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# Standard operating procedure for soil available phosphorus

*Olsen method*



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## **SOIL AVAILABLE PHOSPHORUS Olsen method**

### VERSION HISTORY

<b>No.</b>	<b>Date</b>	<b>Description of the modification</b>	<b>Type of modification</b>
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## 1. Brief introduction to Olsen method

Phosphorus (P) exists in the soil as organic and inorganic P forms. The inorganic P forms are more available for plant uptake than the other forms. Inorganic P forms are primarily mixtures of aluminium (Al-P), iron (Fe-P), and calcium (Ca-P) phosphates; the relative percentages between these three forms are a function of soil pH, with higher percentages of Al-P and Fe-P in acid soils, and higher percentages of Ca-P in neutral to alkaline soils (Jones, 2001).

In soil analysis of P, we distinguish two types: a) total analysis and b) fractional analysis. The P-Olsen corresponds to the second group. The fractions of this element present in the soil must be related to the response of plants to the application of a phosphate fertilizer. There are numerous methods for extracting P fractions with different sets of generated values. However, these only have meaning when they are associated with the response of the plants. In the Olsen method, phosphorus is extracted using a 0.5 M NaHCO<sub>3</sub> solution adjusted at a pH of 8.5.

## 2. Scope and field of application

This procedure is suitable for calcareous, alkaline, neutral, and slightly acidic soils containing CaPO<sub>4</sub>, since the calcium concentration in the solution is suppressed by the precipitation of CaCO<sub>3</sub>, increasing PO<sub>4</sub> concentration in solution. Neutral and slightly acid soils (pH 6.0 to 7.0) may contain both Ca- and Al-phosphates. The NaHCO<sub>3</sub> extractant can remove Ca-phosphates and phosphate adsorbed on the surface of calcium and magnesium carbonates along with Al-phosphates and is considered the most suitable extractant for these soils.

## 3. Principle

The Olsen method uses NaHCO<sub>3</sub> extractant that can remove Ca-phosphates and phosphate adsorbed on surfaces of calcium and magnesium carbonates along with Al-phosphates. Phosphorus is extracted from the soil with 0.5 M NaHCO<sub>3</sub> at a nearly constant pH of 8.5. The OH<sup>-</sup> and CO<sub>3</sub><sup>2-</sup> in the NaHCO<sub>3</sub> solution controls the activity of Ca<sup>2+</sup>, Al<sup>3+</sup> and Fe by precipitation of calcium as carbonate, and aluminium and iron as hydroxides. Soil extraction is carried out for 30 min at a soil/solution ratio of 1:20. P extraction is affected by several analytical factors: soil/solution ratio, shaking time, shaker type, shaking bottle position, capacity and type of shaking bottles, contact time of the soil in the extractant and temperature. Colour interference of the organic matter dissolved by the extractant must be removed (e.g. adding phosphate-free activated charcoal). There is no prior adjustment of the soil pH, or any attempt to remove possible interferences such as arsenate and silicate.

The measurement or determination of phosphorus with spectroscopic methods is based on colour development. In the coloration process, molybdenum blue methods are the most sensitive and, as a result, they are widely used for soil extracts containing small amount of phosphate, as well as for total P determination in soils. These methods are based on the principle that, in an acid molybdate solution containing orthophosphate ions, phosphomolybdate complex is formed, which can be reduced by ascorbic acid or other reducing agents (e.g. SnCl<sub>2</sub>) in the presence of potassium antimony tartrate to form a blue coloured heteropolymolybdic complex. Antimony accelerates the development of the blue colour and stabilizes it for up to 24 hours, and no interference of Si should be expected. If such

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interference does occur (blue coloured zero standard), then procedure should be repeated using deionized/distilled water (Van Reeuwijk, 2002). The intensity of the blue colour varies with the P concentration (following Beer's Law) but is affected by other factors such as acidity, arsenates, silicates and substances which influence the oxidation-reduction conditions of the system. The concentration of P is determined by spectrophotometrically at 882 nm.

## 4. Apparatus

- 4.1. Erlenmeyer flasks: 125 mL
- 4.2. Funnel
- 4.3. Beakers: 5L, 1L, 500 mL
- 4.4. Polyethylene bottles with lid, wide-mouth type : 125 mL capacity
- 4.5. Volumetric flasks : 1L, 250 mL, 100 mL, 50 mL
- 4.6. Graduated cylinder : 100 mL, 50 mL
- 4.7. Test tubes : 20 mL capacity
- 4.8. Adjustable pipette : 1–10 mL, 0.1-1 mL
- 4.9. Volumetric pipette : 50 mL, 20 mL, 10 mL
- 4.10. UV-VIS spectrophotometer capable of measuring absorbance at 882 nm and adjustable cuvette parameters for optical density.
- 4.11. Analytical balance, with an appreciation of 0.0001 g for the preparation of reagents.
- 4.12. Reciprocating shaker, capable of 60-260 oscillations/min
- 4.13. Vortex Mixer
- 4.14. Graduated pipette: 10 mL
- 4.15. Filter Paper Whatman No. 42 or equivalent

## 5. Materials

All chemicals and reagents should be of at least Analytical Grade.

- 5.1 Deionized water/distilled water, it should have an EC < 0.001 dS m<sup>-1</sup> (ASTM D1193-91 and ISO 3696:1987).
- 5.2 Extracting solution - Sodium bicarbonate solution, 0.5 M, pH 8.5. In a 1 L volumetric flask, dissolve 42 g of NaHCO<sub>3</sub> in deionized/distilled water. Adjust pH to 8.5 by adding 1 M NaOH (4 g/100 mL) and adjust to volume.
- 5.3 Sulphuric acid, 4 M. Slowly add 56 mL of conc. H<sub>2</sub>SO<sub>4</sub> (96% or 18M) to about 150 mL deionized/distilled water in a graduated beaker under constant stirring. After cooling, make up the volume to 250 mL with deionized/distilled water.
- 5.4 Ammonium molybdate solution, 4 percent. Dissolve 4 g of (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O in deionized/distilled water and make up to 100 mL. Store in a polyethylene or glass bottle.

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5.5 Potassium antimony tartrate solution, 0.275 percent. Dissolve 0.275 g of  $\text{KSbOC}_4\text{H}_4\text{O}_6 \cdot 1/2 \text{H}_2\text{O}$  in deionized/distilled water and bring the volume to 100 mL.

5.6 Ascorbic acid solution, 1.75 percent. Dissolve 1.75 g of ascorbic acid in deionized/distilled water and make up to 100 mL. Prepare fresh daily.

5.7 Mixed reagent, prepare fresh for every batch. Add successively with a graduated cylinder to a 500 mL beaker and homogenize after each addition:

- 200 mL deionized/distilled water
- 50 mL of 4 M sulphuric acid
- 15 mL of ammonium molybdate solution
- 30 mL of ascorbic acid solution
- 5 mL of potassium antimony tartrate solution

5.8 Standard Phosphate Solution, 100 mg P L<sup>-1</sup>. Pipette 50 mL of NIST or other equivalent traceable 1000 mg P L<sup>-1</sup> phosphorus stock solution into a 500 mL volumetric flask and make up to volume with the extracting solution.

Alternatively, dissolve 0.4390g of  $\text{KH}_2\text{PO}_4$  (dried for 2 h at 110 °C) in the extracting solution in a 1L volumetric flask and make up to the final volume.

5.9 Secondary standard phosphate solution, 4 mg P L<sup>-1</sup>. Pipette 10.0 mL of 100 mg P L<sup>-1</sup> standard solution into a 250 mL volumetric flask and make up to the final volume with the extracting solution.

5.10 Working phosphate standard series. Pipette into 100 mL volumetric flasks 0, 10, 20, 30, 40 and 50 mL of the 4 mg P L<sup>-1</sup> standard solution. Make up to the final volume with the extracting solution. The standard series is then 0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg P L<sup>-1</sup>

**Remarks:**

The range for working phosphate standards can be adjusted according to the equipment specification and the expected concentration of P in the analysed soil samples.

5.11 Activated Charcoal, phosphate-free (see 8.2.3).

## 6. Health and safety

This procedure involves the use of hazardous chemicals. Refer to the laboratory safety guidelines or the Safety Data Sheet (SDS) before proceeding.

### 6.1. Personnel safety

Wear proper personal protective equipment. Use laboratory coat, closed shoes, gas or dust mask, and appropriate gloves and safety glasses when performing chemical analysis to mitigate the harmful effects of chemical exposure. Wash hands and clean other exposed areas with mild soap and water after using all chemical reagents.

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## 6.2. Chemical hazard

- 6.2.1 Concentrated sulphuric acid is a clear, colourless and odourless liquid. It is extremely corrosive and can cause serious burns if not handled properly. Always dilute the sulphuric acid by adding a small portion of acid to a large amount of water and carry out the work under a fume hood to avoid inhaling the fumes.
- 6.2.2 Ammonium molybdate solution is a corrosive liquid. Contact with the eyes or body can cause serious health hazard. Reaction with metals may produce hydrogen gas and oxides of sulphur may be produced in a fire.
- 6.2.3 Ascorbic acid has no known effect on the skin/body but should be stored in light-resistant containers because it is light sensitive. Keep away from incompatibles materials, such as oxidizing agents.
- 6.2.4 Potassium antimony tartrate is hazardous in case of skin/body contact. Do not discharge the waste into the drain. It is incompatible with strong acids, strong bases and strong oxidizers. Do not expose to direct sunlight.
- 6.2.5 Activated Charcoal is hazardous in case of skin/body contact. It causes eye irritation and may cause respiratory irritation if inhaled. It should be kept away from heat/spark/open flames/hot surfaces.

## 7. Sample preparation

Air dry the soil sample (or dry in an air forced oven below 35 (+-5) °C), then grind and sieve to ≤ 2.0 mm size.

## 8. Procedure

### 8.1. Preparation of standards curve

From the working standards prepared as described in *section 5.8-5.10*, pipette 3.0 mL of standard series into test tubes. Slowly add 3.0 mL of the mixed reagent by pipette. Homogenize using a vortex mixer and allow the solutions to stand for at least 1 hour for complete blue colour development.

### 8.2. Preparation of samples

- 8.2.1. Weigh 5 g of dry soil (accuracy, 0.01g) into a wide-mouth 125 mL capacity shaking bottle. Include two blanks and three quality control materials (QCMs) or check samples.
- 8.2.2. Add 100 mL of the extracting solution and place the bottle caps.
- 8.2.3. Add a half teaspoon of phosphate-free activated charcoal (approx. 0.5g).
- 8.2.4. Mechanically shake for 30 min at 180-200 oscillations/min, with shaking bottles placed horizontally.
- 8.2.5. Filter through a filter paper Whatman No. 42 or with an equivalent acid-treated, identical porosity filter paper.

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- 8.2.6. Pipette 3 mL of the blanks, QCMs and the sample extracts into test tubes.
- 8.2.7. Slowly add 3 mL of the mixed reagent by pipette and homogenize using a vortex mixer.
- 8.2.8. Allow the solutions to stand for at least 1 hour for the blue colour to develop its maximum.

**Remarks:**

1. The analysis must be carried out at room temperature, between 20 and 25 °C.
2. It is necessary to use optimal concentrations of reagents for colour development: acid, ammonium molybdate, ascorbic acid. Their quantity and concentration allow the maximum colour per unit of phosphorus present and the highest stability of the complex. It is therefore not advisable to modify the procedures without first studying the effect that this could have on the development of the colour and its stability.

### 8.3. Measurement

Read the absorbance of the calibration standards, blanks, QCMs and samples in a spectrophotometer set at 882 nm wavelength. If the absorbance of the samples is too high, dilute the extract with the extraction solution and record the dilution factor.

When the correlation coefficient of the calibration curve is  $\geq 0.995$ , proceed with sample analysis. Otherwise, verify that the standards and reagents have been correctly prepared, the instrument is functioning properly, and the instrument set-up is correct. Corrective actions must be taken and details of the corrective action must be recorded.

### 8.4. Reporting

Compute the concentration of phosphorus in mg P kg<sup>-1</sup> with the calculation in *section 9* and report the oven-dry basis to two (2) decimal places.

## 9. Calculation

$$\begin{aligned} \text{mg P kg}^{-1} &= (a - b) \times \frac{V \times 1\text{L} \times 1000 \text{ g} \times \text{DF} \times \text{mcf}}{W \times 1000 \text{ mL} \times 1\text{kg}} \\ &= (a - b) \times \frac{V \times \text{DF} \times \text{mcf}}{W} \end{aligned}$$

Where :

- a = concentration of P in sample extract, mg L<sup>-1</sup>
- b = concentration of P in blank, mg L<sup>-1</sup>
- V = volume of extractant, mL
- W = weight of soil sample, g
- DF = dilution factor = total volume of diluted sample solution/aliquot of extract, mL mL<sup>-1</sup>
- mcf = moisture correction factor

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Conversion factor for reporting:  $P_2O_5 = 2.29 \times P$

## 10. Quality Assurance/Quality Control

### 10.1. Accuracy test

- 10.1.1 Participate in an Inter-Laboratory Proficiency Test at least once a year. The PT z-score should be less than 2. If not, identify possible causes, perform corrections or develop corrective action plans to solve the problem. Record the actions taken.
- 10.1.2 Perform replicate analyses of the Certified Reference Material (CRM). Compare the result of your own laboratory with those of other laboratories, as provided in the performance analysis report or the CRM certificate. The result from your own laboratory is considered accurate when it falls within the reported 95 percent confidence interval or the target value.

### 10.2. Precision test

Perform replicate analysis of 10 percent of the samples in a test batch. Calculate the Percent Relative Standard Deviation (RSD percentage) to determine if the precision of replicate analyses is within specifications. Compare the result with the target precision for the analyte concentration (Table 1).

$$\% RSD = \frac{s}{\bar{x}} \times 100$$

Where:  $s$  = standard deviation of the replicate result  
 $\bar{x}$  = mean

Table1. Expected precision (repeatability) as a function of analyte concentration

Analyte, %	Analyte ratio	Unit	RSD, %
100	1	100%	1.3
10	10 <sup>-1</sup>	10%	1.9
1	10 <sup>-2</sup>	1%	2.7
0.01	10 <sup>-3</sup>	0.1%	3.7
0.001	10 <sup>-4</sup>	100 ppm (mg/kg)	5.3
0.0001	10 <sup>-5</sup>	10 ppm (mg/kg)	7.3
0.00001	10 <sup>-6</sup>	1 ppm (mg/kg)	11
0.000001	10 <sup>-7</sup>	100 ppb (µg/kg)	15
0.0000001	10 <sup>-8</sup>	10 ppb (µg/kg)	21
0.00000001	10 <sup>-9</sup>	1 ppb (µg/kg)	30

**Source:** AOAC Peer Verified Methods Program. Manual on Policies and Procedures (AOAC, 1998)

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### 10.3. Control chart

Analyse at least a triplicate of the Quality Control Material or Check Sample for every batch analysis. Plot the result in the control chart. Monitor for out of specified limits. If out of specified limit is observed, identify possible causes, perform corrections or develop corrective action plan, and solve the problem. Record the actions taken.

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## 12. Appendix I – Acknowledgements

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## 14. Appendix III - Contributing laboratories

GLOSOLAN would like to thank the following laboratories for completing the GLOSOLAN form on the method and providing information on their standard operating procedure for soil available phosphorous, Olsen method. This information was used as a baseline for the global harmonization.

From the Asian region:

- Soil Research Directorate, Agriculture Research Institute of Afghanistan, Ministry of Agriculture Irrigation and Livestock, **Afghanistan**
- Soil Resource Development Institute, **Bangladesh**
- Soil and Plant Analytical Laboratory, **Bhutan**
- Royal University of Agriculture, **Cambodia**
- ICAR-Indian Institute of Soil Science, **India**
- International Crop Research Institute for the Semi-Arid Tropics, **India**
- Indonesian Soil Research Institute, **Indonesia**
- Institute for Agro-Environmental Sciences, NARO (NIAES), **Japan**
- Soil and Agrochemistry Laboratory, School of Agroecology, Mongolian University of Life Sciences, **Mongolia**
- Soil, Agro-Chemistry Laboratory of Institute of Plant and Agricultural Sciences, **Mongolia**
- Department of Agricultural Research, **Myanmar**
- Soil Science Division, NARC, **Nepal**
- Fauji Fertilizer Company Limited, **Pakistan**
- Regional Soils Laboratory, Department of Agriculture, Regional Field Office 3, **Phillippines**
- Laboratory Services Division - Bureau of Soils and Water Management, **Phillippines**
- Horticultural Crops Research and Development Institute, Department of Agriculture, **Sri Lanka**
- Office of Science for Land Development, Land Development Department, **Thailand**
- Soils and Fertilizers Research Institute (SFRI), **Viet Nam**

From the Pacific region:

- DES - Chemistry Centre, **Australia**
- Fiji Agriculture Chemistry Laboratory, **Fiji**
- Laboratoire des Moyens Analytiques Nouméa, **New Caledonia** University of the South Pacific, Alafua Campus, **Samoa**
- Scientific Research Organisation of Samoa, **Samoa**

From the Near East and North African region:

- Soil and Fertilizer Laboratory, **Bahrain**
- KIMIA AB Environmental and Agricultural Consulting Laboratory, **Islamic Republic of Iran**
- Soil and Water Research Institute laboratory, **Islamic Republic of Iran**
- Ministry of Science and Technology, Directorate of Agricultural Researches, Soil and Water Resources Centre, **Iraq**
- National Agricultural Research Center, **Jordan**
- Kuwait Institute for Scientific Research, **Kuwait**

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- Lebanese Agricultural Research Institute, **Lebanon**
- Land and Water Research Center, **Sudan**
- Natural Resources, Land Use, Conservation and production Administration Central Laboratory, **Sudan**
- General Commission for Scientific Agricultural Research, **Syrian Arab Republic**

From the African region:

- International Institute of Tropical Agriculture, **Cameroon**
- Laboratoire Central de l'Institut de Recherche Agronomique de Guinée, IRAG, **Guinea Bissau**
- Kenya Agricultural & Livestock Research Organization (KALRO), **Kenya**
- Laboratoire des Radiosotopes, **Madagascar**
- Phosphorus Lab (P-Lab), Department of Soil Science I.A.R., Ahmadu Bello University, **Nigeria**
- National Soil, Fertilizer and Water laboratory, Kaduna, **Nigeria**
- Togolese Agricultural Research Institute, **Togo**
- Fertilizers Seed and Grain, **Zimbabwe**
- Marondera University of Agricultural Sciences and Technology, **Zimbabwe**

From the European region:

- Aarhus University, AGRO University laboratory, **Denmark**
- Natural Resources Institute Finland, **Finland**
- National Research Institute for Agriculture, Food and Environment (INRAE), **France**
- Chemisch Biologisch Laboratorium Bodem, Wageningen University, **Netherlands**
- Laboratório de Solos e Fertilidade da Escola Superior Agrária de Castelo Branco, **Portugal**
- Soil and Fertilizer Laboratory, Dept of Soil Sci and Plant Nutrition, Faculty of Agriculture, Ankara University, TURKEY (SOFREL-TR), **Turkey**
- Soil, Fertilizer and Water Resources Central Research Institute, **Turkey**
- Rothamsted Research, **United Kingdom of Great Britain and Northern Ireland**

From the Eurasian region:

- Scientific-Research Centre of Agriculture, **Georgia**
- Laboratory of Chemical and Biological Factors, **Ukraine**

From Latin America:

- Centro de Estudios Ambientales Integrados, Facultad de Ingeniería, Universidad Nacional de la Patagonia San Juan Bosco, **Argentina**
- Laboratorio de Suelos y Tejidos Vegetales, Universidad de Concepción, **Chile**
- Agencia de Regulación y Control Fito y Zoonosanitario. AGROCALIDAD, **Ecuador**
- Laboratorio Nacional de Suelos, Ministerio de Agricultura, de recursos Naturales y de Desarrollo Rural, **Haiti**
- Colegio de Postgraduados (LABFER-CPM), **Mexico**

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- Soil and water laboratory Comandante Fidel Castro Ruz, Nicaraguan Institute of Agricultural Technology (INTA-Nicaragua), **Nicaragua**

From North America:

- Kellogg Soil Survey Laboratory, **United States of America**

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The Global Soil Partnership (GSP) is a globally recognized mechanism established in 2012. Our mission is to position soils in the Global Agenda through collective action. Our key objectives are to promote Sustainable Soil Management (SSM) and improve soil governance to guarantee healthy and productive soils, and support the provision of essential ecosystem services towards food security and improved nutrition, climate change adaptation and mitigation, and sustainable development.

**GLOSOLAN  
GLOBAL SOIL LABORATORY NETWORK**

GLOSOLAN is a Global Soil Laboratory Network which aims to harmonize soil analysis methods and data so that soil information is comparable and interpretable across laboratories, countries and regions. Established in 2017, it facilitates networking and capacity development through cooperation and information sharing between soil laboratories with different levels of experience. Joining GLOSOLAN is a unique opportunity to invest in quality soil laboratory data for a sustainable and food secure world.

Thanks to the financial support of

