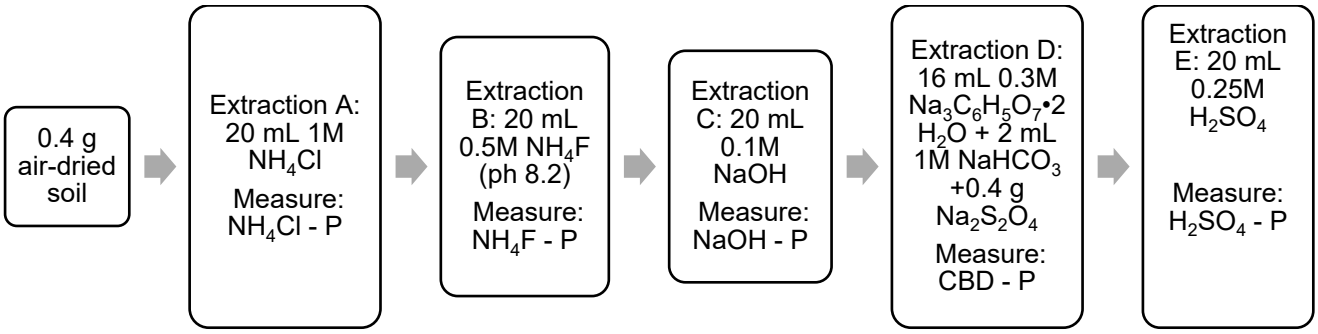
SOP: **Inorganic P fractionation**

Overview:

This standard operating procedure (SOP) describes a protocol for commonly used sequential extraction of soils to estimate pools of ***inorganic*** phosphorus (P) of soils and sediments. A key caveat is that if organic P is present in the sample, colorimetric analyses must be used since ICP-OES/ICP-MS will quantify total P in the extract, thus overestimating inorganic P of fractions. These operationally defined fractions (solubility) are hypothesized to vary by chemical speciation and availability (i.e., pools). The method was originally reported by Chang and Jackson (1957) and modified by Zhang and Kovar (2009). Soils/sediments that are ground to pass a <2 mm sieve are typically used on an air-dried mass basis.



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: Gloves
Particularly hazardous substances: Concentrated sulfuric acid should be handled in the fume hood. 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

Reagent preparation

- pH meter
- Analytical balance (at least two decimal places sensitivity)

- NH_4Cl (ammonium chloride)
- NH_4F (ammonium fluoride)
- NaOH (sodium hydroxide)
- $Na_3C_6H_5O_7 \cdot 2H_2O$ (sodium citrate)
- $NaHCO_3$ (sodium bicarbonate)
- $Na_2S_2O_4$ (sodium dithionite)
- H_2SO_4 (sulfuric acid)

Sequential Extraction

- Oscillating (or horizontal) shaker capable of at least 120 oscillations per minute
- Centrifuge
- Dispensette
- 50 mL centrifuge tubes
- Pipette and tips (100-1000 µL)

ICP analysis

- Determination of phosphorus concentration needs to be done by **ICP** for materials ***that do not have organic P***.
- Colorimetry for these extracts has not been successful and is not a recommended practice.

Detailed Procedure:

I. **Sample Preparation**
   1. Measure 0.4 g of air-dried soil into 50 mL centrifuge tube. Falcon tube is recommended for avoiding leak during extraction. Record exact weight of soil to at least 1/100th of one gram (1.XX g)

II. **Reagent preparation**
   1. Extractants
      i. 1 M NH_4Cl
         a. Dissolve 53.3 g of NH4Cl in 1 L 18.2 MΩ water
      ii. 0.5 M NH_4F at pH 8.2
         a. Dissolve 18.5 g of NH4F in 1 L 18.2 MΩ water and adjust pH to 8.2 with 4 M NH4OH.
      i. 0.1 M NaOH
         a. Dissolve 4.0 g of NaOH in 1 L 18.2 MΩ water
      ii. 0.3 M $Na_3C_6H_5O_7 \cdot 2H_2O$
         a. Dissolve 88.2 g of $Na_3C_6H_5O_7 \cdot 2H_2O$ 1 L 18.2 MΩ water
      iii. 1 M $NaHCO_3$
         a. Dissolve 84 g of $NaHCO_3$ in 1 L 18.2 MΩ water
      iv. 0.25 M H_2SO_4
         a. Dilute 14 mL of concentrated H_2SO_4 to 1 L 18.2 MΩ water (Note: *Always* add acid to water)
      v. Saturated NaCl

a. Add ~300 g of NaCl to 1 L 18.2 MΩ water

2. Standards
    i. Calibration standards (ranging from 0 – 20 mg P/L) need to be made in each extracting solution. Dilute commercial standard (1000 mg P/L) in each 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 because molybdate colorimetry of P is sensitive to pH (color development and intensity, precipitation).

III. Sequential Extraction

1. Extract the pre-weighed soil in the 50 mL centrifuge sequentially in the order of 1 M NH_4Cl, 0.5 M NH_4F (pH 8.2), 0.1 M NaOH, 0.3M $Na_3C_6H_5O_7 \cdot 2H_2O$ + 2 mL 1M $NaHCO_3$ +0.4 g $Na_2S_2O_4$, and 0.25 M H_2SO_4.
   Note: Omit 0.5 M NH_4F for calcareous soils as it reacts with $CaCO_3$.
    i. Add 20 mL 1M NH_4Cl to pre-weighed soil and place on horizontal shaker (low or 120 rpm) for 30 min, centrifuge and decant the supernatant into a *new* 50 mL Falcon tube (Extract A) and bring to volume (20 mL).
    ii. Add 20 mL 0.5M NH_4F to residue from Extract A and place on horizontal shaker (low) for 1 h, centrifuge and decant the supernatant into a *new* 50 mL Falcon tube (Extract B). Add 10 mL saturated NaCl to the residue two separate times, add the washings to Extract B and bring to volume (40 mL).
    iii. Add 20 mL 0.1M NaOH to residue from Extract B and place on horizontal shaker (low) for 17 h, centrifuge and decant the supernatant into a *new* 50 mL Falcon tube (Extract C). Add 10 mL saturated NaCl to the residue two separate times, add the washings to Extract C and bring to volume (40 mL).
    iv. Add 16 mL 0.3M $Na_3C_6H_5O_7 \cdot 2H_2O$ and 2 mL 1M $NaHCO_3$ to residue from Extract C and heat suspension for 15 min in a water bath at 85°C (uncapped). Remove samples from water, add 0.4 g $Na_2S_2O_4$ (will create a smelly odor) and stir rapidly. Place samples back into water bath for 15 more minutes (uncapped), centrifuge and decant supernatant into a *new* 50 mL Falcon tube (Extract D). Add 10 mL saturated NaCl to the residue two separate times, add the washings to Extract D and bring to volume (38 mL). Expose to air for 15 minutes before capping.
    v. Add 20 mL 0.25M H_2SO_4 to residue from Extract D and place on horizontal shaker (low) for 1 h, centrifuge and decant the supernatant into a *new* 50 mL Falcon tube (Extract E). Add 10 mL saturated NaCl to the residue two separate times, add the washings to Extract E and bring to volume (40 mL).

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; 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 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 original extract volume (solution added + NaCl washings) (e.g., 0.04 L for NH_4F-P and 0.02 L for NH_4CL-P) and divide by soil mass (1 g = 0.001 kg) to yield concentration in mg P kg^{-1} soil

Example calculation for raw data from ICP:

P concentration in given fraction (mg kg^{-1}) =
[Conc. of P (mg L^{-1}) x Volume of extractant (L)] ÷ mass of soil (kg)

Example for NH_4F:
Absorbance from ICP = 4.46
Dilution = 1
Extraction volume = 40 mL = 0.040 L
Soil mass = 0.4 g = 0.0004 kg

Concentration in soil basis = 4.46 mg L^{-1} * 0.04 L / 0.0004 kg = 178 mg P kg^{-1} soil

References:

Zhang, H.; Kovar, J.L. Fractionation of Soil Phosphorus. In *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*, Second Edition ed.; Kovar, J.L., Pierzynski, G.M., Eds. SERA-IEG 17: Southern Cooperative Series Bulletin No. 408, 2009.

Suggested reading:

Chang, S.C., and M.L Jackson. 1957. Fractionation of soil phosphorus. Soil Sci. 84:133-144.

Citation:

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