METHODS OF SOIL AND TISSUE ANALYSIS USED IN THE ANALYTICAL LABORATORY

by

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ABSTRACT

The methods and equipment described in this report are those currently in use in the Soils and Tissue Analytical Laboratory, Maritimes Forest Research Centre, Canadian Forestry Service.

All samples for analyses are from the Maritime region and consist of soil, peat, foliage, roots, and water.

RESUME

Les méthodes et les installations que décrit ce rapport sont celles présentement en usage dans le laboratoire d'analyse des sols et tissus, au Centre de recherches forestières des Maritimes, Service Canadien des Forêts.

Les analyses sont faites sur des échantillons de sol, tourbe, feuillage, racines et eau, qui parviennent tous de la région des Maritimes.

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INTRODUCTION

The Soil and Tissue Analytical Laboratory at the Maritimes Forest Research Centre carries out chemical and physical analyses of soils and chemical analyses of tissue and water on a service basis for investigators at the Centre.

To facilitate reference to methodology, the standard routines for analysis of samples are given in this report. The methods and procedures described are those in use in 1977. Where applicable, the originator of a specific method is given. Unpublished methods referred to are on file at the Maritimes Forest Research Centre. Some of the reagents and procedures have been modified to adapt them to maritime forest soils and to the particular equipment used. Such changes are noted.

All glassware is of Pyrex or Corning quality and only analytical reagent and reagent grade reagents are used.

As a precaution against systematic errors, each batch of samples analysed (normally 12 samples) contains one sample of standard material. The appropriate amount of the standard material is subjected to preliminary treatment, e.g. ashing and dilution, in precisely the same way as the materials submitted for analyses. Basic standard materials with known values for every element analysed in this laboratory were obtained from another laboratory. Using these as benchmarks, the values for standard materials collected and prepared locally are determined. As a further check, samples of the local standard materials were sent to two other laboratories for analysis, and the results of the three analyses were compared. The analytical results obtained for the standard sample(s) are included in the laboratory book and in the report to the researcher along with the results for the other samples in the same batch.

With the exception of total nitrogen analysis, all samples are weighed to the nominal weight, plus or minus 100 milligrams. The actual weight is recorded to the nearest tenth of a milligram. For example, if

the nominal weight is 2.5 g, samples may weigh from 2400 to 2600 mg and the actual weight is recorded as either 2.4763 g or 2476.3 mg, 2.5177 g or 2517.7 mg etc.

In total nitrogen analysis, organic materials are weighed to approximately 100 mg but not less than 100.0 mg. Mineral soils are weighed similarly according to the recommended weights for each horizon as stated in the Table, page 12.

Part I - SOIL ANALYSIS

A. Preparation of Soil Samples

The soil samples are air dried in open trays and then ground in a mortar. The ground sample is passed through a 2-mm sieve.

After drying, grinding, and sieving, the samples are placed in labelled covered containers.

B. Chemical Analysis

(1) Available Phosphorus

Olsen, S.R., *et al.* 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. Agric. Circ. 939.

Principle

Available phosphorus is extracted and the amount determined colorimetrically after the formation of a blue complex of molybdate and orthophosphate.

As the available phosphorus content in forest soils is relatively low and the method is very sensitive, extra precautions must be taken to ensure that glassware is chemically clean and free of contaminants. Washing soaps and detergent powders are not recommended and, if used, they must be removed completely by cleaning with a strong acid. Reagents and filter paper must be free of phosphorus. (Contamination with dust, saliva, perspiration, and tobacco ashes must be prevented.)

Equipment

1. Spectronic "20" colorimeter.
2. Rotary shaker.
3. Burette.
4. 25-ml volumetric flasks.
5. 50-ml Erlenmeyer flasks.

Reagents

1. Sodium bicarbonate - 42.01 g of 0.5 *M* $NaHCO_3$ made up to 1 litre with distilled water. Adjust pH to 8.5 with 1 *N* NaOH.
2. Ammonium molybdate - 15 g of $(NH_4)_6MO_7O_{24}\cdot 4H_2O$ in 300 ml of distilled water at 60°C. Filter and add 342 ml of conc HCl while mixing. After cooling, make up to 1 litre with distilled water.
3. Stannous chloride - *Stock solution* - 10 g of crystalline $SnCl_2\cdot 2H_2O$ in 25 ml of conc HCl. Prepare fresh every 2 months.

   Dilute solution - Add 0.5 ml of stock solution to 66 ml of distilled water. Prepare fresh for each set of determinations.
4. Standard phosphate solution - 0.2195 g of KH_2PO_4 dissolved in distilled water and made up to 1 litre. This solution contains 50 ppm of phosphorus. Dilute to appropriate concentration to establish a standard curve.
5. Darco G-60 Activated Carbon - before use, test for phosphorus.

Procedure

1. Weigh 2.5 g of mineral soil or 1.25 g of organic soil and place in a 50-ml Erlenmeyer flask.
2. Measure about 1/2 teaspoon of Darco G-60 Activated Carbon for mineral soils or 1 rounded teaspoon for organic soils and add to the sample to decolorize the extract.
3. With a volumetric pipette, transfer 25 ml of sodium bicarbonate solution to the sample.
4. Stopper the flask and shake for 30 min on a rotary shaker.
5. Filter the solution through Whatman No. 40 filter paper.
6. Pipette 5 ml of the extract into a 25-ml volumetric flask.

7. Using a burette, add 1 ml of ammonium molybdate and swirl solution *gently*. Then add a further 4 ml and swirl solution. This method of adding the ammonium molybdate is necessary to prevent frothing which may occur especially in organic samples, causing the solution to rise up the neck of the flask with a possible loss of sample material.
8. Wash down neck of flask with about 10 ml of distilled water and swirl.
9. Add 1 ml of the dilute stannous chloride and mix well.
10. Add distilled water to volume (25 ml).
11. After 10 min, read color at 660 μ using an infra-red sensitive tube and an infra-red filter.

(2) Exchangeable Cations

Jackson, M. L. 1964. Ammonium acetate method. *In:* Soil chemical analysis. Prentice-Hall Inc.

Principle

Extraction is achieved with an ammonium acetate solution. Potassium and sodium are determined by emission spectroscopy. Calcium and magnesium are determined in the extract by atomic absorption spectroscopy at a selected wave length using a specific cathode lamp.

In this laboratory, little or no interference has been encountered on neighboring wave lengths on the Pye Unicam SP1900 Atomic Absorption and Emission Spectrophotometer, therefore pure standards are used as they require less time to prepare and they compare favorably in periodic checks with the composite standard and samples of known concentrations.

Equipment

1. Pye Unicam SP1900 Atomic Absorption and Emission Spectrophotometer.
2. Analytical balance.
3. Vacuum flasks.
4. Büchner funnels.
5. 200-ml volumetric flasks.

Reagents

1. Ammonium acetate - 154.5 g of CH_3COONH_4 dissolved in distilled water, made up to 2 litres in a volumetric flask, and adjusted to pH 7.0.
2. Standard solutions - each containing 100 ppm of specific cations.
   A. *Calcium* - 0.36682 g of $CaCl_2$ made up to 1 litre.
   B. *Magnesium* - 0.83606 g of $MgCl_2 \cdot 6H_2O$ made up to 1 litre.
   C. *Potassium* - 0.19070 g of KCl made up to 1 litre.
   D. *Sodium* - 0.25423 g of NaCl made up to 1 litre.

A composite standard solution is used as a control for the pure standards. Suitable dilutions of the standards are prepared to establish a standard curve for each cation to be determined.

Procedure

1. Weigh between 3 and 5 g of sample into a 250-ml Erlenmeyer flask. Record the exact weight.
2. Add 150 ml of ammonium acetate of pH 7.0.
3. Shake well and allow to stand overnight.
4. Pour this mixture through a Büchner funnel and leach slowly.
5. Add 30 ml of ammonium acetate in 10 ml aliquots.
6. Pour the extract into a 200-ml volumetric flask and make up to volume with distilled water.
7. Set aside Büchner funnel containing the sample as it is used further in determining the exchange capacity (see below).
8. Keep extracts refrigerated in polyethylene bottles until analyzed.
9. Calibrate atomic absorption and emission spectrophotometer, e.g. with magnesium standard.
10. Introduce sample into the spectrophotometer, read the relative emission or absorption in ppm concentration.
11. Repeat steps 9 and 10 for each cation requested.

Calculations

$$\text{Exc cat in m.e./100 g} = \frac{(\text{ppm}/5) \times 100}{(\text{sample wt}) \times (\text{m.e. wt of element})}$$

(3) Exchange Capacity

Jackson, M. L. 1964. Soil chemical analysis. Prentice-Hall Inc.

Principle

In the method described here, the cation exchange capacity determination, which involves measuring the total quantity of negative charges per unit weight of the material, is accomplished by washing the soil with calcium chloride in excess. Further washing with 80% acetone removes excess salt. This is followed by a final six washings with ammonium acetate to replace the absorbed calcium. The calcium is then determined by atomic absorption spectrophotometry.

Equipment

1. Pye Unicam SP1900 Atomic Absorption and Emission Spectrophotometer.
2. Vacuum flasks.
3. Büchner funnels.
4. 250-ml volumetric flasks.

Reagents

1. Calcium chloride - 111.0 g of $CaCl_2 \cdot 2H_2O$ dissolved in distilled water and made up to volume in a 2-litre flask. Adjusted to pH 7.0.
2. 80% acetone - 1600 ml of acetone mixed with 400 ml of distilled water.
3. Ammonium acetate - See *Reagents* for Exchangeable Cations (page 5).
4. Calcium standard - See *Reagents* for Exchangeable Cations (page 5).

Procedure

Using the soil samples that were retained in the Büchner funnels from analysis for Exchangeable Cations (page 5 Procedure 7.), connect the vacuum filtering system.

1. Wash each sample with 200 ml of calcium chloride solution (pH 7.0) in five aliquots. Discard filtrate.
2. Wash sample with 400 ml of 80% acetone in 10 aliquots. Discard filtrate.
3. Wash sample with 200 ml of ammonium acetate solution (pH 7.0) in six portions. Retain filtrate.

4. Make up volume of filtrate to 250 ml with distilled water.
5. Using calcium cathode lamp calibrate atomic absorption and emission spectrophotometer with calcium standards.
6. Read concentration in ppm.

Calculations

$$\text{Exc cap in m.e./100 g} = \frac{(\text{ppm}/4) \times 100}{(\text{sample wt}) \times (\text{m.e. wt of Ca})}$$

(4) Organic Matter

Principle

Total organic carbon as defined here includes:

1. Highly condensed, nearly elemental, organic carbon (charcoal, coal, and graphite).
2. Altered and rather resistant organic residues of plants, animals, and microorganisms, sometimes termed humus or humate, but not, as these latter terms tend to suggest, a single compound.
3. Little altered organic residues of plants and animals, living and dead microorganisms subject to rather rapid decomposition in soils.

Two methods of determination are available:

Method A is normally used because of the large number of samples to be analyzed. It consists of dry combustion and calculation of weight loss; this is a rough measurement.

Method B is a more demanding procedure than Method A and is used only when specifically requested.

The conventional factor to convert carbon to organic matter is 1.724 based on the assumption that soil organic matter is 58% carbon. The factors to convert carbon content to organic content have been found to be 1.9 for many surface soils and 2.5 for many subsoils. The variation in the carbon/organic matter ratio makes it desirable to report the organic content rather than the carbon content for comparisons between different horizons or between dissimilar soils (Jackson, 1964).

A. Dry Combustion Method

Anon. 1965. Loss of weight upon ignition. *In*: Methods of soil analysis, Part 2. Amer. Soc. Agron., Madison, Wisc.

Equipment

1. Drying oven.
2. Muffle furnace.
3. Analytical balance.
4. Porcelain crucibles.

Procedure

1. Oven dry samples for 24 hours at 105°C.
2. Transfer samples to desiccator to cool.
3. Weigh about 5 g of organic soil or 15 g of mineral soil into a tared porcelain crucible. Record exact weight.
4. Place the crucibles in a muffle furnace, bring the temperature to 550°C, and hold for 24 hours.
5. Transfer crucibles to a desiccator and allow to cool.
6. Reweigh crucibles. Record exact weight.

Calculation

% organic matter = [(wt loss)/(oven-dry wt)] x 100.

B. Walkely-Black Method

Walkely, A., and I. A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29-38.

Equipment

1. Analytical balance.
2. Burette.
3. 500-ml Erlenmeyer flasks.
4. 1000-ml volumetric flasks.

Reagents

1. Potassium dichromate, 1 *N* - dissolve 49.04 g of $K_2Cr_2O_7$ in distilled water and make up to 1 litre.

2. Ferrous ammonium sulphate, 0.4 *N* - dissolve 159.6 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water containing 40 ml of conc H_2SO_4 and make up to 1 litre. Determine normality by titrating against the potassium dichromate solution.

3. O-phenanthroline ferrous sulphate complex - 0.025 *M* solution of Ferroin.

4. Phosphoric acid - 14.6 *M* solution (85%).

Procedure

1. Pass soil through a 0.5-mm sieve and weigh 0.5 g into a 500-ml Erlenmeyer flask.

2. Add 10 ml of potassium dichromate and 20 ml of conc H_2SO_4. Mix rapidly and thoroughly for 1 min. Let stand for at least 20 min or until cool.

3. Dilute to 150 ml with distilled water and add 10 ml of conc H_3PO_4.

4. Titrate with 0.4 *N* ferrous ammonium sulphate. Use six drops of O-phenanthroline indicator.

5. Simultaneously, run a blank using the same procedure.

Calculations

$$\% \text{ O.M.} = (T-B) \times (N/0.4) \times 0.545.$$

Where T = $Fe(NH_4)_2(SO_4)_2$ solution used in titration of sample, ml;
B = solution used in titration of blank, ml; and the factor 0.545 is derived as follows:

$$(0.4 \text{ N}) \times (12/4000) \times (1.72/0.76) \times (100/0.5) = 0.545$$

and m.e. wt of carbon = 12/4000; 1.72 is a factor based on the assumption that organic matter is 58% carbon; 0.76 is % recovery factor; and 0.5 is weight of sample.

(5) pH - Soil-Water Paste Method

Doughty, J. L. 1941. The advantages of a soil paste for routine pH determinations. Sci. Agr. 22: 135-138.

Principle

The pH is the negative logarithm of the hydrogen ion concentration in the soil. This concentration is determined indirectly by measuring the electromotive force of a cell consisting of hydrogen and calomel electrodes (old method) or by measuring the potential across a membrane of special glass.

Equipment

1. pH meter.
2. Appropriate electrode.
3. Disposable paper portion cups, e.g. Lily portion cups No. 100.

Reagents

1. Standard buffer solutions.

Procedure

1. Fill paper cups 2/3 full with soil.
2. Add distilled water to make a smooth paste which glistens and closes the indentation made by a stirring rod. This is about a 1:2 ratio of soil to water. Stir well.
3. Allow to stand for 20 min.
4. Read pH with pH meter using a combination glass electrode.

(6) Total Nitrogen

Bremner, J. M. 1965. Total nitrogen; Inorganic nitrogen. Amer. Soc. Agron. Monogr. 9. 1189 pp.

Principle

This procedure does not determine the nitrate and nitrite-nitrogen. In most forest soils, however, these can safely be neglected in the determination of total nitrogen.

The ammonium-nitrogen produced in the digestion is liberated by making the solution strongly alkaline with sodium hydroxide and distilling the liberated ammonia into a boric acid solution. The ammonium borate is then back-titrated with standard acid to form boric acid. A methyl red-bromocresol green mixture is used for the indicator of this reaction.

Equipment

1. Drying oven.
2. Analytical balance.
3. Aminco rotary digestion apparatus.
4. Aminco Kjeldahl distillation assembly.
5. Standard burettes.
6. 5-ml micro-burette.
7. 1-mm sieve.
8. 100-ml Kjeldahl digestion flasks.

Reagents

1. Boric acid indicator solution - dissolve 20 g of pure H_3BO_3 in 700 ml of hot distilled H_2O, then cool the solution. Add 200 ml of ethanol and 20 ml of mixed indicator solution (prepared by dissolving 0.330 gm of bromocresol green and 0.165 g of methyl red in 500 ml of ethanol). Adjust to pH 5.0 using 0.05 *N* NaOH, transfer to 1000 ml volumetric flask and make up to 1 litre with distilled H_2O. Mix thoroughly. When 1 ml of indicator solution and 1 ml of distilled H_2O are mixed a colour change from pink to pale green should be detected.
2. Digestion catalyst - mix K_2SO_4, $CuSO_4$, and Se in the ratio 100:10:1.
3. 10 *N* NaOH
4. 0.01 *N* HCL
5. Conc H_2SO_4

Procedure

1. Pass soils samples, oven dried at 65°C, through a 1-mm sieve.
2. Dry samples again before analysis.
3. Weigh soil on a piece of glazed paper that will fold and fit into the neck of the Kjeldahl flask.

The minimum weights of forest soil used for analysis are:

Horizon	Approx wt (g)	Horizon	Approx wt (g)
H	0.100	Ah	0.300
LFH	0.100	Ae	0.500
BFH	0.200	B	0.500
BF	0.200	BC	0.500
		C	0.500

4. Record exact sample weight.
5. Add 5 ml of conc H_2SO_4 to samples, standards, and blank. Stopper and set overnight.
6. The following morning, add 1 g of catalyst.
7. Digestion
   (a) Place flasks on digestion rack and start digestion at a low temperature.
   (b) After the danger of frothing or splatter has ceased, increase the heat so that the contents of the flask boil smoothly.
   (c) Continue digestion at this temperature until white fumes stop forming and the reaction subsides. Slowly increase heat until maximum heat of digestion rack is reached and the solution clears.
   (d) After clearing, digest for an additional 3 h. Allow cooling while still on digestion racks.
   (e) Remove flasks from racks, stopper, and permit further cooling before adding 20 ml of distilled H_2O.
   (f) Cool before distillation.
8. Distillation
   (a) Turn on the tap for the condenser.
   (b) Start preheating water in the boiling chamber.
   (c) Close stopcock on distillation apparatus until steam has built up in the boiling chamber.

(d) After steam has built up, open stopcock on distillation apparatus and permit steam to pass through to clean interior of apparatus before distillation of blanks and samples.

(e) Attach digestion flask containing blank to the distillation apparatus.

(f) Add 20 ml of 10 *N* NaOH through stopcock on top of apparatus.

(g) Place 50 ml Erlenmeyer flask containing 15 ml of indicator solution under the condenser.

(h) Collect for 5 min or until the volume of liquid in the Erlenmeyer flask is a little more than double.

(i) Stopper and store until titration.

(j) Repeat steps (e) to (j) for the standards and samples.

Titration

Titrate the distillate back to its original colour with 0.01 *N* HCL.

Calculation

$$\text{mg N} = (T-B) \times No \times (\text{m.e. wt of N})$$

$$\% \text{ N} = [(\text{mg N})/(\text{sample wt, mg})] \times 100$$

Where T = standard acid used in the back-titration of the sample, ml;

B = standard acid used in the back-titration of the blank, ml;

No = normality of the standard acid.

C. Physical Analysis

(1) Moisture Content

A. Air Dry

Principle

The air-dry moisture content is the percentage soil moisture remaining after the soil has been dried in the open. The weight loss after drying in a well ventilated oven is expressed as a percentage of the air-dried weight.

Equipment

1. Analytical balance.
2. Soil-moisture cans.
3. Forced-air drying oven.

Procedure

1. Weigh sample into a tared aluminum soil-moisture can and dry in the oven for 24 h at 105°C.
2. Transfer can to a desiccator to cool.
3. Reweigh sample.
4. Calculate weight loss.

Calculations

% moisture content = [(wt loss)/(oven-dry wt)] x 100.

B. Field Capacity

van Groenewoud, H. Suction plate picnometer apparatus. Marit. For. Res. Cent. Fredericton, N.B. Unpubl. paper.

Principle

The suction plate picnometer is used at 1/3 atmosphere pressure for 24 h to simulate field conditions. The apparatus was designed for undisturbed samples, but it can be used equally well for disturbed samples. Samples delivered to the laboratory are usually not core samples.

Equipment

1. Suction plate picnometer.
2. Soil-moisture cans.
3. Analytical balance.
4. Drying oven.

Procedure

1. The suction plate picnometer consists of 20 numbered porous plates. Number the samples to correspond with the plates.
2. Saturate the soil samples with water and allow to stand overnight.
3. Wet the porous plates.

4. Place about 12 to 20 mm of soil on the plate. Add a little water before covering the sample.
5. Turn on the apparatus and apply 1/3 atmosphere suction for 24 h.
6. Remove samples to covered, tared, soil-moisture cans and weigh. This represents the (soil + moisture) weight at field capacity.
7. Remove covers and dry at 105°C for 24 h.
8. After cooling in a desiccator, replace covers and weigh samples to obtain the dry weight.
9. Calculate weight loss by subtraction.

Calculations

% moisture at field capacity = [(wt loss)/(oven-dry wt)] x 100.

C. Wilting Point

Anon. 1966. Operating manual for fifteen-bar ceramic plate extractor. Soil Moisture Equipment Co., Santa Barbara, Calif.

Principle

The permanent wilting point is that percentage soil moisture at which the first two bottom leaves of plants growing in that soil reach a wilted condition from which they cannot recover in a saturated atmosphere. The 15-bar ceramic plate extractor is used to simulate this condition in the laboratory.

Equipment

1. 15-bar ceramic plate extractor.
2. Analytical balance.
3. Covered moisture cans.
4. Desiccator.
5. 250-ml beakers.

Procedure

1. Saturate each sample in a 250-ml beaker and allow to stand overnight.
2. Wet ceramic plates with water.
3. Number positions on each plate and number soil samples correspondingly.

4. At each position, place a soil-retaining ring, fill with soil sample, and saturate with water.
5. Place the plates in the extractor. Close and bolt down the lid as tightly as possible by hand.
6. Turn on the compressor and apply 15-bar pressure for 24 h.
7. Remove samples to tared soil-moisture cans and weigh. This weight is the (soil + moisture) weight at the wilting point.
8. Remove covers and dry samples at 105°C for 24 h.
9. After cooling in a desiccator, replace covers and weigh samples. Calculate weight loss by subtraction.

Calculation

% moisture at wilting point = [(wt loss)/(wt at wilting point)] x 100.

(2) Mechanical Analysis

Black, C. A., *et al.* 1965. Hydrometer method of particle-size analysis. *In*: Methods of soil analysis, Part 1. Amer. Soc. Agron., Madison, Wisc.

Principle

The determination of the particle-size distribution depends on the settling rates of different size particles from suspension in water. The particles are therefore dispersed in an aqueous suspension so that they will be detached from one another and suspended in the liquid. Soils that contain soluble salts, gypsum, or organic matter may not disperse adequately. Dispersal in such cases is accomplished by treating the soil with hydrogen peroxide to destroy organic matter, followed by filtration and washing with enough water to dissolve the gypsum. Dispersion usually requires the addition of a reagent such as $Na_4P_2O_7 \cdot 10H_2O$ (Sodium pyrophosphate) or $NaPO_3$ (sodium metaphosphate) or various mixtures in dilute concentrations. The particles must be separated by shearing action or turbulent mixing by means of a mechanical shaker or electric mixer. The mixing must not be too vigorous because rupture of the individual particles should be avoided.

A hydrometer is used to measure the density of the solution at prescribed time intervals as sedimentation takes place.

In this laboratory, two methods of analysis are available for samples containing no organic materials. Method A, normally used, determines % sand, % silt, and % clay. Upon specific request Method B is used and determines % sand fractions, % silt, and % clay.

Method A

Equipment

1. Soil dispersion mixer.
2. Soil-testing cylinders.
3. Brass plunger.
4. Soil hydrometer.
5. Desiccator.
6. Drying oven.
7. Electric timer.
8. Thermometer.

Reagents

1. Calgon solution 10% - 10 g of Calgon dissolved in distilled water and made up to 100 ml volume with distilled water. Adjust to pH 8.5.

Procedure

1. Weigh 60 g of air-dried soil.
2. Dry in the oven at 105°C for 24 h.
3. After drying, cool the sample in a desiccator.
4. After cooling, weigh 50 g of the oven-dried soil into a porcelain evaporating dish.
5. Add 5 ml of 10% Calgon. Stir and let stand for 15 min.
6. Wash all the material into a dispersing cup and add water to half-fill the cup. Mix for 10 min on the electric mixer (5 min for fine material).
7. Pour the contents of the cup into a soil-testing cylinder. Rinse the cup well to obtain all of the sample.
8. Add water to the contents of the cylinder making it up to 1000 ml.
9. Make up a blank cylinder containing the 10% Calgon.

10. Mix the suspension with the plunger using strong upward strokes. Wait 30 sec and carefully insert the hydrometer. At exactly 40 sec record the reading at the top of the meniscus (if any foam occurs, use a drop or two of amyl alcohol to disperse it). This reading indicates the silt + clay fraction. Record the temperature of the suspension.
11. Record the reading and temperature of the blank.
12. Allow the suspension to stand completely undisturbed for 2 hr and then take another reading. Again, record the temperature of the sample and of the blank. This reading indicates the clay fraction.

The calculated percent result obtained from the clay reading is subtracted from the percent result obtained from the silt + clay result to give the percent silt.

The percent result of silt + clay is subtracted from 100% to give the % sand. Theoretically, % sand + % silt + % clay = 100%.

Calculations

A correction factor (C) of 0.2 must be added to the hydrometer reading (H) for each 1° above 67°F, or subtracted for each 1° below 67°F.

% silt + clay = 2 x (soil H ± C) ± (blank H ± C)[40-sec reading]

% clay = 2 x (soil H ± C) ± (blank H ± C)[2-hr reading]

% silt = % (silt + clay) - % clay

% sand = 100% - % (silt + clay)

Method B

Equipment

1. Soil dispersion mixer.
2. Soil-testing cylinders.
3. Brass plunger.
4. Soil hydrometer.
5. Desiccator.
6. Drying oven.
7. Electric timer.
8. Thermometer.

9. 270-mesh (0.053 mm) sieve.
10. 2.00 mm., 1.00 mm, 0.5 mm, 0.25 mm, and 0.10 mm sieves for the sand fractions.

Reagents

1. Calgon solution 10% - 10 g of Calgon dissolved in distilled water and made up to 100 ml volume with distilled water. Adjust to pH 8.5.

Procedure

1. Weigh 60 g of air-dried soil.
2. Dry in the oven at 105°C for 24 h.
3. After drying, cool the sample in a desiccator.
4. After cooling, weigh 50 g of the oven-dried soil into a porcelain evaporating dish.
5. Add 5 ml of 10% Calgon. Stir and let stand for 15 min.
6. Wash all the material into a dispersing cup and add water to half-fill the cup. Mix for 10 min on the electric mixer (5 min for fine material).
7. Place a very large funnel in the soil-testing cylinder and place a 270-mesh sieve in the funnel.
8. Pour the contents of the dispersing cup into the sieve. Rinse the cup well to obtain all of the sample. Wash the sieve with water until all the silt and clay is removed, i.e., until the water running through the sieve is clear, leaving only the sand in the sieve.
9. Place the sieve in the drying oven. Later, after the sand has dried, carefully transfer it from the sieve to a small tared dish and put it in a desiccator to cool. When cool, fractions can be determined by using the other sieves listed under equipment, according to the U.S. Dept. of Agriculture scheme (p. 56 of reference.).
10. Add water to the contents of the cylinder making it up to 1 litre.
11. Make up a blank cylinder containing the 10% Calgon.

12. Mix the suspension with the plunger using strong upward strokes. Wait 30 sec and carefully insert the hydrometer. At exactly 40 sec record the reading at the top of the meniscus (if any foam occurs, use a drop or two of amyl alcohol to disperse it). This reading indicates the silt + Clay fraction. Record the temperature of the suspension.
13. Record the reading and temperature of the blank.
14. Allow the suspension to stand, completely undisturbed, for 2 h and then take another reading. Again, record the temperature of the sample and of the blank. This reading indicates the clay fraction.

Calculations

% sand fractions are determined by weighing each fraction and calculating its percent of total oven-dried sand weight.

For calculating % silt and % clay, a correction factor (C) of 0.2 must be added to the hydrometer reading (H) for each 1° above 67°F, or subtracted for each 1° below 67°F.

% silt + clay = 2 x (soil H ± C) ± (blank H ± C)[40-sec reading]

% clay = 2 x (soil H ± C) ± (blank H ± C)[2-h reading]

% silt = % (silt + clay) - % clay.

Theoretically, % sand + % silt + % clay = 100%.

Part II - TISSUE ANALYSIS

A. Preparation of Tissue Samples

Foliage and bark samples are delivered to the laboratory for analysis.

1. The samples are dried at 65°C in a convection oven for 24 h.
2. After drying, the samples are cleaned both by hand and with compressed air: the samples are retained in sieves.
3. The samples, ground fine enough to pass through a 1-mm sieve, are packaged and stored in labelled covered containers.

It is important that the mill be cleaned *thoroughly* between the grinding of individual samples. This is done with compressed air, acetone, and a brush.

B. Chemical Analysis

(1) Dry Ashing

van Groenewoud, H. Marit. For. Res. Cent. Unpubl. paper.

Principle

The dried ground sample to be analysed is ashed in a muffle furnace, cooled, dissolved in dilute acid, and made up to volume for analysis.

Equipment

1. Analytical balance.
2. Convection drying oven.
3. Muffle furnace.
4. 100-ml volumetric flasks.
5. Funnels.
6. Porcelain crucibles.
7. Desiccator.

Reagents

1. HCl, 1 *N*.
2. Strontium chloride 15,000 ppm - weigh 45.6437 g $SrCl_2 \cdot 6H_2O$ and make up to 1 litre.

Procedure

1. Remove the dried ground samples from storage and dry at 65°C for 12 h.
2. After cooling in a desiccator, weigh 1.25 g ± 100 mg (if trace elements are to be determined) into a porcelain crucible.
3. Place the crucibles in a muffle furnace; bring the temperature slowly to 550°C and maintain for a total ashing time of 10 h.
4. Allow the material to cool quickly outside the furnace with the crucibles covered.
5. Using a squeeze bottle, wet the ash with a small amount of distilled water directed down the sides of the crucibles.
6. Add 5 ml of 1 *N* HCl to each sample.
7. Transfer all the contents of each crucible to a 100-ml volumetric flask through a funnel. Wash any material left in the crucible into the flask with distilled water from a squeeze bottle.
8. Add distilled water to each sample until the flasks are about half-full; stopper, then shake the contents vigorously for a few moments.
9. Add 10 ml of strontium chloride stock solution to give a final concentration of 1500 ppm.
10. Add distilled water to bring the total volume to 100 ml.
11. Filter the contents through Whatman No. 40 filter paper into polyethylene bottles.
12. Place the samples in the deep freeze to prevent possible deterioration before analysis.

(2) Total Ash

Anon. 1962. Chemical method of plant analysis. Can. Dep. Agric. Publ. 1064.

Principle

After drying and grinding the sample, weigh and place in a muffle furnace, cool, and then reweigh. The percent ash is then determined.

Equipment

1. Muffle furnace.

Procedure

1. Place numbered crucibles in drying oven overnight.
2. Take crucibles from drying oven and place in dessicator to cool.
3. After cooling weigh crucibles and record the oven dry weight of each numbered crubible.
4. Dry samples in drying oven for 12 h at 55-60°C.
5. Remove samples from drying oven and place in dessicator to cool.
6. After cooling in a desiccator, weigh 3 g ± 100 mg of sample into a weighed crucible and place in a muffle furnace. Record exact wt of sample plus crucible.
7. Bring the temperature slowly to 550°C and maintain for 10 h.
8. Remove crucibles from furnace and place in a desiccator to cool. Cover crucibles.
9. Reweigh the sample plus crucible. This is the weight of the ash plus crucible.
10. Subtract the weight of oven dried crucible to give the weight of the ash. The % ash can be calculated.

Calculations

$$\% \text{ ash} = [(\text{wt of ash})/(\text{oven-dry sample wt})] \times 100.$$

(3) Total Cations (Ca, Cu, Co, Fe, Pb, Mg, Mn, Zn, K, and Na)

Principle

Total cations of the extract of the ashed residue (see step 12 of *Procedure* for Dry Ashing, page 22) by atomic absorption and by emission (K and Na) spectroscopy.

The extract is sprayed into the flame of the spectrophotometer. This produces the element in the atomic state. A hollow cathode lamp, for the element being determined, is used to emit a line spectrum containing a sharp resonance line characteristic of that element. Radiation from the lamp is passed through the flame and into the

Radiation from the lamp is passed through the flame and into the monochromator. Although some atoms in the sample are excited and emit radiation, most of the atoms remain in the ground state and are able to absorb energy from the radiation emitted by the lamp. One advantage of the atomic absorption technique is that a very high proportion of the atoms in the flame are available to absorb radiation. Whereas the intensity of emission varies greatly with flame temperature, the degree of absorption will not vary to the same extent. The use of the monochromator to remove adjacent spectral lines results in light of a high spectral purity being received by the detector.

Postassium and sodium are determined by emission spectrophotometry, all other cations in the list are determined in this laboratory by absorption spectrophotometry.

Equipment

1. Pye Unicam SP1900 Atomic Absorption and Emission Spectrophotometer.

Reagents

1. Standards, containing 1500 ppm of strontium chloride, for each element in the range of concentration present in the material being analysed.

Element	Source for pure standard	Element	Source for pure standard
Ca	$CaCl_2$	Mg	$MgCl_2 \cdot 6H_2O$
Cu	$CuCl_2 \cdot 2H_2O$	Mn	$MnCl_2 \cdot 4H_2O$
Co	$CoCl_2 \cdot 6H_2O$	Zn	$ZnCl_2$
Fe	$FeCl_2$	K	KCl
Pb	$PbCl_2$	Na	NaCl

2. Composite standard - either to be used directly in suitable concentrations or for a check in pure standards.

Procedure

1. Set spectrophotometer for either absorption or emission as required.
2. Allow 5 or 10 min to warm up the specific cathode lamp.
3. Set spectrophotometer at the appropriate wave length and power for element to be determined.
4. Introduce the standard concentrations and calibrate the spectrophotometer.
5. Pour extracts into a 10-ml beaker.
6. Introduce the sample and record the reading (the read out in this instrument is directly in ppm concentration).

For some of the cations, it is necessary to dilute the extract before measuring as the concentration is greater than can be measured on the spectrophotometer.

Calculations

% total cation = ppm x (F)

When (F) represents conversion factor to % dependent upon actual oven-dried sample weight in 100 ml.

(4) Total Nitrogen

Bremner, J. M. 1965. Total Nitrogen; Inorganic nitrogen. Amer. Soc. Agron. Monograph 9. 1189p.p.

Principle

See (6) Total Nitrogen (p. 10).

Equipment

1. Drying oven.
2. Analytical balance.
3. Aminco rotary digestion apparatus.
4. Aminco Kjeldahl distillation assembly.
5. Standard burettes.
6. 5-ml micro-burette.
7. 100-ml Kjeldahl digestion flasks.

Reagents

1. Boric acid indicator solution - dissolve 20 g of pure H_3BO_3 in about 700 ml of hot distilled H_2O, then cool the solution. Add 200 ml

of ethanol and 20 ml of mixed indicator solution (prepared by dissolving 0.330 g of bromocresol green and 0.165 g of methyl red in 500 ml of ethanol). Adjust to pH 5.0 using 0.05 *N* NaOH, transfer to 1000 ml volumetric flask and make up to 1 litre with distilled H_2O. Mix thoroughly. When 1 ml of indicator solution and 1 ml of distilled H_2O are mixed a colour change from pink to pale green should be detected.

2. Digestion catalyst - mix K_2SO_4, $CuSO_4$ and Se in the ratio 100:10:1.
3. 10 *N* NaOH.
4. 0.05 *N* HCl.
5. Conc H_2SO_4

Procedure

1. Samples that have been previously dried, ground, and packaged are dried at 65°C for 12 h immediately before analysis.
2. Cool in a desiccator.
3. Weigh approximately 100 mg (but not less than 100 mg) and record the exact weight.
4. Place weighed samples in a 100 ml Kjeldahl flask.
5. Add 5 ml of conc H_2SO_4 to samples, standards, and blank; stopper and set overnight.
6. The following morning, add 1 g of catalyst.
7. Digestion
   (a) Place flasks on digestion rack and start digestion at a low temperature.
   (b) After the danger of frothing or splatter has ceased, increase the heat so that the contents of the flask boil smoothly.
   (c) Continue digestion at this temperature until white fumes stop forming and the reaction subsides. Slowly increase heat until maximum heat of digestion rack is reached and the solution clears.
   (d) After clearing, digest for 1 h more and then allow cooling while still on digestion racks.
   (e) Remove flasks from racks, stopper, and permit further cooling before adding 20 ml of distilled H_2O.
   (f) Cool before distillation.

8. Distillation

(a) Turn on the tap for the condenser.

(b) Start preheating water in the boiling chamber.

(c) Close stopcock on distillation apparatus until steam has built up in the boiling chamber.

(d) After steam has built up, open stopcock on distillation apparatus and permit steam to pass through in order to clean interior of apparatus before distillation of blanks and samples.

(e) Attach digestion flask containing blank to the distillation apparatus.

(f) Add 20 ml of 10 *N* NaOH through stopcock on top of apparatus.

(g) Place 50-ml Erlenmeyer flask containing 15 ml of indicator solution under the condenser.

(h) Collect for 5 min or until the volume of liquid in the Erlenmeyer flask is a little more than doubled.

(i) Stopper and store until titration.

(j) Repeat steps (e) to (j) for the standards and the samples.

Titration

Titrate the distillate back to its original colour with 0.05 *N* HCl.

Calculations

$$\text{mg N} = (T-B) \times No \times (\text{m.e. wt of N})$$

$$\% \text{ N} = [(\text{mg N})/(\text{sample wt mg})] \times 100.$$

Where T = standard acid used in the back-titration of the sample, ml;

B = standard acid used in the back-titration of the blank, ml;

No = normality of the standard acid.

(5) Total Phosphorus

Vanadate-Yellow Method

Jackson, M.L. 1958. Soil chemical analysis. Prentice-Hall, Inc.

Principle

Phosphate ions form a yellow complex with vanadate and molybdate ions. This reaction is used for the colorimetric determination of phosphorus.

The procedure is simple and practically free of interferences. The yellow colour is very stable and the sensitivity of phosphate detection is about 10 times less than that of the phosphomolybdo-blue method (available Phosphorus, page 2). For these reasons, the vanadate-yellow method is well suited to determination of phosphorus in solutions of ashed plant materials.

Equipment

1. Spectronic "20" spectrophotometer or colorimeter.
2. 10- and 25-ml transfer pipettes.
3. 50-ml volumetric flasks.

Reagents

1. Standard phosphorus solution, 50 ppm - dissolve 0.2195 g of KH_2PO_4, dried at 40°C in 800 ml of distilled water and dilute to 1 litre.
2. Perchloric acid-vanadate-molybdate solution - prepare solutions A and B as follows:

   A. Dissolve 12.5 g of ammonium molybdate, $(NH_4)_6MO_7O_{24}\cdot 4H_2O$, in 150 ml of distilled water.

   B. Dissolve 0.625 g of ammonium metavanadate NH_4VO_3, in 200 ml of boiling water. Cool. Add 107 ml of 72% perchloric acid, $HClO_4$. Allow to cool. Pour solution A into solution B. Mix. Adjust volume to 500 ml with distilled water. This solution is 2.5 *N* with respect to $HClO_4$.
3. Perchloric acid, 1 *N* - dilute 85.5 ml of 72% $HCLO_4$ to 1 litre with distilled water.

Preparation of the Standard Curve

1. Add 2 ml of the phosphorus standard solution to a 50-ml volumetric flask. To four additional flasks, add 4, 6, 8, and 10 ml of standard solution.
2. Add 15 ml of 1 *N* $HClO_4$ to each flask containing the phosphorus standard solution and to one additional empty 50-ml volumetric flask.
3. Adjust the volume of liquid in each flask to 25 ml with distilled water.

4. Add to each flask 10 ml of perchloric acid-vanadate-molybdate reagent.
5. Mix and dilute contents to 50 ml with distilled water. The solutions contain 0, 2, 4, 6, 8, and 10 ppm of phosphorus.
6. Allow 10 min for color development.
7. Determine the percent transmittance for each solution in the spectrophotometer at a wave-length of 440 μ. Use the solution without the phosphorus addition to adjust the meter to 100% transmittance.
8. Plot percent transmittance against phosphorus concentration on semi-logarithmic paper.

Procedure

1. Transfer 5 ml of the solution containing the residue of ashed plant material (see step 12 of *Procedure* for Dry Ashing, page 22) to a 50-ml volumetric flask using a volumetric pipette.
2. Add 10 ml of perchloric acid-vanadate-molybdate solution using a volumetric pipette.
3. Dilute the contents of the flask to 50 ml.
4. Allow 10 min for colour development.
5. Determine the percent transmittance with a spectrophotometer or colorimeter at a wave-length of 440 μ. Use a reagent blank solution to adjust the meter to a 100% transmittance.
6. Determine the concentration (ppm) of phosphorus in the test solution from the standard curve.

Calculation

% P = (ppm P in test solution) x 10 x (F)

10 = dilution factor in procedure

(F) = factor to convert to % depending on sample weight and volume.

(6) Sulphur

Blanchar, R. W., Rehm, G., and Caldwell, A. C. 1964. Sulfur in Plant Materials by Digestion with Nitric and Perchloric Acid. Soil Sci. Soc. Proc., 1965, Vol. 29, Nos. 1-6, pp 71-72.

Principle

The material is oxidized by nitric and perchloric acid using a heating block to reduce perchloric acid loss. The SO_4 content of the solution is determined turbidometrically as $BaSO_4$.

Equipment

1. Analytical balance.
2. Aluminum block digestor.
3. Explosion proof fume hood.
4. Magnetic stirrer.
5. Spectronic "20" spectrophotometer or colorimeter.
6. 50-ml graduated test tubes.
7. Glass beads.
8. Small funnels.
9. 150-ml beakers.
10. 50-ml burette.
11. 1- and 2-ml volumetric pipettes.

Reagents

1. Conc HNO_3.
2. 60-70% $HClO_4$.
3. I *N* HCl.
4. Salt buffer solution - containing 40 g $MgCl_2 \cdot 6H_2O$, 4.1 g sodium acetate, 0.83 g potassium nitrate, and 28 ml of 95% ethanol. This is made up to 1 litre.
5. $BaCl_2$ - 20-30 mesh crystals.
6. Sulphur standards containing 10-40 ppm S - weigh 768.71 mg of $MgSO_4 \cdot 7H_2O$ and make up to 1 litre giving a concentration of 100 ppm. From this solution pipette appropriate amounts to give 10-40 ppm in 50 ml. Prepare a standard curve.

Procedure

1. Weigh 0.50 g or enough plant material to give a final concentration of 20-40 ppm S in 50 ml and place the sample in a 50 ml graduated test tube.
2. Add 2 glass beads and 3 ml of conc HNO_3.
3. Place small funnels in the mouth of the tubes and allow them to stand overnight.
4. Place the tubes in the aluminum block digestor and heat for 1 h at 150°C.
5. Add 2 ml of 60-70% $HClO_4$ through the funnels.
6. Gradually raise the temperature to 235°C and digest for 2 h.
7. After the digestion is completed remove the funnels and add 1 ml of HCl.
8. Heat for 20 min at 150°C.
9. Remove the tubes from the digestion block, allow to cool slightly, and add 35 ml of distilled H_2O and 10 ml of salt buffer solution.
10. Adjust the volume of the solutions in the digestion tubes to 50 ml with distilled H_2O.
11. The solutions are filtered into 150 ml beakers.
12. Place the solutions on a magnetic stirrer and allow the stirring action to come to a constant state.
13. Add 0.30 g of 20-30 mesh $BaCl_2$ crystals and continue to stir the solution for exactly 1 min.
14. Transfer the turbid solution to a colorimeter and read the transmittance at a wave length of 420 μ exactly 2 min after the addition of $BaCl_2$ crystals.
15. The readings are compared to standards having SO_4 concentrations from 10 to 40 ppm S.
16. Standards are run with each group of samples.
    The standard curve should be verified with each new technician.

Calculations

The ppm concentration is read directly from the standard curve except with samples that require dilutions and then dilution factors must be applied.

Part III - WATER ANALYSIS

A. Preparation of Water Samples

Water analyses performed in this laboratory are determined on samples of rain water collected at the bases of trees (throughfall and stemflow) and on samples of well water used to irrigate nurseries. The most common requests are for the Ca, Mg, K, Fe, and Na content of these samples.

The samples are treated with 1 *N* HCl to make them 5% acid.

B. Chemical Analysis

(1) Total Cations

Principle

See Total Cations (page 23).

Equipment

1. Pye Unicam SP1900 Atomic Absorption and Emission Spectrophotometer.

Reagents

1. 1 *N* HCl.
2. Standards for each element to be determined in suitable concentrations.
3. Strontium cloride 15,000 ppm - weigh 45.6437 g $SrCl_2 \cdot 6H_2O$ and make up to 1 litre.

Procedure

1. Half-fill a 100-ml volumetric flask with sample.
2. Pipette 5 ml of 1 *N* HCl and shake.
3. Add 10 ml of strontium chloride stock solution.
4. Make up to volume with sample and shake to mix well.
5. Follow the *Procedure* for Total Cations (page 25).

Calculations

Concentration in ppm is a direct read out from Pye Unicam SP1900.

(2) Total Phosphorus

Principle

See *Principle Vanadate-Yellow method* (page 27).

Equipment

1. Spectronic "20" spectrophotometer or colorimeter.
2. 10-ml transfer pipettes.
3. 50-ml volumetric flasks.

Reagents

1. Standard phosphorus solution, 50 ppm - dissolve 0.2195 g of KH_2PO_4, dried at 40°C, in 800 ml of distilled water and dilute to 1 litre.
2. Perchloric acid-vanadate-molybdate solution - prepare solutions A and B as follows:

A. Dissolve 12.5 g of ammonium molybdate, $(NH_4)_6MO_7O_{24}\cdot 4H_2O$, in 150 ml of distilled water.

B. Dissolve 0.625 g of ammonium metavanadate, NH_4VO_3, in 200 ml of boiling water. Cool. Add 107 ml of 72% perchloric acid, $HClO_4$. Allow to cool.

Pour solution A into solution B. Mix. Adjust volume to 500 ml with distilled water. This solution is 2.5 *N* with respect to $HClO_4$.

3. 1 *N* $HClO_4$, - dilute 85.5 ml of 72% $HClO_4$ to 1 litre with distilled water.

Preparation of the Standard Curve

See *Preparation of the Standard Curve* for *Vanadate-Yellow Method* (page 28).

Procedure

1. Transfer 10 ml of water sample to a 50-ml volumetric flask using a volumetric pipette.
2. Add 10 ml of perchloric acid-vanadate-molybdate solution using a volumetric pipette.
3. Dilute the contents of the flask to 50 ml.
4. Allow 10 min for color development.

5. Determine the percent transmittance with a spectrophotometer or colorimeter at a wave-length of 440 μ. Use a reagent blank solution to adjust the meter to 100% transmittance.
6. Determine the concentration (ppm) of phosphorus in the test solution from the standard curve.

Calculations

Read concentration (ppm) directly from the standard curve.