Biogeochemistry **47:** 25–38, 1999.

A comparison of fractionation methods for forms of phosphorus in soils

ERIC T. LEVY[1]* & WILLIAM H. SCHLESINGER[2]
[1] *Department of Biological Sciences, Stanford University, Stanford, California 94305, U.S.A. (e-mail: eric.levy@stanford.edu);* [2] *Department of Botany, Duke University, Durham, N.C. 27708, U.S.A.*

Accepted 22 October 1998

Key words: Hedley fractionation, phosphorus, Ruttenberg fractionation, soil phosphorus

Abstract. We used 16 soils to compare the Hedley method for soil phosphorus fractionation to an alternative method recently developed by Ruttenberg to differentiate among P fractions in marine sediments. For forms of labile and Fe-bound P in soils, these methods were poorly correlated, with the Hedley fractionation showing a greater ability to discriminate among variations in plant-available P. For Ca-bound P, total organic P, and total P, the methods were well correlated (r^2 = 0.93, 0.48, 0.74, respectively), although the sum of P measured in the Ruttenberg extractions is only 45% of the total P recovered by the Hedley fractionation. The Hedley fractionation seems superior when an index of plant-available phosphorus and a separation of organic and inorganic forms is needed, whereas the Ruttenberg method allows a separation of $CaCO_3$-bound P from apatite-P, which is potentially useful in calcareous soils.

Introduction

Investigations of phosphorus (P) biogeochemistry often must partition the pool of P into its labile and refractory components, especially with regard to soil P availability for plant growth. Many researchers have used the sequential fractionation scheme developed by Hedley et al. (1982), in which the pool of soil P is partitioned into soluble, aluminum/iron-bound, calcium-bound, and residual forms (see a review of studies in Cross and Schlesinger (1995)). Each of these fractions can be analyzed separately for inorganic and organic forms of phosphorus.

Marine geochemists have also developed sequential fractionations, with the goal of understanding the origin and stability of sedimentary phosphorus compounds (Martin et al. 1987). For example, Ruttenberg (1992) developed a sequential fractionation scheme for P in marine sediments. Her method, developed independently of the Hedley fractionation, uses a different set of reagents to isolate many of the same forms. It offers a welcome separation

Table 1. Tested soils.

Number	Soil order	Vegetation	Location	References to Collection and site description
1	Histosol	*Typha domengensis* marsh	Everglades, FL	Qualls and Richardson (1995)
2	Histosol	*Taxodium distichum* forest	Durham, NC	Collected new for this study
3	Aridisol Typic Haplargid	*Larrea tridentata* shrubland	Chihuahuan Desert, Jornada LTER, NM	Schlesinger et al. (1996)
4	Mollisol Aridic Arquiustolls	*Bouteloua gracilis* grassland	Shortgrass Steppe, CPER, CO	Hook et al. (1991)
5	Entisol Torripsamment	*Larrea tridentata* shrubland	Mojave Desert, Coxcomb Mts., CA	Schlesinger et al. (1989a)
6	Alfisol A Vertic Hapludalf	*Quercus alba* forest	Duke Forest, Durham, NC	Gallardo and Schlesinger (1991)
7	Alfisol B Vertic Hapludalf	*Quercus alba* forest	Duke Forest, Durham, NC	Gallardo and Schlesinger (1991)
8	Oxisol	Tropical rainforest	Kalimatan, Borneo, Indonesia	Lawrence, unpub.
9	Entisol	Bare	Coastal, NC	Collected new for this study

Table 1. Continued.

Number	Soil order	Vegetation	Location	References to Collection and site description
10	Entisol Spodic Udipsamment	*Ammophila breviliqulata* beachgrass	Western Michigan	Lichter (1998)
11	Entisol Spodic Udipsamment	*Pinus strobus* forest	Western Michigan	Lichter (1998)
12	Entisol Spodic Udipsamment	*Pinus strobus* forest	Western Michigan	Lichter (1998)
13	Entisol Andeptic Troporthents	*Neonauclea calycina* forest	Krakatau, Indonesia	Schlesinger et al. (1998)
14	Entisol Andeptic Troporthents	*Neonauclea calycina* forest	Krakatau, Indonesia	Schlesinger et al. (1998)
15	Aridisol Xerollic Haplargid	*Artemisia tridenata* shrubland	Sierra Nevada, NV	Schlesinger et al. (1989b)
16	Entisol Lithic Entisol	*Pinus ponderosa* woodland	Sierra Nevada, NV	Schlesinger et al. (1989b)

of the Ca-bound forms of phosphorus into carbonate and apatite associated fractions, which are common in marine sediments. The Ruttenberg fractionation does not, however, allow the investigator to isolate the amount of organic P associated with each extraction. Here, we compare the fractionation techniques developed by Hedley and Ruttenberg using soils with a wide range of physical and chemical characteristics.

Methods

Sixteen soils were used in this comparison (Table 1). These included young (#10, 13, 14) and highly weathered (#7, 8) soils; acid (#16) and alkaline (#3, 5) soils; and soils with a wide range of organic content (#1 and 2 vs #9 and 10). Most of these soils were available from earlier studies, which have published their characteristics. All soils were originally collected from the plant rooting zone under areas of natural vegetation. These 16 soils allowed us to compare the Hedley and Ruttenberg fractionation schemes across a wide range of soil types in undisturbed ecosystems.

All soils were oven-dried and passed through a 2-mm sieve. Replicate 0.5-g samples of unground soil were used to complete the Hedley and Ruttenberg fractionations, as illustrated in Figures 1 and 2. All extraction solutions were analyzed on a Traacs 800 autoanalyzer using the phosphomolybdate blue method (Murphy & Riley 1962). In all cases, standards and blanks were mixed in the extractant solutions to account for matrix interferences during the analysis. The citrate-dithionate bicarbonate (CDB) extract of the Ruttenberg method was diluted before analysis to eliminate a chemical interference with the autoanalyzer reagents.

To measure organic P in the Hedley fractionation, each fraction was digested using ammonium persulfate (D'Elia et al. 1977), and organic P was determined by subtraction of the concentration in the undigested from the digested sample. In previous work, we have found 85 to 95% recovery of P from fructose-6 phosphate using this digestion (Schlesinger et al. 1998). In the Ruttenberg fractionation, organic P is not determined on the $MgCl_2$, CDB fractions, or HCl fractions, but it is determined on residual material after ashing the sample at 500 °C.

Results and discussion

Linear regressions were calculated to compare the Hedley vs the Ruttenberg fractionations for labile-P, Fe-bound P, Ca-bound P and organic P (Tables 2 and 3). In the Hedley fractionation, labile, or plant-available, P is considered

Table 2. Phosphorus concentrations (ug P/g soil) of the 16 tested soils for each step of the Hedley fractionation.

	Soil order	Resin	Bicarbonate Inorganic	Bicarbonate Organic	NaOH Inorganic	NaOH Organic	1M HCl Inorganic	Conc. HCl Inorganic	Conc. HCl Organic	Total Organic	Residual	Total P
1	Histosol	33.1	14.0	54.0	7.5	17.3	84.3	79.6	2.6	74.0	20.0	312.3
2	Histosol	0.3	37.3	0	4.9	0.4	7.1	216.2	0	0.4	37.5	303.7
3	Aridisol	20.5	11.0	0	8.7	0.7	286.3	174.7	0	0.7	24.0	525.9
4	Mollisol	18.5	27.4	9.4	9.0	1.9	65.6	146.4	0	11.2	34.6	312.8
5	Entisol	66.9	6.2	0	3.8	0	306.6	49.7	53.8	53.8	12.6	499.6
6	Alfisol A	1.0	9.0	16.6	9.9	0	0.6	1.8	9.8	26.4	10.8	59.6
7	Alfisol B	0	10.6	0	7.1	1.3	1.7	105.3	0	1.3	19.1	145.0
8	Oxisol	0	83.2	0	89.3	0	1.8	136.5	0	0	39.7	350.5
9	Entisol	0	0	0	0	4.1	0	0	7.3	11.4	0	11.4
10	Entisol	1.4	0.7	0	0.6	0	22.0	6.2	0	0	1.3	32.2
11	Entisol	0.7	1.7	0	0	1.5	22.3	0	2.4	3.9	0.3	28.9
12	Entisol	0	3.1	6.3	1.8	2.3	0	0	0	8.7	1.0	14.5
13	Entisol	26.6	93.1	57.5	12.2	0	66.4	191.5	0	57.5	19.9	467.2
14	Entisol	76.9	59.1	0	36.6	0	253.2	66.2	0	0	6.3	498.4
15	Aridisol	1.7	13.9	0	0	0	0.5	14.9	0	0	7.7	38.7
16	Entisol	52.1	65.8	0	30.2	0	144.4	775.5	0	0	60.9	1128.9

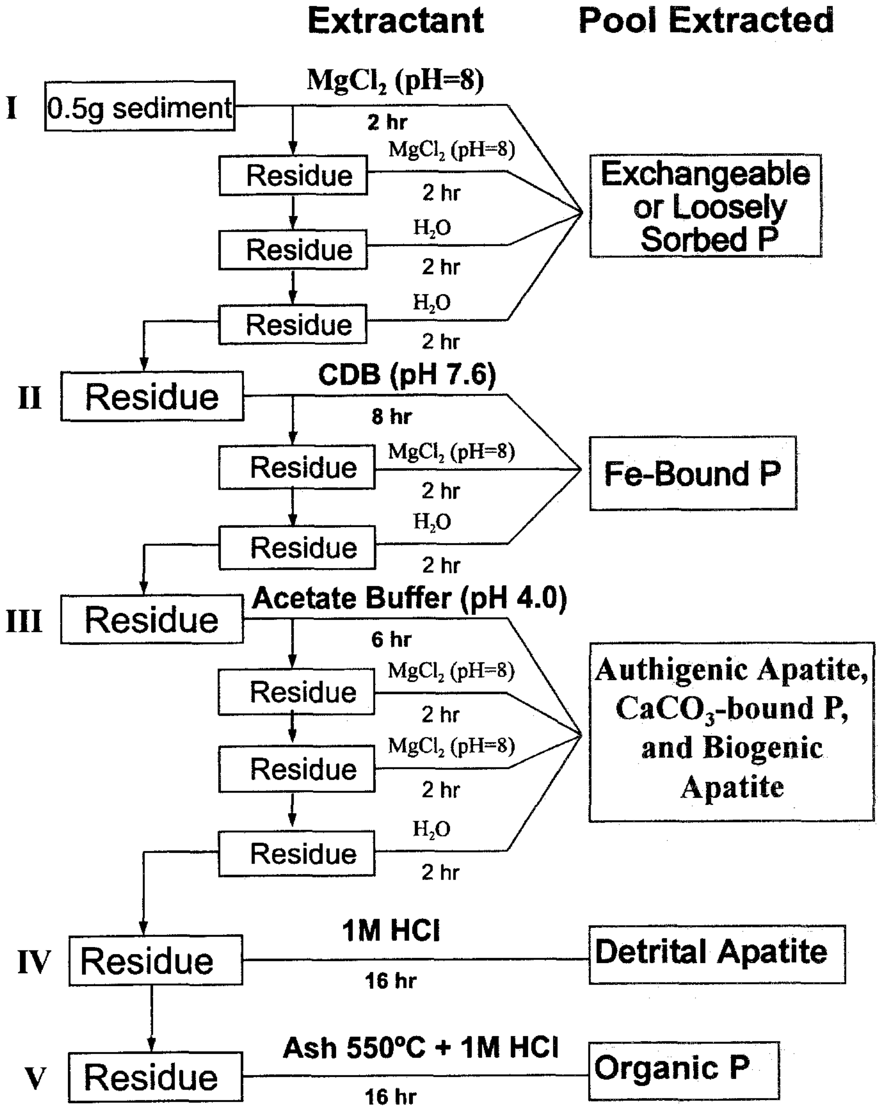

Figure 1. Phosphorus fractionation scheme of Ruttenberg (1992).

to be the sum of resin-extractable and HCO_3-extractable P (Kumar et al. 1992), which spanned a wide range of values among the soils tested (Figure 3). The use of unground soils may underestimate labile P (Saggar et al. 1992; Potter et al. 1992), but we felt that unground soils were more representative of the normal environment of plant roots. With the Ruttenberg fractionation, only a few samples contained any labile P, as measured by the $MgCl_2$ extraction (Figure 3). The one sample that showed a relatively high phosphorus

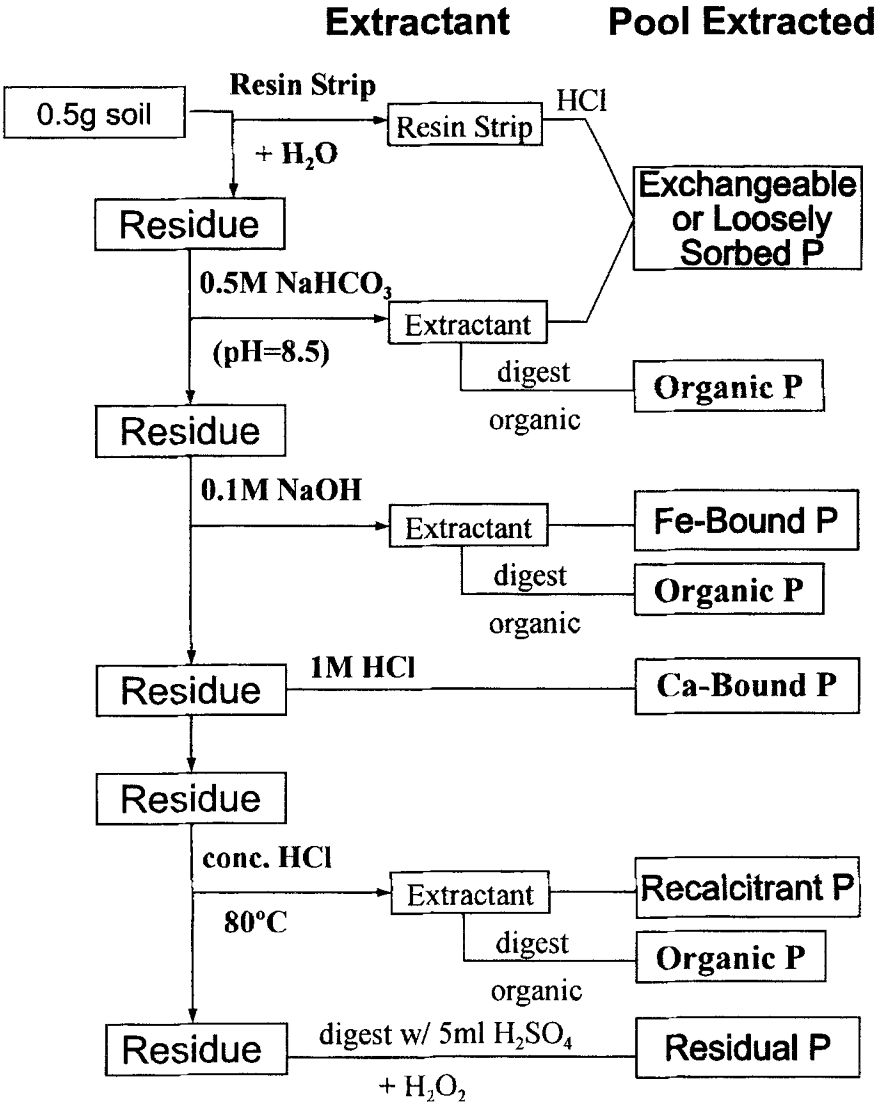

Figure 2. Phosphorus fractionation scheme of Hedley et al. (1982), as modified by Tiessen and Moir (1993).

concentration in the Ruttenberg fractionation was the soil from the Everglades (#1) which has recently been exposed to phosphorus pollution (Qualls & Richardson 1995). Overall, labile P measured by the Ruttenberg and Hedley fractionations were not correlated ($r^2 = 0.02$). Previous studies have shown that HCO_3-extractable P of the Hedley fractionation is well correlated to plant growth (e.g., Bowman et al. 1978). Among our 16 soils, the HCO_3-

Table 3. Phosphorus concentrations (ug P/g soil) of the 16 tested soils for each step of the Ruttenberg fractionation.

	Soil order	$MgCl_2$	Acetate w/ Acetic acid	1M HCl	CDB	Organic P	Total P
1	Histosol	70.6	19.4	6.8	48.7	151.4	296.9
2	Histosol	0.8	20.0	10.7	110.1	26.3	167.9
3	Aridisol	0.4	104.2	150.4	95.5	38.5	388.9
4	Mollisol	7.4	9.9	51.2	9.9	74.1	152.5
5	Entisol	6.6	65.8	233.7	58.9	9.4	374.3
6	Alfisol A	3.1	0	5.6	3.4	7.0	19.0
7	Alfisol B	0	35.1	9.2	0	17.1	61.3
8	Oxisol	2.5	12.7	5.3	8.9	6.9	36.3
9	Entisol	2.2	8.7	6.8	1.3	0	18.9
10	Entisol	0	0	24.3	0	34.7	59.0
11	Entisol	1.9	1.8	24.4	2.4	4.7	35.2
12	Entisol	0.6	0	5.1	0.9	0	6.6
13	Entisol	6.0	38.5	49.3	18.7	40.7	153.3
14	Entisol	0	56.4	259.5	0	10.0	325.9
15	Aridisol	1.9	10.3	6.5	13.6	0	32.4
16	Entisol	20.9	73.1	111.4	130.0	109.1	444.5

extraction seems superior to the Ruttenberg fractionation if the objective is to differentiate among soils for the content of labile P that might be available for plant uptake.

Values of Fe-bound P obtained by the Ruttenberg and Hedley techniques were also poorly correlated (Figure 4). The NaOH-extraction of the Hedley fractionation is thought to remove P bound to the surface of Fe and Al minerals (Chang & Jackson 1956; Pettersson & Istvanovics 1988). We expected higher values from the citrate-dithionate-bicarbonate (CDB) extraction of the Ruttenberg method, which is thought to more completely dissolve iron oxide minerals in the sample (Chang & Jackson 1956). Low values for the CDB extraction of some samples may stem from the need to dilute these samples before analysis. In two tropical soils (#8 & 14), the NaOH-extraction of the Hedley method removed more P than the CDB extraction of the Ruttenberg fractionation. In contrast, the CDB removed more P from several arid and semiarid soils (#3, 5 and 16). In these soils, in which $CaCO_3$ is often abundant, P extracted from Fe and Al minerals by NaOH during the Hedley fractionation may bind to Ca, resulting in an underestimate of Fe and

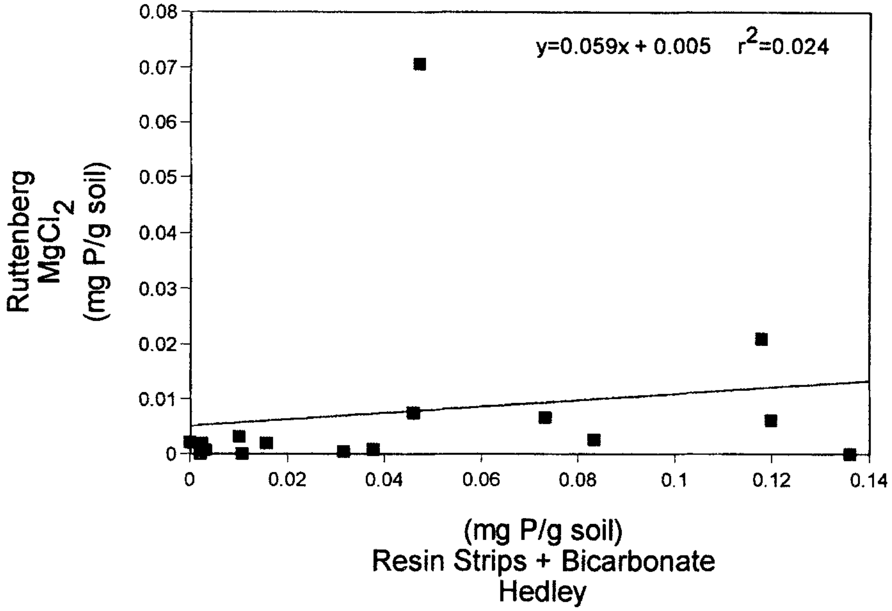

Figure 3. Correlation between labile, or plant-available, P as measured by the Hedley fractionation (sum of resin + bicarbonate-extractable P) and the Ruttenberg fractionation ($MgCl_2$-extractable P).

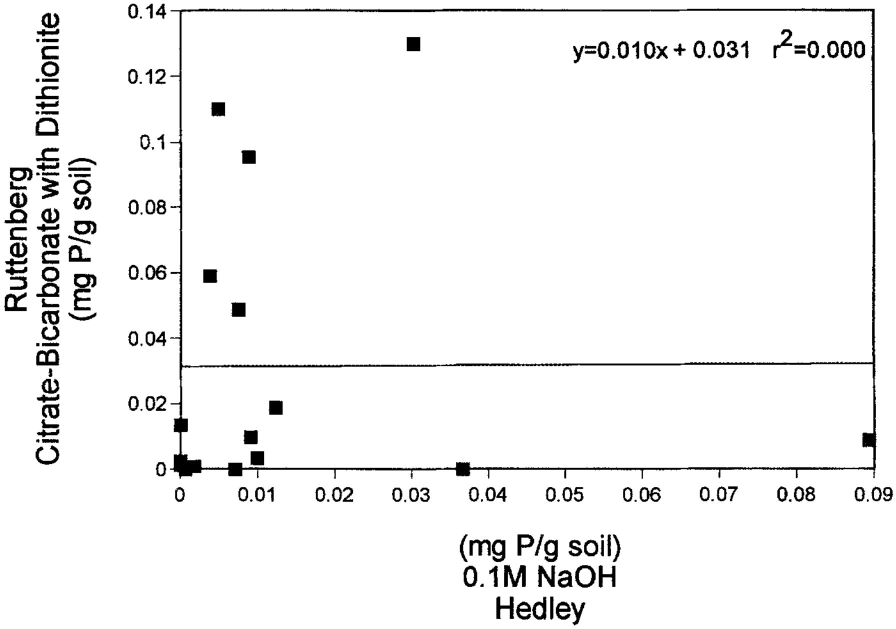

Figure 4. Correlation between the Hedley fractionation (NaOH-extractable) and the Ruttenberg fractionation (CBD-extractable) for Fe- and Al-bound forms of phosphorus.

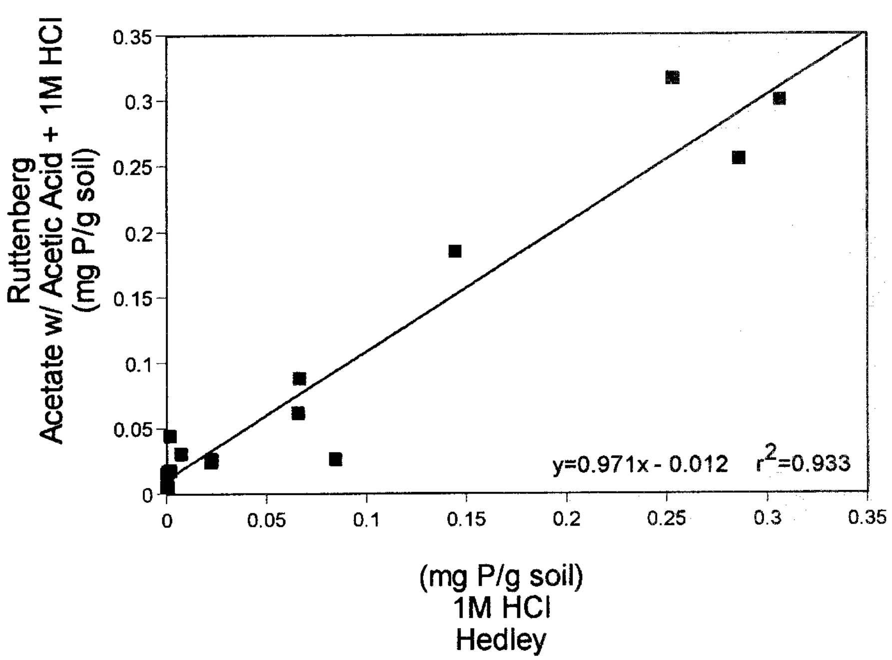

Figure 5. Correlation of the Hedley fractionation (1 M HCl) and the Ruttenberg fractionation (buffered acetic acid + 1 M HCl) for Ca-bound forms of phosphorus.

Al-bound P (Williams et al. 1971). Low values for P in the NaOH extraction may also stem from some P being removed by the previous step (i.e., HCO_3) of the Hedley fractionation.

In the Ruttenberg fractionation, the Ca-bound P is extracted in two steps – the first representing P bound to $CaCO_3$ and authigenic apatite (acetate buffered with acetic acid at pH 4.0) and the second representing P bound in detrital apatite (1 M HCl). The sum of these subfractions is well correlated to the single 1 M HCl extraction, which is taken as Ca-bound P, in the Hedley fractionation (Figure 5). The absolute values obtained by the two methods are very similar. Thus, among the soils tested, only a small amount of Fe or Al-bound P was precipitated with Ca during the NaOH-extraction, which would otherwise result in an overestimate of Ca-bound P in the HCl extraction of the Hedley fractionation. These results indicate that the Ruttenberg method successfully divides the total pool of Ca-bound P into two fractions of differing solubility.

In the Hedley fractionation, total organic P is obtained by the summation of organic P measured in digested samples at each stage of the fractionation, whereas in the Ruttenberg method organic P is obtained by ashing the material that remains after $MgCl_2$, CDB, and HCl extractions. Following

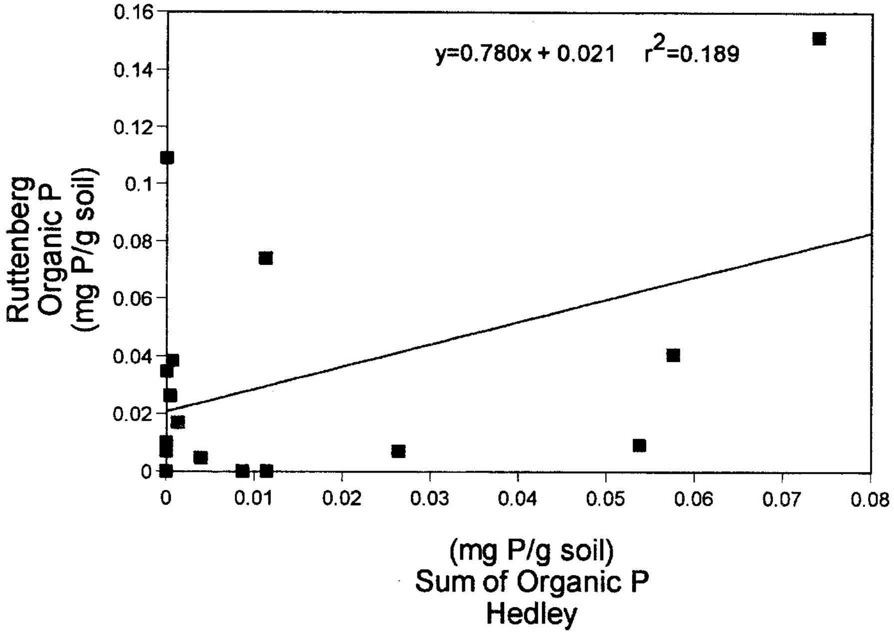

Figure 6. Correlation between the sum of organic P measured in the bicarbonate, NaOH, and HCl extracts of the Hedley fractionation to organic P measured in the residual materials of the Ruttenberg method.

this protocol, organic P measured by the two methods is poorly correlated (Figure 6), with the Hedley method showing zero values for many samples. If, however, the content of residual P in the Hedley method is added to the traditional sum of organic P values, the result is correlated to organic P as measured by Ruttenberg (Figure 7). The slope of the regression is close to 1.0. This relationship suggests that in both methods most organic P is contained in the residual material and that much of the residual P in the Hedley fractionation is organic.

Total P, obtained by summation of the measured fractions in each method, is well correlated between the Hedley and Ruttenberg fractionations (Figure 8). Values are uniformly higher for the Hedley fractionation (slope = 0.448), suggesting that the Ruttenberg method must underestimate the total amount of P in many soils. Some of the underestimate is likely to derive from not measuring organic P in the early steps of the Ruttenberg fractionation. Tiessen and Moir (1993) found that the Hedley fractionation underestimated total P when the various extractions were not analyzed for organic P following persulfate digestion. Although we did not find large values of organic P in these fractions, it would be easy to add persulfate digestion steps to the Ruttenberg fractionation scheme. The underestimate of total P in the

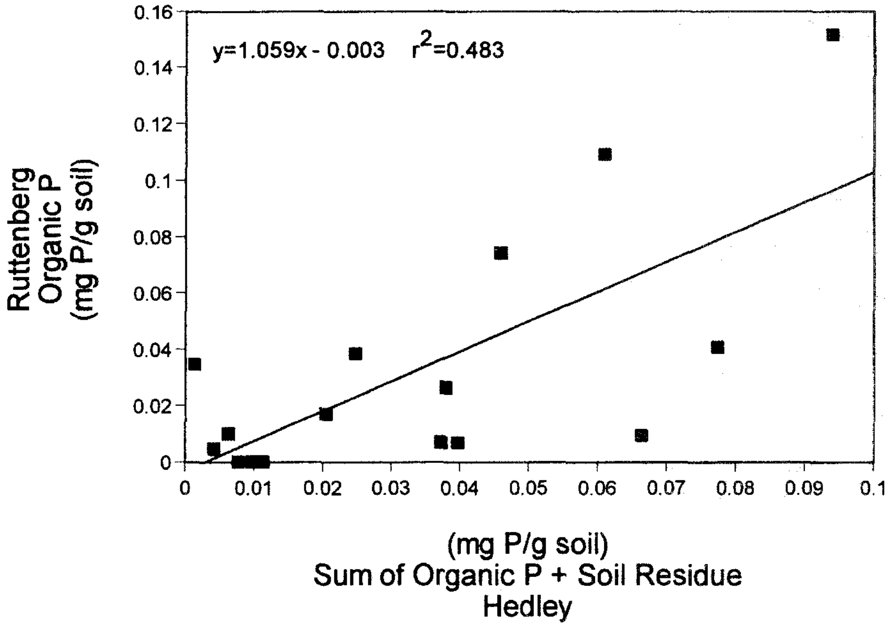

Figure 7. Correlation between the sum of organic P measured in the bicarbonate, NaOH, HCl and residual extracts of the Hedley fractionation to organic P measured in the residual materials using the Ruttenberg method.

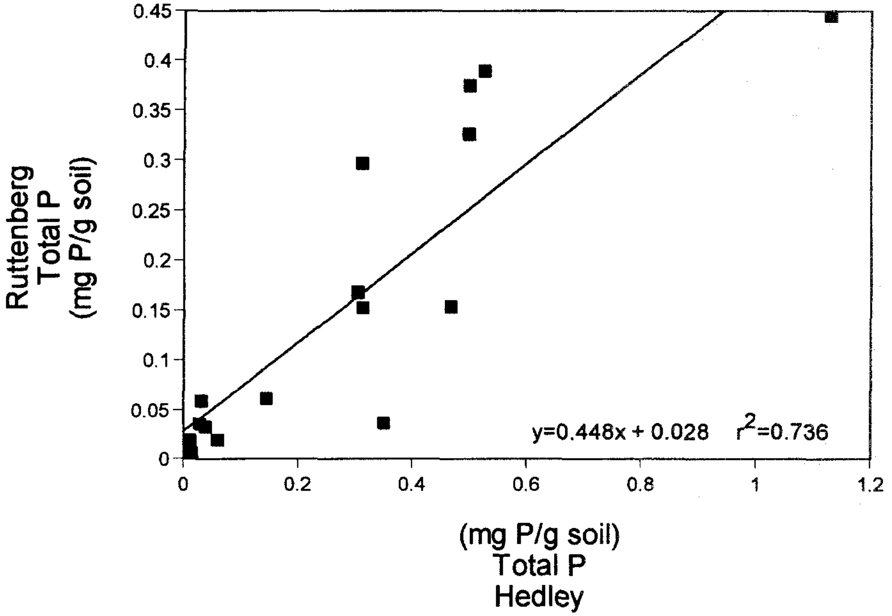

Figure 8. Correlation between total P as measured by the sum of individual fractions using the Hedley or the Ruttenberg fractionations.

Ruttenberg method may also derive from an incomplete digestion of residual P. Ruttenberg (1992) reported that her method extracted 14% less total P than a single extraction of an ashed sample with 1 M HCl (Aspila et al. 1976) and 18% less than the value found in a total fusion of the samples (Shapiro 1967).

Conclusions

When an index of plant-available P is desired, our results suggest that the Hedley fractionation is effective in differentiating among soils and among organic fractions, which are increasing recognized as controlling plant-available P in soils (Cross & Schlesinger 1995; Schlesinger et al. 1998). An alternative, the Ruttenberg fractionation, offers a useful separation of $CaCO_3$-bound P and apatite-bound P, which may prove especially useful in aridland soils containing both of these forms (e.g., Lajtha & Schlesinger 1988).

Acknowledgements

Special thanks to K. Mace and H. Hemric for invaluable technical assistance. We also thank J. Lichter, D. Lawrence, and C. Richardson for allowing us to use their previously collected soil samples. Thanks to H. Tiessen, M. Hedley, and an anonymous reviewer for helpful comments on this manuscript.

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