TEST METHODS MANUAL

Laboratory Services Division Bureau of Soils and Water Management

Department of Agriculture SECTION : SOIL CHEMISTRY Effective date: April 15, 2024 Issue No.: 3 SUBJECT : AVAILABLE PHOSPHORUS (OLSEN METHOD)

Revision No.: 1

TM-LSD-04-05

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SCOPE

The procedure is suitable for calcareous, alkaline, neutral, and slightly acidic soils containing Ca-PO₄ as the Ca concentration in the solution is suppressed by the precipitation of CaCO₃, thus increasing PO₄ concentration in solution. It can also be applied to acid soils as the carbonate buffer suppresses the solubility of Al and Fe and thus increases the phosphate concentration.

Neutral and slightly acid soils (pH 6.0 to 7.0) may contain both Ca- and Al-phosphates. The NaHCO₃ extractant can remove Ca-phosphates and phosphate adsorbed on surfaces of calcium and magnesium carbonates along with Alphosphates and is considered the most suitable P test for these soils.

PRINCIPLE

Phosphorus is known as the master key to agriculture because lack of available P in the soils limits the growth of both cultivated and uncultivated plants. It has also been established that phosphorus is relatively unavailable for plant uptake in highly weathered soils. It is firmly fixed in soil and its proportion is relatively stable and dependent on soil reaction values. Adequate phosphorus results in higher grain production, improved crop quality, greater stalk strength, increased root growth, and earlier crop maturity.

Phosphorus is extracted from the soil with 0.5 M NaHCO₃ at a nearly constant pH of 8.5. The OH⁻ and $CO_3^{2^-}$ in the NaHCO₃ solution decrease the concentration or activity of Ca²⁺ and Al³⁺ resulting in increased P solubility in soils. The soluble orthophosphate reacts with molybdate using ascorbic acid as reducing agent in the presence of potassium antimony tartrate, to form a blue colored heteropolymolybdic complex. The extractant is useful for both acidic and calcareous soils.

TEST PRECAUTIONS

The amount of soil phosphate extracted is influenced by the pH of the extraction solution, temperature, extraction time and the spectrophotometric measurement. Deviation from the prescribed method for any one of these variables leads to bias in the amount of PO₄ recovered.

The 0.5 M NaHCO₃ adjusted at pH 8.50, is unstable and will increase in pH with time. The extractant solution is typically stable for a period of eight hours and will over 48 to 72 hours increase to a pH of 9.0, dependent on laboratory environmental conditions. Research using NAPT reference soils indicates that an increase in pH of 0.25 units of the NaHCO₃ results in an average 15% increase in the amount of phosphate extracted. An increase of 0.50 pH units resulted in an increase of 20% and was soil dependent. Thus, for bicarbonate extractable PO₄-P it is essential that the bicarbonate extraction solution be prepared fresh, the pH adjusted to 8.50 \pm 0.05 and be checked daily to verify pH. (Miller, 2002)

The glassware to be used must be free of contamination with phosphorus (or arsenic, which gives the same test unless reduced with NaHSO₃). Since Pyrex glass contains 0.7% arsenic oxide, new glassware must be thoroughly weathered before use by treatment with warm sulfuric acid-dichromate solution for at least 24 hours. Soaps and detergent powders, if used, must be completely removed by cleaning in strong acid, as they frequently contain phosphorus.

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Use micropipette when delivering volumes as small as a microliter. This is to provide more accurate means for measuring volumes. Always check the appropriate conditions. The amount of P extracted will vary with temperature and shaking speed.

EQUIPMENT

- a) UV-VIS spectrophotometer capable of measuring absorbance at 882 nm.
- b) pH meter equipped with glass and reference electrode, or combination electrode
- c) Analytical Balance, precision of at least 0.001g
- d) Reciprocating shaker
- e) Magnetic stirrer
- f) Vortex mixer

LABORATORY WARE

- a) Erlenmeyer flasks, 250 mL
- b) Funnel
- c) Beakers, 5L, 1L, 500 mL
- d) Polyethylene bottles with lid, wide-mouth type, 125 mL capacity
- e) Volumetric flasks, 1L, 250 mL, 100 mL
- f) Graduated cylinder, 100 mL, 50mL
- g) Test tubes, 20 mL capacity
- h) Micropipette, 1-10mL, 10-1000 μ L
- i) Volumetric pipette, 50 mL, 20 mL, 10 mL

CHEMICALS AND REAGENTS

- a) Standard buffer solutions of pH 7.0 and pH 10.0
- b) Activated charcoal, phosphate free.
- c) Deionized water/ distilled water.
- d) Sodium Bicarbonate solution, 0.5 M, pH 8.5 (extracting solution). Dissolve 42 g NaHCO₃ in deionized water and make it 1 L. Adjust the pH to 8.5 by adding NaOH 1 M (4 g/100 mL).
- e) Sulfuric acid, 4 M. Slowly add 56 mL concentrated H₂SO₄ (96%) to about 150 mL deionized water in a graduated beaker under constant stirring. After cooling, make 250 mL with water.
- f) Ammonium molybdate solution, 4%. Dissolve 4 g of (NH₄)₆Mo₇O₂₄.4H₂O in deionized water and make to 100 mL. Store in polythene or pyrex bottle.
- g) Potassium antimony tartrate solution, 0.275% (1000 mg/L Sb). Dissolve 0.275 g KSbOC₄H₄O₆ in deionized water and make to 100 mL.
- h) Ascorbic acid solution, 1.75%. Dissolve 1.75g ascorbic acid in deionized water and make to 100 mL. Prepare fresh daily.
- i) Mixed Reagent, prepare fresh for every batch. Successively add with a graduated cylinder to a 500 mL beaker and homogenize after each addition:
 - 200 mL deionized water
 - 50 mL of 4 M H₂SO₄
 - 15 mL of NH₄-molybdate solution

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- 30 mL of ascorbic acid solution
- 5 mL of KSb-tartrate solution
- j) Standard Phosphate Solution, 100 mg/L P. Pipette 50 mL of NIST Traceable 1000 mg/L Phosphorus stock Solution into a 500 ml volumetric flask and make up to volume with the extracting solution.

Alternatively, dissolve 0.4390 g of KH_2PO_4 (dried for 2 hr. at 110 °C) in the extracting solution in a 1L volumetric flask and make up to the final volume.

- k) Secondary standard phosphate solution, 4 mg/L P. Pipette 10 mL of the 100 mg/L P standard solution into a 250 mL volumetric flask and make up to the Limited Preview ng solution.
- I) Working phosphate standard series. Pipette into 100 mL volumetric flasks 0-10-20-30-40-50 mL of the 4 mg/L P standard solution. Make up to the final volume with the extracting solution. The standard series is then 0-0.4-0.8-

For full access, kindly fill out the Document Request Form.

Remarks:

The range for working phosphate stand **Google Form link:** the equipment specification and the expected concentration of P in the analyzed solid schemes.

https://forms.gle/RbCgCdA54prTS6oN7

HEALTH AND SAFETY

Wear proper personal protective equipment. Use laboratory coat, close shoes, gas mask or dust mask and appropriate gloves when performing chemical analysis to mitigate the harmful effects of exposure on chemicals.

Observe careful and proper handling of chemicals when using strong alkali bases, strong acids and oxidizing agents. Potential direct body contact (e.g. ingestion, inhalation) may cause severe irritation and inflammation to the skin, eyes, respiratory and digestive tracts. Severe exposure to these corrosive substances may cause serious health damage or death. **Thank you!**

Avoid mixing incompatible chemicals to reduce risks of fire and explosion inside the laboratory. Sulfuric acid may react violently when mixed with water because of its high affinity for water. Always pour the acid into water to avoid splattering.

PROCEDURE (GLOSOLAN-SOP-10, 2021)

- 1. Weigh 5 g fine earth soil (accuracy 0.01g) into a wide mouth 125 mL capacity shaking bottle. Include two blanks and three quality control materials (QCM) or QRM.
- 2. Add 100 mL of the extracting solution and place the bottle caps
- 3. Add a half teaspoon of phosphate-free activated charcoal (approx. 0.5 g).
- 4. Mechanically shake for 30 minutes at reciprocating shaker capable of 180 oscillation per minute, with shaking bottles placed horizontally
- 5. Filter through a filter paper Whatman No.42 or with an equivalent acid-treated, identical porosity filter paper.
- 6. Pipette into test tubes 3 mL of the standard series, the blanks and the sample extracts.

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