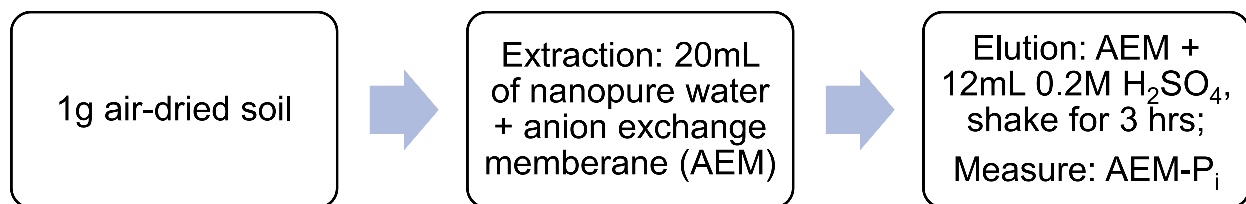
SOP: **Resin-extractable P**

Overview:

This standard operating procedure (SOP) describes a protocol for extraction of soil P to estimate the resin-extractable orthophosphate (resin-P_i) in soil, identified as the form of soil inorganic P (P_i) from which plant normally draw their supply. The method was described by Amer, Bouldin, Black, and Duke (1955) and Bowman, Olsen, and Watanabe (1978), and this protocol is modified from the first step of sequential extraction method developed by Hedley, Stewart, and Chauhan (1982). Air-dried soil that is ground to pass a 2 mm sieve is typically used.



Safety:

All standard safety protocols and online safety training via UIUC [Division of Research Safety (DRS)] are required.
Personal protection (PPE) for this procedure include:
Eye Protection: Safety goggles
Body Protection: Lab coat
Hand Protection: Nitrile Gloves
Particularly hazardous substances: Concentrated sulfuric acid should be handled in the fume hood. Persulfate is an oxidizer, which can cause fire when in contact with combustibles, and exposure to high temperature should also be avoided. Make sure to check Safety Data Sheet if unsure about how to handle these chemicals. Specific details on these substances are incorporated in the Detailed Procedure below.

Instrumentation & Consumables:

Sample preparation

- Analytical balance (two decimal places sensitivity)
- 50 mL centrifuge tube (Falcon brand is preferred for avoiding leak)

Reagent preparation

- Anion exchangeable membrane (AEM, also called resin) strip (1x4 cm, VWR International, West Chester, PA)

- Sodium bicarbonate (0.5M)
- Sodium persulfate
- Sulfuric acid (0.2M and 1.2M)
- Commercial P standard (1000 mg P/L)
- Ammonium molybdate
- Antimony potassium tartrate
- Concentrated sulfuric acid
- Ascorbic acid
- Sodium Hydroxide (5%)

Extraction

- Horizontal shaker
- Dispensette
- 15 mL centrifuge tubes
- Pipette and tips (20-200 uL and 1000-5000 uL)

Colorimetry

- 96-well microplates
- Microplate UV spectrophotometer capable of reading at 882 nm
- Pipette and tips (100-1000 uL)

Detailed Procedures:

I. Sample preparation

1. Measure 1.00 ± 0.02 g of air-dried soil into 50 mL centrifuge tube with proper labeling. Record exact weight of soil to at least 1/100th of one gram (1.XX g). Falcon tube is recommended for avoiding leak during extraction. Note: for soils with low P availability, the soil mass can be adjusted (ranged from 1-6 grams) until the AEM-P_i concentration can be measured through colorimetric method. Up to 600 mg kg^{-1} labile P_i in soil can be effectively extracted by AEM strips.
2. Prepare empty 50 mL centrifuge tube for blanks (no soil, but treated the same way as samples to account for background P throughout the extraction)

II. Reagent preparation

1. Extractants
    i. AEM strip
        a. Shake AEM strips in 0.5 M $NaHCO_3$ for > 3 hours, and then decant the $NaHCO_3$ (careful not to drop any strip) and then shake the AEM strip in water (18.2 MΩ). Repeat this procedure three times and this will ensure that AEM is preloaded with bicarbonate.

   b. AEM strip can be reused until they deform, but make sure to pre-load them before every use.

ii. 0.2 M H_2SO_4 (Molarity Calculator by Sigma-Aldrich can be helpful)

2. Standards

   i. Dilute commercial standard (1000 mg P/L) in the extracting solution and sequentially dilute them to make calibration standards that are within the linear range and cover the concentration you are expecting for your soil samples.

      a. It is essential to use the same extracting solution (for AME-P_i, the standards should be prepared in the background of 0.2M H_2SO_4) because molybdate colorimetry of P is sensitive to pH (color development and intensity, precipitation).
      b. Standards can be prepared using the reagent volumes provided in the following table (total volume 40mL)

Standard [P] (mg P L^{-1})	0	0.5	1	2	5	10	15	20
50 mg/L P standard solution (mL)	0	0.4	0.8	1.6	4	8	12	16
0.4M H_2SO_4 (mL)	20	20	20	20	20	20	20	20
Nanopure water (mL)	20	19.6	19.2	18.4	16	12	8	4

Note: the 0.4M H_2SO_4 used here is to create the background 0.2M H_2SO_4 in the standards. After dilution, all standards will contain 0.2M H_2SO_4 in the background.

3. Colorimetry reagents

   i. Murphy-Riley Solution A (stable for 4 weeks at 4°C)

      a. Dissolve 4.3 g ammonium molybdate in 400 mL of nanopure water in a 1L beaker.
      b. Dissolve 0.40 g antimony potassium tartrate in 400 mL nanopure water, then add to the ammonium molybdate solution in the beaker.
      c. Slowly and carefully, with stirring and while cooling in an ice bath (may be unnecessary but the beaker does heat up when you add sulfuric acid), add 54 mL conc. H_2SO_4.
      d. Allow to cool and make to 1000 mL with deionized water. Mix well and store in a dark bottle in a refrigerator.

   ii. Murphy-Riley Solution B (stable for 24 hours at 4°C)

      a. Ascorbic acid, 1%: Dissolve 1.00 g of ascorbic acid in 100 mL deionized water. Make a fresh solution daily as needed.

   iii. Final Murphy-Riley (MR) reagent (stable for 24 hours at 4°C): combine 56 mL of solution B + 44 mL of solution A, and mix (should turn to light yellow color)
   Note: smaller amount with the same ratio of reagent A and B can be mixed with the same A to B ratio (e.g., 28 mL reagent B + 22 mL reagent A)

iv. 5% NaOH (for adjusting pH for colorimetry)

III. Extraction and colorimetry

1. Procedures
   i. AEM-P_i
      a. Extraction: P_i in this extraction is quantified after desorption from the anion-exchange membrane (AEM) strip.
         1) Extract the pre-weighed soil in the 50 mL centrifuge tube with 20 mL of nanopure water and AEM strip, using a horizontal shaker (120 rpm; “low” for Eberbach E6010.00) for 16 hrs. Wrapping parafilm around cap can help preventing spills while shaking
         2) With tweezers, fish out AEM strip, and attempt to remove as much sediment by shaking strip in the extraction tube
         3) Using a squeeze bottle with nanopure water, use a thin but powerful stream of water to wash sediment off AEM strip
         4) Immerse AEM strip in 12 mL of 0.2 M H_2SO_4 (20-25 mL for soils with high P availability) and shake 3 hrs
            Note1: Concentration of H_2SO_4 used for elution is different from 0.25 M used in Microbial biomass phosphorus (hexanol) protocol. Concentration may be changed as long as full elution can be achieved
            Note2: For soil with low P availability, the volume of 0.2M H_2SO_4 can also be reduced to 6mL to bring up the concentration of AEM-P_i in the elution. If the volume of elution is adjusted, the elution volume used in the later calculation should also be consistent.
      b. Colorimetry: 20 μL 5% NaOH + 120 μL eluent + 140 μL MR
         Notes: The addition of NaOH helps to maintain a neutral pH. Reaction is complete when the color development of standards has stopped increasing. This process takes approximately 30 min.
2. Colorimetry is performed directly in the microplate, which can hold up to 350 μL
3. Absorbance is measured at 882 nm. The blanks should be run for each microplate and the absorbance for blanks should be recorded.
4. Golden Rule: Treat standards the same as samples. This goes for the working standards being made up in the same solution as extracts. For example, working standards for AEM-P_i should be made in 0.2 M H_2SO_4. If colorimetry reactions are diluted to bring absorbance into linear range, do the same for standards. Also, be sure to only use a standard curve with the R^2 higher than 0.99 for analytical chemical method.

IV. Clean up

1. Make sure to clean up dispensette (rinse with dilute sulfuric or hydrochloric acid, followed by water), shaker (especially if tubes leak), and centrifuge (especially if tubes leak).
2. Collect solutions (except those that can go into drain with copious amount of water; check DRS for further information) into chemical waste bottle, clearly labelled with contents and their concentrations. Request pick up when the bottle is almost full. Clean up any spills with absorptive tissues and soap water immediately as needed. For spilled dilute acids, immediately neutralize with sodium bicarbonate and then clean up.

V. Calculations

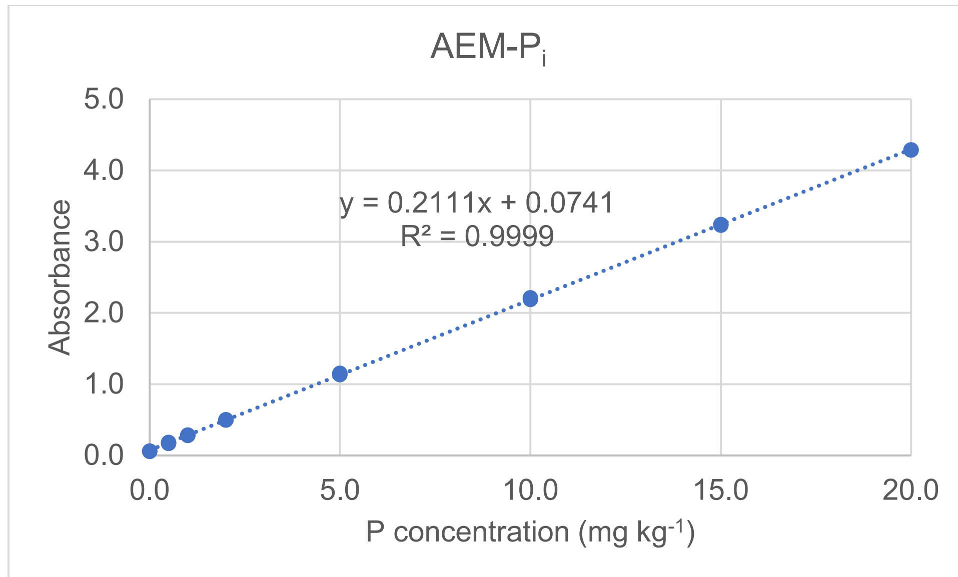
Measurement of P fractions is usually expressed in units of mg P kg^{-1} soil.

1. Convert raw absorbance to concentration (mg P L^{-1}) using calibration curve constructed specifically for each fraction (as noted previously, calibration standards should be treated exactly the same way as samples, so the absorbance can be directly converted to concentrations in the extract, before dilution if samples were separately diluted). Multiply the concentration by dilution factor if diluted.
2. Multiply the concentration by the elution volume (0.012 L for AEM-P_i, **the elution volume should be used instead of the water volume**) and divide by soil mass (1 g = 0.001 kg) to yield concentration in mg P kg^{-1} soil

Example calculation:

Given, for AEM-P_i
Absorbance = 0.385
Dilution = 1
Calibration curve: y = 0.2111x + 0.0741
Elution volume = 0.012 L
Soil mass = 0.001 kg

Concentration in extract = (0.385-0.0741) / 0.2111 × 1 = 1.47 mg L^{-1}
Concentration in soil basis = 1.47 mg L^{-1} × 0.012 L / 0.001 kg = 17.64 mg P kg^{-1} soil



References:

Amer, F., Bouldin, D. R., Black, C. A., & Duke, F. R. (1955). Characterization of soil phosphorus by anion exchange resin adsorption and P 32-equilibration. *Plant and Soil, 6*, 391-408.

Bowman, R. A., Olsen, S. R., & Watanabe, F. S. (1978). Greenhouse Evaluation of Residual Phosphate by Four Phosphorus Methods in Neutral and Calcareous Soils. *Soil Science Society of America Journal, 42*(3), 451-454. doi:10.2136/sssaj1978.03615995004200030015x

Hedley, M., Stewart, J., & Chauhan, B. (1982). Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Science Society of America Journal, 46*(5), 970-976.

Citation:

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