A ROUTINE LABORATORY METHOD TO DETERMINE PHOSPHORUS AVAILABILITY, CAPACITY, AND RELEASE CHARACTERISTICS FOR SOILS Moustafa A. Elrashidi (Research Soil Scientist) and Douglas Wysocki (National Leader), Kellogg Soil Survey Laboratory, National Soil Survey Center, USDA-NRCS, Lincoln, Nebraska

INTRODUCTION

Phosphorus (P) is an essential nutrient for plant growth and is often applied to agricultural land to increase crop production. Many chemical solutions have been proposed to extract available forms of P in soils. The information is used to assess soil P for crop production and as a basis for making recommendations. Water was probably the first extract that scientists applied to measure P in soils. The small amounts of soil P extracted by water and difficulties related to chemical analysis limited the use of water as a soil test.

Actually, soil P testing for crop production did not become common until the mid-20th century. Bray and Kurtz (1945) suggested a soil P test (Bray 1), which is a combination of hydrochloric acid (HCl) and ammonium fluoride (NH₄F) to remove available forms of P from soils. In 1953, Mehlich introduced a combination of HCl and sulfuric (H₂SO₄) acids (Mehlich 1) to extract available P from soils. Both Bray 1 and Mehlich 1 solutions can dissolve Calcium-, Aluminum- and iron-phosphate minerals as well as P adsorbed on colloidal surfaces in soils. In 1984, Mehlich modified his initial soil test and developed a multi-element extractant (Mehlich 3) which is suitable for removing P and other elements in acid and neutral soils (Mehlich, 1984). The Mehlich 3 solution is a combination of acids [acetic (HOAc) and nitric (HNO₃)], salts [ammonium fluoride (NH_4F) and ammonium nitrate (NH_4NO_3)], and the chelating agent ethylenediaminetetraacetic acid (EDTA).

Olsen et al. (1954) introduced sodium bicarbonate (0.5 M NaHCO₃) solution adjusted at a pH of 8.5 to extract P from neutral, alkaline, and calcareous soils. The high pH (8.5) can precipitate calcium carbonate which decreases calcium (Ca^{2+}) ion concentration in solution and enhances the dissolution of Ca-phosphate minerals $[Ca(H_2 PO_4)_2, Ca HPO_4]$ and $Ca_3(PO_4)_2$]. Further, the Olsen test solution can remove both dissolved and adsorbed P forms from soils.

The concept of P-sink was applied to measure the amount of available soil P which can be released in response to such sink. An anion exchange resin (AER) was used in water as a P-sink to determine available P in a wide range of soils. An AER has an advantage over chemical extractants because it uses water and can measure both soil P capacity and rate of P release in addition to available P in soil (Amer et al., 1955; Sibbesen, 1978; Uusitalo and Yli-Halla 1999). The objective of this study was to develop a technique implementing the AER P-sink concept to determine P availability, capacity, and release characteristics for a wide range of soils that differ greatly in chemical properties.

MATERIALS AND METHODS

For this study, 24 samples were collected from the surface horizon of 15 U.S. soil series. The samples were selected to represent a wide range of properties and climatic factors. Soil analyses were performed by methods described in the Soil Survey Investigations Report (SSIR) No. 42 (USDA/NRCS, 2014). The water soluble P, Bray 1 P, and Olsen P were measured for soils.

Development of the AER Method

We measured soil P by using AER bags. Spherical resin beads were placed in a perforated nylon bag which was immersed in soil suspension and shaken for a specific period to release soil P. Phosphorus retained by resin was removed by using a sodium chloride (NaCl) solution and measured by colorimetric method or Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), (Perkin Elmer 3300 DV) (1B2b) as described in USDA/NRCS (2014).

Dowex Marathon A2 AER, strong base, type II, 510-610 µm spherical beads (DOW Chemical Company) were used for all laboratory experiments. The resin is chloromethylated copolymer of styrene and divinyl benzene which is provided in chloride form. Nitex nylon fabric with 300-μm pores (Sefar America Inc.) was used to make the resin bags. Four grams of air-dry AER was confined in each bag. The resin was converted to bicarbonate form before use by soaking the bags overnight in 1.0 M NaHCO₃ solution and washing out the excess salt with d.w.

Three laboratory experiments (a. efficiency of chloride solutions; b. errors associated with using HCl; and c. soil:solution ratio) were performed to develop the AER method to determine phosphorus release characteristics (PRC) for soils as follows:

a. Efficiency of chloride solutions

The objective of this experiment was to investigate the efficiency of three chloride solutions (0.5 M HCl, 0.5 M NaCl, and 1.0 M NaCl) in removing P adsorbed by the AER.

A 100 ml of KH₂PO₄ solution and a resin bag were placed in a 250-ml polyethylene bottle. The bottle was mounted horizontally on a reciprocating shaker (displacement = 4.0 cm, and speed = 100 rpm) and shaken for 24 hours at room temperature (20 \pm 2°C). At the end of the shaking period, the resin bag was rinsed with d.w. and placed in a 125-ml polyethylene bottle containing 50-ml solution of 0.5 M HCl, 0.5 M NaCl, or 1.0 M NaCl. All treatments were replicated 4 times.

The bottle was shaken horizontally in a reciprocating shaker for one hour at room temperature. The 1st hour solution was transferred into a 60-ml polyethylene bottle. The resin bag was shaken for an hour with another 50 ml of the respective solution. The 2nd hour solution was transferred into a separate 60-ml bottle. Phosphorus concentration in solutions was determined by an appropriate method.

The results indicated that the 1st hour of shaking the resin bag with either 1.0 M NaCl or 0.5 M HCl solution removed > 96% of P retained by resin. Most of P removed by the 2nd hour of shaking was associated with solution entrapped in the resin bag. When P in this solution (about 3.0 ml/resin bag) was considered, almost all retained P could be accounted for. Only 93% of retained P was removed by 0.5 M NaCl solution after the 1st hour of shaking. It appeared that the 2nd hour was necessary for 0.5 M NaCl to remove all P from resin.

b. Errors associated with using HCl

The previous experiment indicated that either 0.5 M HCl or 1.0 M NaCl could be used to remove P adsorbed by AER. The objective of this experiment was to investigate why using HCl to remove P adsorbed by AER (from soil suspension) produced higher P values than NaCl.

The experiment was performed on Caribou (1) (acidic soil), and Windthorst (4) (alkaline soil). Two g of air-dry soil (< 2 mm) along with 100 ml of d.w., and a resin bag were placed in a 250-ml polyethylene bottle; 4 replicates were used for each treatment. The bottle was mounted horizontally on a reciprocating shaker (displacement = 4.0 cm, and speed = 100 rpm) and shaken for 24 hours at room temperature ($20 \pm 2^{\circ}$ C). At the end of shaking period, the resin bag was taken out of the soil suspension, and rinsed with d.w. Phosphorus retained by resin was removed either by 0.5 M HCl or 1.0 M NaCl solution as described above. Phosphorus concentration in both 0.5 M HCl and 1.0 M NaCl solutions was determined.

The results indicated that the 0.5 M HCl solution resulted in higher P values (162.6±6.5 and 227±5.2 mg/kg) than 1.0 M NaCl solution (142.9±6.9 and 207.5±10.4) for both the acidic and alkaline soils, respectively. A drawback of using nylon resin bag to extract P from soil suspension is the adherence of fine soil particles to the nylon fabric. The amount of adhering particles is usually related to clay and organic matter content of soil. In a preliminary experiment, we found only a few milligrams of adhering particles for most soils investigated and it was independent of the amount of soil used. However, most soil P is associated with fine soil particles.

The 0.5 M HCl solution appeared to dissolve more P from the adhering soil particles, but we could avoid this problem by using the mild NaCl solution to remove P from resin.

c. Soil:Solution ratio

The objective was to investigate the optimal soil:water ratio for soils. The experiment was performed on Caribou (2) (acidic), and Genola (2) (alkaline calcareous). Three soil:solution ratios were implemented where 1, 2, or 4 g of airdry soil sample (< 2 mm) along with 100 ml of d.w. and a resin bag were placed in a 250-ml polyethylene bottle. The method was conducted as explained above to determine the amount of P released for 1 and 24 hours of shaking. But, only 1.0 M NaCl solution was applied to remove P from resin.

For both 1-h and 24-h extraction periods, P released (mg/Kg soil) increased with decreasing the ratio. In the presence of P-sink (AER) in the soil:water system during extraction, the dynamic equilibrium for both precipitation/ dissolution and adsorption/desorption reactions are no longer valid. Under these conditions, dissolution and desorption reactions are dominant in the system and enhanced by decreasing the soil:solution ratio. Both water soluble and adsorbed forms of P are finite sources and their release during the 1st hour should be complete and independent of the soil:solution ratio. The data for the 1-h extraction suggested P was released from sparingly soluble minerals in addition to water soluble and adsorbed P sources in both soils. In light of the results obtained, using a high soil:solution ratio might be preferred for light-textured soils usually low in organic matter (OM) and P content. However, the low soil:solution ratio would be more suitable for heavy soils with high OM and P content. We have adopted the medium soil:solution ratio (2:100), which should be suitable for most soils.

Proposed Phosphorus Method

Based on data derived from these three experiments, the AER method was designed to measure amounts of P released from soil at 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hours of extraction periods. The method has been performed on soils varying greatly in their properties. The method is outlined in three sections (soil extraction, P removal from resin, and P measurement) as follows:



Figure 1. Relationship between extraction period (hours) and P released (mg/Kg soil)



Figure 2. Relationship between Log (extraction period, hours) and P released (mg/Kg soil) for 8 acidic and alkaline soils.





Table 1. Phosphorus measured by Bray1, Olsen, and water method; and 1h, 24h, and 48h AER-extractable P (mg/kg soil) for 24 soils.

Soils	Bray1	Olsen	Water	AER-1h	AER-24h	AER-48h
(mg P/ Kg soil)						
Greenson	133.05	97.60	34.92	113.07	185.67	195.96
Genola (1)	0.10	15.20	10.42	33.29	60.73	62.87
Layton (1)	19.45	9.16	4.65	19.67	36.12	39.43
Layton (2)	23.70	10.98	5.55	17.85	33.08	36.47
Layton (3)	26.70	10.71	5.52	18.83	38.91	42.51
Layton (4)	167.75	154.80	26.06	135.91	255.80	274.01
Windthorst (1)	35.00	12.58	3.15	11.95	26.00	29.98
Windthorst (2)	3.25	1.15	0.90	3.80	14.77	15.89
Windthorst (3)	41.60	32.60	3.52	16.57	42.41	48.64
Colby	0.05	28.25	3.31	33.61	64.25	70.93
Rosebud	7.85	14.03	1.14	6.47	12.25	13.51
Kim	0.05	17.98	1.51	12.57	29.23	32.93
Syrupcreek	331.85	288.00	15.29	43.95	200.07	226.49
Caribou (2)	275.80	138.00	3.57	38.56	185.37	243.06
Charlton	51.20	5.07	1.64	20.73	62.04	67.10
Albia	168.15	66.80	1.88	39.73	125.38	153.69
Pleasant	87.50	33.18	11.79	56.46	108.79	120.07
Caspiana (1)	85.55	26.56	11.36	48.47	112.13	129.26
Caspiana (2)	87.65	25.59	13.15	52.13	108.89	128.51
Marshall (1)	125.90	38.63	14.50	85.29	168.82	187.62
Marshall (2)	96.70	10.53	7.54	65.44	130.82	149.90
Christian (1)	46.40	8.28	3.74	24.44	51.86	56.45
Christian (2)	44.80	12.43	4.11	7.29	39.87	46.98
Hestehave	57 60	8 66	2 56	27.61	65.86	73 92

1-48 h extraction region

USDA is an equal opportunity provider, employer, and lender

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Soil

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Genola (1)

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20	30	40	50
Extract	ion period (I	hours)	
) for 8	R acidic a	nd alkaline	soils

Table 2. Linear regression equations* used to predict P

n exchange resin (mg/Kg soil) for 1-60 min traction regions in the 24 soils investigated						
Intercept (I)	Slope (S1) (1-60 min)	Slope (S2) (1-48 h)				
118.1	66.4	48.6				
35.4	19.9	17.8				
19.9	11.2	11.7				
16.9	9.52	11.4				
18.8	10.54	14.4				
147	82.7	79.6				
12.6	7.1	10.2				
4.32	2.43	7.3				
15.7	8.82	19.3				
33.6	18.9	22.2				
6.47	3.64	4.19				
13.4	7.51	11.7				
43.2	24.3	113.1				
23.4	13.2	120.8				
21.2	11.9	28.5				
33.2	18.7	68.2				
54.2	30.5	38.9				
47	26.4	48.2				
49.1	27.6	45				
83.5	47	61.4				
62.4	35.1	50.4				
26.9	15.1	18.3				
7.29	4.1	23.6				
28.4	16	27.6				

* P = I + S1 x (Log h) for 1-60 min; P = I + S2 x (Log h) for Correlation coefficient (r) between P and (Log h) for all regression equations were higher than 0.99.

PROPOSED METHOD

a. Soil extraction

- 1. Add 2.0 g of air-dry soil sample (< 2 mm) and 100 ml of d.w. in a 250-ml wide-mouth polyethylene bottle. Use at least two replicates for each soil sample in addition to a control treatment where all steps of the extraction process are performed in the absence of soil.
- 2. Place a resin bag (containing 4.0 g of air-dry AER-HCO₃) in each bottle. The bottle is mounted horizontally on a reciprocating shaker (displacement = 4.0 cm, and speed = 100 rpm) and shaken for a specific period at room temperature (20 \pm 2°C). After shaking, remove retained P from resin as described below (b).
- 3. Run step 2 above for all shaking periods. The length of individual shaking period is determined in reference to the beginning of first shaking period at time=0.

b. Phosphorus removal from resin

- 1. Lift the resin bag out of the soil suspension and wait for 2 minutes to drain free water. Rinse the bag with a known volume of d.w. (i.e., 5.0 ml) to remove attached soil particles. Add rinsing water to the soil suspension and keep the soil suspension (when it is necessary) for next extraction.
- 2. Place the resin bag in a wide-mouth 125-ml polyethylene bottle containing 50 ml of 1.0 M NaCl solution and shake the bottle horizontally in reciprocating shaker (displacement = 4.0 cm, and speed = 100 rpm) for one hour at room temperature.
- 3. Transfer the NaCl solution to a 60-ml polyethylene bottle and add 2.0 ml of concentrated HCl to each bottle.

c. Phosphorus measurement

Determine P concentration (μ g/ml) in the acidified 1.0 M NaCl solution by the modified phosphomolybdate/ascorbic acid method or Inductively Coupled Plasma-Optical Emission Spectrometry (Perkin Elmer 3300 DV) (1B2b) as described in USDA/NRCS (2014).

RESULTS AND DISCUSSION

Soil P Tests and Extracted Forms

Soil P adsorbed on surfaces of colloidal CaCO₃ and hydrated oxides of Al and Fe can be replaced by other anions such as sulfate, chloride, hydroxyl, and bicarbonate. Water extraction may remove a small amount of adsorbed P in addition to water soluble forms in soils. On the other hand, bicarbonate solution adjusted at pH 8.5 (Olsen extraction) can remove water soluble, adsorbed P and some amounts of Ca-, Al-, and Fe-phosphate minerals. In alkaline, calcareous, and near neutral soils, Olsen extraction decreases the concentration of Ca in solution which dissolves Ca-phosphate minerals. In acid and near neutral soils, the high pH dissolves AI- and Fe-phosphates.

The Bray 1 test (0.03 M NH₄F and 0.025 M HCl) is an aggressive extraction for acid and near neutral soils where it can remove large amounts of Ca-, Al-, and Fe-phosphates in addition to water soluble and adsorbed P forms. The Bray 1 extraction dissolves Ca-, Al-, and Fe-phosphates by the solvent action of HCl, and by the ability of fluoride ion (NH₄F) to complex Al and Fe which releases P from Al- and Fe-phosphates. Chloride ion (HCl) can also replace P adsorbed on colloidal surfaces.

Both Olsen and Bray 1 extractions can remove water soluble and adsorbed forms of P and portions of Ca-, Al-, Fephosphate minerals for soils. In general, Bray 1 extraction can remove larger amounts of P minerals than those removed by Olsen extraction. As mentioned, mechanisms responsible for dissolving P minerals are more aggressive in Bray 1 than in Olsen extraction. This indicates that Olsen extraction removes P from relatively soluble P minerals compared to Bray 1, which can attack minerals of lower solubility.

The data for the soils investigated (Table 1) show that AER-1h extractable P is always higher than water-P, but it is much lower than Bray 1-P. Meanwhile, AER-1h and Olsen removed similar P concentration for most soils investigated. A significant correlation was observed between AER-1h extractable P and each of water-P (0.89**), Olsen-P (0.46^{*}), and Bray 1-P (0.49^{*}). These data suggested that the AER-1h extractable P was mainly driven from water soluble, adsorbed forms, and relatively soluble P minerals. The data for most soils also suggested that 24- and 48-h AER extraction could remove all P forms dissolved by Bray 1 and Olsen solution. For 24-h AER extractable P, a significant correlation of 0.84^{**} was observed with Bray 1 and 0.77^{**} with Olsen. Significant correlation was also found for 48-h AER extractable P.

Phosphorus Release Characteristics (PRC) for Soils

Figure 1 illustrates the relationship between different extraction periods (hour) and P released (mg/kg soil) for four acidic (Figure 1a) and four alkaline soils (Figure 1b). Similar relationships were observed for other soils (not shown). For the 24 soils, an average of 39% of P was released during the 1st hour, while the remaining 61% was released by the following 47 hours of extraction. The corresponding values were 33 and 67% for 15 acidic soils, compared to 49 and 51% for 9 alkaline soils. The results reflected, to some extent, the solubility of different P minerals and its adherence to the solid phase in these two groups of soils. The data also suggested a difference in P release behavior between the 1st hour and the following 47 hours of extraction. Accordingly, the two regions would be addressed separately in this study.

A Plotting Log of the extraction period (hour) against the amount of P released (mg/kg soil) for the 1-48 h region gave a linear relationship for all soils investigated. The equation could be written as follows:

P = I + S2 x Log h

Where: P = P released (mg/Kg soil), I = intercept, S2 = slope, and h = extraction period (hour).

When Log h is > 0.0, this relationship could be used to predict P released from soil for any extraction period between 1 and 48 h. The linear relationship is shown for 4 acidic (Figure 2a), and 4 alkaline soils (Figure 2b).

A different relationship was expected for the 1st hour of extraction where water soluble and adsorbed P forms were major contributors for released P. We had to assume the amount of P released = 0.0 at the beginning of extraction time to develop the linear relationship (Log h x P) for the 1^{st} hour region. But, this time should be > 0.0 h because of using the log function. To select the boundary for the 1st hour region, we assumed a beginning time at 0.1, 0.5, or 1.0 minute which produced a slope (S1) = I/2.78, I/2.08, and I/1.78, respectively. The 1.0 minute was selected because the 1-60 min slope (I/1.78) gave better P prediction for the 24 soils investigated. The linear relationship for the 1-60 min region could be written as follows:

(2)

P = I + (I ÷1.78) x Log h

The linear equations to predict P released (mg/kg soil) for both 1-60 min and 1-48 h regions are given in Table 2 for the 24 soils. The intercept (I) at 1-h extraction period reflects mainly water soluble and adsorbed forms of P in soil. For the 24 soils, it varied widely between 12.6 and 147 mg/kg soil. The slope (S2) for 1-48 h region is related to the rate of P release from sparingly soluble P minerals in soil. However, S2 values showed no apparent difference between acidic and alkaline soils investigated. This might reflect a disturbance in P dynamic equilibrium caused mainly by management practices.

An agricultural soil usually contains numerous numbers of P minerals in different physical forms (i.e., amorphous and crystallized) and solubilities. Al-, Fe-, and Mn-phosphates dominate in acidic environments, while Ca- and Mgphosphates are major minerals in alkaline and calcareous soils. Both acidic and alkaline phosphates could co-exist in near neutral soils. Additions of P fertilizer or manure to soil could generate adsorption/desorption or precipitation/ dissolution reactions and disturb the dynamic equilibrium, which would change P solubility and release. High P and OM values for some of the soils investigated suggested additions of P fertilizer and/or manure. This might also explain the wide range of intercept (I) and slope (S2) values observed within the acidic and alkaline soils.

We applied the two linear models (equations 1 and 2) to predict P released at different extraction periods (PRC) for the 24 soils. A good agreement was found between predicted and experimental P release values for the two extraction regions (1-60 min and 1-48 h). The data for 4 acidic soils are shown in Figures 3 and 4. Similar results were obtained for the alkaline soils, their figures were not given in this report. Good agreements between predicted and experimental values were also observed for other soils investigated (not shown). The linear relationship (Log h x P) can be represented by a minimum of two points. Thus, for a routine laboratory AER extraction method, we proposed to use only two extraction periods (1 and 24 h) to determine PRC for soils, named the "Double-Point AER Extraction, DP-AER". The DP-AER method (standard operating procedure #4D1a for soil phosphorus) has been published in two Soil Survey Investigation Reports (SSIR No. 42 and 55) (USDA/NRCS, 2014; 2016).

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