

CHARACTERIZATION OF PHOSPHORUS IN TROPICAL SOILS BY SEQUENTIAL EXTRACTION

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ABSTRACT

Phosphorus fertility is a limiting factor particularly in tropical soils which are characterized by low phosphorus contents. Characterization of soil P into total P and available P only, seems rather inadequate in the proper understanding of the P cycle. In the present study, a sequential extraction scheme of P, which is particularly suitable for highly weathered tropical soils was developed. Labile inorganic phosphorus (P_i), moderately labile P_e , calcium bound P_c , moderately labile organic P (P_o), total P, fractions and total P have been estimated. In addition, residual P_1 and residual P_2 have also been evaluated. The scheme has been applied to soil samples from Sri Lanka (Sinharaja forest and Eladuwa rubber plantation), Colombia, Brazil and Puerto Rico. In general, soils contained very low labile P and calcium bound P, fairly high Fe and Al bound P and a large amount of organic P. Each of the extracts used in the procedure can be assigned some role in the P transformations occurring in soil. These assignments may then be used to characterize various P pools and their transformations in the P cycle. Proposed P fractionation scheme permits a complete account of P fertility status in tropical soils. It is hoped that the results would also be of importance in developing management practices leading to sustenance of phosphorus fertility.

KEY WORDS: Inorganic P, Labile P, Organic P, Phosphorus fractionation, Residual P, Tropical soils

INTRODUCTION

Phosphorus, is an important plant nutrient and it is usually the second most limiting element after nitrogen in agro-ecosystems. The only source of phosphorus for the plant is from the soil itself. P fertility is considered to be a limiting factor particularly in highly weathered tropical soils which are characterized by relatively low total P and extremely low labile P content.

The experimental procedures for the determination of various forms of soil P and their availability to plants are essential in understanding the nature and behaviour of phosphorus in soils. Many methods based on a variety of principles

have been described in the literature for the extraction and estimation of soil P in its different forms.

However, until recently characterization of soil P was limited to the estimation of total P and available P. Available P, a widely used quantity, is a functional concept rather than a measurable quantity. Similarly total P hardly gives any information about different P fractions present in soil. Particularly in tropical soils, P turnover and supply are closely linked, and as such simple extracts are rarely adequate for predicting P fertility. Therefore, available information is rather insufficient to obtain a clear understanding of the P cycle.

To overcome this problem a fractionation scheme (Hedley *et al.*, 1982) was proposed to estimate different P pools in temperate soils. In the Hedley fractionation scheme the most labile form of P is extracted with an anion exchange resin (Amer *et al.*, 1955). More tightly adsorbed P forms are extracted with 0.5M sodium bicarbonate (Olsen *et al.*, 1954) while microbial P is extracted with the same bicarbonate solution after chloroform fumigation (Brookes *et al.*, 1982; Hedley *et al.*, 1982). Phosphorus adsorbed onto sesquioxides is extracted with 0.1M NaOH (McLaughlin *et al.*, 1977) while apatite type minerals are extracted with 1.0M HCl. Finally residual P is removed by sulphuric acid/hydrogen peroxide digestion. This method has been widely tested (Tiessen *et al.*, 1983; Tiessen *et al.*, 1984; Roberts *et al.*, 1985) to infer long term changes of various P pools in calcareous and temperate soils. This method was found to be unsatisfactory for highly weathered tropical soils (Tiessen *et al.*, 1984). The method needs certain modifications and adjustments to suit the chemical and physical nature of tropical soils. Furthermore, Hedley scheme is rather tedious and time consuming and as such it needs further simplification and modification to be applicable to tropical soils.

In the present study a simple sequential extraction scheme of P, which is particularly suitable for tropical soils, was developed. This is considered necessary for a proper understanding of the P cycle. Results of such a fractionation study also would enable us to understand the contribution of various inorganic and organic P fractions to P fertility and the relative importance of organic P (P_o) mineralization in tropical soils.

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MATERIALS AND METHODS

Soil samples

One hundred samples of acid tropical soils (0-15 cm depth) were collected from Sri Lanka, Brazil, Colombia and Puerto Rico. Some important characteristics of these soils are indicated in Tables 1-3. These samples were air-dried at room temperature and screened through a 2 mm sieve prior to sequential extraction.

Table 1. P fractionation of soils from Brazil and Colombia

Sample	Resin	NaOH		HCl	Residual		Total
	P_i	P_i	P_o	P_i	P_i	P_o	P
	←----- mg P/kg soil -----→						
Forest (Sandy loam, pH 4.5-4.8)							
Rio Paila	79	150	99	77	173	307	884
Burriaga	31	29	141	28	173	119	522
Popayan	3	115	187	18	133	424	880
Manaus I	3	8	30	8	37	58	144
Manaus II	6	14	27	3	25	49	125
Una	3	21	63	8	278	41	414
Valenza	3	15	46	7	168	60	298
Cultivated							
Rio Paila	142	116	43	133	251	46	669
Burriaga	26	22	67	25	79	74	293
Popayan	2	112	409	6	75	212	813
Manaus I	6	88	52	1	92	42	218
Manaus II	7	42	35	6	126	35	250

Values are mean of 3 replicates

Sequential extraction procedure

A modified and simplified version of Hedley procedure has been adopted. Sodium carbonate extraction and microbial P estimation have been omitted. The method has been simplified by reducing the number of stages in the extraction procedure and also by estimating residual P by calculation.

Table 2. P fractionation of soils from Puerto Rico (Sandy loam, pH 4.8)

Sample	Resin	NaOH		HCl	Residual		Total
	P_i	P_i	P_o	P_i	P_i	P_o	P
	<----- mg P/kg soil ----->						
Control	14	44	94	4	123	120	400
Fertilized	20	177	97	8	168	230	700
Subsoil (3m depth)	11	45	6	12	418	0	492
Ridge top	13	55	82	2	181	101	434
Mid slope	11	63	73	2	156	132	436
Top slope	10	80	103	8	112	218	530

Values are mean of 3 replicates

Table 3: P fractionation of soils from Sri Lanka

Sample	Resin	NaOH		HCl	Residual		Total
	P_i	P_i	P_o	P_i	P_i	P_o	P
	←----- mg P/kg soil -----→						
Sinharaja Forest (Sandy loam, pH 4.6)							
Forest site 127 (10)	1	47	67	1	233	74	423
Forest site 15 (20)	1	32	45	1	109	38	225
Pinus Plantation							
Ridge (3)	9	28	86	6	94	10	233
Mid slope (3)	7	31	63	4	74	26	205
Valley (3)	3	21	103	2	112	10	251
Rubber Plantation (Sandy loam, pH 4.9)							
Eladuwa site I (3)	55	145	44	7	249	57	556
Eladuwa site II (3)	31	208	55	5	103	59	462

Values within brackets refer to the number of replicates

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0.5 g air-dried soil sample was transferred into a 50 ml centrifuge tube. An anion exchange resin (Bio-Rad Anion Exchange Membrane AG 1-X8) strip of dimension 10 x 65 mm was added to the tube followed by 30ml of deionized water (DI) and shaken for 16 h (overnight). The resin strip was taken out and shaken with 30 ml of 0.5M HCl for 6-8 h and the acid extract was analysed for resin P. Soil - water suspension was centrifuged at 10,000 rpm for 10 min. The supernatant solution was discarded, 30 ml of 0.2M NaOH were added to the residue and shaken for 16 h. The sample was centrifuged and the supernatant solution was decanted for P analysis. Then, 30 ml of 1.0M HCl was added to the residue and shaken for 16 h. The suspension was centrifuged and the supernatant solution was decanted for P analysis. Sequential extraction was carried out as follows:

- Day 1 : Resin P extraction (overnight).
- Day 2 : Removal of resin bound P with acid and P estimation. NaOH extraction (overnight).
- Day 3 : Estimation of inorganic P and total P in NaOH extract. HCl extraction (overnight).
- Day 4 : Estimation of HCl extractable P.
Estimation of total P by NaOH fusion.
- Day 5 : Estimation of total organic P by ignition method.

A flow diagram for the extraction scheme is presented in Fig. 1.

Total P determination

Total P was determined by NaOH fusion method (Smith and Bain, 1982). 0.2 g finely ground (<100 mesh) soil sample was fused with about 2 g NaOH in a nickel crucible. Crucible with the melt was transferred into a beaker with 50 ml of DI water. After 3 h, the solution was quantitatively transferred into a 100 ml volumetric flask and made up to volume with DI water and P was determined.

Total P_o determination

Total organic P was determined by igniting 0.5 g finely ground soil samples in porcelain crucibles for 1 h using a muffle furnace at 550°C (Olsen and Sommers, 1982). Pairs of ignited and unignited samples were then extracted with 30 ml portions of 0.5M sulphuric acid for 16 h. Samples were then centrifuged and P determined

in the supernatant liquid. The total organic P was calculated from the difference in the acid extractable P of the ignited and unignited sample of the same soil.

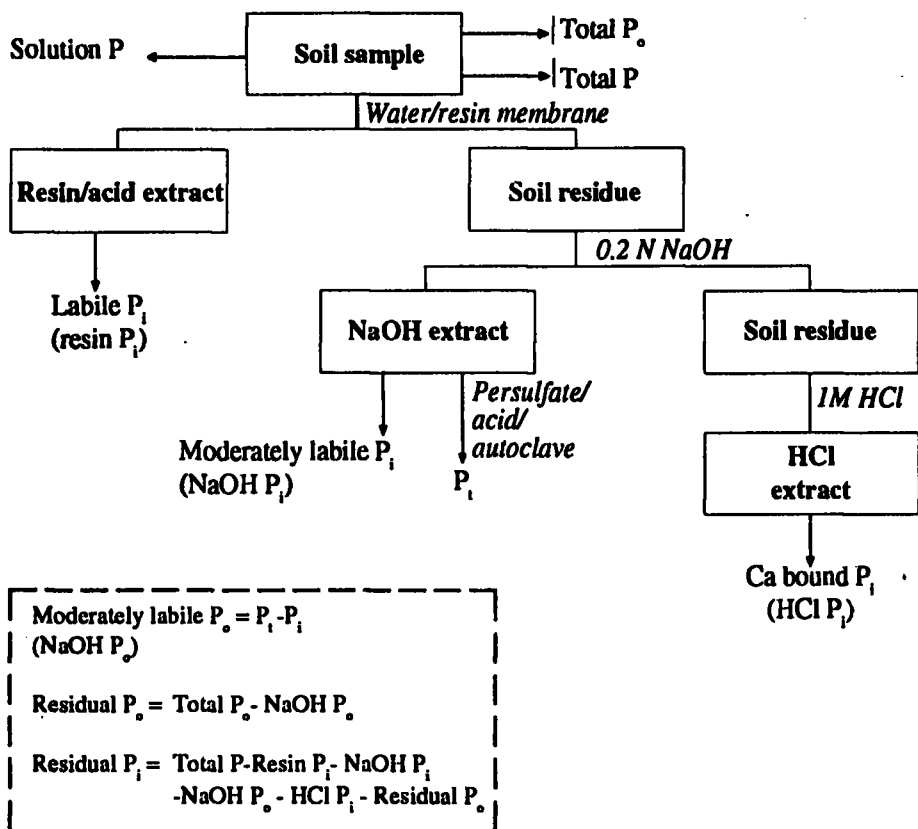


Fig. 1. Flow diagram for P fractionation

Phosphorus analysis

P analyses for all extracts were done using 5 or 10 ml aliquots. pH of the solution was adjusted using p-nitrophenol indicator; thereafter Murphy and Riley (Murphy and Riley, 1962) reagent was added and the resulting blue colour was read at 882 nm using a spectrophotometer.

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Some soil samples gave intensely coloured NaOH extracts owing to the presence of high organic matter rendering direct estimation of P_i difficult. In such cases organic matter was precipitated by adjusting the pH to 1.0 - 1.5 with 3M sulphuric acid and allowing it to stand at 0° C for 30 min. The resulting suspension was centrifuged prior to P_i estimation.

Organic P in the NaOH extract was estimated as the difference between total P and inorganic P in the extract. Total P in NaOH extract was determined by potassium persulphate / sulphuric acid digestion of an aliquot in an autoclave at 121°C and 15-20 psi for 45 min., followed by P analysis.

RESULTS AND DISCUSSION

The results of the P fractionation study on the soil samples from Brazil, Colombia, Puerto Rico and Sri Lanka are summarized in Tables 1-3.

Inorganic P fractions

Inorganic forms of phosphorus present in soil may be further fractionated into labile P_i , moderately labile P_i , calcium bound P_i and residual P_i .

Labile P_i

Anion exchange resin acts as a sink for solution P_i disturbing the equilibrium between dissolved and soluble P_i . The exchangeable P_i as well as some of the more soluble precipitated P forms will enter the depleted solution and bind onto the resin. The P_i sorbed by the resin is subsequently removed and measured.

Most reported work on resin P extraction has been carried out using resin bags (Amer *et al.*, 1955; Sibbesen, 1977). However, it has been observed in the present study that the resin membrane strips are more efficient with regard to P adsorption and subsequent recovery of P using acids. In addition, resin strips are more convenient to use than resin bags.

The capacity of a resin strip of dimension 10 x 65 mm was estimated to be 300 µg P. Optimum shaking time for 0.5 g soil/30 ml DI water was found to be about 16 h and the P adsorbed onto the resin could be extracted rapidly within 4 - 6 h using 0.5 M HCl.

Extraction of P by resin is affected by the saturating anion on the resin. In temperate soils bicarbonate is frequently used but in more weathered soils chloride anion is preferred to avoid any pH effects on the extraction. In the present study chloride form was found to be more suitable and effective.

Labile P contents of most soils investigated in the present study, except the ones from fertilized plots, were rather small varying from 1.0 to about 20 mg/kg soil.

Moderately labile P_i

An important feature of the present sequential extraction scheme is that it avoids the use of sodium bicarbonate and uses sodium hydroxide (0.2 M) as the only alkaline extractant. Inorganic P in this extract is estimated directly or after precipitation of organic matter if the extract is strongly coloured. NaOH removes Fe and Al bound P and this fraction is also referred to as secondary P_i . In general, moderately labile P_i content was found to be higher than the labile P_i content in the samples investigated. Furthermore, most cultivated soils contained more moderately labile P_i than the corresponding forest soils.

Calcium bound P_i

This fraction is also called primary P_i and is extracted using HCl. This is derived from the parent material consisting mostly of apatite group of minerals. Calcium bound P is present in very small quantities (Tables 1-3).

Organic P fractions

It has been suggested that organic P plays an important role in plant nutrition particularly in tropical soils (Agboola and Oko, 1976; Tiessen *et al.*, 1984). However, it is rather difficult to estimate organic P fractions directly and the nature of organic P is not completely understood. Organic P fractions are best estimated from the difference between total P and inorganic P in a given extract.

Labile and moderately labile P_o

It has been reported that NaOH extractable organic P is relatively labile (Bowman and Cole, 1978). Thus, labile and moderately labile organic P is extracted

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as one fraction using 0.2 M NaOH in this study. In most soils this fraction was found to be higher than the corresponding P_i fraction.

Total organic phosphorus

Ignition of soil organic matter at 550°C in a muffle furnace renders organic P acid extractable and the difference in the acid extractable P of ignited and unignited samples of the same soil is a measure of the total organic P. Although this method sometimes overestimates total organic P, it is widely used. Nevertheless, total P_o values have been used only for the purpose of evaluating the residual P_o in the present scheme.

Organic forms of soil P are rapidly mineralized in tropical soils to make a substantial contribution to plant nutrition (Cole *et al.*, 1977; Blair and Boland, 1978). P availability to plants is determined primarily by the mineralization rates rather than the absolute amounts of P_o . Therefore, an extensive study of mineralization rates and a search for more precise and direct methods for the estimation of various P_o components is essential in order to understand the prominent role believed to be played by the organic P in these soils.

Residual P

About 20-60% of soil P is unextractable using normal and mild extractants and this fraction is usually described as the residual P. This includes P forms which are rather stable and unreactive. In Hedley scheme (Hedley *et al.*, 1982) an elaborate experimental procedure has been used to estimate residual P. However, residual P can be conveniently calculated from the other experimentally determined P fractions. Residual P_o and residual P_i fractions have been evaluated in the present scheme as shown in Fig.1.

Total P

Methods available for the determination of total P include acid digestion methods (Bray and Kurtz, 1945; Bowman, 1988) and alkali carbonate fusion (Jackson, 1970). Acid digestion uses corrosive acids while sodium carbonate fusion requires the use of expensive platinum crucibles. In the present study a more convenient fusion technique was employed (Smith and Bain, 1982) in which NaOH was used as a flux and fusion carried out in a nickel crucible at a much lower temperature.

Phosphorus cycle

Highly weathered tropical soils investigated in the present study are characterized by very low labile P and calcium bound P, fairly high Al and Fe bound P and a large proportion of organic P. In general, total P values are also relatively low. It appears that Al and Fe oxide/hydroxides are chemically active in these soils fixing a considerable proportion of labile and any added fertilizer P.

Sequential extraction, which first measures the labile P and then the more stable forms of P, is useful in examining the longer term transformations of P in soils. Each of the extracts used in the procedure can be assigned some role in the P transformations occurring in soil. These empirical assignments may then be used to characterize various P pools and their transformations. Various transformations of P in ecosystems combined with the results of P fractionation study can best be synthesized in a conceptual model of soil P transformations or P cycle as shown in Fig.2.

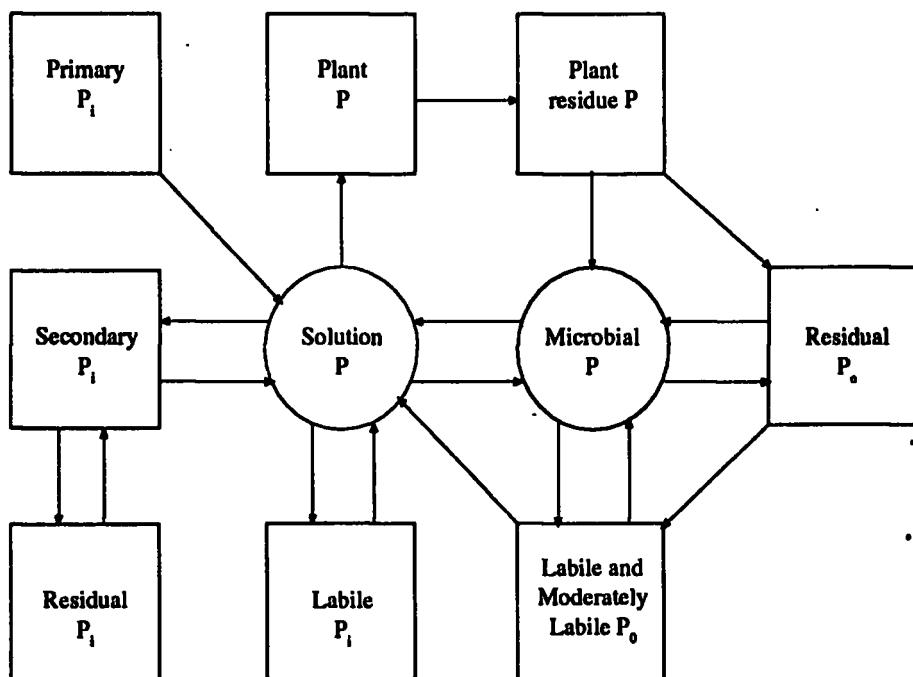


Fig. 2. Soil P cycle

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Plant uptake is from solution P (Fig.2) which is held in equilibrium with labile P. Very slow weathering of calcium bound P (Primary P_i), Fe and Al bound P (secondary P_i) and the mineralization of P_o would supply the solution P pool. Microbial P is also in equilibrium with the solution P pool. Microbial population is responsible for the conversion of P_i to different forms of P_o as well as for the P_o mineralization.

The proposed fractionation scheme deviates from Hadley scheme in that it avoids sodium bicarbonate extraction and the extractions of microbial P and residual P. For acidic highly weathered soils sodium bicarbonate extraction becomes redundant and the residual P could be estimated by a simple calculation, which makes the proposed fractionation scheme simpler and faster avoiding time consuming tedious steps.

The main advantage of the proposed fractionation procedure is that it permits a complete account of the P forms present, thereby providing a complete budget of the different P forms in tropical soils. This would enable a better understanding of the P cycle. In particular, vital information regarding the importance of P_o mineralization and contribution of various P fractions towards P fertility can be obtained. Consequently, the results would be of immense importance in developing management practices for sustenance of P fertility with minimum use of chemical fertilizer.

Nevertheless, much more work is needed to evaluate the proposed sequential extraction scheme on a wide variety of soils and also to relate the estimated P pools to crop response.

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