Comparison of Methods to Estimate Occluded and Resistant Soil Phosphorus

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ABSTRACT

Weathering, pH changes, and biotic interactions through geologic times have created a gradation of easily released (labile) to strongly stabilized (resistant) P pools. While methodologies for fertility indices and labile P are well established, methods for quantifying less labile P, such as the occluded and resistant or residual P, are less well established. We reexamined existing methods for these pools, and suggested new procedures to improve their precision and timeliness. Specifically, we compared two methods for reductant-soluble (occluded) P, and two existing and a new procedure for the resistant P. Occluded P was difficult to reproduce from a sequential extraction procedure because of problems associated with molybdate blue reaction, which required extra molybdate or persulfate oxidation to minimize citrate interference. However, use of inductively coupled plasma (ICP) spectroscopy eliminated this problem. Measurement of P by ICP for surface and occluded P in the total free Fe oxide pool was more quantitative and reproducible [average coefficient of variation (CV) = 5%] than in the sequential extraction (average CV = 7%) procedure. Results for the proposed resistant P method (total soil P minus acid-extractable P in an ignited sample) approximated the two sequential extraction procedures (total soil P − total acid- and base-extractable organic and inorganic P) and is easier, and more reproducible. Both procedures for all soils except the Molokai showed essentially the same amounts of occluded P. An average of about 26% of the total soil P (TP) was resistant, with the more weathered Cecil soil containing about 50% resistant P.

U NLIKE C AND N, which can be added to the soil system from the atmosphere, P levels in natural systems are essentially fixed from levels of the primary apatite minerals. From natural weathering and dissolution, this primary P mineral is converted to secondary P minerals through surface reactions, or to organic fractions through biological uptake and decomposition. For this reason, Walker and Adams (1958) and Walker and Syers (1976) suggested that P transformations and availability exerted major controls on soil formation. They explained that given sufficient time and active weathering conditions, all mineral P not lost to erosion is converted to resistant P in the form of organic and occluded P. Under this scenario, estimated to take ≈22 000 yr, the soil loses its basic cations, and increases in acidity; these conditions become the driving force for further P transformations. Thus, plant-available P under this extreme weathering is generated essentially from the mineralization of organic P and, rather sparingly, from the occluded P protected by oxides of Fe and Al in this acid environment.

From a pedogenic point of view, these various P pools and their profile distribution are used frequently to diagnose soil taxonomic classes. Smeck (1985) showed some

of these relationships between taxonomy and P in its various forms. According to him, TP should decrease from Entisols (young soils rich in Ca P with alkaline to neutral pH) to Oxisols (old soils rich in Fe with acid pH). Similarly, Tiessen et al. (1984) investigated pathways of P transformations in soils of differing pedogenesis, and found various relationships between available and stable P forms and the degree of weathering. In Spain, Trasar-Cepeda et al. (1990) conducted similar studies.

The transition from available to resistant P forms is slow. While methodologies for fertility indices and labile P are well established, methods for quantifying less labile P, like the occluded and resistant or residual P, are less well established. Some questions concerning methodology, timeliness, and reproducibility remain. For instance, is the amount of resistant P fixed regardless of methods of extraction such as the sequential extractions proposed by Hedley et al. (1982), Tiessen and Moir (1993), and Chang and Jackson (1957)? Such comparisons have never been done before, and similarity of results could indicate the presence of a quantitative discrete resistant pool. Also, is the occluded P only relevant in highly weathered, low-pH soils? Can the same analogy be drawn where $CaCO_3$ envelops P under high-pH conditions? Can the P in the free Fe_2O_3 procedure be used as an adequate measure of occluded P when corrected for surface-adsorbed P? Iyamuremye et al. (1996) used incubation studies to assess short-term changes in P chemistry and sorption from the addition of organic amendments, but quantified primarily P pools in the Hedley fractionation. Some reexamination of these pools, because of their use in pedogenic functions (Smeck, 1973), in soil and sediment chemistry (Sharpley and Smith, 1990), and in short- and long-term biological P release (McGill and Cole, 1981), is necessary relative to method simplification and precision.

Present methods for resistant P require multiple extractions, which could take 4 to 6 d. Occluded P is determined with great difficulty with the presently prescribed method; therefore, easier and quicker methods were sought. We reexamined P methodologies in two areas: (i) the extractability of surface and occluded P in acid and calcareous soils, and (ii) the suitability of a proposed method for resistant P and its relationship to other published methods.

MATERIALS AND METHODS

Soils from six different soil orders (Table 1) were selected to accomplish the above objectives. All soil samples were air dried and passed through a 2-mm screen. Soil organic matter was determined from organic C (1.72 conversion factor) with a C–N gas analyzer. The hydrometer method was used to

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Abbreviations: CBD, citrate–bicarbonate–dithionite procedure; CV, coefficient of variation; ICP, inductively coupled plasma; Pi, inorganic P concentration; Pt, total P concentration obtained through persulfate oxidation; Po, organic P concentration as determined by difference (Pt − Pi); TP, total soil P.

Table 1. Selected physical and chemical characteristics of test soils.

Soil series†	Subgroup	pH (1:1)	Soil organic matter	Clay	CaCO₃	Total Fe	Total Al	Total P
					g kg⁻¹			
Ascalon sl	Typic Argiustoll	6.6	13.7	140	0	14.0	40.0	0.385
Cecil sl	Typic Kanhapludult	4.9	8.6	90	0	9.0	21.0	0.175
Duroc l	Pachic Paplustoll	7.3	37.8	130	0	19.0	51.0	0.690
Haverson l	Ustic Torrifluvent	7.8	20.6	260	80	21.0	52.0	0.610
Molokai c	Typic Torrox	6.1	43.0	800	0	148.0	114.0	1.200
Redfeathers sl	Lithic Cryoboralf	5.3	10.3	90	0	48.0	59.0	0.590

† sl, silt loam; l, loam; c, clay.

determine soil texture. Soil reaction was determined with 20 g of soil in 20 mL of distilled water. Total Fe and Al and total free Fe_2O_3 and Al_2O_3 were determined by the Colorado State University Soil Testing Laboratory (Holmgren, 1967). Iron concentrations in dilute acid (1.0 M H_2SO_4) extracts were determined colorimetrically with hydroxylamine hydrochloride for reduction of ferric to ferrous Fe, and α, α-dipyridyl for color development assessed at 520 nm. All analyses were replicated at least twice.

In sequential extractions, all samples were resuspended after centrifugation at 9000 × g before the next extraction. All extracts were adjusted to optimal pH range with p-nitrophenol indicator before addition of acid molybdate and ascorbic acid for colorimetry at 882 nm (Murphy and Riley, 1962). Determinations were conducted in two areas: surface-adsorbed and occluded P, and resistant P. We use both of the terms *resistant P* and *residual P* to mean that pool which is extracted with great difficulty, or by difference from the whole when a soil residue yields essentially no more acid- and base-extractable inorganic P (Pi) and organic P [Po, as determined by difference (Pt − Pi)].

Surface-Adsorbed and Occluded Phosphorus

Surface-adsorbed and occluded P were determined, with some modifications, according to the fractionation procedure of Chang and Jackson (1957), as described by Olsen and Sommers (1982). All quantities were halved, and the extraction was done in 50-mL polypropylene centrifuge tubes. First, the surface nonoccluded P was removed with base (0.25 M NaOH) after a 16-h shaking extraction. Phosphorus (organic and inorganic) and Fe in the extract were measured colorimetrically. Soil residues were then extracted for occluded P with the citrate–bicarbonate–dithionite (CBD) procedure. Persulfate oxidation was used to destroy the citrate and to oxidize any residual dithionite. Both modifications required P standards and blanks in the appropriate matrix for adequate quantification. Besides the colorimetric procedure, P was also determined by ICP in the free Fe_2O_3 extract as a measure of total free P. An indirect measure of the occluded P was thus obtained by correcting for the surface-adsorbed P (P in Fe_2O_3 − P in 0.25 M NaOH).

We also determined available P (labile surface P) by anion-exchange resin (Amer et al., 1955).

Resistant Soil Phosphorus

The resistant soil P was determined by various published methods and by a proposed new method. Resistant P was determined by difference between TP and total acid- and base-extractable Pi and Po by the modified Hedley–Tiessen (Hedley et al., 1982; Tiessen and Moir, 1993) procedure or by the difference between the TP and the sum of extractable P from the Chang and Jackson (1957) procedure plus Po from hot (90°C) 0.5 M NaOH extraction.

For the modified Hedley–Tiessen procedure, 0.75 g soil

was extracted twice for 1 h with 25 mL of 0.5 M NaHCO₃ (pH 8.5). Both extracts after centrifugation and filtration were combined into a 100-mL volumetric flask. The soil residue was then extracted twice for 1 h with 25 mL of 0.5 M NaOH, with both extracts after centrifugation and filtration placed into the original flask. The flask was made to volume, mixed, and Pi and total P concentration (Pt) determined. The soil residue after NaOH treatment was extracted with 30 mL of 0.5 M H_2SO_4 for 16 h, and Pi and Pt were determined on the extracts after centrifugation and filtration. The acid soil residue was then placed into a 90°C water bath for 2 h with 25 mL 0.5 M NaOH. Inorganic P and Pt were determined. The various Pi and Po pools were summed to obtain a value for the total extractable P.

For the modified Chang and Jackson (1957) procedure, soils after occluded P extraction were further extracted with 1 M H_2SO_4 for 16 h for Ca-associated P. After acid extraction for Ca-associated P, soil residue was incubated at 90°C in a water bath with 25 mL of 0.5 M NaOH for 2 h and Pi and Po determined to obtain the more resistant Po. As above, all pools of Pi and Po in the fractionation scheme were added for the total-extractable P pool.

For the proposed method, the resistant P was calculated by difference from the TP and the Pi extracted from a high-temperature (550°C) ignited sample (Saunders and Williams, 1955).

Total soil P was determined by strong acid digestions (Bowman, 1988). The procedure was modified so 0.3 g of soil could be digested with 3.5 mL of 18 M H_2SO_4. Proportions of 8.8 M H_2O_2 and 24 M HF remained the same. After about 20 min of digestion at 200°C in polypropylene beakers, samples were cooled and quantitatively transferred to 50-mL volumetric tubes. After bringing to volume, the contents were mixed and filtered through fine precipitate retention filter paper. A 1-mL aliquot, after appropriate neutralization, was used for P determination. Complete color development usually required ≈20 min.

Statistics

For statistical evaluations, regression and CV analyses were conducted where methods were compared. Analysis of variance was also conducted to evaluate mean differences at the 0.05 level.

RESULTS AND DISCUSSION

The six soil orders represented a variety of soil physical and chemical properties that influence soil P content (Table 1). For the test soils, TP ranged from <0.18 g kg⁻¹ in the highly weathered Cecil sandy loam to about 1.2 g kg⁻¹ in the Molokai clay. The Molokai soil contains about 80% clay and the Haverson soil 26% clay with 8% free CaCO₃ on a w/w basis. Generally, P is highly correlated with clay (Smeck and Runge, 1971), and is

Table 2. Iron and Al oxides and surface–adsorbed and occluded P in test soils.

Soil	Free Fe_2O_3 as Fe	Fe_2O_3 as hematite	Free Al_2O_3 as Al	Al_2O_3 as gibbsite	Fe_2O_3 P	Surface–adsorbed P	Resin P	Occluded P	
	g kg^{1}				mg kg^{-1}				
Ascalon	3.1	4.4	0.5	1.4	72	52	22	40†	20‡
Cecil	5.4	7.7	1.5	4.3	59	26	7	41	33
Duroc	4.8	6.9	1.0	2.9	154	40	59	102	114
Haverson	3.6	5.1	0.8	2.3	94	5	12	68	89
Molokai	109.2	156.2	8.7	25.1	799	296	28	256	573
Redfeathers	10.2	14.6	1.5	4.3	219	158	22	57	61

† Occluded P from Chang and Jackson (1957) sequential fractionation.
‡ Occluded P by difference: P in free Fe_2O_3 minus surface-adsorbed P.

adsorbed on the surfaces of $CaCO_3$, and on oxides of Fe and Al (Sample et al., 1980). The Molokai and Red-feathers soils contain the highest amounts of total Fe and Al while the Cecil and Ascalon contain the least. The soil reaction (pH) of the test soils reflect weathering and loss of bases in the Cecil soil (an Ultisol from Georgia), and podzolization and surface acidity from organic acid buildup from the Redfeathers soil (an Alfisol from the Rocky Mountain alpine area) (Duchaufour, 1982). The bottomland Haverson soil had accumulated blown-in lime from nearby chalk bluffs, and is calcareous in its surface. Because of the high clay content in the Molokai (an Oxisol from Hawaii), bases are still retained and pH is only slightly acid. The Ascalon is a typical Mollisol of the semiarid central Great Plains, high in Ca-associated phosphates with an argillic B horizon. The Duroc soil contains relatively high levels of soil organic matter, and the highest available P by anion-exchange resin (Table 2).

Surface-Adsorbed and Occluded Phosphorus Determination

Surface-adsorbed P (Table 2) was obtained with dilute base (0.25 M NaOH) after overnight shaking. Data for P-associated free oxides show that Fe_2O_3 was the dominant oxide surface in all the soils except the Haverson. Aluminum oxide levels were much lower than Fe oxide levels even though total soil Al was higher in all soils except the Fe-dominated Molokai clay. Values for surface adsorbed P ranged from 5 mg kg^{-1} for the calcareous Haverson loam to 226 mg kg^{-1} for the Molokai clay. On a percentage basis, the Redfeathers soil contained the greatest amounts of surface-adsorbed P (27%) while the Haverson soil was essentially free of surface-adsorbed P. The P in the Haverson was intimately associated with free $CaCO_3$ surfaces, and was released only after decomposition of lime with dilute H_2SO_4 (Table 3). On a percentage basis of total soil P, the order of surface-adsorbed P released was: Haverson < Duroc < Ascalon < Cecil < Molokai < Redfeathers.

Phosphorus in the free Fe oxide extracts ranged from 59 mg kg^{-1} in the Cecil soil to 799 mg kg^{-1} in the Molokai (Oxisol). The order of associated P by percentage was: Haverson (16%) < Ascalon (19%) < Duroc (22%) < Cecil (34%) < Redfeathers (37%) < Molokai (67%). The sequence was similar to that for the surface-adsorbed P, except for Molokai, which contained large amounts of associated P on the total free Fe oxides, surpassing Redfeathers.

The occluded P was estimated directly (Table 2) through the sequential extraction procedure of Chang and Jackson (1957), and by difference from the P in the total free Fe oxides (Fe_2O_3–P − surface-adsorbed P). For the extraction of occluded P by the Chang and Jackson method, the physically protected layer (usually ferric oxide envelopes) was removed by dithionite. However, extra molybdate can also be used to eliminate or minimize citrate interference (Weaver, 1974). The P in this fraction was recovered by oxidation with persulfate and measured colorimetrically or by ICP. Some organic P may be oxidized by this procedure, but the initial base extraction for the surface-adsorbed P probably released the bulk of the Po. An alternative method to determine Pi without Po confounding is the isobutyl alcohol separation, which is tedious and not conducive to large sampling analysis. The Molokai clay contained the highest percentage of occluded P while the Ascalon and Redfeathers soils contained the lowest. The Molokai clay showed 48% occluded P by the difference method but only 25% by the modified Chang and Jackson fractionation method. In spite of this difference, both methods were highly correlated ($r^2 = 0.98$) with no significance between slopes. The difference method, however, was easier and more reproducible (CV of 4.0–6.0% for the difference method vs. 6.0–8.0% for the modified Chang and Jackson).

In an analogous manner, P enveloped by $CaCO_3$ (pH = 7.8) can be regarded as occluded. Note the very low percentage of P (2%) extracted by anion-exchange resins (Table 2). The P is released only after the lime has been dissolved with acid (Table 3). Even though just one calcareous soil was used in this study, there is much documentation of the difficulty with which P is extracted under calcareous conditions (Barrow, 1980; Mattingly, 1975).

While the occluded P pool was significant in the Oxi-

Table 3. Acid-extractable P and Fe for ignited and nonignited soil samples.

Soil	Ignited P	Non ignited P	Ignited Fe	Non ignited Fe
	mg kg^{-1}			
Ascalon	294 (76)†	200 (52)	2 101 (15)	1 236 (9)
Cecil	85 (49)	35 (19)	2 490 (28)	1 659 (18)
Duroc	590 (85)	330 (48)	3 800 (20)	2 796 (15)
Haverson	580 (95)	490 (80)	2 831 (13)	2 059 (10)
Molokai	775 (64)	340 (28)	21 440 (14)	7 468 (5)
Redfeathers	450 (76)	350 (59)	11 008 (23)	4 032 (8)
Mean	(74)	(48)	(19)	(12)

† Percentage of total given in parentheses.

sol soil (47% by the proposed method), and to some extent in the Ultisol soil, the need for its quantification in Great Plains soils may be necessary only where mineralogy shows high clay with reactive surfaces.

Resistant Soil Phosphorus Determination

Knowledge about the size of the resistant P pool is important since it essentially represents a pool virtually unavailable to plants. We hypothesized that the P not extractable in acid after dry combustion at 550°C (TP− ignited Pi) would represent such a highly resistant P fraction. This proposed procedure was then compared with the residual P obtained by the Hedley–Tiessen and the Chang and Jackson procedures.

Before evaluating the resistant P, we assessed the P and Fe concentrations in the acid-extractable ignited and nonignited soils (Table 3). The P from the ignited samples reflected the increases in P solubility (Soltanpour et al., 1987) and oxidation Po converted to Pi compared with the nonignited sample (Saunders and Williams, 1955). Phosphorus extractability compared with the TP ranged from 19% for the nonignited Cecil to 80% of TP for the nonignited Haverson soil. With ignition, almost all the P was released in the Haverson soil, but only about 50% from the Cecil. The highly weathered Cecil and Molokai soils released the lowest percentages with acid extraction. Among the different soils, the order of efficiency of Pi extraction was: Cecil (Ultisol) < Molokai (Oxisol) < Ascalon (Mollisol) < Redfeathers (Alfisol) < Duroc (Mollisol) < Haverson (Entisol). With respect to acid-extractable Fe, an average of 19 and 12% of the total soil Fe was extracted by ignition and nonignition, respectively. This was comparable with an average of 74 and 48% of total P, respectively, for the test soils. Only 5% of the total Fe was extracted in the Molokai soil without ignition, while 28% of the total P was extracted.

On a molar ratio basis (moles Fe per moles P extracted in acid), data for the Ascalon, Duroc, and Haverson showed similar extraction efficiencies. The other three soils released much more Fe with the extracted P.

For resistant P, the proposed procedure compared well with the other two established methods (Table 4). The Hedley–Tiessen procedure extracted about 9% more P, while the Chang and Jackson extracted about 3% less. Statistical analysis showed that both established methods were highly correlated with the proposed

method ($r^2 = 0.98$), and that the slopes were not significantly different from zero, and the intercepts from one another. Percentage of resistant P increased according to the following order: Haverson (Entisol) < Duroc (Mollisol) < Redfeathers (Alfisol) = Ascalon (Mollisol) < Molokai (Oxisol) < Cecil (Ultisol).

The proposed resistant P methodology is fairly straightforward, and its reproducibility is only as variable as the methodology for acid-P extraction. In many instances the TP will have already been known, and the procedure would require only the measurement of P in an ignited soil. This procedure could be applicable for the quantification of resistant P along a catenary sequence as an indirect measure of weathering and soil maturation.

CONCLUSIONS

Reactive surfaces such as oxides of Fe and Al and $CaCO_3$ play an important role in determining the levels of transitional or intermediate P. Given sufficient weathering and time, a resistant P pool is formed from these transitional pools that is essentially unavailable to plants. Previous quantification methods for these pools have been difficult or lengthy. The easier quantification of the occluded and resistant P pools of the proposed method may increase their use in assessing P budgets, especially where comparisons are made across soil orders and catenary sequences.

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Table 4. Resistant P pools by modified Hedley–Tiessen (H–T), modified Chang and Jackson (C&J), and proposed method.

Soil	Resistance P			Proposed method
	Proposed	H–T	C&J	% of TP
	mg P kg^{-1}			
Ascalon	84 ± 5	78 ± 6	72 ± 6	22
Cecil	90 ± 4	70 ± 4	55 ± 4	51
Duroc	118 ± 8	134 ± 10	140 ± 13	16
Haverson	30 ± 5	57 ± 6	40 ± 6	5
Molokai	450 ± 15	503 ± 20	420 ± 18	38
Redfeathers	125 ± 8	138 ± 9	143 ± 9	21
Mean	150	163	145	26

† TP is total soil P.

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