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## PREFACE

The purpose of this booklet is to provide standard analysis methods for the different laboratories of the Finnish Forest Research Institute. The original version was published in Finnish (Tiedonantoja 36) in 1981 and this new edition includes a number of additions and modifications to the methods currently in use. This English language version is also designed to provide foreign visitors and forestry research institutes abroad with information about the nutrient analysis methods used by the Finnish Forest Research Institute.

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# 1 INTRODUCTION

The nutrient analysis methods published in this booklet are intended as general instructions which, when followed, give relatively accurate results for sample material with the least amount of effort. Slavishly following these instuctions will no doubt sometimes be difficult. The fact that the laboratory equipment used within the institute is considerably heterogenous means that in certain cases digression from methods will be unavoidable in practice. This is permissible if the chemical principles of such methods are in accordance with the methods published here and if sound judgement is used when making essential changes in the type of equipment to be used.

It is worth pointing out here that even though the work is done carefully, erroneous results sometimes will be obtained. The reason for this may be, for instance, the abnormal composition of the sample or an unnoticed fault which has developed in the measuring equipment. It is therefore essential that the analysis results are checked from time to time. The methods most commonly used for this purpose are the so-called internal standard method and the dilution method. In the latter method, a series of dilutions is made of the solution to be analysed and these are then analysed aginst the same standard series. In most cases, these two methods give some indication of the type of interfering phenomena involved and other analysis errors.

As present-day nutrient analyses are in the main based on atomic absorption spectrophotometric and colorimetric measurements, the following text books are worth studying:

DEAN, J. A. & RAINS, T. C. 1969. Flame emission and atomic absorption spectrometry. Vols. 1 - 3. Marcel Dekker. New York and London.

KOCH, O. G. & KOCH-DEDIC, G. A. 1964 Handbuch der Spurenanalyse. Springer-Verlag. Berlin, Göttingen, Heidelberg und New York.

## 2 PRELIMINARY HANDLING OF SAMPLES

### 2.1 Plant samples

The samples should be dried as rapidly as possible at 70°C. The twigs are removed from leaf and needle samples and the samples then milled as finely as possible. If micro-nutrient analyses are to be carried out on the samples, then the screen in the mill should be made of stainless steel. The samples should be stored in tightly closed containers or packets.

### 2.2 Humus samples

The samples should be dried in the same way as in Section 2.1. Any thick roots or stones should be removed. Continue as in Section 2.1.

### 2.3 Mineral soil samples

The samples should be dried in the same way as in Section 2.1. If there is no danger of ammonia vapour being present in the air, then the samples can also be dried in open plastic containers in a warm, airy place. Any lumps of earth can be broken up in a porcelain mortar. All the material passing through a 2 mm sieve is retained. Samples which are to be analysed for Cu and Zn should be sieved using a stainless steel sieve. The samples should be stored in the same way as in Section 2.1.

# 3 TOTAL ANALYSIS

## 3.1 Metal cations and phosphorus

Plant and humus samples are ignited in a muffle furnace, the ash dissolved in acid and the cation (macronutrients: K, Ca, Mg and also sometimes micronutrients: Mn, Cu, Zn and Fe) content determined on an atomic absorption spectrophotometer. As the hydrogen ion concentration and anion content of the solution often affects the results of AA analyses, the same acid as that used in dissolving up the ash, and of the same concentration as the solutions to be measured, should be used in preparing the standard solutions.

Phosphorus is determined from the same solution prepared from the ashed sample. Depending on the composition of the sample, there are two suitable photometric methods to choose from:

A. Molybdate-hydrazine method
B. Vanado-molybdate method

Large amounts of silica oxide in the solution have an interfering effect in method B, and as it can also choke the burner of the AA instrument during cation determinations, it should be removed at the stage when the ash is being dissolved. The absorption of ferric iron is considerable at the wavelength used for measurements in method B, and reduction of the iron is not possible. The advantages of method A are the excellent stability of the colour and the insensitivity of the method with respect to iron.

If the laboratory glassware has been washed using synthetic detergents, then all the glassware to be used in phosphorus determinations should be washed with a mixture of conc. $H_2SO_4$ and methanol, and then rinsed with distilled water.

Procedure:

Weight out 1 - 3 g of the plant or humus sample into a pre-weighed quartz or platinum dish. The dish is left overnight in a drying oven at 105°C in order to determine the dry weight of the sample. The dish is left to cool in a desiccator and then weighed. The dish is transferred to a muffle furnace, the temperature gradually increased to 550°C and then kept there for 3 hours. The dish is then left to cool in a desiccator and weighed if the ash weight is required.

The ash is carefully moistened with a little absolute alcohol and 2 - 3 ml of HCl (1 part conc. HCl + 1 part $H_2O$) added. The dish is then transferred to a boiling water bath and allowed to boil dry in order to precipitate the silica oxide. When the dish no longer smells of hydrochloric acid, the residue is dissolved up in 10 ml of 1.0 N HCl and left to stand on the waterbath, covered with a watch glass, for about 20 min. The solution is then filtered into a 100 ml volumetric flask and the filtrate washed with 0.1 N HCl until the level in the flask reaches the mark. The silica oxide content of plant samples is usually so small that filtering is unnecessary. After precipitating out the silica oxide and dissolving up the residue, the solution to be analysed can be transferred, along with the preciptitate, into a 100 ml volumetric flask by washing with 0.1 N HCl. Aliquots of the solution can then be carefully pipetted out after the solution has been mixed and the precipitate allowed to settle.

K, Mg, Ca:

A suitable aliquot of the clear solution in the 100 ml volumetric flask is pipetted into a 25 ml volumetric flask, 2.5 ml of the 5 % lanthanide (La) solution added and the flask filled to the mark with 0.2 N HCl. After mixing, the cations are determined on

an atomic absorption spectrophotometer. The results are calculated using a series of standards containing the same amount of the La solution with a final HCl normality of 0.2.

Solutions:

La solution (5 %): 58.64 g $La_2O_3$ + 640 ml 2 N HCL. After the salt has dissolved, the solution is diluted to 1000 ml with water.

Standard solutions: The basic standards are made by diluting standard ampoules with 0.2 N HCl to give solutions containing 1000 ppm. These are then futher diluted to give 100 ppm mixed standards, 10 ppm mixed standards and the standards to be used in the analyses. Enough lanthanide solution is added to the final standards so as to give the same concentration as in the samples to be analysed. All dilutions are done using 0.2 N HCl.

Mn, Fe, Cu, Zn:

The micronutrients are determined directly from the solutions remaining in the 100 ml volumetric flasks by means of atomic absorption spectrophotometry. No lanthanide solution is added.

Solutions:

Standard solutions: 1000 ppm basic standards are prepared from standard ampoules by diluting with 0.2 N HCl. A mixed standard containing 500 ppm Fe and 100 ppm Mn, Cu and Zn is then prepared from the basic standard solutions. The mixed standard is futher diluted to give a mixed standard containing 50 ppm Fe, 10 ppm Mn, Cu and Zn. The standards used in the analyses are prepared from this mixed standards solution. All dilutions are done with 0.2 N HCl. No lanthanide solution is added.

Phosphorus:

A. Molybdate-hydrazine method

Pipette a 1 ml aliquot (must not contain more than 5 mg Fe) of the solution remaining in the 100 ml volumetric flask into a 25 ml volumetric flask. Add 0.2 N HCl until the flask is half full. Add 5 ml of the molybdate solution and mix. Add 1 ml of the hydrazine sulphate solution, make up to the mark with 0.2 N HCl and mix. The standard solutions are prepared in the same way. The flasks are immersed up to the neck in a hot water bath (97 $^{o}$C) and left to stand for about 12 minutes. The flasks are then cooled under running water for about 10 minutes, mixed and left to stand for at least 1/2 hour before measuring on a spectrophotometer at 822 nm.

Solutions:

Molybdate solution: 10 ml $(NH_4)_6Mo_7O_{24}.4H_2O$ is dissolved in 800 ml of water, 120 ml of conc. $H_2SO_4$ (density 1.8) added and then diluted to 1 litre with water.
Hydrazine sulphate: 1 g of hydrazine sulphate is dissolved in 200 ml of water.
Basic P standard (0.5 mgP/ml): 2.197 g of $KH_2PO_4$ is dissolved in 1 litre of 0.2 N HCl. The standards used in the analyses (0.005 mgP/ml) are prepared from this solution.

B. Vanado-molybdate method

Pipette a suitable aliquot from the solution remaining in the 100 ml flask into a 25 ml volumetric flask. Add sufficient 0.2 N HCl to give a volume of about 15 ml and mix well. Add 5 ml of the vanado-molybdate solution, fill to the mark with 0.2 N HCl and mix well. Addition of the 5 ml of reagent should be done as

accurately as possible. An error in pipetting of, for instance, 0.025 ml will result in an absorbance reading error of 0.010. Leave to stand for about 15 minutes. Measure on a spectrophotometer at a wavelength of 336 nm against the 0 standard.

Even small amounts of iron will affect the determination. For instance, 20 g Fe will bring about an increase in the reading which is equivalent to about 1 g of phosphorus in the solution.

Solutions:

Vanado-molybdate solution: 1 g $NH_4VO_3$ is dissolved in about 800 ml hot water. When the solution has cooled down to about 50°C, add 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. After it has dissolved, leave to cool. Add 42 ml conc. $H_2SO_4$ (density 1.8) or 140 ml conc. $HNO_3$ (density 1.4) and cool: Make up to 1 litre with water.
Basic P standard: as in Method A.

3.2 Nitrogen

3.2.1 Nitrogen determination by the Kjeldahl method

The method described here is the classical basic Kjeldahl method which does not require any special equipment. If commercial equipment is available for carrying out the nitrogen determinations,then the method given in the instructions supplied by the manufacturer should be followed. The $K_2SO_4$ and catalyst tablets recommended by many manufacturers can be replaced, for economic reasons, with the chemicals used in the method described here. It is always a good idea to carry out routine checks on the results of nitrogen determinations, for instance, by determining the nitrogen content of pure nitrogenous compounds of

different composition.

More information about the Kjeldahl method and its variations can be found, for instance, in the following:

BRANDSTREET, R. B. 1965. The Kjeldahl method for organic nitrogen. Academic Press. New York and London.

Procedure:

Weigh out 1 - 2 g of the organic material or 5 g of the mineral soil to be analysed into a 300 ml Kjeldahl digestion flask. Add about 10 g $K_2SO_4$ , a small amount of $CuSO_4$ as catalyst, and 20 ml of conc. $H_2SO_4$. The flask is placed on the nitrogen digestion apparatus and heated up slowly over a low heat, taking care not to let it foam over. The heat is gradually increased and the solution allowed to boil until the solution is clear green in colour. The solution is then left to boil for approximately one hour more.

After the solution has cooled, carefully add 100 ml of distilled water and mix. The solution is transferred to a distilling flask, the digestion flask rinsed out a few times with distilled water (altogether about 100 ml). 50 ml of boric acid solution , containing an indicator, are pipetted into a 300 ml graduated conical flask. The flask is placed under the distilling apparatus so that the tube passing from the condenser is below the surface of the liquid in the flask.

Place boiling beads in the distilling flask and add 100 ml of the NaOH solution. After adding the NaOH, the flask is immediately coupled to the distilling apparatus so as to prevent any losses of ammonia. The flask is then heated. After boiling for about 5 minutes, the conical flask can be lowered and the tube passing

from the condenser rinsed with distilled water. Distillation is continued until the level in the conical flask reaches the 200 ml mark. The condensate is then titrated with 0.1 N sulphuric acid. The equivalent point is reached when the solution starts to turn pink (cf. control).

Solutions:

NaOH solution: 6 kg technical NaOH is dissolved in 10 litres of water. Tap water can be used if it is ammonia-free.
Indicator solution: 300 g methyl red + 450 mg bromcresol green + 600 ml of 94 % ethanol.

Boric acid solution: 400 g of boric acid + 50 ml of the indicator solution are dissolved in distilled water and then made up to 10 litres. Boric acid dissolves very slowly so the solution should be shaken daily until it has completely dissolved.

Control: 50 ml of the boric acid solution is diluted to 200 ml. If the solution is red, then 0.1 N NaOH is added from a burette until the solution is slightly pink. This procedure is then repeated on the whole batch of boric acid until the colour corresponding to the required equivalent point is achieved.

3.2.2 Measurement of Kjeldahl-nitrogen using an NH electrode

After digestion, the Kjeldahl flask is cooled and then filled to the 300 ml mark with distilled, ammonia-free water. The solution is mixed and a 5 ml aliquot pipetted into a 150 ml beaker. 95 ml of NaOH (see below) is added, the solution mixed with a magnetic stirrer and the ammonia content measured immediately with an NH electrode. The standards are measured in exactly the same way (5 ml of standard solution).

Solutions:

Distilled water: must be free of all traces of ammonia (e.g. passed through an ion exchange column).
NaOH solution: 12 ml of conc. NaOH (600 g/l litre) are diluted with water to 1 litre.
Standard solutions: 10 ml of 30 % $NH_3$ are diluted with water to 100 ml. The exact ammonia concentration of this solution is determined by removing a number of 2 ml aliquots and titrating then against 0.2 N acid. The NH solution is them diluted so as to give a 1 N solution. The strongest standard (St. 10) is diluted directly from this solution. The other standards are prepared by diluting St. 10. The standards are as follows:

| | |
|---|---|
| 0.02 N $NH_3$ | = St. 10 |
| 0.002 N $NH_3$ | = St. 1 |
| 0.001 N $NH_3$ | = St. 0.5 |
| 0.0002 N $NH_3$ | = St. 0.1 |

A mixture which is approximately equivalent to the solution formed after digestion is used for diluting the actual standards: 10 g $K_2SO_4$ + 0.5 g $CuSO_4$ + 10 ml conc. $H_2SO_4$, all in 300 ml of $H_2O$.

### 3.2.3 Determination of Kjeldahl-nitrogen in samples containing nitrate

Determination of the total nitrogen of samples containing nitrates cannot be done by the ordinary Kjeldahl method. Part of the nitrates are lost as nitric oxide from the digestion flask before the ammonia-forming reaction is completed. Nitrate thus has to be first reduced to ammonia before proceeding according to the Kjeldahl method.

Procedure:

2 g of the sample are weighed out into a digestion flask (300 ml). 3 g of Devarda's alloy are added and mixed well. If the sample is dry, then water is added and the sample left to absorb the water overnight. If it is a solution, then an aliquot is added to the digestion flask, followed by the Devarda's alloy.

In both cases, deionised water is added to the flask so as to give a final volume of about 50 ml. 2 ml of Antifoam solution are added.

The flask is stoppered with a rubber cork fitted with a U-tube and an extraction funnel. 10 ml of $H_2SO_4$ (10 vol. %) are added to the U-tube and the mouth of the tube stoppered with a filter paper plug. 5 ml of NaOH (density 1.4) are added via the extraction funnel which is then rinsed with a little water to remove all the NaOH. Shake the flask gently so as to ensure that the foam does not reach the neck of the flask. The reaction is allowed to continue for 1/2 an hour. The flask is then heated up to boiling point and the reaction continued at this temperature for another hour.

After the mixture has cooled, 20 ml of $H_2SO_4$ (50 vol. %) are added via the extraction flask, while gradually rotating the flask so that the neck and sides of the digestion flask are rinsed with the acid. Mix well and leave to cool. The filter paper is removed from the U-tube and the contents of the U-tube flushed into the flask, the paper plug being added to the mixture. The excess water in the flask is evaporated off on a boiling water bath. The rest of the procedure is the same as for normal Kjeldahl digestion except that potassium sulphate and copper sulphate are not added.

DYER, H. & HAMENCE, J. H. 1938. The determination of nitrogen in mixed fertilizers containing nitrates and chlorides. The Analyst 63 (1938) 866-870.

3.3 Boron

The determination of boron is affected by the fact that boric acid is easily vaporised when an aqueous solution of boron is boiled. This also accounts for the fact that distilled water usually contains boron. Furthermore, the fact that boric acid is a weak acid means that it is not removed by ion exchange resins. When determining rather small amounts of boron, the same batch of purified water should be used for preparing the reagents and the standard solutions.

Procedure:

1 g of sample is weighed out into a pre-weighed quartz dish and then left to dry overnight at 105 °C. The sample is then left to cool in a desiccator and finally weighed to determine the dry-matter content. The sample is then burnt in a muffle furnace ar 550 C for three hours. The ash is extracted with a mixture of phosphoric and sulphuric acid. After adding the acid, the mixture is immediately stirred using a magnetic stirrer, the dish covered with a lid, and then left to stand overnight. 5 ml of 1N NaOH are added, stirring continously, and the solution then filtered. 4 ml of the filtrate are then pipetted into a plastic bottle (10 ml) fitted with a screw cap. 2 ml of the buffer solution are added, the solution mixed, and 2 ml of the azomethine H reagent added. The solution is mixed well and the absorbance of the colour which develops is measured after one hour on a spectrophotometer at a wavelenght of 425 nm. Water samples (in 1 % acetic acid) are treated in the same way as the exrtact made from the ashed sample.

Solutions:

All the solutions should be made up and stored in plastic containers.
Boron standard: 571.5 mg of boric acid are dissolved in 0.2 N hydrochloric acid and the solution made up to one litre with the same acid. This solution contains 100 ppm B. This is again diluted to give a solution cantain 10 ppm B.
Standard solutions (0 - 0.6 ppm B) are then prepared from this solution. When making these standards, a mixture of 1 part 1 N NaOH and 2 parts phosphoric/sulphuric acid is used for diluting. In order to improve the storage life of these standard solutions, buffer solution is added: 100 ml standard solution + 50 ml buffer. 6 ml of these final solutions are used for the determinations.

The standards used in determining the boron content of water samples are diluted using 1 % acetic acid. Buffer does not have to be added.

PHOSPHORIC-SULPHURIC ACID MIXTURE: 40 ml of phosphoric acid (85 %, density 1.71) are diluted to one litre using distilled water. This gives a solution of about 0.6 M $H_3PO_4$. This is then mixed with 0.4 N $H_2SO_4$ in the ratio 1:1. The mixture is checked by removing a 10 ml aliquot, which is then titrated against 1 N NaOH to pH 4.5. 5 ml of NaOH should be required.

Buffer solution: 7 g of EDTA are dissolved in about 200 ml of ammonium acetate (pH = 4.65) buffer (Solution C, p.00) on a boiling water bath. The solution is made up to 250 ml using the same buffer solution. Filter if necessary.

Azomethine H reagent: 1 g of azomethine H and 3 g of ascorbic acid are dissolved in 200 ml water. The solution is filtered if

necessary and stored in a plastic bottle. The bottle should be filled as full as possible so as to leave the minimun of air space at the top. The solution should be stored in a refrigerator. It is warmed up to room temperature before use.

Preparation of azomethine H from H-acid: 18 g of H-acid (8-amino-1-naphthol-3.6-disulphonic acid) are dissolved in 1 litre of distilled water in a beaker at about 40 °C. The solution is neutralised to pH 7.00 using 10 % KOH. The temperature is raised to 80 °C - 85 °C and concentrated HCl added dropwise, with stirring, until the pH reaches 1.5. If a precipitate develops, leave to stand until clear before adding more acid. Transfer the solution to a flask fitted with a reflux condenser. Add 20 ml of salicylaldehyde and maintain at a temperature of 85 °C - 90 °C for one hour, stirring continously. Transfer the solution back to the original beaker and leave to stand covered by a watch glass for 16 hours. The orange precipitate which develops is removed by filtering on a Buchner filter, the precipitate washed five times with ethanol and then dried for 3 hours at 100 °C. Store in a brown bottle in a desiccator. This compound is also available commercially.

### 3.4 Ash residues from boiler furnaces

The ash samples should be ground as finely as possible using a pestle and mortar. A small part of the material is transferred to a 100 ml beaker and dried overnight at 105 °C in a drying oven. After cooling in a desiccator, approx. 500 mg are weighed out into a quartz dish for analysis. The ash is moistened with 2 - 3 ml of HCl (1 part conc. HCl + 1 part dist. $H_2O$) and transferred to a boiling water bath. After all the HCl has evaporated off, the sample is dissolved in 10 ml of 2 N HCl and left to stand, covered with a watch glass, on a boiling water bath for about 20 minutes. The sample is then filtered into a

500 ml volumetric flask. The dish is washed carefully (dist. $H_2O$) using a glass policeman and the washings transferred to the flask. 40 ml of 2 N HCl are added to the flask trough the filter paper and the filter paper then washed with distilled water until the total volume in the flask is about 350 ml. The flask is then filled to the mark with dist. $H_2O$ and shaken well.

Determination of Ca, K, Mg

A suitable aliquot (1 - 2 ml) of the solution in the 500 ml volumetric flask is transferred to a 25 ml volumetric flask. 2.5 ml of 5 % lanthanide solution are added and the flask filled to the mark with 0.2 N HCl.

Determination of Cu, Zn, Mn, Fe

Micronutrients are measured directly from the solution in the 500 ml volumetric flask.

ALL GLASSWARE SHOULD BE ACID WASHED

Determination of phosphorus

A suitable aliquot (1 - 5 ml) of the solution in the 500 ml volumetric flask is transferred to a 25 ml volumetric flask. The flask is filled to the half-way mark with 0.2 N HCl. 5 ml of the molybdate solution is added, the flask shaken and 1 ml of the hydrazine sulphate solution added. The flask is then filled to the mark with 0.2 N HCl and shaken. The standards are prepared in the same way. The flasks are then immersed up to the neck in a water bath (97 $^{o}$C) for about 12 minutes. After cooling under running water for about 10 minutes, the flasks are again shaken. The solutions are then measured on a spectrophotometer at a wavelength of 822 nm after standing for at least half an hour.

Determination of carbon, sand and ash content

Carbon: The quartz dish is kept in the drying oven (105°C) for at least 3 hours and then left to cool in a desiccator and weighed. About 500 mg of the sample is weighed out into a quartz dish and burnt for 3 hours in a muffle furnace at 550°C. Leave to cool in a desiccator and weigh.
Carbon content = initial weight - weight after burning

Sand: The filter paper used for filtering the extract in the first half of this section is placed in a quartz dish and burnt in a muffle furnace for 3 hours at 550°C. Leave to cool in a desiccator and weigh.
Sand content = weight after burning

Ash:

Ash content = weight after burning in the carbon determination - sand content

Solutions:

2 N HCl: 172 ml of conc. HCl/1000 ml distilled water
Lanthanide solution: see p.
Molybdate solution: see p.
Hydrazine sulphate solution: see p.

## 4. EXCHANGEABLE AND EASILY SOLUBLE NUTRIENTS

### 4.1 Exchangeable metal cations and soluble phosphorus

The amount of nutrients present in plant available form is determined by means of an exchange reaction in which a particular cation group is used to displace the cations adsorbed on the

surface of the soil particles. The position of the state of equilibrium in the exchange reaction, i.e. the amount of cations which have passed into the solution, is primarily dependent on the acidity of the extractant. However, in the case of clay minerals and schists especially, it is also dependent on the diameter of the exchanging cation.

The extractant most commonly used in Finland for determining K, Ca and P, and nowadays also Mg, is acidic ammonium acetate. Rather small amounts of micronutrients, especially copper, are extracted by this solution. It is thus recommended that ethylene diamine tetraacetic acid (EDTA) which, owing to its strong chelating ability, also extracts micronutrients that are bound to the organic material, be added to the acidic ammoniumacetate used as the extractant. However, in order to make the analyses simpler and straightforward, the micronutrients are determined from the same extract as the macronutrients in the methods described here.

LAKANEN, E. & ERVIÖ, R. 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agr. Fenn. 128: 223 - 232.
VUORINEN, J. & MÄKITIE, O. 1955. The method of soil testing in use in Finland. Agrogeol. Publ. 63: 1 - 44.

Procedure:

K, Mg, Ca:

15 g of mineral soil or 5 g humus (air-dry samples) are weighed out into a plastic bottle (200 - 300 ml) fitted with a screw cap. 150 ml of ammonium acetate (extraction acetate, solution E) are added, mixed and left to stand overnight. The following morning the bottle is shaken for 1 hour and the solution then filtered.

After a clear filtrate has been obtained, suitable aliquots are taken for the cation determinations. 5 ml (humus samples) or 23 ml (mineral soil samples) of the filtrate are pipetted into a 25 ml volumetric flask, 2 ml of the La solution added, and the flasks then made up to the mark with extraction acetate solution (E). The flasks are mixed and then measured on an atomic absorption spectrophotometer.

Mn, Fe, Cu, Zn:

The cations in the filtrate are measured by atomic absorption spectrophotometry. No lanthanide is added.

Solutions:

La solution: 73.3 g $La_2O_3$ + 891 ml 6 N HCl. Made up to 1 litre with distilled water.
6 N HCl: 516 ml conc. HCl diluted to 1 litre with distilled water. Extraction acetate (solution E): Weight out about 5 kg of ammonium acetate ($CH_3COONH_4$) and dissolve in about 7 litres of distilled water. This is the starting solution S. Take a 4 ml aliquot from this solution for the determination of the normality of the starting solution and dilute to 100 ml with distilled water. This is the determination solution D. Pipette a 5 ml aliquot from solution D into a Kjeldahl distilling flask. Add 200 ml $H_2O$ and 10 ml NaOH, distill into boric acid solution using the same technique as for the Kjeldahl determination of nitrogen. Titrate with 0.1 N $H_2SO_4$ (consumption about 20 ml).

$$v_1 \times n_2 = v_1 \times n_2 \quad \text{or} \quad 5 \times n_D = v_{H_2SO_4} \times 0.1$$

The normality of the starting solution S is $n_S = 20 \times n_D$. In order to prepare 10 litres of concententrated extraction acetate

(C), the following amount of starting solution S is needed:

$$v \text{ (litres)} = \frac{10 \times 5}{n_S}$$

3 litres of concentrated acetic acid are added to the solution v and enough distilled water to give, after mixing, a total volume of 10 litres. This is strong extraction acetate C.

Control: An aliquot of solution C is diluted 10 times with water and the pH of this solution, which is extraction acetate solution E, measured. The pH should be 4.65 (4.60 - 4.70).

Standards: The standard solutions required in the exchangeable nutrient determinations are prepared from the same 1 000 ppm basic standards and in the same way as the standards for the total nutrient analyses. However, all the dilutions are done with the extraction acetate (E) instead of 0.2 N HCl.

P:

Soluble phosphorus is determined by the molybdate-hydrazine method.

Procedure:

A suitable aliquot (2 - 5 ml for humus samples, 10 ml for mineral soil samples) of filtrate is pipetted into a 25 ml volumetric flask. Add 9 ml of 1.7 N $H_2SO_4$, 5 ml of the molybdate solution, and i ml of hydrazine sulphate. Fill to the mark, if necessary, with extraction acetate buffer and mix well. The standards are prepared in the same way. The flasks are kept in a hot water bath (97$^{o}$C) for about 12 minutes. The water should reach the neck of the flasks. Cool under running water for about

10 minutes, mix and leave to stand for about half an hour. Measure on a spectrophotometer at a wavelength of 822 nm.

Solutions:

Molybdate and hydrazine sulphate: as for total analysis.
1.7 N $H_2SO_4$ : 47 ml of conc. $H_2SO_4$ (density 1.8) is diluted to 1 litre with distilled water.
P standards: the 0.5 mg P/ml standard (see total analysis) is diluted with extraction acetate (E) to give a standard containing 0.005 mg P/ml.

4.2 Exchangeable aluminium

This method is based on the extraction of aluminium from soil samples using a solution of KCl. If there are only small amounts of aluminium present in the sample, then the aluminium is best determined by atomic absorption spectrophotometry using a mixture of nitric oxide/acetylene or a graphite oven attachment. In most cases, however, the aluminium content of humus or mineral soil samples is high enough to permit determination by the classical titration method. One advantage of this method is that the first of the two titrations provides a measure of the total exchangeable acidity of the soil sample, in accordance with the following reaction:

$$HCl + NaOH = H_2O + NaCl$$
$$AlCl_3 + 3NaOH = Al(OH)_3 + 3NaCl$$

The method is based on the fact that addition of a solution of NaF to the titrated solution results in the formation of the stable complex ion of fluoaluminate and the relaease of hydroxyl ions:

$$Al(OH)_3 + 6NaF = 3NaOH + Na_3AlF_6$$

The hydroxyl ions are then titrated with standard acid.

Procedure:

Weigh out 20 g of fresh sample into a stoppered flask and add 100 ml of 1 N KCl. Leave to stand overnight and the next morning shake for 2 hours. Filter and transfer 50 ml of the filtrate into a beaker. Titrate with 0.05 N NaOH to pH 8.0. Then add 10 ml of NaF and back titrate with 0.02 N $H_2SO_4$ to pH 8.0. A blank titration should be carried out on the NaF solution.

Solutions:

0.05 N NaOH
0.02 N $H_2SO_4$
1 N KCl: 74.6 g of KCl in 1 litre $H_2O$
NaF solution: 4% NaF, 10 g of NaF in 250 ml $H_2O$. Filter the solution if cloudy.

4.3 Exchangeable ammonium nitrogen

20 g of soil are weighed out into a 400 ml beaker and 250 ml of KCl added. Mix and leave to stand overnight. Mix and filter. An aliquot (10 - 50 ml containing about 0.05 - 0.25 mg N ) of the filtrate is pipetted into a distillation flask and enough water added to give a total volume of about 150 ml. A 100 ml volumetric flask, containing 1 ml of gelatine solution and enough water to cover the tip of the condenser tube, is used as the receiving vessel. 2 g of MgO are added to the distillation flask. Distillation is then carried out until the liquid in the volumetric flsk reaches the mark. 1 ml of Nessler's reagent is then added and the flask mixed. The colour which develops is

measured 5 minutes after adding the reagent on a spectrophotometer at a wavelength of 368 nm.

If the original sample contains large amounts of ammonia, then the distillation can be carried out in the same way as in the Kjeldahl method. MgO is added and the amount of ammonia determined by titration. The ammonia in the KCl extract can also be determined directly using an NH electrode. In this case the standards should be prepared using the same KCl solution.

Solutions:

KCL solution: A saturated solution of KCl is diluted with water in the ratio 1 : 10.
Gelatine solution: 500 mg of gelatine, 10 ml of 0.2 N $H_2SO_4$ and 90 ml of water are boiled gently for about 10 minutes in a beaker covered with a watch glass. The solution is poured, while still hot, into a heated (105 °C) storage bottle. The solution is stored in the refrigerator. The solution should be discarded immediately any cloudiness appears.
Nessler's reagent: 100 g $HgI_2$ and 80 g KI are dissolved in 100 ml of water. When the solution has clarified add 200 g of NaOH dissolved in 900 ml of water. Store in a brown bottle.

4.4 Extractable nitrate

The remainder of the KCl extract prepared for determining ammonium nitrogen is pipetted (150 - 200 ml) into a distilling flask, 1 ml of saturated NaOH added and the free ammonia distilled off into the collecting vessel. When the solution in the distilling flask has boiled down to about 50 ml, remove from the heat, rinse the end of the tube leading from the condenser with water and leave the flask to cool. Then add about 100 ml of ammonia-free water, 10 ml of saturated NaOH and about 3 g of

Devarda's alloy. The flask is closed immediately after adding the alloy. The nitrate is reduced to ammonia, which is then distilled off into a 100 ml volumetric flask. The ammonia is determined using Nessler's reagent as in Section 4.2, or in the case of large amounts of ammonia titrated or measured using an $NH_3$ electrode.

## 5 WATER ANALYSIS

These procedures are intended for water samples containing 1 % acetic acid to prevent microbial spoilage of the samples.

Determination of P, Ca, Mg, Cu, Zn, Fe, K:

1000 ml of the water sample is transferred to a 1 litre beaker. The sample is then evaporated to dryness on a boiling water bath. 10 ml 2 N HCl are added, the beaker covered with a watch glass and then left to stand on the bath for about 1 minute.

The sample is filtered into a 100 ml volumetric flask. The beaker is washed with distilled water using a glass policeman and the washings poured through the filter paper into the flask. This is repeated with small amounts of water until the total volume in the flask is about 90 ml. The flask is then made up to the mark with distilled water and mixed well.

Ca, K, Mg:

Pipette 0.5 ml (leachate) or 2 ml (rainwater) into a 25 ml volumetric flask. Add 2.5 ml 5 % lanthanide solution. Fill to the mark with 0.2 N HCl and mix. These cations are determined on an atomic absorption spectrophotometer using the same standards as for total analysis.

Mn, Cu, Zn, Fe:

The cations are determined directly from the solutions in the 100 ml volumetric flasks. (Note. Lanthanide is not added).

ALL GLASSWARE SHOULD BE ACID WASHED

P:

By the molybdate-hydrazine method. 10 - 15 ml of the sample in the 100 ml volumetric flask is pippetted into a 25 ml volumetric flask. The flask is filled about half full with 0.2 N HCl. 5 ml of the molybdate solution is added and mixed well. 1 ml of the hydrazine solution is then added and the flask filled to the mark with 0.2 N HCl. Mix well. The standards are made in the same way. The flasks are placed up to the neck in a boiling water bath (97 $^{o}$C) and left there without the corks for about 12 min. The flasks are then cooled in running water for about 10 min. The flasks are mixed and left to stand for at least half an hour. The absorbance is measured on a spectrophotometer at a wavelength of 822 nm.

6 CARBON DETERMINATION BY THE TIURI METHOD

1.00 - 0.25 g (usually 1.00 g from the C horizon, but 0.25 g if the humus content is high) of air-dried, carefully-mixed sample is weighed out into a dry 300 ml concical flask. 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of conc. $H_2SO_4$ are added, the mixture carefully mixed by shaking the flask and then placed on a boiling water bath, covered with a watch glass, for one hour. After cooling the solution is diluted to 150 ml with cold tap water. 2.5 ml of conc. $H_3PO_4$ (density 1.70) are added and 5 drops of diphenylamine solution (5 %) as indicator. The excess $K_2Cr_2O_7$ is then back titrated with a 0.4 N solution of $FeSO_4$. (The

solution changes from an intense blue colour to a dirty green at the equivalence point). The consumption of chromate can also be determined by potentiometric titration. If the sample contains a lot of roots or other debris which make homogenisation difficult, then parallel determinations should be done.

10 ml of 1 N $K_2Cr_2O_7$ corresponds to 25 ml of 0.4 N $FeSO_4$

$a = n \times v \times Ep$ or $a(mgC) = 0.4 \times (25-v) \times \frac{12}{4}$

where v = consumption of $FeSO_4$ in ml.

Solutions:

1 N $K_2Cr_2O_7$ : 49.036 g of $K_2Cr_2O_7$ are dissolved in water and diluted to 1000 ml. The normality of the solution is checked by titrating with
0.4 N $FeSO_4$. e.g. 5 ml $K_2Cr_2O_7$

$$\text{normality} = \frac{FeSo_4 \text{ consumption} \times 0.4}{5}$$

0.4 N $FeSO_4$ : weigh out exactly 156.856 g of $(NH_4)_2Fe(SO_4)_2 6H_2O$ dissolve in water and dilute to 1000 ml.

5 % diphenylamine indicator: 5 g diphenylamine/100 ml 75 % $H_2SO_4$.

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