SOP: **Total P in Soils by Lithium Metaborate Fusion**

Overview:

This standard operating procedure (SOP) describes a protocol commonly used for quantifying total P in soils and can also be used for quantifying several other major and minor nutrients. The method is derived from Robertson et al. (1999) and was modified by Chunhao Gu and Maia Rothman. Samples should be air-dried and ground to a powder (<0.5 mm) for best results as a small particle size allows for rapid dissolution and is essential for complete recovery.

Wet acid digestion may also be used for total P determination, though many of these methods suffer from incomplete recovery in certain soil types and are best used in soils that have low iron and aluminum oxide content and few silicate inclusions. Popular wet digestion methods include nitric-peroxide digestion (Soils Lab 2021), sulfuric-peroxide digestion (Parkinson and Allen 1975), perchloric acid digestion (Olsen and Sommers 1982), and hydrofluoric acid digestion (Bowman 1988). The hydrofluoric acid digestion method is the exception to this rule, as HF is capable of fully dissolving the more recalcitrant components of soil but is extremely hazardous and should only be used with proper precautions.

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: Acid resistant nitrile gloves
Particularly hazardous substances: Concentrated hydrochloric acid should be handled in the fume hood. Make sure to check Safety Data Sheet if unsure about how to handle this chemical. Heat resistant gloves and properly fitted lab coat should be worn whenever furnace is being used. Exercise particular caution when adding and removing samples from heated furnace.

Instrumentation & Consumables:

Instrumentation

- Analytical balance (at least two decimal places sensitivity)
- Muffle furnace capable of reaching 1000°C
- Furnace gloves, wire rack for cooling crucibles, tongs
- Horizontal shaker

Consumables

- 9 mL graphite crucibles* – these last 3-5 heating cycles each (Fig 2)
- 50 mL centrifuge tubes
- Concentrated (37%) hydrochloric acid
- Lithium metaborate powder or pre-fused granules
- Recommended: Soil reference with known P content, such as a NIST reference material (US) or NRC reference material (Canada)

*SCP Science supplies 9mL conical graphite crucibles at a reasonable price compared to other major suppliers. We have found “regular” purity graphite to be sufficient for P determination. SCP graphite products: https://www.scpscience.com/pdfs/Catalogues/English/Graphite_Products.pdf

Detailed Procedure:

1. Preheat muffle furnace to 1000°C
2. Mix 1M hydrochloric acid. Fill a 1L volumetric flask about 1/3 full with nanopure water. Measure 83 mL of HCL in fume hood and add to flask. Bring to volume with more nanopure water.
3. Prepare data sheet to record sample ID, sample mass (g), and acid volume
4. Prepare crucibles by lightly tapping upside down on a paper towel to remove loose graphite
5. Prepare samples
    a. Weigh 0.2±0.01 g of lithium metaborate into crucible and distribute to cover the bottom of the vessel. Soil that comes into direct contact with the crucible may not dissolve fully.
    b. Tare crucible, and weigh 0.25 g of finely ground soil, making sure to record exact sample weight.
    c. Tare crucible and add an additional 0.2±0.01 g of lithium metaborate to cover the exposed soil.
    d. Prepare as many samples as can comfortably fit in muffle furnace (typically 9-12 for benchtop models). Be sure to include your reference soil as well as one blank. Crucibles can also be prepared in advance, though keeping track of a large number of samples can be challenging as the crucibles themselves cannot be labeled.
6. Carefully place graphite crucibles into furnace using tongs and heatproof gloves. Most gloves cannot handle these temperatures for more than a few seconds at a time, so it is important to work quickly. Taking breaks to change gloves and let

them cool every 4 crucibles or so can also help. Once the furnace is fully loaded, wait for temperature to return to 1000°C before setting a timer. This is a good time to prepare the next batch of crucibles, clean, or label centrifuge tubes for later.

7. Carefully remove the samples from the furnace and place on wire rack to cool. It takes at least a few hours for the furnace to cool down completely and it is often impractical to wait this long in between batches, but you can turn the furnace off temporarily and let it cool for a few seconds to make removing the samples easier.
8. Once the samples have cooled (~5 min), inspect them to make sure there are no visible soil particles remaining (Fig 1). If particles are present, repeat steps 4 and 5. The final cooled bead should be translucent with a color ranging from blue-green to yellow.
9. Label centrifuge tubes and fill with 45 mL of 1M HCl using a dispensette. If using a less precise measuring method, weigh the tubes before and after the addition of acid to obtain the exact volume used.

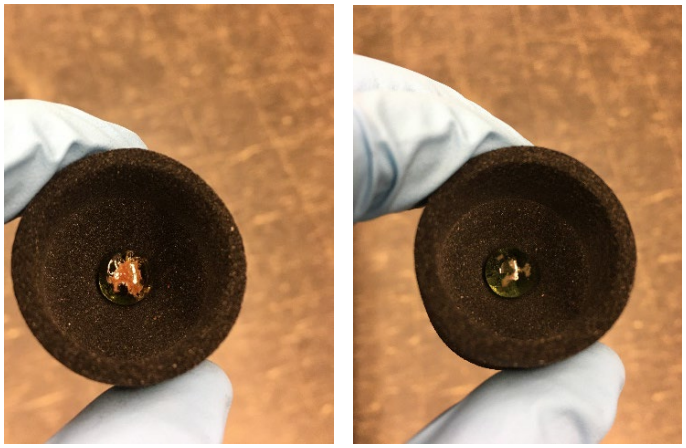

Fig 1. Two examples of an incomplete fusion, indicated by visible brown particles suspended in the bead.

10. Add the bead to the filled centrifuge tube, capping tightly, and immediately place on shaker. Shake on low speed for 6-8 hours or overnight, so that the bead fully dissolves. The resulting solution can be analyzed colorimetrically or by ICP and can be stored indefinitely at room temperature.

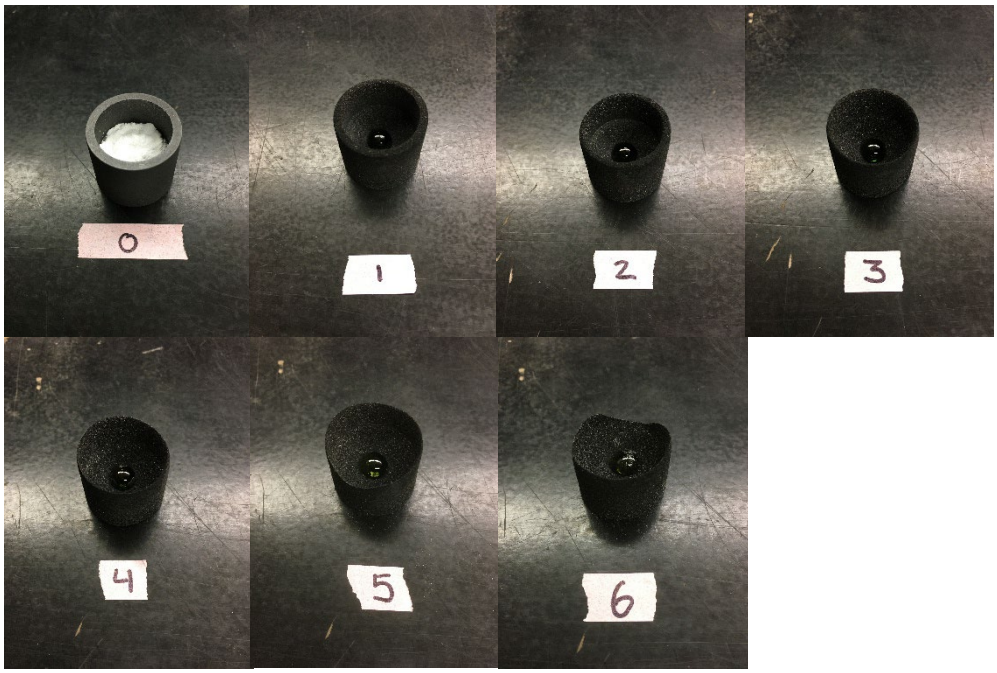

Fig 2. Progression of crucible degradation over the course of six firings. Clear glass beads indicate complete dissolution of the soil.

P Colorimetry

Instrumentation & Consumables:

Instrumentation

- Microplate spectrophotometer
  - Note: this SOP's colorimetry procedures are based on microplates, but can be scaled for cuvettes while keeping same ratio of reagents and extracts
- Pipette and tips (100-1000 µL)

Consumables

- 15 mL centrifuge tubes
- 1000 mg/L P standard
- 2M hydrochloric acid
- ammonium molybdate
- deionized water
- antimony potassium tartrate
- concentrated sulfuric acid
- ascorbic acid
- 96 well microplates
- 5% and 10% sodium hydroxide (w/v)
- 4-nitrophenol or pH paper

Detailed Procedure:

1. Standards
   a. Calibration standards (ranging from 0 – 40 mg P/L) need to be made in each extracting solution and undergo the same neutralization process as the samples. Dilute commercial standard (1000 mg P/L) in each extracting solution and sequentially dilute them to make calibration standards that cover the concentration you are expecting for your soil samples. It is essential to use the same extracting solution because molybdate colorimetry of P is sensitive to pH (color development and intensity, precipitation).
   b. To prepare standards from a 1000 ppm P stock solution: Create a 50 ppm aqueous stock solution by combining 5 mL 1000 ppm stock with 95 mL nanopure water. Add 20 mL 2M HCl to all centrifuge tubes. Then, add required amount of 50 ppm stock solution to each tube and bring to a total volume of 40 mL with nanopure water. Example ratios are below:

	0 ppm	0.5 ppm	1 ppm	2 ppm	5 ppm	10 ppm	15 ppm	20 ppm	40 ppm

P stock solution (50ppm)	0 ml	0.4 ml	0.8 ml	1.6 ml	4 ml	8 ml	12 ml	16 ml	20 ml
HCl (2M)	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Nanopure water	20 ml	19.6 ml	19.2 ml	18.4 ml	16 ml	12 ml	8 ml	4 ml	0 ml

2. Colorimetry reagents
    a. Murphy-Riley Solution A
        I. Dissolve 4.3 g ammonium molybdate in 400 mL of deionized water in a 1 L volumetric flask.
        II. Dissolve 0.40 g antimony potassium tartrate in 400 mL deionized water, then add to the ammonium molybdate solution in the beaker.
        III. Slowly and carefully add 54 mL conc. H_2SO_4. This step will create an exothermic reaction.
        IV. Allow to cool and make to 1000 mL in the volumetric flask with deionized water. Mix well and store in a dark bottle in a refrigerator. The reagent is stable for 4 months at 4°C.

    b. Murphy-Riley Solution B
        I. Dissolve 0.56 g of ascorbic acid in 56 mL deionized water to form a 1% ascorbic acid solution. Make a fresh solution daily as needed.

    c. Final Murphy-Riley (MR) reagent: combine all 56 mL of solution B + 44 mL of solution A, and mix (should turn to light yellow color)

3. Plating and P determination in solution
    a. Test varying ratios of the sample solution and 5% and 10% NaOH to create a solution that is approximately neutral with a combined volume of 80 µL. A 1% aqueous solution of 4-nitrophenol may be used as a pH indicator.
    b. Pipette samples, NaOH, and Murphy-Riley reagent into well plates being sure to include standards, blanks, and replicates (if used). A ratio of 47 µL sample: 38 µL 10% NaOH : 80 µL MR reagent has worked for soils in the past.
    c. After pipetting, set plates aside to develop for 15-30 minutes. Absorbance is measured at A882.
    d. Plot a standard curve(r^2>.98) and use the resulting linear equation to convert from raw absorbance values to [P] in solution. To convert to [P] in soil, see below section on calculations.

Calculations

[P] in solution (mg L^{-1}) x volume of HCl (mL) ÷ soil mass (g) = [P] in soil (mg kg^{-1})

e.g. (4.75 mg L^{-1}) x (45 mL) ÷ (0.250 g) = 855 mg kg^{-1}

References:

Bowman, R.A. 1988. A rapid method to determine total phosphorus in soils. Soil Soc. Am. J. 52:1301-1304.

G. Philip Robertson, David C. Coleman, Caroline S. Bledsoe, & Phillip Sollins. (1999). *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press.

Olsen, S.R. and Sommers, L.E. 1982. Perchloric Acid Digestion. In: Carter, M.R. and Gregorich, E.G., editors. 2008. Soil Sampling and Methods of Analysis, Second Edition. Canadian Society of Soil Science, Pinawa, MB.

Parkinson, J.A. and Allen, S.E. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Soil. Sci. Plant Anal. 6:1-11.

SOP: Soil or Plant Material Total P Digestion. 2021. Soils Lab, University of Illinois Urbana-Champaign. Urbana, IL. Accessed at: https://margenot.cropsciences.illinois.edu/methods-sops/

Suggested reading:

Church, C., Spargo, J., and Fishel, S. 2017. Strong acid extraction methods for “total phosphorus” in soils: EPA method 3050b and EPA method 3051. Agric. Environ. Lett. 2:160037.

Citation:

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