4 Protocol for bicarbonate extractable P (Pt or P-number)

4.1 Principle

Phosphorus is extracted from soil with a sodium bicarbonate solution at pH 8.5 for exactly 30 minutes at 20°C ± 1°C, after which soil and solution are immediately separated. In the clear filtrate/supernatant, the concentration of the blue phosphomolybdate complex is measured by spectrophotometry after adding sulphuric acid, ascorbic acid, and ammonium molybdate reagent to the extract.

This method extracts only a modest proportion of soil total P and can therefore be very sensitive to small deviations in extraction time and temperature and intensity of shaking. It is therefore very important that the temperature should be kept at 20°C ± 1°C from initiation of the extraction until soil and solute are separated. The bicarbonate extractant can produce coloured soil extracts, which may precipitate upon acidification of the extract during the colorimetric determination of P. These problems are handled by addition of polyacrylamide to the extracting solution as described by Banderis et al. (1976).

4.2 Apparatus

- Rotating shaking apparatus “end-over-end”, shaking intensity  20 ± 2 rounds per minute.
- Scale for measuring 1-5 grams with two decimal places.
- Analytical scale with 5 decimal places.
- Acid-washed bottles and lids and glassware (or similar of material suitable for soil and analysis of phosphorus i.e. materials which do not adsorb phosphate).
- Spectrophotometer or similar for determination of light absorbance at wavelength 880 nm.

4.3 Reagents

All reagents shall be analytically graded and water should be purified (resistivity at 25°C of maximum 18.2 MΩ cm.

4.3.1 4M sodium hydroxide solution

Dissolve 160.0 g sodium hydroxide (NaOH) pellets in 700 ml water. Cool and dilute to 1000 ml with water.
Store the solution in an inert and hermetically sealed bottle.

4.3.2 Polyacrylamide solution

Polyacrylamide (Granular powder MW over 5,000,000, BDH Laboratory supplies prod. no. 297883N or similar) approx. 0.05% water solution. Dissolve 0.10 g polyacrylamide in 200 ml water. Note that it takes several hours to dissolve the polyacrylamide.
4.3.3 Extracting solution
Dissolve 210 g of sodium hydrogen carbonate (NaHCO₃) in 4500 ml water. Add 25 ml of the polyacrylamide solution (4.3.2). Adjust the pH to 8.50 ± 0.02 with the 4.0 M sodium hydroxide solution (4.3.1) while stirring. Add water to 5000 ml volume. The solution should be prepared and sealed within 10–15 minutes. If hermetically sealed, it can be kept for weeks. However, pH should be controlled daily and a new solution should be prepared if pH deviates from 8.50 ± 0.04.

4.3.4 4M Sulphuric acid
In a fume hood, pour ca. 350 ml of water into >1000 ml container, add 110.0 ml concentrated (95-97%) sulphuric acid (H₂SO₄) while stirring, cool to room temperature, and add up to 500 ml volume.

4.3.5 0.1M Sulphuric acid
Dilute 4.0 M sulphuric acid (4.3.4) 40 times with water, by adding 25 ml 4.0 M sulphuric acid to approx. 900 ml water and fill up to 1000 ml volume with water.

4.3.6 Ammonium molybdate potassium antimonyl tartrate solution (Sulfomolybdic reagent)
a. Dissolve 13.0 g ammonium heptamolybdate-tetrahydrate ((NH₄)₆Mo₇O₂₄ • 4H₂O) in 100 ml water
b. Dissolve 0.35 g potassium antimonyl tartrate (K(SbO)C₄H₄O₆ • 0.5 H₂O) in 100 ml water
c. In a fume hood, add approx. 120.0 ml concentrated sulphuric acid (95-97%) into approx. 170 ml water while stirring, and cool to room temperature. Mix solution “a” and “b” into the diluted sulphuric acid and fill up to 500 ml with water. Store the reagent cool (2-5°C) and protect against sunlight.

4.3.7 Ascorbic acid solution
Dissolve 5.00 g ascorbic acid (C₆H₈O₆) in water and dilute to 100 ml volume.

4.3.8 Stock solution
Stock solution of 200 mg P/l. Dissolve 1.7573 ±0.0002 g dried potassium dihydrogen phosphate (KH₂PO₄) in 2000 ml volume of 0.1 M H₂SO₄ (4.3.5).

4.3.9 Standard solutions
Prepare standard solutions with concentrations of PO₄-P ranging from 0 to 8 ppm as suggested in table A4 by appropriate dilution of the stock solution with the extracting solution (4.3.3).
Table A2: Concentrations of P in standard curve solutions and the amount of stock solution (4.3.8) to transfer to 100 volumes to obtain these concentrations.

<table>
<thead>
<tr>
<th>PO4-P concentration (mg/l)</th>
<th>Amount of stock solution (3.8) (µl) to dilute with extracting solution (3.3) to 100 ml volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>50</td>
</tr>
<tr>
<td>0.2</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>250</td>
</tr>
<tr>
<td>1.0</td>
<td>500</td>
</tr>
<tr>
<td>3.0</td>
<td>1500</td>
</tr>
<tr>
<td>5.0</td>
<td>2500</td>
</tr>
<tr>
<td>8.0</td>
<td>4000</td>
</tr>
</tbody>
</table>

4.4 Procedure

4.4.1 Extraction
Weigh 5.0 ±0.1 g soil (dried at max. 40°C until constant weight, sieved <2.0 mm and mixed) into 250 ml flask or container. Alternatively, weigh 5 ±0.30 g dried soil (with two decimals precision) and correct the final result for the actual amount of soil used in the extraction). In special cases with little soil available for the extraction, the amount of soil can be down-scaled to 1.00 g soil, but soil weight to container volume ratio has to be 1:50 and soil to solution ratio has to be 1:20 (w:v). The temperature of the mixture of soil and extractant (4.3.3) has to be 20°C ± 1°C from the start of the extraction to end of separation. Close flasks immediately and mount them on the shaker for exactly 30 minutes at 20 ± 1°C. Separation of soil and solute by either centrifugation of samples at minimum 1800 g for 5 minutes at 20 ± 1°C or by filtration. Handling time between end of shaking and end of separation must not exceed 30 minutes. Suspensions which are not immediately separated must not be re-suspended prior to separation. When separation is carried out by filtration, the first milliliters of filtrate should be discarded.

Prepare blanks following the same procedure, but excluding soil.

4.4.2 Measurement
Transfer 1 ml of extract quantitatively to a beaker large enough to handle foaming and bubbles upon acidification (25 ml Erlenmeyer flasks work well. Handling in racks makes work easier). Add 9 ml of water and 125 µl 4.0 M H₂SO₄ (4.3.4). Swing flask and leave for CO₂ evolvement and foaming to cease. Then add 400 µl ascorbic acid solution (4.3.7) and swing. Add 400 µl of the sulfomolybdic solution (4.3.6) and swing.

A standard curve is produced by transferring 1 ml of each standard solution and adding water, acid, and reagents the same way as to the samples.
Flasks are left for 10-15 minutes at room temperature for color development to complete. The blue color is typically stable for up to 24 hours. The color intensity of the samples and standards are measured on a spectrophotometer at 880 nm. Use the zero standard for setting zero. A path length of 1 cm of the cuvette is appropriate for most measurements, but at concentrations of less than 0.25 mg/l the path length should be 4 cm or more. Make sure that bubbles of CO_2 do not obstruct the measurement.

If blanks do not produce zero absorbance or very close to (less than 0.004), the analysis should be repeated. A thorough check for contamination of reagents, bottles, and glassware can be necessary.

If the soil extract is highly colored, it should be tested if this color absorbs light at 880 nm and if it does corrections for this absorbance will be necessary.

Automated procedures for measurements are accepted, as long as they rely on the principle described above of measuring the intensity of the blue color developed after addition of the above-mentioned reagents.

### 4.5 Calculations

Carry out a linear regression of measured absorbance of standard solutions against their known concentrations of P according to this equation:

\[
Abs_{st} = \alpha * C_{Pst}
\]

Where:

- \(Abs_{st}\) is the measured absorbance for each standard solution,
- \(C_{Pst}\) is the known P concentration in each standard solution,
- \(\alpha\) is the constant derived from the regression line crossing the origin.

P concentration in the soil extracts can then be calculated as:

\[
P_{\text{cons, extract}} = (Abs_{\text{sample}} - Abs_{\text{blank}})/\alpha
\]

The amount of bicarbonate-extractable P in mg P kg\(^{-1}\) dry soil can then be calculated as:

\[
P_{\text{extracted}} = P_{\text{cons, extract}} \times 20
\]

Detection area is 2 to 160 mg Olsen P kg\(^{-1}\) soil.
If the result is requested as the Danish Ptal, the result should be divided by 10 and the unit is then mg P extracted per 100 g of soil.

4.6 Repeatability

Reference soils should be included in every analytical run. The standard deviation of independent measurements on the reference soils measured at different times in the same laboratory with the same equipment should be less than 10% of the measured value or less than 2 mg P kg\(^{-1}\) soil (0.2 P-tal units).

4.7 Test report

A test report shall contain the following:

a. A reference to this method description
b. All information necessary for complete identification of the sample
c. Results of the determination in whole numbers in milligram per kilogram calculated on the basis of dried sieved soil (dried at max. 40°C)
d. Any details of operations not specified in this method description as well as any other factors, which may have affected the results.

4.8 Comments

This method description is an update of the former Danish method description (Plantedirektoratet, 1994) and of the preliminary protocol published in Rubæk (2015). It corresponds in major aspects to the ISO 11263:1994 and to the original method description by Olsen et al. (1954).

4.9 References


5 References

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