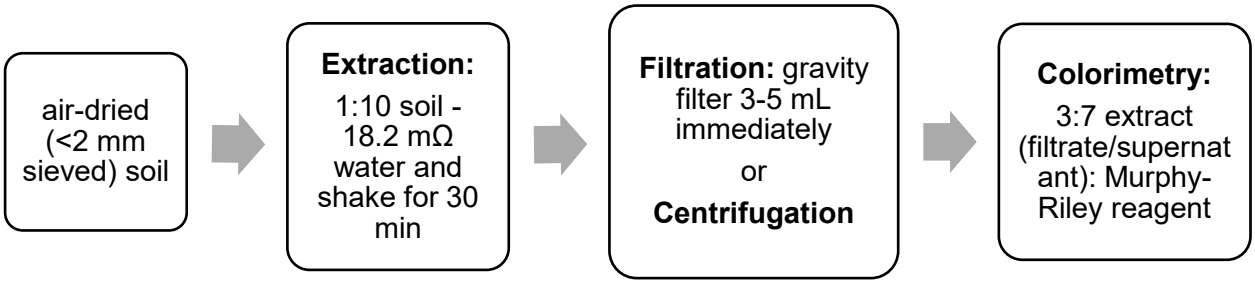
# SOP: **Water-extractable Pi**

## **Overview**:

This standard operating procedure (SOP) describes a protocol for extraction of soil P to estimate the water-extractable orthophosphate (Pi) pool in soil, used as an indicator of runoff P loss. The method was developed by Olsen and Sommers (1982) (unrelated to the commonly used Olsen P test), and this protocol is modified from Sharpley et al (2007), which is a variation of Olsen and Sommers (1982) method. Air-dried soil that are 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: Gloves
Particularly hazardous substances: Concentrated sulfuric acid should be handled in the fume hood. Make sure to check Material Safety Data Sheet (MSDS) 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

- Analytical balance (at least two decimal places sensitivity)
- Class A volumetric flasks

- Filter funnels
- Assorted stir bars
- Stir plate
- Assorted pipettes with adjustable volumes
- Pipette tips (1 mL – 10 mL)
- Concentrated Sulfuric acid ($H_2SO_4$ 95-98% ACS grade)
- Commercial P standard (1000 mg P/L)
- Ammonium molybdate tetrahydrate (CAS number: 12054-85-2)
- Antimony potassium tartrate
- Ascorbic acid
- 1 gallon glass jug

**Extraction**

- Horizontal shaker (low setting – 120 rpm required)
- Macro-centrifuge
- Dispensette
- 50 mL centrifuge tubes
- Pipette and tips (40-1000 uL)

**Filtration/centrifugation**

- 15 mL centrifuge tubes (*only if filtration is done*)
- Filter funnels (*only if filtration is done*)
- Whatman 42 filter paper (preferably 15cm round) (*only if filtration is done*)
- Microcentrifuge
- Microcentrifuge tubes and racks
- Cold storage (4°C for short-term storage)

**Colorimetry**

- Cuvettes (or 96 well microplates)
- Beckman Spectrophotometer capable of reading at 882 nm (or microplate spectrophotometer)
- Pipette and tips (100-1000 uL)

## Detailed Procedure:

I. **Sample Preparation**
   1. Measure 3.00 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 three grams (3.XX g)

II. **Reagent Preparation**
   1. Extracting solution preparation: No preparation is needed as extracting solution is 18.2 mΩ water. Use 1 gallon glass jug (dispensette fits nicely on

these) for storing the extractant and further throughput extraction with the dispensette.

2. Standards
    a. Calibration standards (ranging from 0 – 20 mg P/L) need to be made in the same extracting solution as used for samples. Dilute commercial standard (1000 mg P/L) in 18.2 mΩ water 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. It is essential to use the same extracting solution because molybdate colorimetry of P is sensitive to pH (color development and intensity, precipitation).

3. Colorimetry reagents
    i. Murphy-Riley Solution A
        a. Dissolve 4.3 g ammonium molybdate in 400 mL of deionized water in a 1-litre beaker.
        b. Dissolve 0.40 g antimony potassium tartrate in 400 mL deionized water, then add to the ammonium molybdate solution in the beaker.
        c. Slowly and carefully, with stirring, 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. *The reagent is stable for 6 weeks at 4°C
    ii. Murphy-Riley Solution B
        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: combine 56 mL of solution B + 44 mL of solution A, and mix (should turn to light yellow color).

## III. Extraction

1. Extract the pre-weighed soil in the 50 mL centrifuge tube by adding 30 mL of 18.2 mΩ water (soil to solution ratio of 1:10). *note: can increase soil concentration to reduce variability if soils are not <2 mm sieved; also advisable for non-air dried soils)
2. Shake immediately on horizontal shaker at 120 rpm (low setting) for exactly 30:00 min. The idea is to have all samples in contact with the extracting solution the same amount of time.

## IV. Filtration/centrifugation

1. Filtration
    i. Remove samples from shaker, centrifuge (macro centrifuge - 5000 rpm, 3 minutes at 25 °C) and filter through Whatman 42 filters into clean 15- or 50-mL centrifuge tubes. Labels on flasks can be transferred to the tubes as the samples are poured.

ii. Gravity filter at least 3-5 mL and proceed to colorimetry immediately.

2. Centrifugation *(works well and is efficient)*
    i. Immediate after shaking, quickly uncap and pipette out 1 mL of soil + extract suspension into labelled micro-centrifuge tubes (hold up to 1.5 mL) and centrifuge at 15000 rpm for 1.45 min at 25 °C in a microcentrifuge.
    ii. Immediately post centrifugation, pipette out 60 µL of the clear supernatant into a 96-well plate (wells can hold 400 µL volume, but a usual recommended working volume is 350 µL), to avoid any further contact with soil.

## V. P – Colorimetry

1. Colorimetry is performed directly in the well plates (standard disposable polyacrylamide or another cheap polymer). 96 well plates hold up to 350 uL. Or cuvettes (standard disposable [polyacrylamide or another cheap polymer]) may be used. Cuvettes marked as 1.5 mL cuvettes can hold up to 2.5-3 mL.
2. Conduct colorimetry using a 3:7 ratio of water extracts (and standards!) and Murphy-Riley reagent. The reaction generally takes 15-20 minutes.
    i. Example volumes that have worked in the past:
        a. Cuvette: 300 µL water extract: 700 µL Murphy-Riley reagent
        b. 96-well plate: 60 µL water extract: 140 µL Murphy-Riley reagent
3. Absorbance is measured at A882 in a spectrophotometer (or microplate spectrophotometer). Use 0 mg/L standard as blank reading in the Beckman spectrophotometer (abs = 0.000).
4. To prepare standards from a 1000 ppm P stock solution: Create a 100-mg/L (can also pick a lower concentration like 20 mg/L) aqueous stock solution by combining 1 ml 1000 mg/L stock with 9 ml nanopore water. Then, combine the new 100 mg/L stock with the extracting solution in 15 ml centrifuge tubes to produce a range of standards. An example with 100 mg/L stock solution:

| Standard | 100 mg/L stock (ml) | 18.2 mΩ water (ml) |
|---|---|---|
| 0 mg/L | 0 | 10 |
| 0.25 mg/L | 0.025 | 9.975 |
| 0.5 mg/L | 0.05 | 9.95 |
| 1 mg/L | 0.1 | 9.9 |
| 2 mg/L | 0.2 | 9.8 |
| 2.5 mg/L | 0.25 | 9.75 |
| 5 mg/L | 0.5 | 9.5 |
| 10 mg/L | 1 | 9 |
| 15 mg/L | 1.5 | 8.5 |
| 20 mg/L | 2 | 8 |

## VI. Clean up

1. Make sure to clean dispensette (with nanopure water – fill up to 50 mL twice and dispense) and bring to the original maximum volume of 50 mL, shaker (especially if tubes leak), and centrifuge (especially if tubes leak). Clean analytical balance with brush and kimwipe after each use.
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 (≥75%). 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.

## VII. Calculations

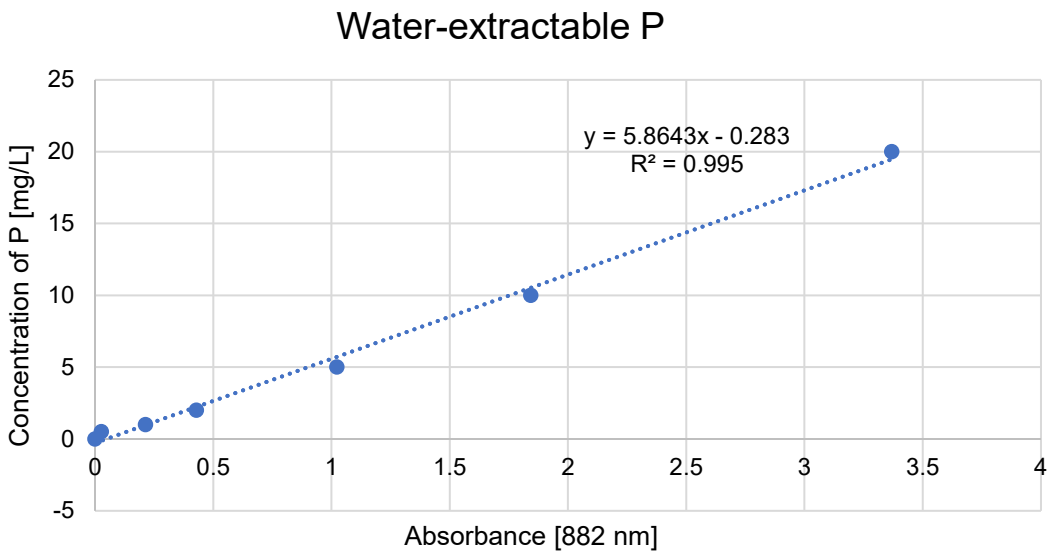
Measurement of water-extractable P is usually expressed in units of mg P $kg^{-1}$ soil. To calculate.

1. Convert raw absorbance to concentration (mg P $L^{-1}$) using calibration curve (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 extract volume (e.g., 30 mL = .03 L) and divide by soil mass (3.00 g = 0.003 kg) to yield concentration in mg P $kg^{-1}$ soil.

## Example calculation:

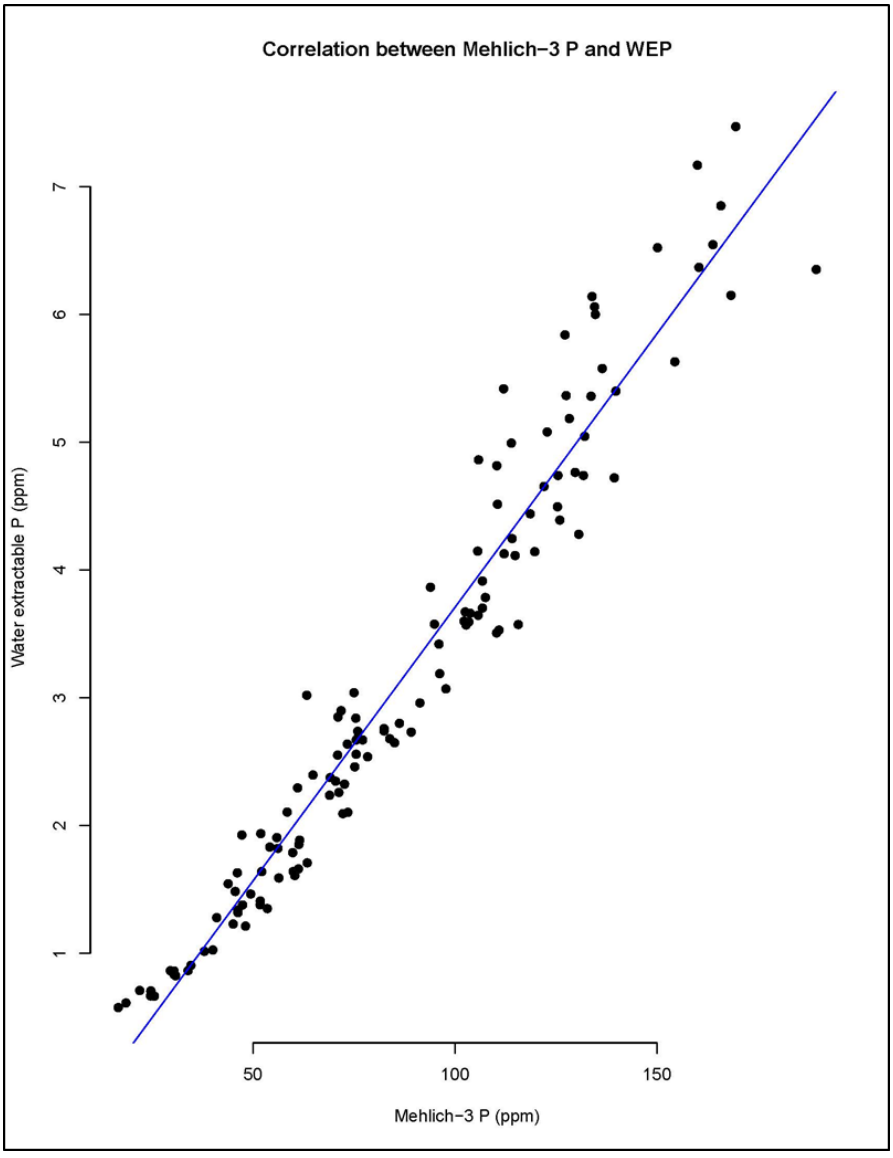
Given,
Absorbance = 0.074
Dilution = 1
Calibration curve: y = 5.8643x - 0.283
Extraction volume = 0.03 L
Soil mass = 0.003 kg

Concentration in extract = [5.8643 * 0.074 – 0.283] * 1 = 0.151 mg $L^{-1}$
Concentration in soil basis = 0.151 mg $L^{-1}$ * 0.03 L / 0.003 kg = 1.51 mg P $kg^{-1}$ soil



## Correlation between Mehlich-3 P and Water-extractable Pi

For Drummer the soil series that is common throughout north-central Illinois, there is a strong correlation between Mehlich-3 P and WEPi (**Pearson correlation coefficient = 0.97**). Mehlich-3 P is 23.3 fold higher than WEPi, hence for these soil types, WEPi can be measured and converted to Mehlich-3 P values by multiplying by 3.




## References:

Olsen, S.R. and Sommers, L.E. 1982. Phosphorus. In A.L. Page et al., Eds. Methods of Soil Analysis. Agronomy 9, 2nd edn. American Society of Agronomy, Inc., Madison, WI, pp. 403–430.

Sharpley, A.N., Kleinman, P.J.A. and Weld, J.L. 2007. Environmental Soil Phosphorus Indices. In: Soil sampling and methods of analysis / edited by M.R. Carter and E.G. Gregorich. -- 2nd ed. p. 167-171.

## Suggested reading:

Sharpley, A.N. 1995. Dependence of runoff phosphorus on extractable soil phosphorus. J. Environ. Qual. 24: 920–926.

## Citation:

SOP: Water-extractable P. 2021. Soils Lab, University of Illinois Urbana-Champaign. Urbana, IL. Accessed at: https://margenot.cropsciences.illinois.edu/methods-sops/

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