FACTORS INFLUENCING THE ISOTOPICALLY EXCHANGEABLE PHOSPHATE IN SOILS

PART I. THE EFFECT OF LOW CONCENTRATIONS OF ORGANIC ANIONS

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INTRODUCTION

The rate of isotopic exchange of phosphate ions between the soil solution and the soil has been used to divide the total labile phosphorus into 'rapid' and 'slow' fractions by an examination of the curve giving the decrease in P^{32} activity of the solution with time (Talibudeen ¹⁰). Earlier work on soil suspensions by other investigators referred to previously, had indicated the possibility of such a sub-division but no quantitative work had been described. Amer *et al.* ¹ examined the rates of removal of phosphate ions from soils by anion-exchange resins and found that the data are qualitatively similar to those on the isotopic exchange of phosphate ions between the soil and the soil solution. They showed that the observations on the resin-soil experiments "could be fitted by assuming that there were three simultaneous first-order reactions", and state that "the sources of P involved in the three hypothetical reactions are unknown". The reactions are considered as "behavioural patterns characterized by a quantity and a rate. . .". Although data on the rate of P^{32} equilibration are given for two soils, detailed analyses were not attempted. Seatz ⁸ also suggested the existence of three separate reactions in the isotopic exchange of phosphate ions in soil systems without giving any detailed analyses.

In non-soil systems, Govaert *et al.* ⁴ studied the isotopic exchange of Ca^{45} and P^{32} in suspensions of "tricalcium phosphate". With phosphate ions, they observed three separate kinetic reactions with half-lives of exchange 16.7 minutes, 9 hours, and 222 days. Neuman *et al.* ¹¹ obtained similar results with apatite crystals of bone suspended in phosphate buffers. Isotopic exchange of C^{45} -labelled CO with CO adsorbed on iron catalysts

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was used by Eischens³ to study the surface heterogeneity of the precipitated iron; again three reactions were detected.

In our work the results for the isotopic exchange of phosphate ions obtained with two soils were analyzed by methods similar to those of Eischens³ and Amer *et al.*¹. To make this possible the reactions are assumed to be confined to the surface of the solid, and a high speed of agitation of the system is maintained. In this and succeeding papers, we shall investigate the nature of these fractions by a study of their interaction with external conditions. Such measurements and their treatment may be equally valuable in investigating the surface activity of other nutrient ions in the soil, *e.g.* potassium, ammonium, nitrate, calcium and micronutrient elements, if suitable isotopes are available.

When isotopically exchangeable (or total labile) phosphate is determined in soil suspensions containing decreasing concentrations of citrate ions (Talibudeen⁹), a limiting citrate concentration of 0.001 *M* is observed below which the total labile phosphate does not alter as the citrate concentration falls to 0.0001 *M*. However, the phosphate displaced into solution decreases continuously with decreasing citrate concentrations. The behaviour of different soils under such conditions in regard to the total labile phosphate and the phosphate displaced into solution is discussed elsewhere (Talibudeen⁹). In the following sections, the distribution of phosphate ions in the total labile phosphorus determined in the absence of organic anions is compared with the distribution in the presence of this limiting concentration of citrate ions and also in the presence of the same concentration of the non-chelating anion of diethyl barbituric acid.

KINETICS OF ISOTOPIC EXCHANGE

McKay⁶ derived a simple exponential law to predict the course of the isotopic exchange process in a homogeneous stable system. This can be adapted for soil systems in the following way. If *R* is the specific rate of exchange of the labelled ion between the phosphate ions in the soil *P_s* and those in the soil solution *P_{ss}*, then the fractional exchange *F* changes with time according to the equation:

$$\ln(1 - F) = - \frac{P_e}{P_s \cdot P_{ss}} \cdot Rt \quad (1)$$

where P_e is the total labile phosphate in the system and is equal to $(P_s + P_{ss})$ and the fractional exchange F is the ratio of the specific activity at time t in the soil to the asymptotic value. When the labelled ions exchange at two or more different rates because more than one exchanging component occur in the solid, the composite curve can be resolved into its components graphically.

The experimentally determined values of the specific activities of the solution at suitable time intervals are converted to fractional exchange values in the soil F which is given by the equation:

$$F = \frac{\text{Change in specific activity after time } t}{\text{Total change in specific activity value}}$$

METHODS AND MATERIALS

The two soils from Rothamsted Experimental Plots used in this study have been described in detail elsewhere (Talibudeen ¹⁰), essential data are summarized in Table 1.

TABLE 1

Description of soils from Rothamsted Experiments				
Site	pH in 0.02 M KCl	% CaCO ₃	% Clay content	Total phosphorus, mg per 100 g soil
Soil A Highfield	6.9 to 7.1	<0.1	20.3	91
Soil B Exhaustion land, superphosphate plot . .	7.5 to 7.7	1.5	19.3	68

The solutions used were made up from analytical grade reagents of ammonia, potassium chloride, citric acid and diethyl barbituric acid. The pH of the solutions of the organic anions were adjusted to those of the soils.

The solutions used were

- 0.02 M KCl
- 0.02 M KCl + 0.001 M Citrate (NH₄ form)
- 0.02 M KCl + 0.001 M diethyl barbiturate (Na + H form)

(The diethyl barbiturate ion is here-after referred to as the DEB anion). The solution of 0.02 M KCl was used as a reference solution to avoid any effects from electrolytes dissolved from the soil and from the different cations in the organic buffer.

To keep the phosphorus concentration as constant as possible during the isotopic-exchange experiment, preliminary runs were made to determine the rate at which 0.5 g of soil came to equilibrium with 100 ml of the different extracting solutions. After 20 to 24 hours continuous shaking at 150 to 200 oscillations per minute, the systems containing organic anions showed a small increase during the course of the isotopic-exchange experiments (ranging from 150 to 250 hours), whereas those without organic anions showed a larger change after the initial rapid rise in the first 24 hours.

Because of this difference, the procedures adopted after labelling with carrier-free radioactive phosphorus were varied. Labelling was done after the initial rapid rise in the phosphate in solution was over. The amount of carrier-free P^{32} was assayed by adding it to control flasks containing the extracting solution together with the levels of P expected in the equilibrium solution. Levels of carrier-P ranging from $6 \times 10^{-7} M$ to $1 \times 10^{-5} M$ were used without showing any measurable change in radioactivity over 7 to 10 days. A half ml of chloroform was added to all flasks to prevent the growth of microflora; this was renewed whenever it appeared to have evaporated. All experiments were at 25° C. The time intervals for sampling were chosen so that more than half the total number of samplings were done in the first 48 hours. Samples were centrifuged at 16000 rpm in an angle head for 5 minutes, temperature control being either between $\pm 1^\circ$ or between 0° and 5° C. Where the larger temperature changes were encountered, the bulk of the soil was removed by sedimentation under gravity or pre-centrifuged at lower speeds (4000–5000 rpm).

The alternative procedures for studying isotopic exchange were as follows.

(A) With organic ions: Three flasks were set out initially and sampled at suitable times by removing approximately 5-ml portions of the supernatant solution. This was centrifuged and the clear supernatant liquid sampled in duplicate with a 0.5-ml micropipette. The P^{32} -content of this 0.5 ml aliquot was estimated as before (Talibudeen¹⁰, Procedure A). The suspension remaining in the centrifuge tube was returned to the reaction flask. Three to four successive samplings were made from each flask. Each flask was centrifuged for the final sampling and analysed for its pH, total phosphorus and P^{32} -content as before.

(B) Without organic ions: One to two μC of P^{32} were added to each of 10 to 12 flasks set out initially. These flasks were removed from the thermostatic water bath at suitable time intervals consecutively and their contents were centrifuged. The centrifugate was analysed for its pH, total phosphorus and P^{32} -content by methods referred to in a previous paper (Talibudeen¹⁰, Procedure B).

RESULTS

All the radioactivity counts were used after correction for decay, lost counts, and also for slight day-to-day variation in the counting apparatus, by referring them to the same P^{32} -standards.

The results obtained with suspensions without organic anions (Procedure B) were converted to specific activities. Radioactivity data from Procedure A in the presence of organic anions were used without further computation because it was considered that the slight increase in phosphorus content in solution over the period

of the isotopic exchange would not affect the rate of isotopic exchange measurably.

The asymptotic value of the specific activity or radioactivity, with respect to time was obtained by repeated measurements at extended time intervals until results remained constant within experimental error.

The expression $1 - F$ was then calculated from the specific activity or radioactivity f_t at time t and the asymptotic value f_∞ by the expression

$$1 - F = \frac{f_t - f_\infty}{1 - f_\infty} \quad (2)$$

The total labile phosphorus in the soil P_s was obtained from the expression

$$P_s = \left(\frac{1}{f_\infty} - 1 \right) \cdot P_{ss} \quad (3)$$

where P_{ss} is the amount of phosphate in solution.

The semi-logarithmic plot of $\ln(1 - F)$ against time t , was analyzed graphically to give the amounts of phosphorus P_0 , P_1 , P_2 , and P_3 associated with the 'instantaneous', 'rapid', 'medium' and 'slow' exchanging phosphate fractions in the soil such that

$$P_s = P_0 + P_1 + P_2 + P_3 \quad (4)$$

The total labile phosphate in the system is given by

$$P_e = P_s + P_{ss} \quad (5)$$

From the graphical analysis the half-lives of exchange ${}^1t_{\frac{1}{2}}$, ${}^2t_{\frac{1}{2}}$ and ${}^3t_{\frac{1}{2}}$ for the 'rapid', 'medium' and 'slow' fractions could be read off which made it possible to calculate the specific rates of exchange of phosphate ions between those in solution and those in the various fractions by the expression.

$$R_x = \frac{0.693}{x t_{\frac{1}{2}}} \cdot \frac{P_x \cdot P_{ss}}{P_x + P_{ss}} \quad (6)$$

where x refers to the sub-numerals 1, 2 and 3 pertaining to the 'rapid', 'medium' and 'slow' fractions.

Table 2 shows the variation of the total labile phosphate due to the presence of 0.001 M concentrations of organic anions and the effect of the latter on the distribution of the total labile phosphate

TABLE 2

Effect of organic anions on the isotopically exchangeable phosphorus and its fractions All figures in mg P per 100 g soil per 20 litres solution							
	P in solution at time of labelling with P^{32} P_{ss}	P in solid, P_s					Total labile P P_e
		Instan- taneous P_0	Rapid P_1	Medium P_2	Slow P_3	Total in soil $P_0 + P_1 +$ $P_2 + P_3$	
<i>Soil A</i>							
0.02 M KCl	0.80	1.69	0.23	0.42	8.16	10.52	11.32
+ 0.001 M citrate . .	6.58	3.01	1.08	1.69	3.86	9.64	16.32
+ 0.001 M barbiturate	2.25	0.78	0.59	0.77	4.68	6.81	9.06
<i>Soil B</i>							
0.02 M KCl	1.65	1.41	0.28	0.43	7.74	9.86	11.51
+ 0.001 M citrate . .	3.66	1.46		0.32	4.01	5.79	9.45
+ 0.001 M barbiturate	1.04	1.87		0.59	3.64	6.09	7.13

into its components, the 'instantaneous', 'rapid', 'medium' and 'slow' fractions. In the calcareous soil, the total phosphate exchanging isotopically in the system is decreased by the presence of these low concentrations of organic anions despite an increase in the phosphate desorbed into solution by the citrate ions, whereas the DEB anion increases P_{ss} only for the non-calcareous soil A. The decrease in the phosphate left in the soil after extraction by the organic anions is reflected in a considerable decrease in the amount of the 'slow' fraction P_3 . It is also noticeable that the 'instantaneous' and 'rapid'

TABLE 3

Effect of organic anions on the half-life and specific rate of exchange of phosphate ions						
	Half-life of exchange (in hours)			Rate of exchange (in mg P per 100 g soil per hour)		
	Rapid	Medium	Slow	Rapid	Medium	Slow
<i>Soil A</i>						
0.02 M KCl	1.6	8.6	40.0	0.24 *	0.022	0.013
+ 0.001 M citrate	0.5	4.4	38.3	1.21	0.21	0.044
+ 0.001 M barbiturate . .	0.4	2.5	25.8	0.44	0.23	0.04
<i>Soil B</i>						
0.02 M KCl	0.5	3.4	46.1	1.16 *	0.070	0.020
+ 0.001 M citrate	small	2.4	36.2	fast	0.086	0.037
+ 0.001 M barbiturate . .	0.3	1.8	27.2	1.49	0.14	0.02

* The 'rapid' rate is calculated approximately since a large part of the rapid component appears to exchange instantaneously.

fractions are clearly distinguishable from each other in *all* exchanges with the non-calcareous soil. With the calcareous soil in the presence of organic anions, however, only one rapidly exchanging fraction is observed.

Table 3 gives the half-lives of exchange and the specific rates of exchange for each fraction and shows that the half-lives of exchange for all fractions in both soils follow the order

no organic anion > citrate > barbiturate
except the 'rapid' fraction in soil B. By contrast, the specific rates of exchange for each fraction are in the order

citrate > barbiturate > no organic ion.
except the 'medium' fraction in both soils.

DISCUSSION

The constancy of the total labile phosphate in a soil at low concentrations of an organic anion like citrate (which strongly chelates alkaline earth cations and cations of iron and aluminium) suggests that this total labile phosphate is a characteristic quantity of the soil related to the physical micro-distribution of phosphates within it. The large and progressive increase in the total labile phosphate because of high citrate concentrations can be ascribed to the dissolution of crystalline phosphates or phosphate ions chemically bound to sesquioxides or calcareous compounds. With low citrate concentrations, there is no dissolution of a phosphate phase in the calcareous soil, because the total labile phosphate decreases when citrate ions are added; the total labile phosphate in the noncalcareous soil, however, increases in the presence of citrate ions suggesting the dissolution of precipitated phosphates. The phosphate concentration in solution in both soils however is increased over that in the absence of citrate anions due mainly to a decrease in the activity of cations, responsible for phosphate 'retention' in the intracellular solution, by chelation with citrate ions.

A second-order effect responsible for the increase in the phosphate in solution is an anion-exchange process between the adsorbed phosphate ions and the organic anion. The trivalent form of citrate ions is 80 and 93 per cent of the total citrate concentration at pH values of 7.0 and 7.5 respectively, whereas the corresponding values for the monovalent form DEB are only 29 and 56 per cent respective-

ly. Thus the citrate ion would be much more active in an anion-exchange process at those pH values. The specificity of the adsorbing surface plays an important role in deciding whether anion exchange will occur *e.g.* for soil A, DEB anions increase the phosphate in solution, whereas for the slightly calcareous soil B, they decrease it below the value obtained without of organic anions.

Data concerning the two organic anions, relevant to this study, are given in Table 4. These anions are very similar in their size, and their range of buffering overlaps considerably but their chelating power, at least for calcium ions, is vastly different.

TABLE 4

Data on organic anions				
Anion	Molecular weight	Molecular volume (X-ray)	Dissociation pK_0 at 25° C	Chelation Log K_{Ca}
Citric acid (mono-hydrate)	210	284 Å ³	6.4 (3rd dissociation constant)	3.15 (Concentration range .0002—.004 <i>M</i>) (0.66)
Diethyl barbituric acid	184	248 Å ³	7.4	(Concentration range .016—.15 <i>M</i>)

Note; X-ray data are taken from References ² and ⁶, dissociation and chelation data from Reference ⁸.

The effect of organic anions on the labile phosphate left in the soil is to decrease it with the non-chelating monovalent DEB ion to within 62 and 65 per cent of its value in the absence of organic ions, and between 60 and 85 per cent of the original value for the strongly chelating polyvalent citrate ion. As will be shown later, this decrease undoubtedly occurs because the larger organic ions with an average diameter of more than 8 Å block the release of adsorbed phosphate ions (average diameter 4.5 Å) from micropores. The effect of organic anions on the distribution within the total labile phosphate is primarily to decrease the 'slow' fraction. With soil A in citrate solution, over half of this decrease is offset by increases in the phosphate in solution and the 'medium' fraction, and to a smaller extent in the 'instantaneous' and 'rapid' fractions. With the DEB anion, however, the 'instantaneous' fraction is also

decreased whereas only small increases are observed in the remaining fractions. The 'slow' fraction must thus mainly represent adsorbed phosphate buried in micro-pores in the soil of diameter between 5 and 10 Å approximately. As citrate ions can decrease the activity of phosphate-retaining cations in the intracellular solution, they can desorb more phosphate from these micropores before 'blocking' occurs. With the non-chelating DEB anion these phosphate ions are almost completely locked in and become non-exchangeable isotopically without permitting the movement of PO_4 -groups to more easily exchanging sites; the sharp drop in the 'instantaneous' fraction appears to be only partly offset by an increase in the phosphate in solution, suggesting that the DEB anion is able to partly cover PO_4 -groups on 'instantaneously exchanging' sites and prevent them from exchanging isotopically.

With the calcareous soil B, the decrease in the total labile phosphate by the addition of organic anions is less pronounced. This is reflected, as in soil A, in the change in the slow fraction P_3 , offset by a large increase in the phosphate in solution in the presence of citrate ions. However, although the DEB anion affects the 'medium' and 'slow' fractions P_2 and P_3 as in the non-calcareous soil, it depresses the phosphate in solution significantly and depresses the rate of exchange of the 'instantaneous' fraction so as to make it indistinguishable from the 'rapid' fraction. It is of some interest that, (i) despite this internal re-distribution due to the DEB anion, the sum total of $(P_{ss} + P_0 + P_1)$ is the same (2.9 mg per 100 g soil) in the absence and the presence of the DEB anion; (ii) the citrate ion mobilises some of the 'slow' fraction to the more readily exchangeable sites in P_0 , P_1 and P_2 as well as to the phosphate in solution in soil A, whereas in the calcareous soil B the phosphate moves from the 'slow' fraction directly to the soil solution without affecting the more rapidly exchanging fractions, and (iii) whereas citrate invariably increases the phosphate in solution, the DEB anion actually decreases it with the calcareous soil B as noted earlier.

Thus, a comparison of the effects of the two organic anions on the residual phosphate in the soils shows that Soils A and B behave differently. In the CaCO_3 -free soil, nearly 50 per cent more phosphate is labile in the solid with citrate ions as with DEB ions, whereas in the calcareous soil the effect of the two ions differs much less. This could be attributed to the lower lability of phosphate ions

adsorbed on the calcareous material in soil B in the presence of organic anions and perhaps to the specific effects of the DEB anion on the phosphate retaining surfaces in the two soils. This effect is more clearly brought out when the detailed analyses of the rate-curves is considered (Table 3).

The effect of both organic anions is to greatly decrease the half-lives of exchange for all fractions in both soils. However, except the 'rapid' fraction in soil B, these half-periods are always less for the DEB anion than for citrate. This apparent inconsistency is corrected when the specific rates of exchange are worked out which take into account the partition ratio of the total labile phosphate between the phosphate fraction in the solid and the phosphate in solution. The specific rates of exchange in soil A follow the order

citrate $\geq$ barbiturate $\gg$ no organic anion

for the 'rapid' and 'medium' fractions; for the 'slow' fraction the same order is maintained but the effect of the organic anions is small. For the calcareous soil B, however, the order observed for the rapid reaction only is

citrate $\gg$ barbiturate $>$ no organic anion.

For the 'slow' fraction, the order is

citrate $\geq$ barbiturate $\geq$ no organic ion.

It is also observed that the presence of organic anions does not affect the specific rates of exchange so much in soil B as in soil A except for the 'rapid' fraction in the calcareous soil which is only a very small part of the total labile phosphate. This confirms the earlier suggestion that the labile phosphate in the calcareous soil is less mobile than that in soil A.

There is some evidence in the literature that phosphate groups combined with organic molecules can exchange isotopically with orthophosphate ions. Half-lives of exchange varying from a few hours to many days have been observed with glycerophosphates, sugar phosphates and nucleic acids in homogeneous systems where chemical methods are used to separate the orthophosphate and organophosphate components. In soil systems where this physical separation may be achieved by adsorption of the organophosphates on the soil-mineral matrix, it is possible that the characteristics of isotopic exchange with orthophosphate ions in solution may be altered. The net effect would be to reduce the rate of exchange which would increase the half-life under comparable conditions of

the exchange of phosphate ions between the two components. The possibility of such a mechanism occurring in soil systems must be envisaged although the present work does not provide evidence of its existence. It is clear, however, that adsorbed organo-phosphate components, if present, must occur in the 'medium' or 'slow' fractions because of the magnitude of the half-lives of exchange.

SUMMARY

The labile phosphate in a non-calcareous and a slightly calcareous soil was determined by isotopic exchange in the presence and absence of 0.001 molar solutions of a chelating and a non-chelating organic anion. The rate of isotopic exchange curves were analyzed graphically to sub-divide the labile phosphate into 3 or 4 fractions. The half-lives of exchange for the 'rapid', 'medium' and 'slow' fractions were between 0.3 to 1.6 hours, 1.8 to 8.6 hours and 25.8 to 46.1 hours respectively. In addition, an 'instantaneously-exchanging' component was sometimes observed.

In the presence of the citrate ion, the total labile phosphate was increased in the non-calcareous soil and decreased in the calcareous soil, whereas the diethyl barbiturate ion (DEB) anions decreased the total labile phosphate in both soils. In general, the citrate ion increased the 'rapid' and the 'medium' fractions whereas the DEB anion either did not affect them or decreased them. Again, whereas citrate always increased the phosphate in solution, the effect of DEB anions depended on the soil. The major effect of the organic anions was to greatly decrease the 'slowly-exchanging' fraction in both soils.

The half-lives of exchange for the 'rapid', 'medium' and 'slow' fractions were in the order

no organic anion > citrate > barbiturate

and the rate constants for a first-order mechanism were in the order

no organic anion < barbiturate < citrate.

Small but significant differences were observed between the two soils.

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