



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L405

Soil Extractable Phosphorus

Standard Operating Procedure

SOP Number	SOP-L405
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

FOR INTERNAL USE ONLY

This document is the property of the Environmental Analysis Program at Pomona College.
Unauthorized distribution is not permitted.

Table of contents

1 Purpose	2
2 Scope	2
3 Responsibilities	2
4 Definitions	3
5 Safety and Hazards	3
5.1 Required PPE	3
5.2 Emergency Procedures	4
6 Equipment and Materials	4
6.1 Equipment	4
6.2 Reagents – Olsen Extraction	4
6.3 Reagents – Bray-1 Extraction	4
6.4 Reagents – Ascorbic Acid Colorimetric Determination	5
6.5 Reagents – General	5
7 Procedure	5
7.1 Sample Preparation	5
7.2 Selecting the Extraction Method	6
7.3 Olsen Extraction	6
7.4 Bray-1 Extraction	6
7.5 Colorimetric Determination (Ascorbic Acid/Molybdate Blue)	7
7.6 Calibration	7
7.7 Calculations	7
8 Quality Control and Acceptance Criteria	8
8.1 Interpretation of Olsen P	8
8.2 Interpretation of Bray-1 P	8
8.3 QC Requirements	8
9 Waste Disposal	9
10 References	9

11 Training Record	10
12 Appendix A: Example Calculation in R	10
13 Appendix B: Reagent Preparation Quick Reference	11
13.1 Olsen Extractant (0.5 M NaHCO ₃ , pH 8.5)	11
13.2 Bray-1 Extractant (0.03 M NH ₄ F + 0.025 M HCl)	11
13.3 Combined Color Reagent (Murphy-Riley)	11
13.4 P Stock Standard (1000 mg/L P)	11
14 Appendix C: BibTeX Entries	12
15 Appendix D: Troubleshooting	12

1. Purpose

This procedure describes the extraction and colorimetric determination of plant-available (extractable) phosphorus in soils. Two extraction methods are presented: the Olsen method (0.5 M NaHCO₃ at pH 8.5) for neutral to alkaline soils, and the Bray-1 method (0.03 M NH₄F + 0.025 M HCl) for acidic soils (Bray & Kurtz, 1945; Olsen et al., 1954). Extractable P is quantified colorimetrically using the ascorbic acid/molybdate blue method (Kuo, 1996; Murphy & Riley, 1962).

Soil phosphorus availability is a key factor controlling plant productivity, and excess P in runoff is a primary driver of eutrophication in freshwater systems. Routine soil P testing guides fertilizer management and helps assess the environmental risk of P loss from agricultural and urban soils (Sharples et al., 2003).

2. Scope

- **Applies to:** Air-dried, sieved (less than 2 mm) mineral soils from all EA Program field sites. The Olsen method is the default for southern California soils, which are typically neutral to alkaline. Use Bray-1 for soils with pH below 6.5.
- **Does not apply to:** Total phosphorus determination (requires acid digestion); dissolved reactive phosphorus in water samples (see [SOP-L303 Hach Colorimetric](#)); organic phosphorus fractionation
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Prepare and standardize reagents, maintain spectrophotometer
Trained Personnel	Perform extractions and analyses, report results
New Students	Complete training before independent use

4. Definitions

- **Available phosphorus:** Phosphorus in soil solution and loosely sorbed fractions that can be taken up by plant roots; estimated by extraction with reagents that mimic root chemistry (Olsen bicarbonate in alkaline soils; Mehlich-3 or Bray in acid soils).
- **Bray P1 extraction:** An extraction using 0.025 M HCl + 0.03 M NH₄F; removes acid-soluble (Al- and Fe-bound) phosphorus; appropriate for acid and neutral soils (pH < 7.4) but not for calcareous soils.
- **Mehlich-3 extraction:** A multi-element extractant (0.2 M CH₃COOH + 0.25 M NH₄NO₃ + 0.015 M NH₄F + 0.013 M HNO₃ + 0.001 M EDTA); extracts plant-available P, K, Ca, Mg, and several micronutrients simultaneously; widely used in the eastern US and California.
- **Murphy-Riley method:** The standard colorimetric procedure for measuring dissolved reactive phosphorus; reacts phosphate with ammonium molybdate and ascorbic acid to form a blue phosphomolybdenum complex measured at 880 nm (Murphy & Riley, 1962).
- **Olsen extraction:** An extraction using 0.5 M NaHCO₃ (pH 8.5); removes Ca-phosphate forms; the standard method for calcareous and alkaline soils (pH > 7.0); results expressed as mg P kg⁻¹.
- **Sorption:** The attachment of phosphate ions to soil mineral surfaces (primarily Fe and Al oxides in acid soils; CaCO₃ in alkaline soils); limits plant availability and affects the choice of extraction method.

5. Safety and Hazards

Hazards

- **Chemical:** Ascorbic acid, ammonium molybdate, and antimony potassium tartrate are mild irritants. Sulfuric acid (used in the color reagent) is corrosive at concentrated strengths. Ammonium fluoride (NH₄F, Bray-1 extractant) is toxic and corrosive; handle in the fume hood. Sodium hydroxide (NaOH, for pH adjustment of Olsen reagent) is corrosive.
- **Physical:** Standard glassware handling risks.

5.1. Required PPE

- ☐ Safety glasses (splash goggles when handling H₂SO₄ or NH₄F)
- ☐ Lab coat
- ☐ Nitrile gloves

- ☐ Work in fume hood when handling NH_4F

5.2. Emergency Procedures

For acid or NH_4F splashes, flush with water for at least 15 minutes. For NH_4F skin contact, flush with water and apply 2.5% calcium gluconate gel if available. Refer to [SOP-L003 Hazardous Materials Handling](#).

6. Equipment and Materials

6.1. Equipment

Item	Notes
Reciprocating or end-over-end shaker	For extraction
Spectrophotometer or colorimeter	880 nm wavelength; Hach DR3900 (SOP-L303) or equivalent
Analytical balance (0.01 g)	See SOP-L102
pH meter	For verifying extractant pH; see SOP-L300
Centrifuge or vacuum filtration apparatus	For clarifying extracts
Volumetric flasks (100 mL, 500 mL, 1 L)	Class A
Erlenmeyer flasks (125 or 250 mL)	One per sample plus blanks
Graduated cylinders (25, 50, 100 mL)	For measuring extractant volumes
Pipettes (1–10 mL)	Class A volumetric or calibrated micropipettes
Cuvettes (1 cm path length)	Glass or polystyrene, matched
Filter funnels and Whatman No. 42 filter paper	For clarifying extracts

6.2. Reagents – Olsen Extraction

Reagent	Preparation / Notes
Sodium bicarbonate (NaHCO_3), 0.5 M at pH 8.5	Dissolve 42.0 g NaHCO_3 in approximately 900 mL DI water. Adjust to pH 8.5 with 1 M NaOH. Bring to 1 L. Prepare fresh weekly.
Activated charcoal (P-free)	For decolorizing extracts; wash with 0.5 M NaHCO_3 before use to remove any residual P (Olsen et al., 1954)

6.3. Reagents – Bray-1 Extraction

Reagent	Preparation / Notes
Bray-1 extractant (0.03 M NH_4F + 0.025 M HCl)	Dissolve 1.11 g NH_4F in approximately 900 mL DI water. Add 2.1 mL concentrated HCl (12 M). Bring to 1 L. Prepare in fume hood. Store in polyethylene. (Bray & Kurtz, 1945)

6.4. Reagents – Ascorbic Acid Colorimetric Determination

Reagent	Preparation / Notes
Sulfuric acid (H_2SO_4), 5 N	Dilute 70 mL concentrated H_2SO_4 (18 M) into approximately 400 mL DI water; bring to 500 mL after cooling. Add acid to water.
Ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$), 4%	Dissolve 20 g in 500 mL DI water
Antimony potassium tartrate ($\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2\text{H}_2\text{O}$), 0.274%	Dissolve 0.274 g in 100 mL DI water
Ascorbic acid, 1.76%	Dissolve 1.76 g in 100 mL DI water. Prepare fresh daily.
Combined color reagent	Mix in order: 50 mL 5 N H_2SO_4 + 15 mL ammonium molybdate + 5 mL antimony potassium tartrate + 30 mL ascorbic acid. Prepare fresh for each batch. (Murphy & Riley, 1962)
Phosphorus stock standard (1000 mg/L P)	Dissolve 4.394 g anhydrous KH_2PO_4 (dried at 105 °C) in 1 L DI water. Stable for months at 4 °C.
Phosphorus working standards	Dilute stock to 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 mg/L P in the appropriate extractant matrix (NaHCO_3 or Bray-1)

6.5. Reagents – General

Reagent	Notes
Deionized water (Type I or II)	From SOP-L11

7. Procedure

7.1. Sample Preparation

1. Air-dry the soil at room temperature until constant mass
2. Pass through a 2 mm sieve per [SOP-F400 Soil Sampling](#)
3. Determine gravimetric moisture content on a subsample per [SOP-L400](#) to express results on a dry-weight basis

7.2. Selecting the Extraction Method

Soil pH	Recommended Extractant	Reference
6.5 or above	Olsen (0.5 M NaHCO ₃ , pH 8.5)	Olsen et al. (1954)
Below 6.5	Bray-1 (0.03 M NH ₄ F + 0.025 M HCl)	Bray & Kurtz (1945)

Determine soil pH per [SOP-L402](#) before selecting the extractant. If comparing across sites with widely varying pH, consider using Mehlich-3 for all samples (consult the PI).

i Note

The Olsen extractant (NaHCO₃ at pH 8.5) dissolves calcium phosphate precipitates and reduces Fe³⁺ and Al³⁺ activity, releasing adsorbed P. It works across a wide pH range but is the standard for calcareous and alkaline soils. The Bray-1 extractant dissolves Al-bound and Fe-bound P via fluoride complexation and acid dissolution; it underestimates P in calcareous soils because the acid is neutralized by free CaCO₃ (Kuo, 1996).

7.3. Olsen Extraction

1. Weigh 2.50 g of air-dried, sieved soil into a 125 mL Erlenmeyer flask
2. Add approximately 0.25 g of P-free activated charcoal (pre-washed with 0.5 M NaHCO₃)
3. Add 50 mL of 0.5 M NaHCO₃ extractant (pH 8.5)
4. Stopper and shake on the reciprocating shaker at 180 oscillations per minute for 30 minutes
5. Filter immediately through Whatman No. 42 filter paper into a clean flask or tube. The filtrate should be clear and colorless; if yellow-brown, the charcoal was insufficient or not pre-washed.
6. Proceed to colorimetric analysis (Section 6.5) within 24 hours. Store extracts at 4 C if analysis is delayed.

7.4. Bray-1 Extraction

1. Weigh 2.00 g of air-dried, sieved soil into a 125 mL Erlenmeyer flask
2. Add 20 mL of Bray-1 extractant (1:10 soil-to-solution ratio)
3. Shake on the reciprocating shaker at 180 oscillations per minute for 5 minutes (the short extraction time is critical; extended shaking can re-adsorb released P)
4. Filter immediately through Whatman No. 42 filter paper
5. Proceed to colorimetric analysis (Section 6.5) within 24 hours

! Important

The Bray-1 extraction is exactly 5 minutes. Timing begins when the extractant contacts the soil. Over-extraction will underestimate available P due to re-sorption, while under-extraction will miss slowly dissolving forms.

7.5. Colorimetric Determination (Ascorbic Acid/Molybdate Blue)

This procedure follows Murphy & Riley (1962) as adapted by Kuo (1996).

1. Prepare the combined color reagent fresh (see Section 5, Reagents)
2. Pipette a 5 mL aliquot of the filtered extract into a clean test tube or cuvette
3. For Olsen extracts: acidify the aliquot with 1 drop (approximately 0.05 mL) of concentrated H_2SO_4 and swirl to expel CO_2 (the NaHCO_3 matrix effervesces; wait until bubbling stops before adding color reagent)
4. Add 0.8 mL of the combined color reagent to each tube
5. Mix by vortexing or inversion
6. Allow the color to develop for 15 minutes at room temperature. The solution turns blue in proportion to the phosphorus concentration.
7. Read the absorbance at 880 nm (or 882 nm) against a DI water blank within 30 minutes of color development
8. Determine the P concentration from the calibration curve (Section 6.6)

Alternative: Hach DR3900 PhosVer 3 method. If using the Hach spectrophotometer, follow the PhosVer 3 (ascorbic acid) method per [SOP-L303](#), Program 490. Prepare standards in the same extractant matrix as the samples.

7.6. Calibration

1. Prepare a minimum of five working standards (e.g., 0, 0.1, 0.5, 1.0, 2.0, 5.0 mg/L P) by diluting the stock standard with the same extractant used for the samples
2. Treat each standard exactly as the samples (acidification for Olsen, color reagent addition, timing)
3. Read absorbance at 880 nm
4. Plot absorbance versus concentration and fit a linear regression. Acceptable r^2 is 0.995 or higher.

7.7. Calculations

Extractable P in soil (mg P / kg dry soil):

$$P_{\text{soil}} = \frac{C \times V}{M \times \left(\frac{100}{100 + \theta_H} \right)}$$

where C = P concentration in the extract (mg/L), V = volume of extractant (mL), M = mass of air-dried soil (g), and θ_H = hygroscopic moisture (%), determined per [SOP-L400](#)). The term $\frac{100}{100 + \theta_H}$ converts the air-dried mass to an oven-dry basis.

For the Olsen method with the standard parameters (2.50 g soil, 50 mL extractant):

$$P_{\text{soil}} \text{ (mg/kg)} = C \times \frac{50}{2.50} \times \frac{100 + \theta_H}{100} = C \times 20 \times \frac{100 + \theta_H}{100}$$

For Bray-1 (2.00 g soil, 20 mL extractant):

$$P_{\text{soil}} \text{ (mg/kg)} = C \times \frac{20}{2.00} \times \frac{100 + \theta_H}{100} = C \times 10 \times \frac{100 + \theta_H}{100}$$

8. Quality Control and Acceptance Criteria

8.1. Interpretation of Olsen P

Olsen P (mg/kg)	Classification	Agronomic Interpretation
Less than 5	Very low	P deficiency likely; fertilize
5 – 10	Low	P may limit yield; fertilize
10 – 20	Medium	Adequate for most crops
20 – 40	High	No response to added P expected
Greater than 40	Very high	Excess P; environmental risk of runoff losses

Thresholds are approximate and crop-dependent (Olsen et al., 1954; Sharpley et al., 2003). Consult local extension guidelines.

8.2. Interpretation of Bray-1 P

Bray-1 P (mg/kg)	Classification	Agronomic Interpretation
Less than 10	Very low	P deficiency likely
10 – 20	Low	Fertilize to build P levels
20 – 40	Medium	Adequate for most crops
40 – 80	High	No additional P needed
Greater than 80	Very high	Environmental risk

8.3. QC Requirements

- Prepare all standards in the same extractant matrix as the samples (matrix-matched calibration) to account for background color and ionic strength effects
- Run a reagent blank (extractant only, no soil) with each batch. The blank absorbance should be near zero at 880 nm.
- Run a laboratory reference soil with known extractable P in each batch. Recovery should be within +/- 15% of the established value.
- Run duplicate extractions on at least 10% of samples. Acceptable precision: relative percent difference (RPD) less than 15%.
- Calibration curve r^2 must be 0.995 or higher
- Check the mid-range standard after every 20 samples. Recalibrate if the check standard deviates by more than 10%.
- For Olsen extracts, verify the NaHCO_3 pH is 8.5 +/- 0.1 before each batch; pH drift changes extraction efficiency substantially.

9. Waste Disposal

Waste Type	Disposal Method
Olsen extracts (NaHCO_3)	Neutralize to pH 6–8 and drain, or collect for EH&S
Bray-1 extracts ($\text{NH}_4\text{F}/\text{HCl}$)	Collect in fluoride waste carboy; EH&S pickup. Do not drain.
Color reagent waste (molybdate/ H_2SO_4)	Collect in inorganic waste; EH&S pickup
Used filter paper and soil	Standard trash (if non-hazardous soil)
Spent charcoal	Standard trash

Fluoride Waste

Bray-1 extracts contain ammonium fluoride. **Do not pour down the drain.** Collect all fluoride-containing waste in a labeled polyethylene carboy for EH&S hazardous waste pickup.

10. References

- Bray, R.H. & Kurtz, L.T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, 59(1), 39–45.
- Kuo, S. (1996). Phosphorus. In: Sparks, D.L. et al. (Eds.), *Methods of Soil Analysis, Part 3: Chemical Methods*. SSSA Book Series No. 5. pp. 869–919.
- Murphy, J. & Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36.
- Olsen, S.R., Cole, C.V., Watanabe, F.S., & Dean, L.A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. USDA Circular No. 939. U.S. Government Printing Office, Washington, DC.
- Sharpley, A.N., Daniel, T., Sims, T., Lemunyon, J., Stevens, R., & Parry, R. (2003). *Agricultural phosphorus and eutrophication*. 2nd ed. USDA-ARS Publication No. 149.
- Frank, K., Beegle, D., & Denning, J. (2015). Phosphorus. In: Brown, J.R. (Ed.), *Recommended Chemical Soil Test Procedures for the North Central Region*. NCR Publication No. 221 (revised). pp. 21–29.

Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, 59(1), 39–45. <https://doi.org/10.1097/00010694-194501000-00006>

Kuo, S. (1996). Phosphorus. In D. L. Sparks, A. L. Page, P. A. Helmke, & R. H. Loeppert (Eds.), *Methods of soil analysis, part 3: Chemical methods* (pp. 869–919). Soil Science Society of America. <https://doi.org/10.2136/sssabookser5.3.c32>

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)

Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (Circular No. 939). United States Department of Agriculture.

Sharpley, A. N., Daniel, T., Sims, T., Lemunyon, J., Stevens, R., & Parry, R. (2003). *Agricultural phosphorus and eutrophication* (ARS-149; 2nd ed.). USDA Agricultural Research Service.

11. Training Record

Before performing soil extractable P analyses independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) and [SOP-L003 Hazardous Materials Handling](#) training
3. Review the SDS for ammonium fluoride (if using Bray-1)
4. Demonstrate preparation of the extractant, color reagent, and calibration standards under supervision
5. Run a practice batch (at least 5 samples + blank + reference soil + duplicate) with acceptable QC results
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation in R

```
library(tidyverse)

# --- Calibration curve ---
standards <- tibble(
  P_mg_L = c(0, 0.1, 0.5, 1.0, 2.0, 5.0),
  abs_880 = c(0.002, 0.018, 0.075, 0.148, 0.295, 0.740)
)

cal_model <- lm(abs_880 ~ P_mg_L, data = standards)
cat(sprintf("Calibration R-squared: %.5f\n", summary(cal_model)$r.squared))

# --- Sample calculation (Olsen method) ---
sample_abs <- 0.210          # Absorbance at 880 nm
soil_mass_g <- 2.50          # Air-dried soil (g)
extract_vol_mL <- 50         # Extractant volume (mL)
hygroscopic_moisture_pct <- 2.1 # Percent hygroscopic moisture

# Determine C from calibration
C_mg_L <- (sample_abs - coef(cal_model)[1]) / coef(cal_model)[2]

# Calculate soil extractable P (mg/kg dry soil)
P_soil_mg_kg <- C_mg_L * (extract_vol_mL / soil_mass_g) *
  ((100 + hygroscopic_moisture_pct) / 100)

cat(sprintf("Extract P concentration: %.3f mg/L\n", C_mg_L))
```

```
cat(sprintf("Olsen P:                %.1f mg/kg dry soil\n", P_soil_mg_kg))

# Interpret
interpret <- case_when(
  P_soil_mg_kg < 5 ~ "Very low",
  P_soil_mg_kg < 10 ~ "Low",
  P_soil_mg_kg < 20 ~ "Medium",
  P_soil_mg_kg < 40 ~ "High",
  TRUE ~ "Very high"
)
cat(sprintf("Classification:        %s\n", interpret))
```

13. Appendix B: Reagent Preparation Quick Reference

13.1. Olsen Extractant (0.5 M NaHCO₃, pH 8.5)

1. Dissolve 42.0 g NaHCO₃ in approximately 900 mL DI water
2. Adjust pH to 8.5 with 1 M NaOH (dropwise, with stirring)
3. Bring to 1 L with DI water
4. Check pH before each batch; re-adjust if needed
5. Prepare fresh weekly

13.2. Bray-1 Extractant (0.03 M NH₄F + 0.025 M HCl)

1. In a fume hood, dissolve 1.11 g NH₄F in approximately 900 mL DI water
2. Add 2.1 mL concentrated HCl (12 M)
3. Bring to 1 L with DI water
4. Store in polyethylene at room temperature. Stable for several weeks.

13.3. Combined Color Reagent (Murphy-Riley)

Mix in order, swirling between additions:

1. 50 mL of 5 N H₂SO₄
2. 15 mL of 4% ammonium molybdate
3. 5 mL of 0.274% antimony potassium tartrate
4. 30 mL of 1.76% ascorbic acid (freshly prepared)

Total volume: 100 mL. Prepare fresh for each analytical batch.

13.4. P Stock Standard (1000 mg/L P)

1. Dry KH₂PO₄ at 105 C for 2 hours; cool in a desiccator
2. Dissolve 4.394 g in DI water in a 1 L volumetric flask
3. Bring to volume. Store at 4 C. Stable for months.

14. Appendix C: BibTeX Entries

Append these to the project `references.bib`:

15. Appendix D: Troubleshooting

Symptom	Probable Cause	Corrective Action
Yellow-brown Olsen extract	Insufficient charcoal; charcoal not pre-washed	Increase charcoal; pre-wash with 0.5 M NaHCO ₃
Calibration curve non-linear above 5 mg/L	Beer's Law limit exceeded	Dilute high samples; add lower standards
Bray-1 P unexpectedly low on calcareous soil	Acid neutralized by CaCO ₃	Use Olsen method for soils with pH above 6.5
Blank reads above 0.01 absorbance	Contaminated glassware or reagent	Acid-wash glassware (SOP-L101); prepare fresh reagents
Color fades after 30 min	Ascorbic acid degradation	Read within 30 min; prepare ascorbic acid solution fresh daily
Duplicates disagree by more than 15% RPD	Soil heterogeneity; inconsistent extraction timing	Homogenize soil more thoroughly; use a timer for extraction
Reference soil out of range	Extractant pH drift (Olsen); reagent degradation	Verify NaHCO ₃ pH; remake color reagent