Appendix 1 to CSG15 for OF0114

Details of analytical methods

Available P in soil (Olsen P)

Principle

Phosphate ions (HPO_4 and H_2PO_4) are present in soils in association with calcium, aluminium and ferric ions. If the concentrations of these metallic ions is reduced, the concentrations of phosphate ions increases, in order to maintain the various solubility products at their constant values.

An alkaline (pH 8.5) bicarbonate solution can repress the concentration of calcium ions by precipitation as calcium carbonate and of aluminium and ferric ions by precipitation as hydroxides. Thus, phosphate ion concentrations are increased and available phosphate can be extracted from soil by shaking with alkaline sodium bicarbonate. The extraction is dependent on time of contact and temperature.

A disadvantage of this extraction procedure in dissolving organic matter from humus rich soils can be overcome by treating the solution with active charcoal during the shaking or after filtration.

After acidifying the bicarbonate solution, phosphorus is determined by the phospho-molybdenum blue method on the Skalar SAN^{PLUS} System (continuous colorimetric flow analysis).

Apparatus

1 Temperature controlled orbital shaker
2 Polythene bottles 100ml
3 Polythene filter funnels
4 250ml glass conical flasks
5 30ml glass vials
6 42 Whatman filter papers (150mm)

Reagents

1 0.5M sodium bicarbonate solution: dissolve 42g sodium bicarbonate ($NaHCO_3$) and 0.72g sodium hydroxide pellets in 900ml deionised water. Adjust the pH to 8.5 using a saturated solution of sodium hydroxide or concentrated sulphuric acid and make up to 1 litre with water. Mix thoroughly.
2 Activated charcoal

Procedure

1 Weigh 5g of air dried soil, ground to pass a 2mm sieve, into a weighing boat and transfer to a 250ml conical flask.
2 Add approximately 0.1g activated charcoal (tip of a spatula) and cover each flask with cling film.
3 Peel back the cling film and add 100ml 0.5M sodium bicarbonate, reseal, swirl the flask and immediately place on the shaker. These steps must be carried out speedily to avoid delays, which may vary the contact time.
4 Shake the flask in a temperature controlled cabinet at 20°C for exactly 30 mins at 120 rpm.
5 Swirl the flask (to mix the extract and soil) and filter immediately through a Whatman no. 42 filter paper discarding the first 10ml of filtrate. Remove the filter paper 45 minutes after discarding the first 10ml.

IT IS ESSENTIAL TO KEEP THE CONTACT TIME CONSTANT FOR ALL RUNS

6 After filtration the sample should be analysed immediately. DO NOT STORE THE FILTRATE.

7 If the extracts are highly coloured, shake the filtered extract with 1g of activated charcoal and filter through a Whatman no. 42 filter paper.

8 Blanks and in-house standard materials should be included in each batch shaken.

9 Duplicate extractions of at least one in every ten samples should also be included.

10 Sample extracts are analysed on the Skalar continuous flow system.

References

S R OLSEN *et al*, *USDA Circular 939* 1954

Ortho-Phosphate determination by continuous flow colorimetric analysis (Skalar SAN^{PLUS}) **0.1 – 5 ppm P (Olsen) 5 – 50 ppm P**

Principle

In an acidic medium, ammonium molybdate and potassium antimony tartrate react with diluted solutions of phosphate to form an antimony-phospho-molybdate complex. This is reduced with ascorbic acid to form a mixed valence blue coloured complex. The complex is measured at 880nm.

Samples deteriorate quickly and should be run on the same day as extraction.

The presence of copper up to 10 mgl^{-1}, Ferric iron up to 40 mgl^{-1} and silica up to 10 mgl^{-1} do not interfere. Turbid samples should be filtered before determination.

Standard Solutions

1 Potassium dihydrogen phosphate dried at 110°C for 1 hour and stored over a dehydrating agent in a desiccator.

2 1000 ppm stock P solution: dissolve 4.3941g potassium dihydrogen phosphate in 1000ml. Store in a refrigerator.

3 Working standards: dilute various aliquots of 1000 ppm PO_4-P stock solution to produce standard solutions covering the concentration range of PO_4-P expected in the sample.

Reagents

1 0.1N Sodium Hydroxide solution (Olsen extracts only)

Sodium hydroxide (NaOH)	4g
Deionised water	1000ml
FFD6	2ml

2 Sulphuric acid solution (all other PO_4-P extracts)

Sulphuric acid (H_2SO_4 97%	40ml
Deionised water	1000ml

3 Deionised water and FFD6

Deionised water	1000ml
FFD6	2ml

4 Ammonium molybdate solution

Sulphuric acid (H_2SO_4 97%)	40ml
Ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$	4.8g
Deionised water	1000ml
FFD6	2ml

Dilute the sulphuric acid in ± 800ml deionised water. Add the ammonium molybdate and dissolve. Make up to 1 litre and add the FFD6.

5 Stock solution potassium antimony

Potassium antimony tartrate $K(SbO)C_4H_4O_6.0.5H_2O$	300mg
Deionised water	100ml

Dissolve the potassium anitmony tartrate in ± 800ml of deionised water, make up to the mark and mix. The solution is stable for 1 month at 4°C.

6 Ascorbic acid solution

Ascorbic acid $C_6H_8O_6$	18g
Stock solution potassium antimony tartrate	20ml
Deionised water	1000ml

Dissolve the ascorbic acid in ± 800ml deionised water, add the stock solution and make up to 1 litre. The solution is stable for one week at 4°C.

Reagents for Analysis of Other extracts

1 As above but substitute the wash solution with 2M $NaHCO_3$ for Olsen extracts, 2% acetic acid for plant extracts or 0.5% HCl for aqua regia digests of drainage water.

2 Working standards: dilute 1000ppm stock PO_4-P with extracting solutions as above.

Instrument Parameters

1 The sensitivity of the highest standard 5 ppm P is $\pm$ 800 A.U.

2 Sample time: 30 sec., wash time: 60 sec., air time: 0 sec.

3 Connection between the sampler and the sample pump tube is made of 5141 tube.

4 Filter: 880nm, correction filter: 1010nm

5 Flowcells – 0.01 – 5 ppm P – 5cm
5 – 50 ppm P – 1cm

6 Decontaminate the system with 1:10 diluted hypochlorite solution for half an hour when required.

7 If the sample take up is less than 1 ml/min, use a bypass to increase the pull through to 1 ml/min.

References

1 Standard Methods for Examination of Water and Waste water. 15th edition 1980 APHA-AWWA-WPCF page 410-425.

2 D F BOLTZ & M G MELLON Spectophotometric determination of phosphorus as molydiphosphoric acid. *Analytical Chemistry*, Vol. 20, No. 8, August 1948, page 749-751.

3 I WALINGA, W van VARK, V J G HOUBA & LL van der LEE., Plant analysis procedures, Part 7. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University Syllabus 1989, page 138-141.

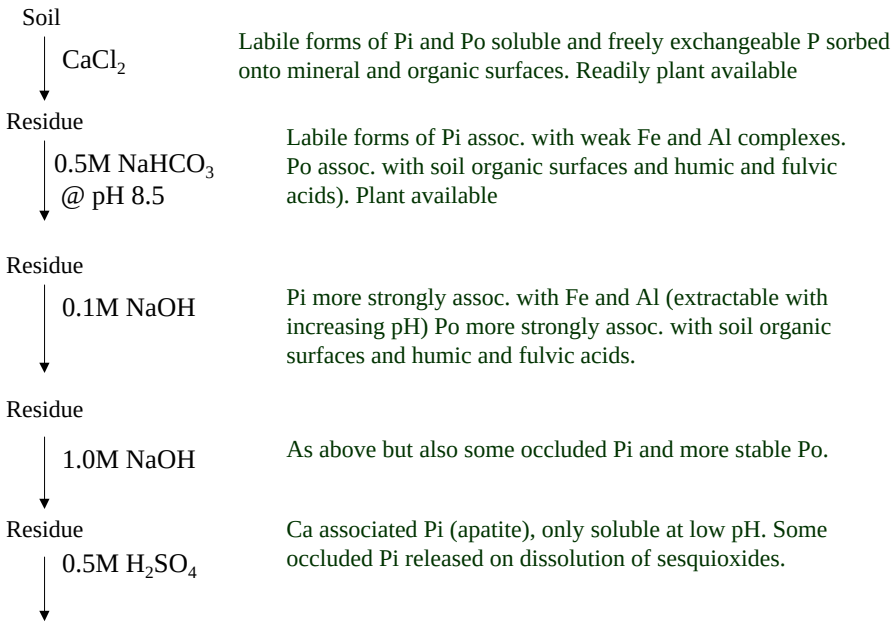
Reserves of P in soil (Hedley fractionation)

All glassware was treated in a 5% HCl v/v acid bath and then rinsed with deionised water prior to use to remove and adsorbed phosphate.

Principle

The $CaCl_2$ extractant removes the most labile phosphate in the soil
The $NaHCO_3$ extractant removes the most labile inorganic phosphate associated with weak Fe and Al-P complexes and organic phosphate associated with soil organic surfaces and humic and fulvic acids. Digestion with potassium persulphate converts organic phosphate to inorganic forms, hence a total phosphate value can be determined. P total – P inorganic = P organic.
The NaOH extract removes inorganic phosphate more strongly associated with Fe and Al-p complexes extracted with increasing pH and organic phosphate more strongly associated with soil organic surfaces and humic and fulvic acids. Digestion with potassium persulphate converts organic phosphate to inorganic forms, hence a total phosphate value can be determined. P total – P inorganic = P organic.
The H_2SO_4 extract removes Ca associated phosphate which is only soluble at low pH. There is some occluded phosphate on the dissolution of sesquioxides. There is little or no organic phosphate extracted.

Figure – Sequential extraction of pools of P from soil



Reagents.

Solution 1 – Ammonium molybdate stock solution.

Add 800 ml deionised water to 1 litre volumetric flask, add 40 ml of concentrated sulphuric acid.
Weigh out 4.8 g ammonium molybdate and add and dissolve in solution.
Make the volume to 1 litre with deionised water.
Note: Do not use metal spoons for ammonium molybdate.

Solution 2 – Potassium antimony tartrate stock solution.
Weigh 0.3000 g of potassium antimony tartrate and dissolve in 80 ml of deionised water in a 100 ml volumetric flask. Make up to 100 ml with deionised water.
Note: This solution is stable for 1 month at 4°C.

Solution 3 – Ascorbic acid solution.
Weigh out 18 g *L*-ascorbic acid and dissolve in 800 ml of distilled water. Add 20 ml of the potassium antimony tartrate solution (solution 2). Make up to 1 litre.
Note: This solution is stable for one week at 4°C.

Solution 4 – Colour developing solution.
Take 50 ml of the ammonium molybdate solution and add to 50 ml of the ascorbic acid solution. Mix well and prepare immediately prior to use.
Note: This solution should be used within 24 hrs. Store in a dark cool place.

Solution 5 – Stock solution 100 ppm P.
Accurately weigh 0.4394 g of potassium dihydrogen ortho-phosphate. Dissolve in 800 ml deionised water in a 1 litre volumetric flask mix well.

Solution 6 – Sulphuric acid.
Add 800 ml of deionised water to a 1 litre volumetric flask. Add 40 ml of concentrated sulphuric acid. Mix and make up to 1 litre.

Solution 7 – Calcium chloride.
Add 800 ml of deionised water to a l litre volumetric flask and add 2.1908 g of $CaCl_2.6H_2O$. Mix and make up to 1 litre.

Solution 8 – Olsen's reagent.
0.5M sodium bicarbonate solution: dissolve 42 g sodium bicarbonate ($NaHCO_3$) and 0.72 g sodium hydroxide pellets in 900 m1 deionised water. Adjust the pH to 8.5 using a saturated solution of sodium hydroxide or concentrated sulphuric acid and make up to 1 litre with water. Mix thoroughly.

Solution 9 – Sodium Hydroxide.
Add 800 ml of deionised water to a l litre volumetric flask and add 20.0000 g of NaOH. Mix and make up to 1 litre. (0.5 M)

Solution 10 – Sulphuric acid.
Prepare a 1 litre solution of 0.5 M solution sulphuric acid by dilution of concentrated sulphuric acid.

Working standards
4 ppm P : Dilute 4 ml of stock solution to 100 ml with deionised water.
3 ppm P : Dilute 3 ml of stock solution to 100 ml with deionised water.
2 ppm P : Dilute 2 ml of stock solution to 100 ml with deionised water.
1 ppm P : Dilute 1 ml of stock solution to 100 ml with deionised water.
0 ppm P: Deionised water.

Day 1
Accurately weigh 0.500 g of sample into a 50 ml centrifuge tube.
Add 30 ml of 0.01 M $CaCl_2$ solution to the tube.
Place the tube on a rotary shaker for 16 hours.

Day 2

Centrifuge the sample at 10,000 rpm for 15 minutes at 0°C. If the supernatant is not free of particulates centrifuge for a further 15 minutes.

Whilst the sample is centrifuging make up fresh 1 - 4 ppm P standards. Take 1, 2, 3 and 4 ml of the 100 ppm stock solution and place in 100 ml volumetric flasks. Make up to volume with deionised water shaking to ensure thorough mixing.

P Determination.

Samples

10 ml of supernatant, place in a 50 ml volumetric flask.
1 ml deionised water.
8 ml sulphuric acid solution (40 ml conc./ l).
8 ml of developing solution.

Standard

3 ml of standard solution.
8 ml of deionised water.
8 ml sulphuric acid solution 40 ml conc./ l).
8 ml of developing solution.

Place the flasks in a 70°C water bath for 10 minutes, remove and allow to cool.
Measure the absorption at 880 nm in a 50 mm cell against a water blank.

Decant the remaining supernatant. Add 30 ml of Olsen's reagent (0.5 M $NaHCO_3$ + NaOH pH 8.5). Place the tube on a rotary shaker for 16 hours.

Day 3

Centrifuge the sample at 10,000 rpm for 15 minutes at 0°C. If the supernatant is not free of particulates centrifuge for a further 15 minutes. Whilst the sample is centrifuging make up fresh 1 - 4 ppm P standards. (Take 1, 2, 3 and 4 ml of the 100 ppm stock solution and place in 100 ml volumetric flasks). Make up to volume with deionised water shaking to ensure thorough mixing.

P Determination.

Samples

3 ml of supernatant, place in a 50 ml volumetric flask.
6 ml deionised water.
10 ml sulphuric acid solution (40 ml conc./ l).
8 ml of developing solution.

Standards

3 ml of standard solution.
8 ml of deionised water.
8 ml sulphuric acid solution 40 ml conc./ l).
8 ml of developing solution.

Place the flasks in a 70°C water bath for 10 minutes, remove and allow to cool.
Measure the absorption at 880 nm in a 50 mm cell against a water blank.

Take 5 ml of the supernatant and place in a 50 ml volumetric flask. Add 10 ml of 0.9 M sulphuric acid solution and 0.5 g of potassium persulphate. Loosely stopper the flask. Place the flask on a hot plate and bring the mixtures up to 100 °C for 90 minutes. Allow the sample to cool before P determination.

P Determination.

Samples

3 ml of reacted supernatant, place in a 50 ml volumetric flask.
12 ml deionised water.
4 ml sulphuric acid solution (40 ml conc./ l).
8 ml of developing solution.

Standards

3 ml of standard solution.
8 ml of deionised water.
8 ml sulphuric acid solution 40 ml conc./ l).
8 ml of developing solution.

Place the flasks in a 70°C water bath for 10 minutes, remove and allow to cool.
Measure the absorption at 880 nm in a 50 mm cell against a water blank.

Decant the remaining supernatant from the centrifuge tubes. Add 30 ml of 0.5 M NaOH. Place the tube on a rotary shaker for 16 hours.

Day 4

Centrifuge the sample at 10,000 rpm for 15 minutes at 0°C. If the supernatant is not free of particulates centrifuge for a further 15 minutes. Whilst the sample is centrifuging make up fresh 1 - 4 ppm P standards. (Take 1, 2, 3 and 4 ml of the 100 ppm stock solution and place in 100 ml volumetric flasks). Make up to volume with deionised water shaking to ensure thorough mixing.

P Determination.

Samples

3 ml of supernatant, place in a 50 ml volumetric flask.
6.5 ml deionised water.
9.5 ml sulphuric acid solution (40 ml conc./ l).
8 ml of developing solution.

Standards

3 ml of standard solution.
8 ml of deionised water.
8 ml sulphuric acid solution 40 ml conc./ l).
8 ml of developing solution.

Place the flasks in a 70°C water bath for 10 minutes, remove and allow to cool.
Measure the absorption at 880 nm in a 50 mm cell against a water blank.

Take 5 ml of the supernatant and place in a 50 ml volumetric flask. Add 10 ml of 0.9 M sulphuric acid solution and 0.5 g of potassium persulphate. Loosely stopper the flask. Place the flask on a hot plate and bring the mixtures up to 100 °C for 90 minutes. Allow the sample to cool before P determination.

P Determination.

Samples

3 ml of reacted supernatant, place in a 50 ml volumetric flask.
10 ml deionised water.
6 ml sulphuric acid solution (40 ml conc./ l).
8 ml of developing solution.

Standards

3 ml of standard solution.
8 ml of deionised water.
8 ml sulphuric acid solution 40 ml conc./ l).

8 ml of developing solution.

Place the flasks in a 70°C water bath for 10 minutes, remove and allow to cool. Measure the absorption at 880 nm in a 50 mm cell against a water blank.

Decant the remaining supernatant from the centrifuge tubes. Add 30 ml of 0.5 M H_2SO_4. Place the tube on a rotary shaker for 16 hours.

Day 5

Centrifuge the sample at 10,000 rpm for 15 minutes at 0°C. If the supernatant is not free of particulates centrifuge for a further 15 minutes. Whilst the sample is centrifuging make up fresh 1 - 4 ppm P standards. (Take 1, 2, 3 and 4 ml of the 100 ppm stock solution and place in 100 ml volumetric flasks). Make up to volume with deionised water shaking to ensure thorough mixing.

P Determination.

Samples

3 ml of supernatant, place in a 50 ml volumetric flask.
11 ml deionised water.
5 ml sulphuric acid solution (40 ml conc./ l).
8 ml of developing solution.

Standards

3 ml of standard solution.
8 ml of deionised water.
8 ml sulphuric acid solution 40 ml conc./ l).
8 ml of developing solution.

Place the flasks in a 70°C water bath for 10 minutes, remove and allow to cool. Measure the absorption at 880 nm in a 50 mm cell against a water blank.

Modified bicarbonate extractions and determination of extracted organic P

Reagents

0.5M sodium bicarbonate solution:
Dissolve 42 g sodium bicarbonate ($NaHCO_3$) and 0.72 g sodium hydroxide (NaOH) pellets in 900 ml of de-ionised water. Adjust the pH to 8.5 using a saturated solution of NaOH or concentrated H_2SO_4 and make up to 1 litre with de-ionised water.

Oxidising solution:
Freshly prepared solution containing 13.4g di-potassium peroxodisulphate (potassium persulphate) dissolved in 1 litre 0.3*M* sodium hydroxide (NaOH).

Standards were made up using potassium hydrogen phosphate (K_2HPO_4) – 0.5 and 1 mg dm^{-3} P

Methods

The soils were extracted using either the standard Olsen-P extraction method or a modified 16hr $NaHCO_3$ extraction method.

Olsen extraction:
5g air dried soil (<2mm sieved) was shaken with 100ml $NaHCO_3$ for 30 minutes at 20°C. (See full method, page 1).

16hr extraction:
The method used for this extraction procedure was based on the Hedley fractionation (Tiessen *et al.,* 1984, Cross and Schlesinger, 1995). For the Hedley fractionation, 0.5 g soil is shaken with 30 ml. We used 2 g of soil and 120 ml in order to give us better replication. The soil was only 2 mm sieved to allow comparison with the standard Olsen; this is in contrast to a lot of the fractionation methods which use more finely sieved soil. The soil was shaken for 16 hrs at 20°C and then filtered in the same way as the Olsen extracts.

Oxidation procedure:
Aliquots (5 ml) of solution were transferred to autoclave bottles and 7.5 ml of persulphate solution added. Lids were placed loosely on the bottles and the samples were autoclaved for 30 min at 110°C (See Williams *et al.* (1995) for the oxidation procedure). Once cool the samples were analysed on a Skalar continuous flow analyser. NB: samples need to be acidified before oxidation.

References

Williams, B. L., Shand C. A., Hill M., O'Hara C., Smith S. and Young M. E. (1995) A procedure for the simultaneous oxidation of total soluble nitrogen and phosphorus in extracts of fresh and fumigated soils and litters. *Commun. Soil Sci. Plant Anal.,* **26**(1&2), 91-106.
Cross A. F. and Schlesinger W. H. (1995) A literature review of the Hedley fractionation…*Geoderma* **64**, 197-214

Biomass P (fumigation-extraaction)

Available K in soil (Ammonium nitrate extractable K)

All material/containers should be either polythene, polypropylene or teflon/PTFE and acid washed prior to use.

Apparatus

1 Roller bed/orbital shaker
2 150 ml polythene extraction bottles
3 Sterilin vials 30 ml

Reagents

1 1M Ammonium Nitrate AR: dissolve 80.04g in deionised water and make up to 1 litre. (400.2g in 5 litres)

Procedure

1 Weigh 10g air dried soil (2mm) into a weighing boat and transfer to a 150ml polythene extraction bottle.
2 Add 50ml 1M ammonium nitrate solution, cap the bottles securely and transfer to shaker.
3 Shake/roll the samples for 30min at 20 C (shakers without temperature control should be used in a 20 C room). For Orbital shakers, set the RPM to 100.
4 Remove samples from the shaker and filter through Whatman no. 40 filter papers into vials. Discarding first 10ml of filtrate.
5 Blanks and in-house standard materials should be taken through the complete procedure. Duplicate samples of at least one in every ten samples should be included in every batch.
6 Samples should be presented for the analysis of potassium and magnesium by ICP.

References

1 From the Analysis of Agricultural Materials MAFF RB xxx, 1986

Slowly available K (boiling nitric acid)

Reagents:
1) Nitric acid (HNO_3), 1.0*N*
2) Nitric acid (HNO_3), 0.1*N*

Protocol:
- Add 2.5g of finely ground soil to a digestion tube.
- Add 25ml of 1.0*N* HNO_3 and place in a water bath/digestion block.
- Raise temperature of block until boiling starts. Reduce the temperature and gently boil for 10 minutes.
- Remove the digestion tube from the block, cool slightly, and pour the contents into a filter, collecting the filtrate in a 100ml volumetric flask.
- Wash the soil with four 15ml portions of 0.1N HNO3.
- Cool the solution and dilute it to volume with de-ionised water.
- Analyse solution using ICP.

NB: The time taken between finishing the 10 minute boiling period and pouring the samples (i.e. the cooling time) has an influence on the amount of K extracted. Therefore, if you are going to use this method, the samples need to be either poured straight away or the time recorded so that the method is standardised across all the samples.

The procedure we have used is a modified version of the method given in: **D. Knudsen, G. A. Peterson and P. F. Pratt. Lithium, Sodium, and Potassium. In: Methods of soil analysis. Part 2 – Chemical and Microbiological properties, Second Edition. Agronomy No. 9 Part 2 pp. 225-243.**

The method in the above publication involves placing a flask with 2.5 g soil and 25 ml of 1.0*N* HNO_3 over a gas burner and boiling. We opted for the water bath option in order to allow us to run more samples at once and to provide more controlled conditions. Pratt and Morse (1954) also used a modified method to run more samples. They placed their samples (10 g soil and 25 ml of 1.0*N* HNO_3) in a beaker in an oil bath and boiled at 113 °C for 25mins.

'Total' P and K in soil (Aqua regia digest)

Principle

Digestion of soils with aqua regia acid is used for the determination of major and trace elements. The samples are digested in tubes, which are heated in time/temperature controlled heating blocks, filtered and analysed by ICP.

This method agrees well with the reflux method but is easier to operate, requiring less attention. **This method does not give absolute total contents**, it does give results sufficiently close to accepted values for different types of soils and sludges for it to be of value in the routine monitoring of metal contents. The method can be modified to suit different types of soil, eg. to overcome excessive frothing when digesting highly calcareous soils, extra nitric acid is added before the aqua regia and heating stages. Peaty soils should be ashed at 450°C before digestion for 3 hours.

Apparatus

1. Carbolite heating block with time/temperature controller.
2. Digestion tubes, 150 x 22mm, graduated to 25ml, with stopper.
3. Optifix variable volume 0-10ml dispenser.
4. Vortex tube mixer (Whirlimixer)
5. Polythene vials - 25ml (Sterilin)
6. Scrubbed fume cupboard

Reagents

1. Hydrochloric acid AR s.g.1.18
2. Nitric acid AR s.g. 1.42
3. 25% hydrochloric acid: add 250ml HCl AR to water, mix and make up to 1000ml with water.

Procedure

- Weigh 0.250g of finely milled, air dried soil into a weighing boat and transfer to a 25ml graduated digestion tube.
- To each tube add 4ml hydrochloric acid and 1ml nitric acid (ie. 5ml aqua regia) and leave to stand at room temperature for at least 2 hours.
- Place in either:

Gerhardt heating block (40 hole) and heat using the following programme:

Programme 3

Time	**Temp.**	**Time**	**Temp.**
Mins/hrs	°C	Mins/hrs	°C
2-00 hr	35	15	85
15	40	15	90
15	45	15	100
15	50	1-00 hr	105
15	55	15	110
3-00 hr	60	15	115
15	65	15	120
15	70	2-00 hr	125
15	75	0-00	0
15	80	cooling time – 10 minutes	

Eurotherm silver heating block (54 hole) and heat using the following programme:

Ramp No.	Temp. Rise °C/min	Dwell Time (mins)	Dwell Temp.°C
1	2	120	25
2	2	180	60
3	2	60	105
4	2	120	125

NB. It may be necessary to whirlimix occasionally at or below 60°C to prevent a cap of partly digested material rising up the tube.
The above heating regime is carried out overnight and the tubes should be cool by morning.

- Using a dispenser add 5ml 25% hydrochloric acid to each tube, whirlimix and reheat at 80°C for 1 hour.
- Whirlimix and add approx. 18ml of deionised water, reheat at 80°C for 30 mins. Remove from the block and allow to cool before making up to volume.
- Whirlimix the contents of the tube then filter through a Whatman no. 40 filter paper in to a Sterilin vial, discarding the first 5 ml of filtrate.
- Blanks and appropriate in-house standard materials should be included in each block digested.
- Duplicate sample digestions of at least one in every ten samples should be included in each batch.
- Samples should be presented for analysis by ICP.

Notes

- Use suitable protective clothing (spectacles and gloves).
- When working with calcareous soils add 2ml hydrochloric acid and whirlimix to reduce frothing, add a further 2ml of hydrochloric acid, whirlimix, when the frothing has ceased, add 1ml of hydrochloric acid and 1ml nitric acid. The additional 1ml of hydrochloric acid makes up for the acid used in reducing the calcium carbonate.
- Peaty soils with high organic matter should be weighed into a silica crucible, placed in a cold muffle furnace and ashed at 450°C for 3 hours. When cool the ash should be washed into a graduated 25ml tube with 8ml of 50% hydrochloric acid followed by 1ml nitric acid. Then proceed from step 3. Under these conditions, copper may be lost by volatilisation during ashing.

References

1 S P MCGRATH & C H CUNLIFFE, A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. *J. Sci. Food Agric.* 1985.36 794-798.
2 T T GORSUCH, The Destruction of Organic Matter. *Pergamon Press, Oxford* 1970.

Total P, K and other trace elements in plant material (Nitric perchloric digest)

Principle

The organic matter of plant material is destroyed during digestion with nitric and perchloric acids. The acids are removed by volatilisation and the residue dissolved in hydrochloric acid.

Digestion with nitric acid alone is preferred when analysis for Mn is required; Mn is oxidised by perchloric acid to permanganate which on heating decomposes into insoluble manganese dioxide and other oxides. This is only necessary if looking at low level manganeese or if the digest is to be analysed by graphite furnace atomic absorption. If Fe analysis is required, the digestion with nitric/perchloric acid mixture is preferred because low recoveries of Fe occur when nitric acid alone is used.

The digestion must be carried out in an approved fume cupboard.

Apparatus

1 Carbolite heating block with time/temperature controller.
2 Digestion tubes, 150 x 22mm, graduated to 25ml, with stopper.
3 Optifix variable volume, 0-10ml dispenser.
4 Vortex tube mixer (Whirlimix)

Reagents

1 Nitric acid AR s.g. 1.42
2 Nitric acid/perchloric acid mixture: add 15 volumes of 60% perchloric acid AR to 85 volumes of nitric acid AR (s.g. 1.42) and mix well.
3 25% hydrochloric acid: add 250ml hydrochloric acid AR (s.g. 1.18) to water, mix and make to 1000ml with water.

Procedure

1 Dry the plant material for a minimum of 4 hours at 80°C, then place in a desiccator to cool.
2 Weigh 0.250g (0.500g of grain) of oven dried plant material (ground to pass 0.5 mm mesh sieve) into a weighing boat, and transfer to a 25ml graduated digestion tube.
3 Add 5ml of the nitric/perchloric acid mixture (or nitric acid for Mn determination), whirlimix immediately and allow to stand at room temperature for at least two hours.
4 Place in either
   1) Eurotherm silver heating block (54 hole) and heat using the following programme:

Ramp No.	Temp. Rise °C/min	Dwell Time mins	Dwell Temp °C
1	1	180	60
2	2	60	100
3	2	60	120
4	1	150	200

2) Gerhardt heating block (40 hole) and heat using the following programme:

Programme 1

Time Mins/hrs	**Temp.** °C	**Time** Mins/hrs	**Temp.** °C
15	35	15	130
15	40	15	140
15	50	15	150
3-00 hr	60	15	160
15	70	15	170
15	80	15	180
30	90	15	190
1-00 hr	100	2-30 hr	200
30	110	0-00	0
1-00 hr	120	cooling time -10 minutes	

NB it may be necessary to whirlimix occasionally at or below 60°C to prevent a `cap' of partly digested material rising up the tube.

5 The above heating regime is carried out overnight and tubes should be cool by morning.
Residual perchloric may interfer with analysis by ICP, the tubes should be near to dryness after the digestion programme.

6 Add 5ml of 25% HCl using a dispenser then whirlimix.

7 Warm the tubes in the heating block for 1 hour at 80°C.

8 Whirlimix again and add water to approx 20-23ml, re-warm for a further 30 mins at 80°C.

9 Remove from the block and allow to cool. Make to 25ml with water, stopper, mix well by shaking (invert fully) and leave to settle for a minimum of 3 hours.

10 Samples should be presented for analysis by ICP, with the samples first, followed by the repeats, standards and blanks.

Hazards and Risk Assessment

1 This method involves the use of concentrated acids. Wear suitable protective clothing (gloves and eye protection) and use an appropriate fume cupboards at all times.

2 Read the notes pertaining to the use of perchloric acid. Read and sign the COSHH regulations before using this method.

3 Do not use this method for plant material containing more than 10% oil.

4 Ensure the sample is `wetted' quickly with the acid by mixing immediately using the whirlimixer to avoid danger of spontaneous combustion.

Quality Control Measures

1 1 `in-house' standard and 1 blank must be digested with each batch of samples at a minimum frequency of 1 standard and 1 blank per 25 samples.

2 Repeat digestions must be included in each batch of samples. For large batches repeats should be at a minimum frequency of 1 per 10 samples. (2R 12R 22R ....)

Reference

1 B A ZARCINAS *et al., Commun. in Soil Sci. Plant Anal.* (1987) 18(1), 131-146.

Soil pH

Principle

On drying, the pH value of a soil is probably modified, so to estimate the true pH of the soil in equilibrium with soil water, the dried and milled soil is wetted with water. The soil suspension used has a soil:water ratio of 1:2.5.

Apparatus

1 pH meter, temperature compensated (Jenway)
2 Combination pH electrode
3 Glass bottles with screw top lids (60ml)
4 Variable volume 0-30ml dispenser

Reagents

1 Buffer solutions, pH 4.0 and pH 7.0 at 20°C (Whatman high resolution pH buffer solution) (two bottles one for pre-conditioning and one for calibration)
2 Distilled water, freshly boiled and cooled.

Procedure

1 Weigh 10g of air dried soil (ground to pass 2mm sieve) and transfer to a glass vial.
2 Using a dispenser, add 25ml of boiled, cooled distilled water, replace lid and shake, leave to stand for 30 minutes, shake once again and leave for a further 30 minutes.
3 Calibrate the pH meter with the pH 7.0 and 4.0 buffers.
   - Switch on the pH meter and leave for 5 minutes for the temperature to stabilize.
   - Rinse the electrode with distilled water, blot off excess water and place electrode and temperature probe into the pre-conditioning pH 7.0 buffer, remove and place directly into the calibration pH 7.0.
   - Allow the meter reading to stabilize and press CALIBRATE. Rinse the electrode and repeat previous 2 steps with pH 4.0 buffer. (pre-conditioning and calibration solutions).
4 Shake the solution again and measure immediately. Lower the electrode and temperature probe into the sample supernatant, wait 30 secs and note the pH reading when the meter has stabilized. Remove the electrode from the sample and rinse with distilled water, blotting the excess water.
   - Check the calibration with pH 7.0 after every 10 samples. If pH 7.0 varies by more than ± 0.1 - 0.15 then re-calibrate the meter.
   - Re-read every 10th sample at the end of the batch to check the pH values.

Notes

1 Duplicate samples of one in 10 should be included in every batch. (eg. 2R, 12R)
2 Two in-house standard soil samples should be included in every batch, one at the beginning and one at the end.
3 Care of the electrode - RAPID RENEW ELECTRODE - the electrode should be stored in electrode storage solution, (if unavailable use 200ml pH 4.0 + 1g KCL).
   Between batches analysed on the same day the electrode may be stored in distilled water. The electrode should be filled with 3.5M KCl solution.