contain somewhat more copper than the average (20 p.p.m.), while podsol swamps and serozems are below average. He also reports the non-uniform distribution of the element in the soil profile and takes it as a sign of the presence of the metal combinations such as carbonates or humates in the soil. Shiha (1951) from Japan reported that the total copper content of the soils he examined, ranged from 26.4 to 150.8 p.p.m., while available copper was 0.5 to 8.8 p.p.m. Total copper was less in soils derived from shales than in those from andesite. Acock (1941) reports that the HCl soluble copper in soils from Kangaroo Island (South Australia) ranged from 0.3 to 22.0 p.p.m., while a red brown earth from the Waite Institute property contained 20 p.p.m. Riceman, Donald and Piper (1938) have indicated that the calcareous sands, which extend for hundreds of miles along the South Eastern Coast of South Australia, have extremely low total copper content. Consequently, the sheep grazing on the pastures grown on these soils suffer from coast disease - a copper-cobalt deficiency disease. Cunningham (1950) from New Zealand, states that there are about 250,000 acres of drained and reclaimed peat where the cattle and sheep are prone to suffer from <u>peat scours</u> because of the comparatively low copper and high molybdenum content of the pasture. The same author goes on to say that these soils have 3 to 7 p.p.m. of total copper, whereas the normal soils contain about 18 to 22 parts of copper per million parts of soil. Mitchell (1948) in Scotland, by spectrochemical analysis of soils derived from granites, norite, serpentine, slate, sandstone and granitic gneiss, found that they contained $<$ 10, 10, 25, 30, 40 and 40 parts of copper per million parts of soil, respectively. Thomas (1940) and Carroll (1941, 1947) in Australia, on geochemical considerations have attempted to correlate the copper deficiency of soils with the nature of the rocks, but no critical values of soil copper content have been given. Generally it is assumed that the soils derived from more basic rocks are less likely to show copper deficiency, in comparison with others formed from acidic parent material. The importance of a geochemical approach to the fertility problems of soils in general and trace elements in particular, hardly needs any emphasis, but it is dangerous to over-simplify and generalise, unless sufficient analyses of the parent material and soils are available to establish such correlations.

From the foregoing, it can be concluded that the amount of copper present in a soil is very small. In most cases, where crops and animals exhibit deficiency symptoms, the soil may be inherently deficient in the nutrient, due to the nature of the parent material or subsequent weathering. Most often the determination of the total copper content of the soil, or even the geochemical considerations as Mitchell in Scotland and Thomas in Australia have pointed out, can be useful in predicting the copper status and its likely consequences, but the difficulty arises in another group of soils where the total copper content may be sufficient and animals and crops may still be suffering from inadequate availability of the nutrient or may be due to improper balance with respect to other nutrients. Such problems of induced deficiency in contrast to the inherent deficiency can only be solved by proper understanding of the factors affecting fixation and release of copper and its interactions with other nutrients in relation to plants.

2. Ability of soils to supply Copper to plants

(Fixation and availability of Copper)

The ability of soils to supply copper to plants depends on the total content of the nutrient and perhaps much more on its forms of association with the soil components. Besides the climatic and plant factors, the other factors influencing the fixation and availability of copper in the soil are:-

i. Soil reaction or pH
ii. Calcium carbonate
iii. Organic matter or humus content
iv. Microbial activities
v. Amount and nature of clay minerals and other inorganic colloids
vi. Fertiliser practices such as the application of phosphates

(i) Soil Reaction or pH

According to Britton (1932) copper is precipitated as the hydroxide at pH5.3 - 5.4. This is in accord with the experimental evidence that the availability of copper is greater in soils more acid than this pH (Piper, 1942; Piper & Beckwith, 1949). Beckwith (reported by Piper, 1947) observed that precipitation of copper as hydroxides, basic carbonates, phosphates or sulphides, always left traces of copper in solution. According to Piper (1942) even traces of copper, i.e. 1 part in 10^9 in nutrient solution, are sufficient for the normal growth of the plants. It seems that precipitation alone cannot account for the lack of availability of copper in a soil, though the soil reaction determines the availability to a great extent. Peech (1941), Lucas (1948), Steenbjerg & Boken (1950) have confirmed the high fixation of copper with increase in pH. Steenbjerg (1940) in an earlier paper reported that there is minimum solubility of copper in a soil at pH 5.5. Lundblad et al. (1949) concluded that in acid soils of Sweden losses of copper in drainage water are marked and liming, due to its effect on pH, may prevent such losses. Likewise, Bryan (1940) observed that copper deficiency in citrus is more prevalent on acid soils than on neutral soils in Florida. Harmer (1941) reported that the muck soils of Michigan gave a greater response to copper when pH is below 6.0, although a few crops responded to added copper when pH exceeded 7.0. On the contrary, Williams (1930) obtained plant injury from an application of 50 lb. of copper sulphate per acre, on an unlimed soil in North Carolina, whereas an application of 2 tons of lime removed the injurious effect, and 6 tons per acre created a deficiency. Purvis & Davidson (1948) conclude that in soils limed in excess of neutrality copper becomes fixed and in more acid soils gets lost through leaching. Thus it seems that the effect of soil reaction on the availability of copper is difficult to generalise because in highly acid soils it may become deficient through leaching, or may become toxic because of high solubility. However, in neutral or alkaline soils the problem may be of its precipitation, and thus becoming less available.

(ii) Calcium Carbonate

Boischot & Quillon (1952) concluded that in calcareous soils ($pH > 7.0$) copper in solution could not reach a higher concentration than 4 mg. per litre. Solutions containing 0 - 1.4 mg. copper per litre were treated for different periods with varying amounts of lime, and the percentage of copper fixed was found to rise with the increasing concentration. Fixation was complete within one hour. They also observed that the uptake of copper was 10 times higher in non-calcareous soils than in calcareous ones. Likewise, Donald, Passey & Swaby (1952) hold the calcium carbonate responsible for fixing copper and other trace elements in the Robe soil ($CaCO_3 > 50\%$). Steinberg (1935) advocated the use of $CaCO_3$ for effectively removing traces of heavy metals like copper from cultures, and both Stout & Arnon (1939) and Piper (1942) have adapted this technique for the removal of traces of micronutrients from nutrient solutions for higher plants.

The fixation effect attributed to $CaCO_3$ may partly be due to the effect of high pH favourable to precipitation of $Cu(OH)_2$ and basic copper carbonate and partly due to its fine particles providing locii for the precipitating particles to gather around. Donald et al. (1952), however, do not subscribe to this view that the fixation of copper by calcium carbonate is due to its high pH, because from a medium more alkaline than the one containing $CaCO_3$ the fungus (A. niger) could extract copper quite readily. On the other hand, Beckwith (1953), in these laboratories, could not find any evidence of the irreversible fixation of copper by $CaCO_3$.

as the copper adsorbed was readily extractable with a solution of ammonium chloride. Thus it is still an open question how $CaCO_3$ renders copper unavailable to A. niger (Donald et al., 1952) and to plants (Boischot et al., 1952).

(iii) Organic matter or humus content

Many investigators (Smith, 1927; Rademacher, 1936; Meizer, 1934; Hoffmann, 1939; Brandenburg, 1933) believe the cause of reclamation disease of cereals - a copper deficiency disease so prevalent on newly reclaimed heaths, moor, muck and peat soils - is the unavailability of copper due to its fixation by the organic matter. Lees (1950) reports that the humus seperated from a Trinidad soil fixed 140 m.e.% of copper. Vermaat et al. (1950) attributed the copper retaining power of soils to the circulating (coarse organic matter) organic matter. In order to immobilise copper and to render it unavailable to rubber plants, they suggested the heavy applications of organic matter. Svanberg et al. (1949) have observed that fixation of copper in soils is a function of organic matter. Soils more acidic than pH 5.5 showed a lower coefficient, but the effect was still a function of organic matter. Allison (1931) reported that the copper fixing power of peats was so great that an application of 10,000 lb. of copper sulphate per acre caused but a little temporary injury. Similar are the observations of Bobko & Panova (1945). Vegetable farmers on the Carolina coastal plain usually apply 200 lb. of copper sulphate per acre to newly cleared peats and may apply 50 lb. per acre annually (Gilbert, 1952), whereas on the other hand Teakle (1942), in Western Australia, observed toxic effects with as little as 10 lb. application of copper sulphate per acre in some sandy mineral soils. Three pounds per acre were found to be the optimum dressing for such soils. However, this appears to be rather an exceptional case, but the fact remains that the organic soils require high dressings of copper sulphate to produce good crops (Browne, 1950; Comin, 1944; Harmer, 1941, 1945). This has been attributed to the lower availability of copper from organic soils (Antipov Karataev, 1947; Steenbjerg et al. 1950; Vermaat et al. 1950), but Swedish workers Lundblad et al. (1949) could not find any evidence to subscribe to this view; they hold that the question is not of copper fixation but rather of the absolute deficiency of the nutrient in such soils. They have gone to the extent to say that in peats copper is deficient not because peat is there, but the peat is there because the copper is deficient. Donald et al. (1952) using A. niger technique, also could not find any evidence that humus or peat rendered copper unavailable. Thus it is still a disputed point whether the peats are the cause of copper deficiency or the effect thereof.

The knowledge about the other aspect as to how the copper is held by the organic matter is also very scanty. Bremner et al. (1946) are of the opinion that copper is fixed by organic matter in metallo organic complex forms. Dawson & Nair (1950) recognise some combination of protein and lignin, involving oH group, for the formation of both copper and copper ammonia complexes, by the peat soils. The groups reacting with copper to form the primary copper peat complexes are RSH and RCooH, provided they occur in the same molecule to form these complexes. The RNH_2 group is considered to be necessary for the formation of secondary copper ammonia peat complexes. Smith (1927) attributed the fixation of copper by the organic matter to the presence of gliedine. Recent work by Kletz, Fuller & Urquhart (1950), Fraenkel et al. (1950) shows that certain proteins are quite avid for heavy metals. It is well-known that a number of microbial products like histidine, cystine, riboflavine are capable of chelating with heavy metals. Eriksson (1949) considers the binding of copper to organic matter is through oxygen, sulphur and nitrogen groups.

Besides the above-mentioned aspects, there are three other aspects of the relationship of organic matter and copper on which information is lacking.

(a) Effect of the high exchange capacity of organic matter and consequently low degree of saturation with a given amount of copper on the fixation and release of the nutrient.

(b) Nature of the complexes of organic matter with copper, which may not be easily broken by conventional electrolyte solutions and even by plants, but may be easily breakable with chelating compounds.

(c) Quality of organic matter and its relation to copper fixation.

(iv) Microbial activities

It is generally assumed that microbes decomposing organic matter may compete with plants by locking up copper atoms in their cells. However, Donald et al. (1952) in Australian soils could find no evidence of the fixation of trace metals like copper and zinc by microbes.

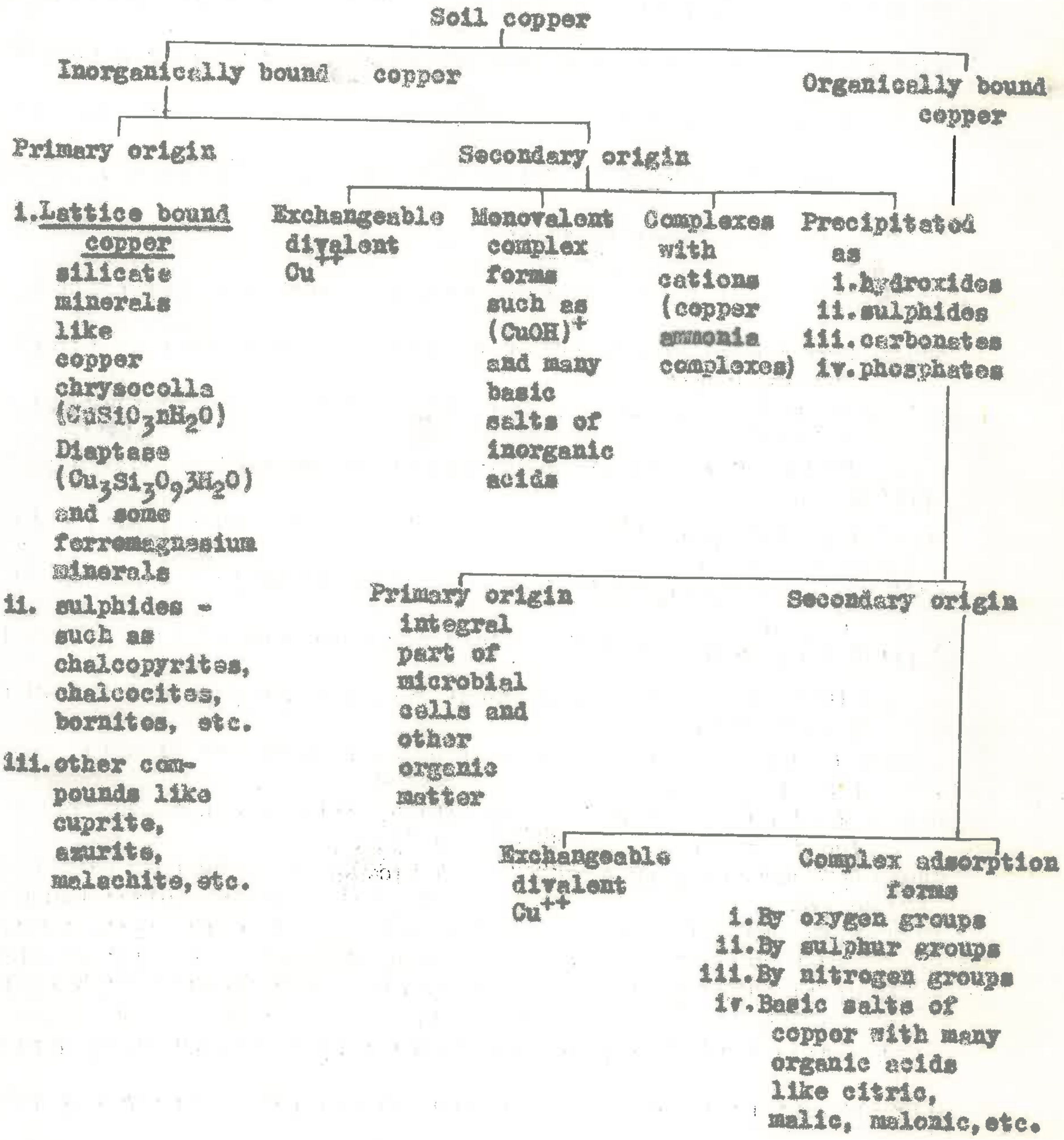
(v) Amount and nature of clay minerals and other inorganic soil colloids

Steenbjerg & Boken (1950) have concluded that besides pH and humus clay content is another very important factor which affects the availability of copper. Antipov Karataev (1947) noted that bentonite and montmorillonite held copper ions very tightly. Menzel & Jackson (1950) observed that hydroxy cupric ions constitute at least 50% of the copper sorbed by kaolinite or montmorillonite from cupric solutions. Hydroxy cupric ion sorption is increased by increasing the cupric ion concentration or the pH of the cupric solution. It is more pronounced with kaolinite than with montmorillonite. Epstein & Stout (1951) using radioactive isotopes, concluded that when Fe, Mn and Cu are less than 0.1% of the exchangeable cations, the amount available is almost proportional to their concentration. Above a degree of saturation of 0.1% varying proportions of Ca and H, as complementary ions, affect the uptake of micro-nutrients very little. Unfortunately no work has been done on the role of inorganic soil colloids like sesquioxides and silica in fixing and affecting availability of copper, but as Donald, Swaby & Passey (1952a) have found that aluminum oxide (chromatographic brand) is very effective in removing traces of heavy metals, the part played by iron and aluminum oxides and other colloids in fixing copper in soil may not be insignificant. However since this brand of Al_2O_3 has alkaline reaction, its activity may even be a pH effect causing precipitation.

(vi) Fertiliser practices such as the application of phosphates

Jamison (1943) in laboratory studies noted a rapid drop in the solubility of copper in the presence of phosphates in the region of pH 5 to 6, the fall continues to pH 10.0. But in most of the soils of Florida which are known for copper and zinc fixation he found little difference in the fixation of both these elements in the presence or absence of superphosphate. In the Norfolk sand moderate applications of superphosphate were found to increase the solubility of copper. Reuther and associates (1952) in fertiliser experiments with oranges on acid sandy soils in Florida, observed that even seven years of heavy application of phosphate did not produce any marked increase in the accumulation of copper, zinc and manganese.

From the foregoing review of the factors effecting the fixation of copper and the probable forms in which copper distributes itself in the soil, it is evident that the information on these aspects is very scanty, but the following tentative scheme may illustrate the forms in which copper exists in the soil.



- Soil copper
  - Inorganically bound copper
    - Primary origin
      - i. **Lattice bound copper** silicate minerals like copper chrysocolla ($CuSiO_3nH_2O$) Diaptase ($Cu_3Si_3O_9 3H_2O$) and some ferromagnesium minerals
      - ii. sulphides - such as chalcopyrites, chalcocites, bornites, etc.
      - iii. other compounds like cuprite, azurite, malachite, etc.
    - Secondary origin
      - Exchangeable divalent Cu^{++}
      - Monovalent complex forms such as $(CuOH)^+$ and many basic salts of inorganic acids
      - Complexes with cations (copper ammonia complexes)
      - Precipitated as
        - i. hydroxides
        - ii. sulphides
        - iii. carbonates
        - iv. phosphates
  - Organically bound copper
    - Primary origin integral part of microbial cells and other organic matter
    - Secondary origin
      - Exchangeable divalent Cu^{++}
      - Complex adsorption forms
        - i. By oxygen groups
        - ii. By sulphur groups
        - iii. By nitrogen groups
        - iv. Basic salts of copper with many organic acids like citric, malic, malonic, etc.

3. Methods of assessing the available copper in soil

The factors effecting fixation of copper in soils determine the availability of the nutrient to plants. It is hard to define what the available copper is, because the availability of a nutrient is generally measured by the increases in growth or yield obtained by the application of the nutrient under investigation. The growth or yield being a resultant of so many independent and some inter-dependent variables, can hardly be relied upon for this purpose.

The second criterion is the percentage amount of the nutrient in plants. Piper et al. (1943) determined the availability of copper in different soils by estimating the copper content of Algerian oats grown on them, but as Steenbjerg (1948) has pointed out the leaf analysis or the copper content of the plant is not an infallible guide in the diagnosis of copper deficiency. When under severe conditions of copper deficiency the growth is stunted, the copper content of the plant material as percentage of dry matter may not be unduly low. Swanback (1950) observed that, with one exception, there was less copper deposited in the leaves of tobacco, with copper sulphate application, than without it. It indicates that copper content of the plant is not the index of the available copper in the soil.

The third approach is the analysis of the soil for total copper or part thereof, and its correlation with either the copper content of the plant or total copper uptake by the plant. The total copper content, particularly in soils with absolute deficiency of the element, is fairly reliable an index of their copper-supplying power, but there are other soils where the total copper content may not be unduly low but the crops may still respond to copper application, as happens in many organic soils. To solve such problems efforts have been made to get an estimate of some fraction of total copper which may serve as an index of its availability.

There are two types of laboratory methods which aim to measure the available copper in soils:

(a) Chemical extractants

(b) Biological methods

Chemical methods of extraction are of two types:

(a) Single extractions

(b) Fractionation

The common solvents used for single extractions are water, acetate buffer pH 5.0 (Morgan, 1941; King, 1947), HCl of such a concentration as to give final pH of the suspension approximately 2.0 (Steenbjerg et al., 1950), $0.5N.HNO_3$ (Antipov Karataev, 1947), 1N.HCl or HNO_3 (Lucas, 1948), Nitric + Perchloric acids (concentrated) (Lundblad et al., 1949). In these methods a correlation is established between the amounts of copper extracted from the soil and its response to crops. Steenbjerg et al. (1950) take an average copper value of 1.6, 3.1 and 14.8 p.p.m. of copper in the soil, as a sign of severe deficiency, deficiency and sufficiency of copper, respectively.

Out of the methods of fractionation, Wood's (1945) and Vermaat et al.'s (1950) are important. Wood recognises three categories:

(a) Readily available copper - extractable with 1N. ammonium acetate

(b) Slowly available copper - extractable with $1N.HNO_3$

(c) Extremely slowly available copper - soluble in $5N.HNO_3$

He assumes that these forms exist in equilibrium with one another, though the nature of the compounds of copper in these forms is not known. Vermaat et al. (1950) also divide copper into three forms:

(a) Exchangeable (ionic form) - soluble in 2% H_2SO_4

(b) Exchangeable + organically bound - (ash copper) - extractable with HNO_3 + H_2SO_4, after igniting the soil

(c) Lattice bound or silicate copper - determined by fusion of the residue from (b) with bisulphite.

These authors consider the ionic form of copper derived from both inorganic and organic combinations, as available copper.

Besides these, the use of complexing agents such as alkali pyrophosphate for extracting available copper has also been suggested by Eriksson (1949) and Heintze and Mann (1949), but the usefulness of such extractants has got to be yet established.

Out of the biological methods, the <u>Aspergillus niger</u> bio-assay method suggested by Mulder (1937, 1938) has been tried by many investigators such as Mulder (1948) and Gerretsen (1948) in Holland, Nicholas (1950) and Nicholas and Fielding (1951) in Britain, Wright and Johnston (1950) in New Zealand, Acock (1941) and Donald et al. (1952) in Australia. The fungus fails to develop black spores in copper deficient medium and the intensity of sporulation can be compared with the standards and thus copper available to the fungus estimated. The soil to be tested is sterilised before adding copper free medium and autoclaving with A. niger inoculum. Most of these investigators have asserted that the results obtained with A. niger method are better correlated with the field responses than those obtained by many chemical or spectro-chemical methods, but this bio-assay method also suffers from three main defects:

(a) The fungus is expected to simulate the action of plant roots, which is impossible. Being very sensitive to copper it can only distinguish copper deficient soils from the non-deficient ones.

(b) In the case of organic soils the fungus extracts more copper than is actually available to crops. Thus it fails to indicate copper deficient soils (Wright and Johnston, 1950).

(c) The sterilisation of the soil, which is a pre-requisite of the bio-assay method, makes the prediction of availability by this technique unreliable, as Piper (1942) observed, that sterilisation of a copper deficient soil made it non-responsive to any applications of copper sulphate.

From the above review of the methods, it is evident that so far there is no clear cut answer to

(a) what forms or compounds of copper in the soil constitute the available copper;

(b) how to measure the available copper.

At the present state of knowledge, none of these laboratory methods can be expected to determine the absolute amounts of available copper, they can only rate the soils on some empirical basis standardised with the field observations.

III. METHOD AND MATERIAL

Materials

For the most of the investigations outlined in this part of the Thesis, the same soils were used, as have been described in Table I, Part I, in connection with the phosphate studies. As the Seddon .V. soil contains minerals such as gibbsite, goethite and kaolinite, samples of these minerals, along with bentonite (Wyoming bentonite) and aluminium oxide (A.R.B.D.H. chromatographic brand) were also included in these studies.

Preparation of samples

The soils were carefully subsampled, ground to pass through 0.5 mm. mesh sieve of stainless steel, and kept in glass jars for use. In the case of minerals whose X-ray diffraction patterns were recorded by K. Norrish of C.S.I.R.O., Soils Division, fifty grams of material were ground in a porcelain pestle and mortar and sieved on a 0.2 mm. sieve (B.S. No.80). An amount of 10-15 gms. of the material passing through was further pulverized for one hour, in a mechanically driven agate pestle and mortar. This well ground material was used for all the studies reported here.

Separation of the coarse organic matter

Coarse particles of organic matter along with coarse sand were separated from the Seddon .V. (0-4") soil. The soil was dispersed in water and sieved on 0.2 mm. sieve. The material thus separated was washed thoroughly to remove as much of the adhering colloidal clay particles as possible. It was further transferred to a 500 ml. dish containing water and heated on the water bath. The coarse organic particles floating on the surface were removed by hand. After evaporating the water, the remaining organic particles which had formed a scum on the coarse sand were removed and freed from any adhering sand grains by refloating on water. It was air dried and gently crushed before use.

Separation of humus from the soil

Humus was extracted from the Seddon .V. (0-4") soil, using the similar technique as adopted by Vermaat et al. (1950) for removing the Matière Noire from the soil. 40 gms. of soil were shaken over-night, with 200 ml. of 0.5N. copper free ammonia. After centrifuging, the aliquot was collected in a Winchester. This process was repeated a number of times till no more coloured extract was obtained. The combined extracts were acidified with HCl and filtered through Whatman no.44 filter paper. The precipitate was washed free of chlorides and dried on the water bath, followed by drying in the air. It was crushed before use.

Methods

(i) Equilibrium studies

To 0.5 gm. duplicate samples of each mineral in centrifuge tubes, 35 ml. of copper sulphate solution of appropriate concentration, namely $^N/_{2000}$, $^N/_{1000}$, $^N/_{500}$, $^N/_{250}$, and $^N/_{100}$, were added and shaken for 16 hours at a constant temperature of 20°C. It was centrifuged and the clear supernatant liquid was collected in pyrex bottles and used for the determination of the non-absorbed copper. To remove the remaining copper sulphate solution from the solid phase, the residue was washed with two separate 25 ml. lots of 60% alcohol by shaking for 5 minutes followed by centrifuging. All washings were rejected and the final washing was done with 25 ml. of 60% redistilled alcohol. Three washings were found adequate to remove traces of the copper sulphate solution, occluded by the solid particles. For extracting the copper adsorbed by the minerals or soils, the washed residue was shaken for 16 hours with 35 ml. or 100 ml. of HCl of appropriate concentration, and centrifuged, and in the extracts thus obtained copper was estimated. In some cases, further extractions were also done after removing the centrifugate of the previous extractions. Duplicate blanks were carried out in the same way. In the case of soils, the rest of the procedure was the same except that 1.4285 gm. of soil and 100 ml. of copper sulphate solution were used for equilibrium studies. However the ratio of soil : copper sulphate solution was the same (1:70) as in the case of minerals.

For the study of the exchange of copper with neutral solutions of electrolytes, a batch of soils and minerals were brought into equilibrium with copper equivalent to 3.5 m.e.% (1106 p.p.m. Cu^{++}), which was added as 100 ml. of $^N/_{2000}$ copper sulphate solution, to 1.4285 gm. of material. After centrifuging and washing, the adsorbed copper was extracted successively either with 100 ml. of 0.005N.NaCl, 1N.NaCl and 0.5N.NaCl + 0.05N.HCl or with calcium chloride solutions of the same concentrations as the sodium chloride solutions. The copper recovered with each extraction was estimated separately. The solutions of NaCl and $CaCl_2$ were purified by using the technique of Donald, Passey and Swaby (1952a) and the reaction of the copper free solution was adjusted to pH 7.0.

(ii) Estimation of copper from solutions

The method used for copper estimation was essentially that of Piper (1950) except for a few modifications wherever necessary.

In the centrifugates obtained from minerals after equilibrium with copper sulphate solution, copper was determined directly without prior extraction with dithizone, but before adding ammonia, in order to avoid precipitation of aluminum and iron hydroxides, an addition of 2 ml. of 10% solution of ammonium citrate was found necessary. The same procedure was followed for the determination of the copper exchanged, with weak solutions of HCl. In certain cases, where the amount of copper adsorbed represented only a small fraction of that applied, it was found more appropriate to determine the amount of copper adsorbed not by difference, as in other cases, but actually by extracting it with 6N.HCl. The copper saturated minerals or soils were shaken for 16 hours with 35 ml. 6N.HCl and the copper dissolved was extracted with dithizone and estimated by the carbamate method, as outlined by Piper (1950, pp.334-337).

For all the studies with soils, and in some with minerals also, the extracts were digested with 2 ml. H_2SO_4 in a microkjeldahl flask. Subsequent procedure was the same as described above.

IV. EXPERIMENTAL

(Equilibrium Studies with Copper.)

(i) Adsorption of Copper from Copper Sulphate Solutions by Minerals, Soils and Soil Separates.

The object of these equilibrium studies was to investigate the copper fixing power of the Seddon V. (0-4") soil, and to trace its relationship with some of its major mineral components. From Tables 35, 36 it may be seen that the amount of copper adsorbed by these minerals and soils is roughly related to their exchange capacity.

TABLE 35

Showing adsorption of copper from copper sulphate solutions by the minerals

Copper added Cu^{++} m.e.%	Amount of copper adsorbed				
	Kaolinite	Gibbsite	Goethite	Bentonite	Aluminium oxide (Chromatographic brand)
	pH6.8	pH6.8	pH7.06	pH10.26	pH8.98
			m.e.%		
3.50 (N/2000 $CuSO_4$)	1.69	2.90	1.10	3.50	3.50
6.40 (N/1000 $CuSO_4$)	1.80	3.30	1.20	6.40	6.40
13.90 (N/500 $CuSO_4$)	2.42	5.50	-	13.90	13.20
27.80 (N/250 $CuSO_4$)	2.70	5.60	1.30	27.80	26.90
69.50 (N/100 $CuSO_4$)	2.86	6.00	1.31	53.40	37.00
200.00 (N/100 $CuSO_4$) *	3.54	-	-	81.30	36.60
Exchange capacity (m.e.%) at pH7.0 as determined by N.ammonium acetate	4.1	1.90	1.00	95.2	2.2
	Percentage adsorbed $\left(\frac{Cu^{++}\text{ adsorbed}}{Cu^{++}\text{ added}} \times 100\right)$				
m.e.%			%		
3.50	48.7	82.8	31.5	100.0	100.0
6.40	28.0	51.2	18.6	100.0	100.0
13.90	17.2	39.6	-	100.0	95.0
27.80	9.7	20.0	4.7	100.0	96.8
69.50	4.3	8.5	1.9	76.8	53.3
200.00	1.72	-	-	40.65	9.15
	Copper adsorbed expressed as percentage of the exchange capacity $\left(\frac{Cu^{++}\text{ adsorbed}}{\text{Exch. capacity}} \times 100\right)$				
m.e.%				%	
3.50	41.3	152.6	110.0	3.7	159.1
6.40	43.9	173.7	120.0	6.7	291.0
13.90	59.0	289.5	-	16.4	600.0
27.80	66.0	400.0	130.0	32.8	1201.2
69.50	70.8	421.0	131.0	63.0	1681.7
200.00	-	-	-	85.4	1663.7

m.e.% = milligram equivalent per 100g. of the material.

* Mineral: Copper sulphate solution = 1:200, in all other cases it is 1.70.
(g) (m.e.)

But in none of them except goethite, gibbsite and aluminium oxide (chromatographic brand) has the copper adsorbed been equal to or more than

TABLE 36.

Adsorption of copper from the copper sulphate solutions by the soils
(Soil = solution ratio = 1:70)

Copper added Cu^{++} m.e.%	Amount of copper adsorbed m.e.%				Percentage adsorbed $\left(\frac{Cu^{++}\text{ adsorbed}}{Cu^{++}\text{ added}} \times 100\right)$				Copper adsorbed expressed as percentage of the exchange capacity $\left(\frac{Cu^{++}\text{ adsorbed}}{\text{Exch. capacity}} \times 100\right)$			
	Seddon V. (0-4") soil (pH6.19)	Seddon V. (4-8") soil (pH6.02)	Seddon (E.B.) soil (pH6.05)	Urrbrae V. (0-4") soil (pH5.91)	Seddon V. (0-4") soil	Seddon V. (4-8") soil	Seddon (E.B.) soil	Urrbrae V. (0-4") soil	Seddon V. (0-4") soil	Seddon V. (4-8") soil	Seddon (E.B.) soil	Urrbrae V. (0-4") soil
			m.e.%				%				%	
3.50m.e.%(N/2000 $CuSO_4$)	3.09	2.15	3.30	3.14	88.6	61.5	94.3	89.7	30.0	32.5	28.9	31.7
7.00 " (N/1000 $CuSO_4$)	4.50	2.50	5.40	4.90	64.3	35.7	77.2	70.0	39.6	37.9	47.3	49.5
13.90 " (N/500 $CuSO_4$)	5.70	3.60	8.60	6.20	40.5	25.7	61.1	44.4	56.0	54.5	75.5	62.5
69.50 " (N/100 $CuSO_4$)	6.80	3.80	9.10	7.70	9.7	5.5	13.0	11.0	66.5	57.6	79.8	77.6
Exchange capacity by ammonium acetate pH7.0	10.20	6.60	11.40	9.90	10.2	6.60	11.40	9.90	10.20	6.60	11.40	9.90

the exchange capacity, despite the fact that the amount of copper applied in N/100 copper sulphate solution has been much higher than their exchange capacity. Another noticeable feature is the high percentage retention from low concentrations of copper sulphate solutions and the decrease in the percentage adsorbed from stronger solutions. As is evident from Fig.X bentonite adsorbed all the copper below 27.8 m.e.% and aluminium oxide up to 6.4 m.e.%. The Seddon V. (0-4") soil adsorbed more copper than the Seddon V. (4-8") soil. When the percentage of the copper adsorbed is plotted against the logarithm of the copper added (Fig.XI) the plots of gibbsite and the Seddon V. (0-4") soil lie quite close to each other, while the Seddon V. (4-8") soil lies closer to kaolinite and below gibbsite. Aluminium oxide (chromatographic brand) and bentonite seem to be unrelated to any of these soils.

When the soil separates* of the Seddon V. (0-4") and (4-8") soils were treated with N/2000 copper sulphate solution, corresponding to 3.5 m.e. per 100g. of separates, the amount of copper adsorbed (Table 37) indicates that the coarse sand and fine sand fractions of the surface soil, adsorbed approximately twice as much copper as their counterparts from the deeper horizons.

TABLE 37

Copper adsorbed by soil separates from an addition of 3.5 milliequivalents of copper per 100g. of the separate.

Soil	Coarse Sand	Fine Sand	Silt	Clay
		Copper adsorbed m.e.%		
Seddon V. (0-4") soil	3.17	3.39	3.48	3.42
Seddon V. (4-8") soil	1.90	1.60	3.48	3.39

At this low level of copper the silt and clay fractions showed no differences, but when 70 milliequivalents of copper per 100 g. of the material were added, the differences in the copper adsorbing powers of these separates also became evident (Table 38).

TABLE 38

Copper adsorbed by silt and clay fractions from an addition of 70 milliequivalents of copper per 100 g. of the separate.

Method of extimation	Seddon V. (0-4") soil		Seddon V. (4-8") soil	
	Silt	Clay	Silt	Clay
		m.e.% Cu^{++} adsorbed		
Direct determination by difference	27.2	39.9	18.4	27.4
By extraction with 6N HCl	27.6	40.1	19.5	27.3

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* Separated by the ammonium carbonate dispension method of Puri (1935) without the removal of organic matter.

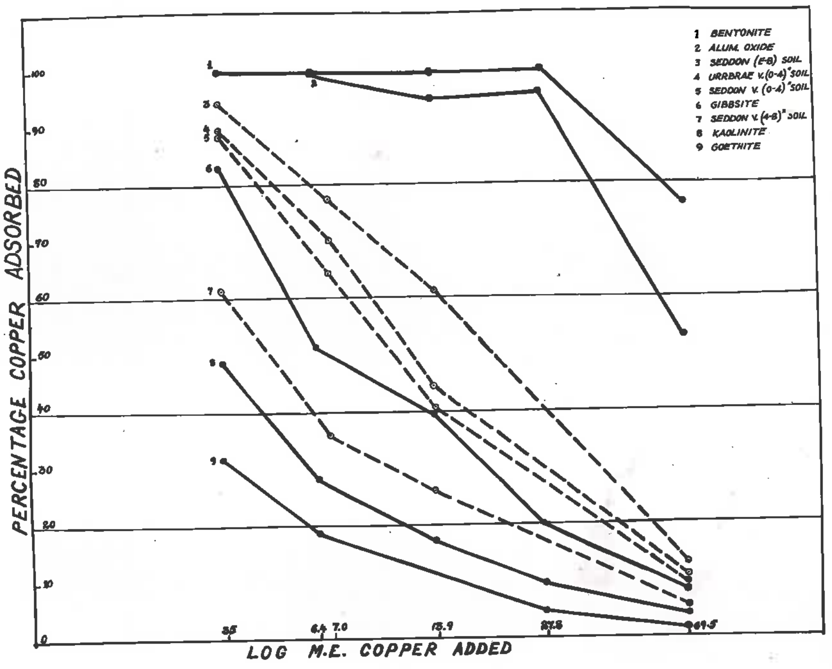

FIGURE X.

Showing percentage of the added copper adsorbed by soils and minerals.

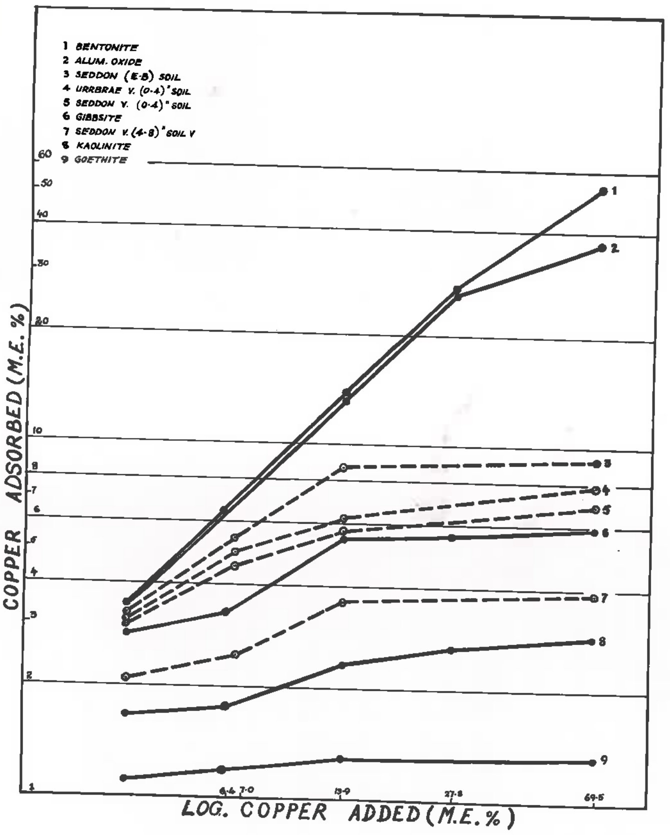

FIGURE XI.

Showing relationship between copper added and copper adsorbed by soils and minerals. (Both logarithmic scales.)

(ii) Exchange of copper with HCl

To replace the copper adsorbed by the minerals, an amount of HCl corresponding to the concentration and amount of copper sulphate solution originally used for adsorption studies, was added. The results (Table 39) show that within the range of these studies H^+ ions are quite effective in displacing most of the copper adsorbed by goethite, kaolinite and gibbsite, but not from aluminium oxide, and bentonite.

TABLE 39
Exchange of copper with HCl (from minerals)

Mineral	Copper added	Copper adsorbed	HCl added for exchange (m.e.%)	Copper ex-changed	Percentage copper exchanged $\left(\frac{Cu^{++}\text{exchanged}}{Cu^{++}\text{adsorbed}} \times 100\right)$	
Kaolinite	3.5	1.69	3.5 (N/2000)	1.59	93.5	
	6.4	1.80	7.0 (N/1000)	1.69	96.0	
	13.9	2.42	14.0 (N/500)	2.26	91.0	
	27.8	2.70	28.0 (N/250)	2.23	90.0	Mean
	50.0	2.64	50.0 (N/100)	2.60	93.0	93.1
	69.5	2.86	70.0 (N/100)	2.69	94.0	
	140.0	3.30	140.0 (N/100)	3.00	91.0	
	200.0	3.54	200.0 (N/100)	3.37	96.0	
Gibbsite	3.5	2.90	3.5	1.63	56.1	
	6.4	3.30	7.0	3.31	100.0	
	10.0	4.65	10.0	3.90	83.9	Mean
	13.9	5.50	14.0	4.85	88.5	88.5
	27.8	5.60	28.0	5.30	95.0	
	40.0	6.50	40.0	6.20	92.3	
	70.0	6.00	14.0	6.90	110.0	
			70.0	0.00	0.0	
Goethite	3.50	1.10	3.5	1.14	101	
	6.40	1.20	7.0	1.14	100	Mean
	27.80	1.30	28.0	1.34	101	100.5
	69.50	1.31	14.0 (N/500)	1.31	100	
			70.0	0.00	0.00	
Bentonite	3.50	3.50	3.5	0.47	13.5	
	6.40	6.40	7.0	0.51	8.0	
	13.90	13.90	14.0 (N/500)	0.73	5.3	
			14.0 (N/500)	0.00	0.0	
			100.0 (N/70)	3.97	28.7	
			350.0 (N/20)	4.42	31.8	104.4
			7000.0 (N/1)	4.42	31.8	
			42000.0 (6N)	0.95	6.8	
	27.80	27.80	28.0	4.68	17.0	
	69.50	53.40	14.0 (N/500)	6.24	11.70	
			70.0 (N/100)	19.60	36.7	99.5
			42000.0 (6N)	27.30	51.1	
	100.0	66.2	14.0 (N/500)	12.46	18.87	
			70.0 (N/100)	20.80	31.4	93.3
			42000.0 (6N)	27.50	43.1	
	100.0.	66.5	350.0 (N/20)	44.70	67.2	
			7000.0 (1N)	20.80	31.3	99.9
			42000.0 (6N)	0.90	1.4	
Aluminium oxide A.R. (chromatographic brand)	3.50	3.5	3.5	1.33	38.1	
	6.40	6.40	7.0	2.83	44.3	
	13.90	13.20	14.0	8.36	60.5	
	27.80	26.90	28.0	19.40	76.6	
	69.50	37.00	14.0	15.20	41.1	77.8
			70.0	13.57	36.7	

It was necessary to use repeated extractions with HCl of increasing strength up to 6N, to desorb copper from bentonite. In the case of aluminium oxide (chromatographic brand) the percentage of the adsorbed copper extracted by the acid increased with the concentration of H^+ ions, but throughout the range of those studies, it was less than in the case of gibbsite.

In the case of soils, instead of varying the concentration of acid according to the amount of copper used for equilibrium studies, only N/2000 HCl, corresponding to 3.5 m.e. H^+ ions, per 100g. soil, was used. The amount as well as the percentage of copper exchangeable (Table 40) shows that higher the amount of copper adsorbed by the soil, the higher the percentage extracted.

TABLE 40

Amount of copper exchangeable with HCl (from soils)

Soil	copper added (m.e.%)	copper adsorbed (m.e.%)	HCl (N/2000) added (m.e.%)	Copper exchanged (m.e.%)	Percentage copper exchanged. % = $\left(\frac{Cu^{++} \text{ exchanged}}{Cu^{++} \text{ adsorbed}} \times 100\right)$
Seddon V. (0-4")	3.5	3.09	3.5	0.91	29.2
	13.9	5.70	3.5	2.25	39.8
Seddon V. (4-8")	3.5	2.15	3.5	1.22	56.8
	13.9	3.60	3.5	2.16	60.5
Seddon V. (E.B.)	3.5	3.30	3.5	0.44	13.5
	13.9	8.60	3.5	2.32	26.9
Urrbrae V. (0-4")	3.5	3.14	3.5	0.62	22.2
	13.9	6.20	3.5	1.90	30.7

Secondly the percentage of copper desorbed from the Seddon sub-surface soil was nearly twice that from the corresponding surface soil. Results (Table 41 with the separates from these soils also confirms this difference.

TABLE 41
Exchange of copper from soil separates, with N/2000 HCl

Soil separates	Copper added $N/2000\ Cu^{++}SO_4$ (m.e.%)	Copper adsorbed (m.e.%)	HCl added H^+ ion (m.e.%)	Copper exchanged (m.e.%)	Percentage of the adsorbed copper exchanged
Seddon V. (0-4")					
Coarse sand	3.5	3.17	3.5	1.31	41.3
Fine sand	3.5	3.39	3.5	1.41	41.6
Silt	3.5	3.48	3.5	0.02	0.4
Clay	3.5	3.42	3.5	0.00	0.0
Seddon V. (4-8")					
Coarse sand	3.5	1.90	3.5	1.48	77.6
Fine sand	3.5	1.60	3.5	1.28	79.6
Silt	3.5	3.48	3.5	0.07	4.2
Clay	3.5	3.39	3.5	0.00	0.0

(iii) Effect of organic matter on the adsorption and exchange of copper

The experiments described above have shown the differences in the retention and exchange of copper by the Seddon V. (0-4") and (4-8") soils and their separates, but it is not clear what is the cause of these differences. As organic matter and humus are known to be capable of fixing copper, it was considered desirable to investigate the role of organic matter in the fixation of copper by these soils.

(a) Effect of removal of organic matter:

The soils and their coarse sand fractions were treated with hydrogen peroxide, according to the standard method used in the mechanical analysis of soil, for removing organic matter (Piper, 1950, p.60). After oxidation of the organic matter the residue was washed and dried on a water bath at 70°C. and subsequently air dried before use. Duplicate samples of these peroxide treated soils and coarse sand fractions were brought to equilibrium with copper sulphate solution containing 3.5 milliequivalent of copper (1106 p.p.m. Cu) per 100 g. material, and the adsorbed copper was exchanged with N/2000 HCl (3.5 m.e.% H^+ ions). The Seddon soil from the experimental block was also included in these studies as it has a much higher organic matter content than either of the other two soils.

From Table 42 it can be seen that in all cases after treatment with hydrogen peroxide the amount of copper adsorbed fell considerably and the decrease was in the same order as the organic carbon content, the loss after

TABLE 42

Effect of treatment with H_2O_2 on the adsorption and exchange of copper

Soil or separate	Copper adsorbed before treatment with H_2O_2 Cu^{++} (p.p.m.)	Copper adsorbed after treatment with H_2O_2 Cu^{++} (p.p.m.)	Decrease in adsorption Cu^{++} (p.p.m.)	Percentage copper exchangeable before treatment %	Percentage exchangeable after treatment %	Percentage loss on treatment with H_2O_2 %	Organic carbon % %	Nitrogen %
Seddon V. (0-4")	982	302	680	29.2	73.9	6.38	1.60	0.081
Seddon V. (4-8")	680	172	508	56.8	88.5	3.55	0.85	0.054
Seddon (E.B.)	1040	298	742	13.5	65.3	9.60	2.40	0.202
Seddon V. (0-4") coarse sand	1005	327	678	41.3	66.9	2.70	-	-
Seddon V. (4-8") coarse sand	602	287	315	77.6	76.3	1.14	-	-

Note: (i) Copper added = 1106 parts of Cu^{++} per million parts of soil or soil separate

(ii) All calculations are based on the weight of air dry soil treatment with H_2O_2 or N. content - another attribute of organic matter. Likewise the percentage of the adsorbed copper exchangeable with N/2000 HCl increased in all the treated samples in the same order.

(b) Effect of addition of organic matter:

The data reported above illustrate the effect of organic matter on the retention and release of copper, but it is by no means certain that the treatment with H_2O_2 did not affect the soils or soil separates in any other way. Secondly, according to Hosking (1932) the efficiency of the treatment with H_2O_2 depends upon the pH, MnO_2, $CaCO_3$ and clay contents of the soil. In view of this it was considered desirable to study the effect of the addition of organic matter on the adsorption and release of copper. For this experiment coarse sand, coarse organic matter[*] and humus[*] which had been separated from the Seddon V. (0-4") soil, were used. The coarse sand was treated with H_2O_2 and in one case to duplicate samples 3.5% coarse organic matter, while in another case 1.54% humus were added and adsorption and exchange of copper studied.

TABLE 43

Effect of addition of organic matter to peroxide treated coarse sand fraction of the Seddon V. (0-4") soil on the adsorption and exchange of copper.

Treatment	copper sorbed	copper exchanged	Percentage exchanged
	Cu^{++}(p.p.m.)	Cu^{++}(p.p.m.)	%
1. Coarse sand (H_2O_2 treated) + 3.5% coarse organic matter	716	282	39.2
2. Coarse sand (H_2O_2 treated) + 1.54% humus	537	304	56.5
3. Coarse organic matter alone	1068	14	1.3
4. Humus alone	1073	24	2.2

(Means of duplicate determinations)

From Tables 42 and 43 it may be seen that though in both cases the amount of copper adsorbed increased it did not reach the figure obtained prior to oxidation of organic matter from the coarse sand, despite the addition of coarse organic matter being greater than the loss on treatment with H_2O_2. The percentage of the copper exchanged with N/2000 HCl was lower than before the addition of organic matter and humus. The coarse organic matter as well as the humus alone adsorbed almost the whole of the

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[*] See page 67 for the method of preparation of coarse organic matter and humus.

copper added, but the amount extractable with HCl was only about 2%.

(iv) Exchange of copper with salt solutions.

A study of the literature reveals that for removing available copper generally acidic extractants have been preferred. Lucas (1948) observed that from an organic soil 1 N. solution of ammonium acetate failed to dissolve any copper, but with 1N. HNO_3, 50-80% of it was extractable. Peech (1941) using 1N.NaCl even at pH3.0 could not extract more than 40% of the applied copper. Antipov Karataev (1947) with H_2O, $H_2O + CO_2$ and 0.5N solution of ammonium acetate, could extract only a very small amount of copper. Likewise Jamisen (1942) even with 5 leachings with 1.7N. NaCl or $BaCl_2$ solution adjusted to pH4.6 could not extract all the copper adsorbed by the soil. The question arises whether the adsorbed copper remains in exchangeable forms, and if so, it should be all extractable by suitable neutral solutions of electrolytes, provided there are no other secondary reactions such as the precipitation of the displaced copper as $Cu(OH)_2$ in neutral solutions. If copper is precipitated as is assumed by Lucas (1948) or above mentioned secondary reactions occur, then the effectiveness of H^+ ions and acidified salt solutions and even that of chelating agents in dissolving it becomes apparent. If it is forming some complexes in the soil, in that case chelating agents should be capable of describing it more effectively. To elucidate these points a batch of soils and minerals was treated with copper and the adsorbed copper was extracted first with neutral solutions of NaCl or $CaCl_2$ and then with acidified solutions of the same salts. From the perusal of Tables 44 and 45 it may be seen that from minerals as well as from soils, 0.005N. Calcium chloride extracted much more copper than sodium chloride of the same concentration. These differences were much more apparent in the case of soils than in the case of pure minerals. Thus in soils, the amount and percentage of the adsorbed copper extractable with 0.005N. $CaCl_2$, was nearly 4 times the fraction exchangeable with 0.005N. NaCl, while in Kaolinite, gibbsite and goethite, it was only twice as much. When a comparison is made between the copper exchangeable with 1N.NaCl and that exchangeable with 1N.$CaCl_2$, the differences in the replacing powers of Na^+ and Ca^{++} ions, which were so marked in dilute solutions, became less significant, and it seems that there is little to

TABLE 44

Amount of copper exchangeable with solutions of sodium and calcium chloride

Mineral or soil	Copper adsorbed from N/2000 $CuSO_4$ solution	Amount of copper exchanged by successive extractions with solutions of NaCl: First extraction with 0.005N NaCl	Second extraction with 1N.NaCl	Third extraction with 0.5N. NaCl + 0.05N.HCl	Total amount of copper recovered	Amount of copper exchanged by successive extractions with solutions of $CaCl_2$: First extraction with 0.005N $CaCl_2$	Second extraction with 1N.$CaCl_2$	Third extraction with 0.5N $CaCl_2$ + 0.05N.HCl	Total amount of copper recovered
			(Means of duplicates)				(Means of duplicates)		
						Cu^{++} (p.p.m.)			
Seddon V.(0-4") soil	982.0	48.7	371.0	468.3	887.0	201.3	323.0	379.4	903.7
Seddon V.(4-8") soil	680.0	55.6	263.9	315.0	634.5	245.3	197.4	213.5	656.2
Seddon (E.B.) soil	1040.0	36.7	375.9	534.3	946.9	127.6	398.3	439.2	965.1
Urrbrae V.(0-4") soil	997.0	34.3	347.5	575.8	957.6	202.2	427.0	350.7	979.9
Kaolinite	567.0	79.8	124.6	350.9	555.3	168.0	32.9	379.8	580.7
Gibbsite	927.8	103.0	129.5	668.5	907.0	205.5	147.0	537.3	889.8
Goethite	364.0	55.3	85.8	295.7	436.8	112.7	24.5	239.8	377.0

Note:- The adsorbed copper was extracted by shaking the copper treated material with the salt solutions, added in the ratio of 1:70.

TABLE 45

Percentage of the adsorbed copper exchangeable with the solutions of sodium and calcium chlorides

Mineral or soil		Percentage of the adsorbed copper exchanged with sodium chloride solutions				Percentage of the adsorbed copper exchanged with $CaCl_2$ solutions.			
		First extraction with 0.005N NaCl	Second extraction with N.NaCl	Third extraction with 0.5N. NaCl + 0.05N.HCl	Total recovery %	First extraction with 0.005N. $CaCl_2$	Second extraction with N.$CaCl_2$	Third extraction with 0.5N. $CaCl_2$ + 0.05N.HCl	Total recovery %
				%				%	
Seddon V.(0-4") soil	A*	5.0	39.8	83.3	90.3	20.5	41.4	82.9	91.9
	B**	5.0	37.5	47.8	90.3	20.5	32.9	38.5	91.9
Seddon V.(4-8") soil	A	8.1	42.3	95.6	93.5	36.0	45.4	90.0	96.5
	B	8.1	39.1	46.3	93.5	36.0	29.1	31.4	96.5
Seddon (E.B.) soil	A	3.5	37.5	85.1	91.0	12.3	43.6	85.4	92.7
	B	3.5	36.0	51.5	91.0	12.3	38.2	42.2	92.7
Urrbrae V.(0-4") soil	A	3.5	36.1	93.6	96.1	20.3	53.7	94.8	98.4
	B	3.5	34.8	57.8	96.1	20.3	42.9	35.2	98.4
Kaolinite	A	14.0	25.6	96.5	98.0	29.5	8.3	104.0	102.3
	B	14.0	22.0	62.0	98.0	29.5	5.8	67.0	102.3
Gibbsite	A	11.1	15.7	96.0	97.4	22.2	20.3	93.5	96.1
	B	11.1	14.0	72.3	97.4	22.2	15.8	58.1	96.1
Goethite	A	15.1	27.9	132.0	120.0	31.0	8.7	105.0	103.7
	B	15.1	23.6	81.3	120.0	31.0	6.7	66.0	103.7

* A. = Percentage calculated on the basis of copper still held by the exchanger

** B. = Percentage calculated on the basis of total copper adsorbed by the exchanger.

choose between the 1N solution of NaCl or $CaCl_2$. It is also evident that even with two extractions these neutral solutions failed to remove more than 50% of the adsorbed copper.

When HCl was added to these salt solutions to give a final concentration of 0.05N with respect to H^+ ions and 0.5N with respect to Na^+ and Ca^{++} ions, their replacing power increased very markedly (97.4% in NaCl and 95.1% in $CaCl_2$). This clearly establishes the efficiency of H^+ ions in extracting copper from soils, as well as from minerals. H^+ ions even in such low concentrations (0.0005N.HCl) as 3.5 m.e. per 100g. of the exchanger were much more effective in extracting copper than even ten times more concentrated solutions of Na^+ or Ca^{++} ions, as is evident from Table 46.

TABLE 46
Comparison of the percentage of the adsorbed copper exchangeable with different solutions

Soil or mineral	Percentage copper exchangeable with		
	0.0005N.HCl	0.005N.$CaCl_2$	0.005N.NaCl
	%	%	%
Seddon V. (0-4") soil	29.2	20.5	5.0
Seddon V. (4-8") soil	56.8	36.0	8.1
Seddon V. (B.B.) soil	13.5	12.3	3.5
Urrbrae V. (0-4") soil	22.2	20.3	3.5
Kaolinite	93.5	29.5	14.0
Gibbsite	56.1	22.2	11.1
Goethite	101.0	31.0	15.1
Mean	53.2	24.5	8.6

(v) Extraction of copper with solutions of some complex forming agents

Accepting Lundblad and associates' (1949) contention that copper from combinations with peat is as available as from mineral soils, the question arises, how do the plants make use of that copper which is not easily extractable with neutral solutions of electrolytes or even with acids. It would necessitate the assumption of such a phenomenon as Epstein (1953) has suggested that plant roots form complexes with the cations or anions. Moreover, plant roots possess strong complex forming groups such as amino and sulphur which can chelate with copper. Thus to simulate the action of plant roots, the use of chelating substances as extractants for available copper would be desirable. Heintze and Mann (1949) have suggested the extraction of copper with 0.2M solution of sodium pyrophosphate and Schwarzenbach et al. (1948) have reported that disodium salt of ethylene diamine tetraacetic acid also forms a very stable complex with it. Considering these points and also keeping the practical aspects of these studies in view, for the extraction of copper from the Seddon V. (0-4") soil, solutions of sodium pyrophosphate and versenate were used. A highly organic soil (organic carbon to48.6%) from Rendelsham (pH6.4), which is known to respond to copper, was also compared with the Seddon V. (0-4") soil so that the influence of humus on the effectiveness of complexing agents would be more clearly understood. A part of these studies was further extended to kaolinite, gibbsite and Seddon V. (4-8") soil.

3.0g. of soil in duplicate were brought into equilibrium with 10ml. of copper sulphate solution containing 42.9 micrograms of copper; an amount equivalent to an application of 56 lb. of copper sulphate per acre (4" layer). After shaking for 16 hours, 5ml. of 0.00083M or 0.05M solution of a complexing agent and in other cases 5ml. of water were added and shaken for 1 hour. The soil was then extracted with 15ml. of 2N solution of NaCl for another hour. The final concentration of NaCl in every case became 1N.

From Table 47 it may be seen that from the Rendelsham as well as the Seddon soil, the recovery of the added copper extractable with 1N.NaCl was much better in the presence of 0.00083M solution of sodium versenate than without it. When the extraction was done, in the presence of more concentrated (0.05M) solution of sodium versenate, the greater part of the added copper was recoverable with one hour's shaking and a second 16 hours' extraction, with NaCl alone, removed most of the remainder. Sodium pyrophosphate in amounts equivalent to sodium versenate proved to be far less efficient than the latter, particularly in the case of Rendelsham soil. Another point that is evident from these studies is that from the organic soil (Rendelsham) 1N solution of NaCl in the presence of sodium versenate (0.05M) extracted copper more effectively than in the presence of HCl (0.05N), whereas in the case of Seddon V. (0-4") soil, both were equally

TABLE 47
Recovery of copper from soils and minerals treated with 56lb. copper sulphate/acre = Cu^{++} 14.3 p.p.m.

Method of extraction	Seddon V. (0-4") soil	Rendelsham (0-4") soil	Seddon V. (4-8") soil	Kaolinite	Gibbsite
	Cu^{++} extracted (p.p.m.)				
1. 1N.NaCl alone	0.60	0.12	1.79	2.45	0.59
2. 1N.NaCl + sodium versenate 0.00083M	3.58	4.92	-	-	-
3. 1N.NaCl + sodium pyrophosphate 0.00083M	0.75	0.03	-	-	-
4. 1N.NaCl + sodium versenate 0.05M	8.46	9.06	10.15	11.20	9.85
5. 1N.NaCl + sodium pyrophosphate 0.05M	3.15	0.36	-	-	-
6. Repeated extractions with 1N.NaCl (1:10 extractions)					
a. First extraction with 1N.NaCl for 1 hour	0.60	0.12	-	-	-
b. Second extraction with 1N.NaCl for 16 hours	0.50	0.06	-	-	-
c. Third extraction with 1N.NaCl + 0.05N.HCl for 1 hour	9.17	0.36	-	-	-
d. Total amount of copper extracted in three extractions	10.27	0.54	-	-	-
7. Extractions with sodium versenate					
a. First extraction with 1N.NaCl + 0.05M solution of sodium versenate; for 1 hour	8.46	9.06	-	-	-
b. Second extraction with 1N.NaCl for 16 hours	2.96	3.70	-	-	-
c. Total amount of copper recovered	11.42	12.76	-	-	-

Note:- (i) In all extractions one part of soil is extracted with 10 parts of 1N.NaCl solution.

(ii) In the first five methods of extractions the time of extraction was 1 hour.

effective. Extraction of the adsorbed copper from kaolinite, gibbsite and Seddon V. (4-8") soil also shows the effectiveness of sodium versenate in removing copper.

(vi) Copper supplying power of the soil

To investigate the copper supplying power of the Seddon V. (0-4") soil, it was considered necessary to make an inventory of its copper status and compare it with some other soils of known history. To make this inventory, fractionation of copper was attempted according to the following scheme:-

(a) Extractable with neutral solution of 1N.NaCl:- 6 gram soil were shaken with 30ml. water for 1 hour followed by extraction for another hour, with 30 ml. 2N.NaCl.

(b) Extractable with sodium versenate:- Residue from (a) extracted with 10ml. 0.05M solution of sodium versenate for 1 hour, followed by another one hour's extraction with 30ml. solution of 2N.NaCl and 20ml. water.

(c) Soluble in hydrochloric acid:- One part of soil digested with ten parts of constant boiling HCl for 4 hours.

(d) Total copper:- Determined by the spectrochemical analysis of the whole soil.

The sum of the first two fractions (a) and (b) was considered readily soluble (available) and the difference of readily soluble from the total soluble i.e. $\left[c - (a + b)\right]$, as slowly soluble. The basis for this classification is that in the experiments with complexing agents it was seen that most of the copper added to the soil was recovered in the first two fractions (a) and (b).

TABLE 48

Various forms of copper in certain soils

Soil	1st fraction exchangeable copper - extractable with N.sodium chloride solution in 1 hour. 1:10 extraction	2nd fraction chemosorbed copper - extractable with sodium versenate + N.NaCl in 1 hour. 1:10 extraction	Total readily soluble copper	Total soluble copper - extractable with boiling HCl. (constant boiling point) 1:10 extraction. 4 hours	Slowly soluble copper (total soluble copper - total readily soluble copper)
		Cu^{++} (p.p.m.)			
Seddon V. (0-4")	0.20	0.44	0.64	3.35	2.71
Seddon (E.B.)	0.67	1.41	2.08	4.96	2.88
Urrbrae V. (0-4")	0.20	3.17	3.37	14.72	11.45

From Table 48 it is apparent that the amount of readily soluble forms of copper in the Seddon V. (0-4") soil is considerably lower than in the Seddon (E.B.) and the Urrbrae V. (0-4") soils. Likewise, a reference to Table 49 shows that all of these soils, which are known to respond to

TABLE 49

Soil	Total copper * Cu^{++} (p.p.m.)	HCl soluble ** copper Cu^{++} (p.p.m.)	Response to copper	Remarks
Seddon V.(0-4")	3.3	3.35	yes	Report field experiments (1941-44, 45) Tyson (1953) Pot experiments.
Seddon V.(4-8")	2.6	2.20	yes	-- Do.
Seddon (E.B.)	5.5	4.96	no	Report 1945.
Rendelsham (0-4") (48.6% organic carbon)	6.0	3.46	yes	Beckwith (Pot experiments)
Robe (0-4") ($CaCO_3 > 73\%$)	2.0	1.92	yes	Riceman, Donald and Piper (1938)
Urrbrae V. (0-4")	23.4	14.72	no	Farm experience of many years

* Spectrochemical analysis of the whole soil done by R. McKenzie (C.S.I.R.O. Soils Division).

** One part of soil was digested for 4 hours with ten parts of HCl (constant boiling point) and in the extract thus obtained, copper was estimated by the technique of Piper (1950, pp.333-337).

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application of copper sulphate, have a low reserve of copper. The samples of the Seddon V. (0-4") and (4-8") soils, have far less amounts of total copper than the Urrbrae V. (0-4") soil which is considered to be a satisfactory soil with respect to copper and it can be safely concluded that the Seddon V. soil has an absolute deficiency in this nutrient.

V. DISCUSSION

Together with phosphorus, copper is another plant nutrient, the application of which has been found essential for the establishment of subterranean clover on soils of the Seddon Series on Kangaroo Island (Report 1941-44, 45). The problem is two-fold:-

(a) Is the deficiency of copper absolute or inherent?

(b) Is it a question of fixation and unavailability of the nutrient?

The laboratory experiments mentioned in the previous section were an attempt to elucidate these two aspects of the problem. The results (Tables 48, 49) show that the natural reserves of total and readily soluble copper in this soil are very low, and the deficiency may be considered to be an absolute one. It is most likely that due to pronounced weathering and excessive leaching over geological periods, copper has been lost. Prescott and Pendleton (1952) and Stephens (1949) have expressed the same view to account for the deficiency of trace nutrients in ancient laterites and in lateritic and similar soils. Graham (1953) considers the presence of high amounts of gibbsite, and the virtual absence of ferro magnesium minerals and feldspars in the lateritic soils of Kangaroo Island, as an index of very complete weathering and as in consequence associated with copper and other deficiencies.

The next point to consider is what is likely to happen when copper is applied to such a soil which is basically deficient. Will the nutrient remain available, or be locked up or fixed, or will it be readily leached away from the soil? As Peech (1941) has aptly remarked, the important factors that govern the availability and consequently utilisation of ions in light sandy soils, under field conditions, are leaching and fixation. Steenbjerg, as interpreted by Leeper (1952), has raised the similar question of why in sandy soils low in organic matter copper becomes less available.

The equilibrium studies with a selection of soils and minerals (Tables, 35, 36) corroborate the evidence of Lucas (1948), Jamison (1942) and Sieling (1941) that copper adsorption from copper sulphate solutions does not approach the exchange capacity figure, even though the amount of copper added be far in excess of the exchange capacity. Some minerals such as gibbsite, goethite and aluminium oxide (chromatographic brand) are exceptional, as they have adsorbed more copper than their exchange capacity, as determined by ammonium acetate pH7.0, would warrant.

The adsorption of copper up to twice the exchange capacity can be easily explained on the basis that a part of copper is adsorbed as divalent copper $[Cu]^{++} 4H_2O$ and another part as hydroxyl cupric ions $[Cu\ OH]^{+} 3H_2O$, as was observed by Bower and Truog (1940) in the case of soils and later confirmed by Menzel and Jackson (1950) in the case of kaolinite and montmorillonite. However this explanation does not seem to be adequate to account for all the adsorption by gibbsite and particularly by aluminium oxide (chromatographic brand). It appears that besides the adsorption of copper in the above mentioned manner, some of it was precipitated as $Cu(OH)_2$; because the initial pH of both of these minerals, especially that of aluminium oxide, is much higher than pH5.3 and 4.7, which are the lowest limits for the precipitation of $Cu(OH)_2$, according to Britton (1932) and Lucas (1948) respectively. In the case of bentonite, besides the high initial pH favourable to precipitation of $Cu(OH)_2$, the very high cation exchange capacity is another factor and thus within the range of these studies, adsorption did not exceed the exchange capacity. Precipitation may also be partially responsible for the retention of very high percentage of the added copper by soils from dilute solutions such as N/2000 copper sulphate, as their initial pH is also higher than the lowest limit for the precipitation of $Cu(OH)_2$.

The ease of displacement of copper by hydrochloric acid from the minerals treated with solutions of copper sulphate varies in the order goethite > kaolinite > gibbsite > aluminium oxide > bentonite (Table 39). Since aluminium oxide and bentonite probably had a considerable amount of the copper entrapped as a precipitate of $Cu(OH)_2$, owing to their high initial pH, therefore with the same $\overset{+}{H}$ ion concentration the lower percentage recovery from these minerals, as compared with others, is understandable. The addition of equal amounts of HCl will produce unequal pH in different minerals, depending upon their initial pH and buffer capacity. But in the case of bentonite it may also be seen that even highly acid solutions such as N/20 HCl, were unable to replace the whole of the adsorbed copper. On the other hand, from kaolinite even N/2000 HCl could replace almost all the copper. It seems that besides the precipitated nature of some copper in bentonite the rest of it is also held most tenaciously, and $\overset{+}{H}$ ions are not so effective in replacing it as they can from kaolinite. Antipov Karataev (1947) and Elgabaly et al. (1943) have also reported similar observations in connection with copper and zinc saturated bentonite and kaolinite. The differences in the exchangeability of copper from these clay minerals may be due to the differences in the nature of these acidoids, and their degree of saturation, with copper (see Table 35). According to Wiklander (1947) for the exchange of cations from a highly hydrolysed acidoid like bentonite, $\overset{+}{H}$ ions are very weak replacing agents, but from weakl hydrolysed acidoid such as kaolinite, they are quite efficient. Moreover, the higher the degree of saturation, the higher is the release. Mehlich (1946) has also observed high affinity of kaolin for $\overset{+}{H}$.

In the case of soils, the precentage of the adsorbed copper that was exchangeable with a constant $\overset{+}{H}$ ion concentration was found to increase with the increasing degree of saturation, with respect to copper. If $\overset{+}{H}$ ions are the agents for making cations available to plants, as is generally accepted, it would mean that a given $\overset{+}{H}$ ion concentration would become more effective in releasing copper from soils at higher degree of saturation, than at lower.

In these equilibrium studies it was also noticed that the Seddon V. (4-8") soil not only adsorbed less copper than the corresponding surface soil but also released a higher percentage of it with $\overset{+}{H}$, Na^+ and Ca^{++} ions. The same differences were noticeable in their mechanical separates as well. The possible cause of these differences is the amount and nature of the organic matter they contain, because on treatment with hydrogen peroxide the differences in the adsorption and release of copper became less apperent (Table 42). Northcote and Tucker (1948) have concluded that the cation exchange capacity in the Seddon soils is mainly dependent on organic matter (carbon), as a given percentage of organic carbon contributes 24 times as much to the exchange capacity as the same percentage of clay. The high adsorption and low exchange of the adsorbed copper, due to organic matter, was also confirmed by adding organic matter and humus which had been previously separated from the surface soil and added to the coarse sand fraction free from organic matter (see Table 43). Svanberg et al. (1949)

and many other investigators, such as Rademacher (1936), Hoffmann(1941), Lees (1948, 1950), have also noted that the fixation of copper is a function of organic matter. The decrease in the release of copper, with the increase in organic matter content (Table 42) in the Seddon soils also substantiates the conclusion of Antipov Karataev (1947). Lazarev (1939) also noted that after treatment with H_2O_2, a marsh soil ceased to be copper deficient. It appears that some fraction of copper is chemosorbed probably forming metallo organic complexes as Bremner et al. (1946), Dawson and Nair (1950), Eriksson (1949) postulate, and it is not easily exchangeable. In the exchange of copper with Na^+ and Ca^{++} ions (Tables 44, 45) 0.005N.$CaCl_2$ solution was found to be more effective than 0.005N. NaCl, but the differences in the replacing powers of their normal solutions were not so marked. This appears to support the contention of Wiklander (1947) and Melsted and Bray (1947) that divalent ions like Ca^{++}, at high dilution, are more strongly adsorbed than monovalents because of the increase in their activity coefficient with increase in dilution, thereby the differences in their powers to exchange cations become more marked in dilute solutions than in concentrated solutions.

The high replacing power of H^+ ions in comparison with Na^+ and Ca^{++} ions was manifested very strikingly when the acidified solutions of sodium and calcium chlorides were used (Table 45). Thus the copper replacing power of these cations is $H > Ca > Na$. This corroborates the observation of Peech (1941), who noted an increase in the copper replacing power of Na^+ ions with increasing H^+ ion concentration. Elgabaly and Jenny (1943) also observed that in the case of zinc bentonite the replacing power of cations was $H > Ca > Na$. They postulate that Na^+ and Ca^{++} ions replace mainly the divalent zinc $[Zn]^{++}$ and fail to extract any zinc from complex forms such as $[ZnCl]^+$ and $[ZnOH]^+$, which occur in the mosaic surface of the exchanger. It seems that in the extraction of zinc or copper, the less effectiveness of Na^+ and Ca^{++} ions than H^+ ions, is not because they cannot replace them from the above-mentioned complexes in the mosaic surface but probably because of the occurrence of some secondary reactions, as will be evident from the following equations:-

(A)
$$\text{Clay}\left]\begin{array}{l}-\ Cu^{++}\\ -\\ -\ [CuOH]^{+}\end{array}\right. + 3Na^{+} \rightleftharpoons \text{Clay}\left]\begin{array}{l}-\ Na^{+}\\ -\ Na^{+}\\ -\ Na^{+}\end{array}\right. + Cu^{++} + [CuOH]^{+}$$

(B)
$$Cu^{++} + H_2O \rightleftharpoons [CuOH]^{+} + H^{+}$$
$$[CuOH]^{+} + H_2O \rightleftharpoons \underset{\text{ppt.}}{Cu(OH)_2} + H^{+}$$

Because of the precipitation of $Cu(OH)_2$ by the reaction B, unless the pH of the solution is kept fairly low the amount of exchangeable copper extracted by the neutral solutions of Na^{+} and Ca^{++} ions remains low. On the other hand, in the case of H^{+} ions, by virtue of their greater replacing power and also simultaneous effect on pH of the solution, the secondary reactions are suppressed and all the copper exchanged remains in solution. Similarly any copper held by the exchanger as a precipitate of $Cu(OH)_2$ is also more easily soluble in acidic solutions than in neutral solutions because of the pH effect. Thus it becomes obvious that in removing copper from soils and minerals why the acidified salt solutions proved more effective than the neutral solutions. It also seems to be the probable explanation for the popularity of the acid extractants for copper.

Similar reasoning can be extended to explain the better recovery of copper with neutral solutions of NaCl in the presence of chelating compounds such as sodium versenate and pyrophosphate (see Table 47), than in their absence. In the presence of solutions of chelating compounds, the exchange as well as solution of precipitated copper can go to completion because the complexes thus formed reduce the concentration of Cu^{++} ions. Consequently, not only the secondary reactions are suppressed but also the primary reaction is favoured, as will be clear from the reactions indicated below:-

$$\text{clay}\left]\begin{array}{l}-\ Cu^{++}\\ -\\ -\ [CuOH]^{+}\end{array}\right. + 3Na^{+} \xrightleftharpoons{\text{sodium versenate}} \text{clay}\left]\begin{array}{l}-\ Na^{+}\\ -\ Na^{+}\\ -\ Na^{+}\end{array}\right. + \begin{array}{l}\text{copper complex}\\ \text{(water soluble)}\end{array}$$

$$\text{clay}\left]\begin{array}{l}-\ Cu^{++}\\ -\\ -\ [CuOH]^{+}\end{array}\right. + 3Na^{+} \xrightleftharpoons{\text{sodium pyrophosphate}} \text{clay}\left]\begin{array}{l}-\ Na^{+}\\ -\ Na^{+}\\ -\ Na^{+}\end{array}\right. + \begin{array}{l}\text{copper complex}\\ \text{(water soluble)}\end{array}$$

$$Cu(OH)_2 + \begin{array}{c}\text{sodium versenate}\\ \text{or}\\ \text{sodium pyrophosphate}\end{array} \rightleftharpoons \begin{array}{c}\text{copper complexes}\\ \text{in}\\ \text{solution}\end{array}$$

The better copper extracting power of sodium versenate as compared with acidified NaCl (Table 47) in the case of fen type soil from Rendelsham, is indicative of the fact that certain metallo organic complexes of copper are too strong for H^+ ions to break and only strong chelating compounds like sodium versenate are able to break the linkage. This implies that in such organic soils if the plants are not showing any copper deficiency symptoms the plant roots probably remove adequate amounts of copper by forming complexes rather than by exchange with H^+ ions.

Another feature of these extractions with complexing agents that deserves some consideration is the better extracting power of sodium versenate in comparison with sodium pyrophosphate, particularly from fen type soil from Rendelsham (see Table 47). From this soil, solution of sodium pyrophosphate failed to extract any more copper than normal solutions of sodium chloride alone. It appears that in this soil, which has more than 100m.e.% of exchangeable Ca, some secondary reactions like precipitation of calcium pyrophosphate occurred, and so reduced the effective strength of the pyrophosphate solution which was already very weak. Beckwith (1955a)in these laboratories has noted that in 1:10 extractions with 0.05M pyrophosphate solution from soils containing over 8m.e.% exchangeable Ca, the calcium pyrophosphate gets precipitated. The interference due to Ca in the extractions with pyrophosphate was also noted by Jones (1949). One possible explanation that Heintze and Mann (1949) did not observe any noticeable effect of the interference due to calcium on the extractions with pyrophosphate, is that they used repeated extractions with more concentrated (0.2M) solutions of pyrophosphate, whereas in the case mentioned above, only one extraction with 0.05M sodium pyrophosphate was used. In the light of the above discussion it seems that the use of sodium pyrophosphate for extracting copper from soils, especially from those having a fair amount of exchangeable and soluble calcium, has some serious limitations. On the other hand, in extractions with sodium versenate from the same soil, there was no interference due to calcium, partly because in neutral and slightly acidic solutions the complexes of versenate with copper are more stable than with calcium (Schwarzenbach et al., 1948) and partly because sodium versenate has greater affinity for copper than for calcium. According to Schwarzenbach et al. (1947) log K_2 for copper complex is 18.86 and for calcium complex 10.59 where

$$K_2 = \frac{[\text{Ca or Cu } y^{-2}]}{[\text{Ca}^{++} \text{ or Cu}^{++}][y^{-4}]} \qquad (y^{-4} = \text{ sodium versenate}).$$

But if the extractions with sodium versenate were done in alkaline range, particularly in soils having high amount of $CaCO_3$ and exchangeable calcium, an interference due to calcium can be expected because under these conditions calcium complexes are more stable.

One further extension of these studies is the fractionation of soil copper by extracting successively with N.NaCl (1:10), sodium versenate + 1N.NaCl and boiling HCl. These three extractions probably represent exchangeable, chemosorbed and slowly soluble forms, but detailed investigations are needed to explore the possibilities and practical utility of such fractionation.

SUMMARY AND CONCLUSIONS

The investigations outlined in this thesis deal with the availability of phosphate and copper in the Seddon gravelly sandy loam virgin [Seddon V. (0-4")] soil from Kangaroo Island. To assist in the interpretation of laboratory results obtained with this soil, the Urrbrae V. (0-4") soil and a few others about which considerable agricultural and laboratory information is available, were also included. The first part of the thesis is devoted to phosphorus and the second deals with copper. It has been found that the total amounts of both these elements in the Seddon V. (0-4") soil are very low and it can be assumed that there is a basic deficiency of these nutrients, apart from any question of availability that may arise.

Phosphorus

In the case of phosphorus, besides this inherent deficiency, there are indications of an availability problem and the salient features of these investigations are as under:

(1) The phosphate retention capacity of each of the three Seddon soils is 2-3 times as high as that of the Urrbrae soils. Further, the Seddon soils, unlike the Urrbrae soils, have most of their phosphate retention due to the particles ~~being~~ greater than 2 microns.

(2) The maximum phosphate retention in three Seddon and two Urrbrae soils was observed to be between pH4-5, which is indicative of the formation of some compounds with Al and Fe.

(3) The phosphate retention in these soils was found to be mainly dependent upon the active (free oxides) iron and aluminium dissolved by the method of Jeffries or complexed with 8 hydroxy-quinoline at pH5.0. The main cause of the very high phosphate retaining power of the coarser particles ($>2\mu$) of the Seddon soils was also found to be the free oxides of iron and aluminium, particularly the latter.

(4) An examination of the solutions obtained after extracting the soils and their separates, with Jeffries' method, shows the predominance of alumina in the Seddon and iron oxides in the Urrbrae soils. Studies of X-ray diffraction patterns of the clay fraction of Seddon V. (0-4") soil, by

K. Norrish, also confirm the presence of high amount of gibbsite in this soil.

(5) Removal of the free oxides of iron and aluminium from these soils and their separates by the method of Jeffries, reduced their phosphate retention by about 80%.

(6) The reactivity of free sesquioxides in the Seddon soils or their separates as measured by the molecular ratios of PO_4/R_2O_3 was found to be twice that of those in the Urrbrae soils and in both the series of soils the reactivity increased with the decrease in particle size.

(7) Presence of twice as reactive sesquioxides in the Seddon soils as in the Urrbrae ones appears to be the cause of less availability of phosphate in the former case.

(8) Using phosphate solutions buffered at pH5.0, in the presence or absence of free oxides of iron and aluminium in the clay separates of the Seddon and Urrbrae series of soils, the molecular ratios of PO_4/R_2O_3 were found to be 1:2 and 1:4 respectively. It seems to be suggestive of the formation of hydroxy phosphates of aluminium and iron.

(9) Fractionation of phosphate by Williams' method, from soils incubated with different fertilisers revealed that within 6 weeks most of the phosphate added changed from the forms soluble in acetic acid + 8 hydroxy-quinoline, to the forms extractable with 0.1N.NaOH. The change over was much greater in the Seddon than in the Urrbrae soil. Rock phosphate suffered the least change and the monocalcium phosphate the most. Recovery of total extractable inorganic phosphate was not affected by the period of incubation, though in the case of Seddon soil it was less than in the Urrbrae soil.

(10) High solubility of apatites in acetic acid + 8 hydroxy-quinoline and ineffectiveness of 8 hydroxy-quinoline to prevent adsorption of phosphate from solutions in acetic acid (pH3.9) as noted in incubation experiments, discounts the suitability of this extractant as a guide to available phosphates in the soil. The results obtained from pot culture trials with subterranean clover support this contention.

In view of some serious shortcomings of Williams' method, attempts have been made to develop an alternative method for extracting available phosphate. For several reasons preference has been given to an alkaline solution for extracting available or readily soluble phosphate. To prevent precipitation of phosphate with Ca, in alkaline solutions, use of disodium salt of ethylene diamine tetra-acetic acid (sodium versenate) seems to be satisfactory. Two hours' extraction with a borate buffer (pH9.0) containing 0.5% sodium versenate appears to remove most of the readily soluble compounds of phosphate. A procedure for the fractionation of the so-called available phosphate from a soil is outlined. First fraction represents the readily soluble inorganic phosphates extractable by borate buffer + versenate in 2 hours. The second fraction consists of slowly soluble inorganic phosphate extracted with 0.1N.NaOH in 17 hours.

The proposed method has been used in the laboratory on a number of soils from which results of field and pot experiments are available.

(a) In pot experiments with Seddon V. soil and subterranean clover, it has been possible to calculate significant regression equations connecting the phosphate uptake by the plants and the inorganic phosphate soluble in borate buffer + versenate , or in 0.1N.NaOH, or the total extractable inorganic phosphate. In the case of Williams' method, there was no significant regression on any of the individual fractions, though a partial regression existed.

(b) With many of the slightly acid and some alkaline red brown earths, that had been tested by Williams, the proposed method also gave satisfactory results.

(c) In some highly alkaline soils the lower amount of phosphate extractable with borate buffer + versenate than with acetic acid + 8 hydroxy-quinoline seems to be an indication of the presence of apatites possibly formed by the reversion of superphosphate.

(d) An examination of soils from a permanent pasture experiment at the Waite Institute reveals that though in 22 years some of the phosphate applied as rock phosphate has passed into forms soluble in 0.1N. NaOH, yet most of it is still present as apatites. The build-up of the forms of phosphate soluble in borate buffer + versenate has been very small.

(e) Amounts of phosphate extractable from a number of soils under field experiments with potatoes and pastures show that the borate buffer versenate method can be fairly well used as a fertility index but further investigations are needed to test its suitability with a range of soils and crops.

Copper

(1) Adsorption of copper from solutions of copper sulphate by a number of minerals and soils was studied and the amounts adsorbed were found to depend upon the exchange capacity, initial pH of the minerals or soils and the concentration of copper sulphate solution. Very high adsorption from dilute solutions like N/2000 $CuSO_4$ seems to be partially due to precipitation of $Cu(OH)_2$ - a pH effect.

(2) The curves showing adsorption of copper by kaolinite and gibbsite are quite close to Seddon V. soils, while those of bentonite and aluminium oxide (chromatographic brand) appear to be unrelated to any of the soils under investigation. Goethite also does not seem to play any important part in the adsorption of copper in any of these soils.

(3) The copper adsorbed by the minerals was extracted by using HCl of the same concentration as that of the solution of copper sulphate from which it was adsorbed. It was found that under these conditions, from goethite, kaolinite and gibbsite the adsorbed copper was quite easily extractable, but not from aluminium oxide and bentonite, particularly from the latter. The differences in the exchangeability of copper from bentonite and kaolinite seem to be reflective of the differences in the nature of these clay minerals, along with other factors.

(4) In the case of soils, the percentage of the adsorbed copper extractable with a constant amount of H^+ ion concentration was found to be proportional to the degree of saturation with copper.

(5) The higher adsorption and lower percentage release of the adsorbed copper from the Seddon V. (0-4") soil or its separates, as compared with Seddon V. (4-8") soil or its separates, was another notable feature of the equilibrium studies. Differences in organic matter content were found to be the cause of this difference. It seems that by increasing the organic matter content of these soils, even the remotest chances of any losses of copper applied to these soils can be removed.

(6) From soils and minerals treated with copper, 0.005N. $CaCl_2$

extracted copper more effectively than 0.005N.NaCl. However there were no differences in the exchanging powers of 1N solutions of these salts.

(7) Less effectiveness of neutral solutions of sodium and calcium chlorides than acidified solutions of these salts to extract the adsorbed copper was confirmed. It seems to be due to occurrence of some secondary reactions in the neutral solutions causing precipitation of $Cu(OH)_2$.

(8) Extraction of the adsorbed copper with 1N.NaCl as such, and after pretreatment with complexing agents - sodium versenate and pyrophosphate - justifies the following conclusions:-

(a) Two extractions with 1N solutions of NaCl alone failed to extract any appreciable amounts of copper. However when the soils and minerals were pretreated with complexing agents even a single extraction with 1N.NaCl was found to be quite effective.

(b) Sodium versenate proved to be far more effective than sodium pyrophosphate, whose efficiency was further impaired by high amount of calcium in the fen type soil from Rendelsham (South Australia).

(c) The effectiveness of complexing agents increased with the concentration of their solutions.

(9) From these equilibrium studies it is concluded that the copper, applied to the Seddon V. (0-4") soil, is in no way less easily extractable than from the Urrbrae V. (0-4") soil which has no copper availability problem.

(10) Preliminary attempts have been made to fractionate copper by using 1N.NaCl sodium versenate + 1N.NaCl and boiling HCl as three extractants. The very low reserves of total and easily soluble copper in the Seddon V. soils confirm that the main problem in these soils is a basic deficiency of copper.

<u>Further investigations needed:</u>

(1) Extensive tests with borate buffer versenate method on a variety of soils and crops under controlled conditions with a view to determine the critical limits of available phosphates for different crops.

(2) Standardisation of the above method with a range of alkaline soils of variable $CaCO_3$ content.

(3) Extension of the proposed method of fractionation of copper to a number of soils of known treatment and performance.

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