Food and Agriculture Organization of the United Nations

Standard operating procedure for soil available phosphorus

Bray I and Bray II method

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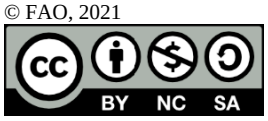

SOIL AVAILABLE PHOSPHORUS
Bray I and Bray II Method

VERSION HISTORY

N°	Date	Description of the modification	Type of modification
01	13 January 2021	All comments by RESOLANs and reviewers to the draft SOP were addressed	Finalization of the SOP
02			
03			
04			

Contents

1. Brief introduction to Bray I and Bray II Method

Phosphorus (P) exists in the soil in the form of organic and inorganic P. The inorganic P forms are more available for plant uptake than the other forms. The 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 occurring in acid soils, and a higher percentage of Ca-P in neutral to alkaline soils (Jones, 2001).

In soil analysis of P, we distinguish two types: a) total analysis and b) extractable analysis. P-Bray 1 and P-Bray 2 correspond to the second type. The fractions of this element present in the soil are 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 have meaning only when they are associated with the plant response.

In the **P-Bray 1 method**, the amount of P is measured using an extracting solution with the combination of 0.03 M NH_4F and **0.025 M** HCl. In acidic soils, phosphorus can be extracted by diluted or strong acid solutions obtaining high correlation values with the plant response. The method presented is close to the original method of Bray 1 P (Bray & Kurtz, 1945). We conserve the soil and extracting solution ratio (1:7) and the concentration of the extracting solution (0.03 M NH_4F + 0.025 M HCl).

The **P-Bray 2 method** was originally designated by Bray and Kurtz (1945) to extract easily acid soluble P as well as a fraction of adsorbed phosphates. It has a similar composition to the Bray I extraction solution, but Bray II has a higher concentration of acid. The method presented is close to the original method of Bray 2 P (Bray & Kurtz, 1945). We conserve the soil and extracting solution ratio (1:7) and the concentration of the extracting solution (0.03 M NH_4F + **0.1 M** HCl).

Both methods consider the deviations from the original method, namely: the shaking method, the optional use of a shaker instead of shaking by hand vigorously, and the reducing agent used in the colorimetric technique ($SnCl_2$ to ascorbic acid). Ascorbic acid produces more stable coloured solutions than $SnCl_2$ (up to 24 hours), and the colour is less affected by interfering substances. The filtration time is adjusted to the type of soil. The changes introduced would increase the range of soils for which the method would be suitable and simplify its adaptation to routine processes.

2. Scope and field of application

P-Bray 1 and P-Bray 2 methods are normally limited to acid soils with water pH values less than 6.8. The P-Bray 1 Method removes a fraction of the “adsorbed” phosphorus (Al-P, Fe-P, Mn-P and Ca-P but less efficient) while the P-Bray 2 Method is best suited to acid soils where rock phosphate has been the primary P fertilizer source and/or the major portion of P exists in the soil in various forms of calcium phosphate. Bray extractants should not be used on alkaline soils because the acid tends to be neutralized and/or excessive calcium phosphates may be extracted, giving a false high test for available P.

3. Principle

The **Bray I method** uses a strong acid in the extracting solution (0.03 M NH_4F + 0.025 M HCl). The high amount of hydrogen ions released by the HCl increases the solubility of calcium phosphates, including basic phosphates such as hydroxyapatite. Under these conditions, it is a fast reaction. Aluminium and iron phosphates are also solubilized, but more slowly than hydroxyapatite. In addition, H^+ ions prevent the precipitation of calcium phosphate. Fluoride ions form complexes with Al^{+3} and Fe^{+3} ions. Fluoride favours the release of these elements that are associated with phosphorus, and are relatively insoluble in water.

These reactions involved in the extraction with Bray I solution are the following:

$$3\ NH_4F + 3HF + AlPO_4 \rightarrow H_3PO_4 + (NH_4)_3AlF_6$$ Aluminium phosphate reaction

$$3\ NH_4F + 3HF + FePO_4 \rightarrow H_3PO_4 + (NH_4)_3FeF_6$$ Iron phosphate reaction

$AlPO_4$ represents the hydrated and hydroxylic phosphates of aluminium, including P adsorbed or precipitated on the surface layers from oxides and aluminosilicates. $FePO_4$ represents iron phosphates and P adsorbed or precipitated on iron oxides. Fluoride ions, in addition, precipitate soluble calcium (CaF_2) extracted from $CaHPO_4$.

The **Bray II method** uses a stronger acid in the extracting solution (0.03 M NH_4F + 0.1 M HCl). As with the Bray I method, the same criteria for soil characteristics apply. The acid HCl concentration is increased to include P that exists in the soil as tricalcium phosphate. At the time this procedure was developed, farmers in the Midwest (Dr. Bray was a Professor at the University of Illinois) were using rock phosphate, which is tricalcium phosphate, as a P fertilizer source. Therefore, P that exists in the soil in this form will be included in the extract using the Bray II extraction reagent.

Both methods use a colorimetric determination of phosphorus using a molybdenum blue complex. This complex is a heteropoliacid. It results in the reaction of the orthophosphate ion with the molybdate ion in an acidic medium. For the blue colour (molybdenum blue), the heteropoliacid must be partially reduced with ascorbic acid or tin as a catalyst.

$$\underset{\text{yellow}}{H_3PO_4} + 12\ H_2MoO_4 \rightarrow \underset{\text{blue}}{H_3P(Mo_3O_{10})_4} + 12\ H_2O$$ (acidic medium)

4. Apparatus

Both Bray I and Bray II methods use the same apparatus in the analysis.

4.1 Erlenmeyer flask, 125 mL

4.2 Funnel

4.3 Beaker; 5L, 1L, 500 mL

4.4 Polyethylene or glass bottles with lid, wide-mouth type, 50 mL capacity

4.5 Volumetric flasks, 1L, 500 mL, 250 mL, 100 mL

4.6 Graduated cylinder, 100 mL, 50 mL

4.7 Test tubes, 20 mL capacity

4.8 Autopipette; 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 (optional)

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^{-1} (ASTM D1193-91 and ISO 3696:1987).

5.2 Ammonium fluoride solution, 1 M. Dissolve 3.70 g NH_4F in deionized/distilled water and make up to 100 mL (store in polyethylene bottle).

5.3 Hydrochloric Acid, 0.5 M. Dilute 8.3 mL 6 M HCl (or 4.3 mL conc. HCl, 37% or 12 M) to 100 mL with deionized/distilled water.

5.4 **Extracting solution Bray 1** (0.03 M NH_4F and 0.025 M HCl). Add 15 mL 1 M NH_4F and 25 mL 0.5 M HCl to a 500 mL volumetric flask and adjust the volume with deionized/distilled water. This solution is stable for more than one year.

5.5 **Extracting solution Bray 2** (0.03 M NH_4F and 0.1 M HCl). Add 15 mL 1 M NH_4F and 100 mL 0.5 M HCl to a 500 mL volumetric flask and adjust the volume with deionized/distilled water. This solution is stable for more than one year.

5.6 Ammonium molybdate solution, 4%. Dissolve 4 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in deionized/distilled water and make up to 100 mL. Store in polyethylene or glass bottle.

5.7 Potassium antimony tartrate solution, 0.275% (1000 mg L^{-1} Sb). Dissolve 0.275 g $KSbOC_4H_4O_6{\cdot}1/2\ H_2O$ in deionized/distilled water and make up to 100 mL.

5.8 Ascorbic acid solution, 1.75%. Dissolve 1.75 g ascorbic acid in deionized/distilled water and bring the volume to 100 mL. Prepare fresh daily.

5.9 Sulfuric Acid, 2.5 M. Slowly add 35 mL conc. H_2SO_4 (18 M or 96%) to 150 mL deionized/distilled water under constant stirring. After cooling, make up to 250 mL with water.

5.10. Mixed reagent, prepare fresh daily. Add successively with a graduated cylinder to a 500 mL beaker and homogenize after each addition:

- 200 mL of deionized/distilled water
- 50 mL of 2.5 M sulfuric acid
- 15 mL of ammonium molybdate solution
- 30 mL of ascorbic acid solution
- 5 mL of potassium antimony tartrate solution

5.11 Boric acid solution, 1%. Dissolve 1 g of H_3BO_3 in 100 mL deionized/distilled water.

5.12 Standard Phosphate Solution, 100 mg P L^{-1}. Pipette 50 mL of NIST or other equivalent traceable 1000 mg P L^{-1} phosphorus stock solution into a 500 mL volumetric flask and make up to volume with extracting solution.

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

5.13 Secondary standard phosphate solution, 12 mg P L^{-1}. Pipette 30.0 mL of the 100.0 mg P L^{-1} standard solution into a 250 mL volumetric flask and make up to the final volume with the extracting solution.

5.14 Working phosphate standard series. Pipette into 100 mL volumetric flasks 0, 10, 20, 30, 40, 50 mL of the 12 mg P L^{-1} standard solution. Make up to the final volume with extracting solution. The standard series is then 0, 1.2, 2.4, 3.6, 4.8, 6.0 mg P L^{-1}.

Remarks:

1. The range of working phosphate standards can be adjusted according to the equipment specifications and the concentration of P in the analysed soil samples.
2. Excess fluorides produce negative interference in the molybdenic-blue reaction. Boric acid is added before the colour development to remove this interference. The boric acid reacts with the fluoride, and a fluoroborate is formed by the following reaction (Kurtz, 1942).

$$4F^- + H_3BO_3 + 3H^+ \rightarrow (BF_4)^- + 3H_2O$$

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.

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 when 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 operation under a fume hood to avoid inhalation of the fumes.

6.2.2 The 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 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 such as oxidizing agents.

6.2.4 Potassium antimony tartrate is hazardous in case of skin or body contact. Do not discharge the waste into the drain. It is incompatible with strong acids, strong bases and strong oxidizers. Do not put in direct sunlight.

7. Sample preparation

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

8. Procedure

8.1. Preparation of standards curve

From the working standards prepared as describe in *section 5.12–5.14*, pipette into test tubes 1.0 mL of standard series. Add 2.0 mL of boric acid solution and 3.0 mL mixed reagent. Homogenize using a vortex mixer and allow solutions to stand for at least 60 mins to allow the blue colour to develop to its maximum.

8.2. Preparation of samples

8.2.1 Weigh 2 g of air-dry (accuracy, 0.01g) soil into a wide-mouth 50 mL capacity shaking bottle. Include at least two blanks and two quality control materials (QCMs) or check samples.

8.2.2 Add 14 mL of extracting solution and place the bottle caps.

8.2.3 Shake by hand vigorously for 1 min (a reciprocating shaker may be used for shaking at 180-200 oscillations/min, with shaking bottles placed horizontally) then filter immediately through a Whatman No. 42 or equivalent acid-treated, identical porosity filter paper.

8.2.4 Filter the samples until enough filtrate is collected for analysis. In case the filtrate is turbid, filter again through the same filter.

8.2.5 Pipette into test tubes 1 mL of the blanks, QCMs and the sample extracts. Add 2 mL boric acid and 3 mL mixed reagent. Homogenize using a vortex mixer.

8.2.6 Allow solutions to stand for at least 60 mins to allow the blue colour to develop to its maximum.

Remarks:

1. The analysis must be carried out at room temperature, between 20 and 25 °C.
2. With the acid molybdate solution, phosphate forms phospho-molybdenic acid which is reduced to phospho-molybdenic-blue with ascorbic acid. The antimony accelerates the development of the blue colour and stabilizes it for up to 24 hours. With this method, no interference of Si is to be expected. Should such interference still occur (blue coloured zero standard), then the procedure would have to be repeated using deionized water/distilled water (Van Reeuwijk, 2002).

8.3 Measurement

For the **Bray I and Bray II method**, read the absorbance of the calibration standards and samples in a spectrophotometer set at 882 nm wavelength.

When the correlation coefficient of the calibration curve is equal to or greater than 0.995, analyse the samples. Otherwise, verify that the standards and reagents were correctly prepared, the instrument functioning properly, and that 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^{-1} with the computation given in *section 9* and report as oven-dry basis with two (2) decimal places.

9. Calculation

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

$$= (\text{a} - \text{b}) \times \frac{\text{V} \times \text{DF} \times \text{mcf}}{\text{W}}$$

Where:

a = concentration of P in sample extract, mg L^{-1}
b = concentration of P in blank, mg L^{-1}
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^{-1}
mcf = moisture correction factor

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 the root cause, perform the correction and develop a corrective action plan to address the problem. Record the actions taken.

10.1.2 Perform replicate analyses of the Certified Reference Material (CRM). Compare the results of your own laboratory with those of other laboratories, as provided in the performance analysis report, or CRM certificate. The result from your own laboratory is considered accurate when it falls within the reported 95% confidence interval or the target value.

10.2. Precision test

Perform replicate analysis of 10% of the samples in a test batch. Calculate the Percent Relative Standard Deviation (%RSD) 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
x̄ = mean

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

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

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

10.3. Control chart

Analyse at least a duplicate of the Quality Control Material or Check Sample for each batch analysis. Record the result in the control chart. Monitor for out of specified limits. If out of specified limit is observed, identify the root cause, perform the correction and develop a corrective action plan, and address 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, Bray I and Bray II methods. This information was used as a baseline for the global harmonization.

From the Asian region:

- Bangladesh Soil Resource Development Institute, **Bangladesh**
- Soil & Plant Analytical Laboratory, **Bhutan**
- Royal University of Agriculture, **Cambodia**
- ICAR-Indian Institute of Soil Science, **India**
- Indonesian Soil Research Institute, **Indonesia**
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- Environmental Analytical Laboratory (CSIR-SARI), **Ghana**
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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.

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