



Applied Microbiology
&
Biotechnology Laboratory

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Standard Operating Procedure

AMBL-208-E

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Prepared by:	Terry E. Baxter
Reviewed by:	

Available Phosphorous Extraction from Soils

METHOD SUMMARY

This SOP describes a procedure for extracting the available phosphorous from a soil using a sodium bicarbonate extracting solution also known as the "Olsen extracting solution." The clarified extraction solution is analyzed for reactive phosphorous. This method is adapted from Method 4D5 Olsen Sodium-bicarbonate Extraction (USDA-NRCS, 2014), method QM-AD002-AP12 Available Phosphorous (IRRI, 2011) and method QM-AD036-MP24 (IRRI, 2011).

ENVIRONMENTAL HEALTH AND SAFETY

Hazards Assessment: This method uses sodium bicarbonate to prepare the extracting solution, but the amounts handled at any one time are unlikely to present a hazard. Potential health hazards associated with using the extracting solution are as follows.

1. Sodium Bicarbonate Extracting Solution is a prepared 0.5 M solution.

Skin Contact: Irritation from prolonged exposure. Use of gloves.

Eye Contact: Mild irritation. Wear eye protection.

Ingestion: Exposure by ingestion of large enough amounts to cause health effects are not anticipated.

Inhalation: Exposure by inhalation of solution is not expected.

2. Sodium hydroxide is used to prepare a 1 M NaOH solution for adjusting pH. Potential health hazards associated with using sulfuric acid are as follows.

Skin Contact: A strong irritant and corrosive agent. Will permeate skin and may cause blistering or inflammation depending on length of contact.

Eye Contact: Serious damage potentially causing corneal damage or blindness.

Ingestion: May cause severe pain, nausea, vomiting, diarrhea and shock, and perforations of the digestive tract. May cause corrosion and permanent damage to the esophagus and digestive tract. Can be fatal if enough swallowed.

Inhalation: Irritation of respiratory tract. A serious over-exposure can produce lung damage, choking, unconsciousness or death.

3. Sulfuric acid is a clear, colorless and corrosive liquid that can burn skin, eyes and clothing. Potential health hazards associated with using sulfuric acid are as follows.

Skin Contact: Will permeate skin causing severe burns, with the destruction of skin tissue within 3 minutes (GHS Category 1A).

Eye Contact: Will cause severe burns and severe eye damage that may not reverse after 21 days (GHS Category 1). Permanent blindness can result if the exposure is severe enough.

Ingestion: Although, unlikely to occur during the performance of this method, ingestion can cause burns to the lips, tongue, throat and stomach. Nausea, stomach cramps, diarrhea and vomiting are all symptoms of an exposure by ingestion.

Inhalation: Exposure by inhalation is not expected unless the acid is heated or misted. This exposure pathway is very hazardous and will cause irritation to the nose and throat, and cause significant damage to respiratory system tissue leading to production and accumulation of fluid in the lungs. Coughing, shortness of breath, breathing difficulty and tightness in the chest are all symptoms of acid vapor or mist exposure by inhalation. Severe exposures can lead to death.

Safety Equipment and Engineering Controls: This method requires that the handling concentrated acids be done in the fume hood and that an eye wash station be located nearby. Preparation of the reduction solution must be done in the fume hood.

Personal Protective Equipment (PPE): This method requires the use of the following PPE.

Gloves (nitrile)

Safety goggles or glasses

Laboratory coat

Analysis-derived Wastes and Disposal:

Waste Generated	Hazardous (Y / N)	Disposal
This procedure generates small volumes (50 mL or less) of sodium bicarbonate extracting solution.	N	May be disposed in a sink.
This procedure generates a saturated soil of 2.5 g (dry mass).	N	Disposal in a waste container destined for the landfill is considered acceptable.

METHOD DESCRIPTION**1.0 Introduction and Applicability**

Soil phosphorous available for plants is determined by using a sodium bicarbonate (0.5 N) or Olsen extracting solution. A 2.5-gram soil sample is mixed with 50 mL extracting solution (20 mL of extraction solution for each 1 g of soil) for 30 minutes. The extraction solution is clarified using centrifugation followed by filtration and reactive phosphorous is determined by the ascorbic acid method. Arsenate is known to interfere with color development and a procedure to remove this interference is included in this method.

This method is applicable for alkaline, calcareous and neutral soils, and is appropriate for Arizona soils.

2.0 Apparatus

- a. Analytical balance capable of weighing to the nearest 0.0001 g or less.
- b. Magnetic stirrer and stir bar
- c. Beaker, 100-mL
- d. Glass stirring rod
- d. Small scoop or spoon
- e. Dish, for drying soil, made of porcelain.
- f. Desiccator containing a desiccant that responds (color change) to moisture or a hygrometer that measures moisture.
- g. Glass-fiber filter, with a 47 mm diameter, nominal pore size $\leq 2.0 \mu\text{m}$ and $\geq 1.0 \mu\text{m}$, and no binders. Pre-rinse and dry the filter as described in AML SOP 105B.
- h. Convection oven operated at $110 \pm 5^\circ\text{C}$ for drying soil samples to a constant weight condition
- i. Filtration funnel assembly for a 47 mm size diameter filter.

- j. Vacuum suction flask, 1000 mL capacity.
- k. glass test tube, 50-mL
- l. Sieves, 10-mesh (2.00 mm) and 100-mesh (0.149 mm)
- m. Mortar and pestle, ceramic
- n. Centrifuge tubes, 50-mL polypropylene
- o. Centrifuge, operated at 2500 rpm

3.0 Reagents

- a. Olsen Sodium bicarbonate extracting solution (0.5 M NaHCO_3): Dissolve 42.008 g NaHCO_3 in approximately 900 mL of reagent water. Adjust pH to 8.5 with 1 M NaOH. Dilute to 1000 mL with reagent water and store in a tightly capped bottle. Check the pH of this solution (AMBL SOP 205A) before each use and adjust to pH 8.5 if necessary.
- b. Sodium hydroxide pH adjusting solution (1M NaOH): Dissolve 4 grams of NaOH in reagent water to 100 mL final volume. Record the actual mass of the NaOH weighed. Store in a tightly capped bottle and label it with the actual molarity of the solution (the actual NaOH mass dissolved multiplied by 0.25 equals the actual solution molarity).
- c. Reagent water, distilled water
- d. Hydrochloric acid rinse solution (1:1), 50 mL concentrated HCl + 50 mL reagent water

4.0 Preparation Soil Sample

- a. Air dry approximately 200 g of soil passing a 10-mesh sieve (<2.00 mm). Break up or grind aggregated soil to pass through the sieve and remove rocks and roots to the extent possible. Continue to grind soil remaining on the 10-mesh sieve until approximately 50 g of soil passing the 10-mesh sieve has been collected. Discard material remaining on the sieve.
- b. Place an evaporating dish with the collected soil in a convection oven at $110 \pm 5^\circ\text{C}$ for a minimum of 3 hours. Remove and cool the dish and sample to room temperature (of balance room).

5.0 Phosphorous Extraction Procedure

- a. Weigh $2.5 \text{ g} \pm 0.005 \text{ g}$ of prepared soil into a 100-mL beaker. Record the exact mass of the soil.
- b. Add 50 mL of the Olsen extracting solution and a stir bar.
- c. Place the beaker on a magnetic stirrer, and stir gently for 30 minutes. Initiate stirring with a glass rod if necessary.

- d. After stirring, remove the beaker from the stirrer, remove the stir bar from the beaker and allow the mixture to settle for another 5 minutes.
- e. Decant the settled solution into a 50-mL centrifuge tube and centrifuge at 2000 rpm for 10 minutes.
- f. Filter the centrate and collect the filtered extract solution in a 50-mL test tube.

6.0 Procedure for Analysis of Phosphorous

Promptly analyze sample by AMBL SOP 208B Reactive Phosphorous, using a calibration curve generated as described in Section 7.0 of SOP 208B.

7.0 Calculation

- a. Calculate the concentration of available phosphorous in soil as follows.

$$\text{Available Soil PO}_4^{3-}\text{-P, mg / Kg} = \frac{A \times 50}{B}$$

where A = concentration of reactive phosphorous as determined from SOP 208B, mg/L

 B = dry mass of soil extracted (in 50 mL of extracting solution), g.

8.0 Quality Control

In addition to the quality control required in SOP 208B, prepare and analyze at least one soil sample in duplicate for each batch of 20 or fewer samples. The soil sample used for the duplicate analysis must be well homogenized and split before weighed and suspended in the extracting solution. If a single sample is being analyzed, this sample must be analyzed in duplicate. Acceptance criteria for duplicate analysis of soil matrix using this extraction method has not yet been established.

9.0 Bibliography

1. Soil Survey Staff (2014) Kellogg Soil Survey Laboratory Methods Manual, Soil Survey Investigations Report No. 42, Version 5.0, Compiled and Edited by Rebecca Burt and Soil Survey Staff. U.S. Department of Agriculture, Natural Resources Conservation Service.
2. International Rice Research Institute (2011) Available Phosphorous (Olsen Phosphorous), Revision 3, IRRI Analytical Service Laboratory, Document QM-AD002-AP12.

3. International Rice Research Institute (2011) Plant and Soil Sample Preparation Procedures, Revision 4, IRRI Analytical Service Laboratory, Document QM-AD036-MP24.
4. Olsen, S.R, C.V Cole, R.S. Watanabe and L.A. Dean (1954) Estimation of Available Phosphorous in Soils by Extraction with Sodium Bicarbonate. USDA Circular No. 939. U.S. Government printing Office, Washington, D.C.