PROCEDURES FOR SOIL AND PLANT ANALYSIS

Edited by
Sahlemedhin Sertsu and Taye Bekele

2000

National Soil Research Center
Ethiopian Agricultural Research Organization

Sponsored by
National Fertilizer Sector Project
NFIA, Addis Ababa, Ethiopia
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FOREWORD

This laboratory manual is prepared for use by all members of the Soil and Plant Analytical Laboratories Network of Ethiopia (SPALNE), including the Regional Soil Testing Laboratories. The manual is intended for standardization of all routine soil testing and plant analytical activities carried out in the country for the purpose of soil classification and assessment and monitoring of the nutrient status of soils and crops for soil fertility and agronomic studies.

The methods included in this manual are extracted from the detailed laboratory manual prepared by the National Soil Research Laboratory (NSRL) for making it handy for use by laboratory technicians. Attempt is made to make the procedures complete and self-contained, so that technicians carry out a given analysis without checking to other references.

Wherever possible, alternative methods are given for a given analysis to accommodate the range of equipment available in most SPALNE member laboratories of Ethiopia. For more detailed and specialized analysis, one has to refer to the detailed manual mentioned above. To be consistent and maintain uniformity with the international norms, most of the results are reported in SI units, but with their non-SI unit equivalents also given in tables and Appendices.

This manual has been prepared with the financial assistance of the NFIA. The editors welcome any suggestion or comment from readers and users for the improvement of a specific procedure or the manual as a whole.
# TABLE OF CONTENTS

Foreword

## PART I

1. **SOIL ANALYSIS**

   1-1. **SAMPLE PREPARATION**
   1-1.1. Registration
   1-1.3. Grinding, Sieving and Storage
   1-1.4. Reference

   1-2. **MOISTURE CONTENT**
   1-2.1. Principle
   1-2.2. Apparatus
   1-2.3. Procedure
   1-2.4. Calculation

   1-3. **PARTICLE-SIZE ANALYSIS**
   1-3.1. Principles
   1-3.2. Apparatus
   1-3.3. Reagents
   1-3.4. Procedure
   1-3.5. Calculations
   1-3.6. Reference

   1-4. **SOIL-PH**
   1-4.1. Principle
   1-4.2. Apparatus
   1-4.3. Reagents
   1-4.4. Procedure
   1-4.5. Reference

   1-5. **EXCHANGEABLE ACIDITY**
   1-5.1. Principle
   1-5.2. Apparatus
   1-5.3. Reagents
   1-5.4. Procedure
   1-5.5. Calculation
   1-5.6. Reference
1-12. CATION EXCHANGE CAPACITY AND EXCHANGEABLE BASES 49
1-12.1. Principle 49
1-12-2. CEC by Ammonium-acetate Method 50
1-12.3. Effective Cation Exchange Capacity (ECEC) 64
1-12.4. Reference 64

1-13. AVAILABLE MICRONUTRIENTS 65
1-13.1. Principle 65
1-13.2. Apparatus 65
1-13.3. Reagents 65
1-13.4. Procedure 67
1-13.5. Calculations 67
1-13.6. Reference 68

PART II

2. PLANT ANALYSIS 69

2-1 PRINCIPLES AND PRACTICES 69
2-2 PLANT SAMPLING 69
2-3 SAMPLE PREPARATION 69
2-3.1. Cleaning (Decontamination) 69
2-3.2. Drying 70
2-3.3. Grinding 70
2-4. LABORATORY ANALYSIS 70
2-4.1. Dry Ashing 70
2-4.2. Wet Digestion 72
2-4.3. Determination of Nutrient Elements 73

PART III

3. APPENDICES 86
PART I

SOIL ANALYSIS PROCEDURES
I. SOIL ANALYSIS

1-1. SAMPLE PREPARATION

The preparation of a bulk soil sample is necessary to make it suitable for analysis. Soil samples coming to the laboratory must first of all be arranged and registered into a "Soil laboratory ledger" and be given identification numbers, which will be used as a reference during the analysis. Sample preparation entails the crushing of the clods by hand, drying, reduction of the aggregate's size to < 2mm, and separation of the coarse fractions from the fine ones by sieving.

1-1.1. REGISTRATION

1. Register samples in the laboratory ledger book, immediately after arrival. Delays in registration can easily cause some mistakes.

2. Follow exactly the order indicated by the client and recopy all information into the soil ledger. The information usually consists of:
   - Name of the sampling area or experiment
   - A sample code given by the client
   - Profile code and horizon
   - Depth (cm)
   - Date of sampling.

3. Add to this information:
   - Date received by the laboratory
   - Person who brought the samples
   - Laboratory number has to be assigned to each sample.
     Laboratory numbers consist of two parts: A number and the year in which the sample was received in the laboratory. The number starts with 001 on the first of January of each year. This way, laboratory numbers such as 001/2000 (first sample received in 2000) or 680/2000 (the 680th sample received in 2000) is assigned.

4. Date of expected completion of analysis.

5. It is preferable to use specially designed application forms, to be completed by the client. These forms should be addressed in duplicate to the Head of the laboratory. The client should retain a 3rd copy.
6. Transfer the information on the forms into the soil Ledger Book, and assign laboratory numbers in the ledger. Copy this information on to the application forms.

7. Copying information from labels into the ledger book is frequently a source of errors. For example, 6 and 9 should always be underlined, 1 and 7 are easily confused for each other. The codes using letters and numbers easily lead to confusion between 5 and S. Take good care and concentrate.

**1-1.2. DRYING**

- Remove the field samples from the bags and spread them out serially on plastic trays.
- Put the labels contained in the bags on the plastic trays and see to it that they do not get missing.
- Check that there is no contamination between samples.
- Remove large plant residues and break the clods into small pieces by hands (generally to less than 2 cm to accelerate the drying process).
- Allow the samples to air-dry on shelves and avoid placing in direct sunlight.
- Mix the soil each day with a clean spoon to expose wet surfaces. This process accelerates drying and makes drying more uniform throughout the sample.
- It is difficult to predict how long it will take to dry a sample. Some samples may take only 3 to 4 days (for example sandy soils during the dry season), where as others require sometimes about 3 weeks (heavy wet clays containing smectite).
- Urgent samples may require fast drying. This can be done by drying it in a ventilated oven, at temperature of less than 35°C. Since heating influences the composition of the soil, this procedure is however discouraged.
- Some soils will require field moist analysis. They should arrive in sealed plastic bags, and are only opened at the time of the actual analysis in the laboratory.

**1-1.3. GRINDING, SIEVING AND STORAGE**

1. Aggregates greater than 2 mm should be ground in a mechanical grinder and sieved through a 2mm sieve. Continue this operation until all fractions pass through the 2mm sieve. Make sure that the friable fragment > 2 mm such as shells, organic debris, schist, weathered rock, calcareous nodules and other concretions of pedogenic nature, gravel, etc., are removed. Also make sure that stones and gravel > 2 mm are not ground (you can also use wooden rollers or a mortar and pestle for grinding if you do not have a mechanical grinder).
2. All fractions greater than 2 mm are weighed separately and are used in the calculation of the percent fine earth fraction and reported in the ledger.

\[
\% \text{ fine earth} = \frac{(\text{total weight} - \text{weight of fraction } > 2 \text{ mm}) \times 100}{\text{total weight}}
\]

3. Homogenize the < 2 mm soil fraction on a piece of paper (50 cm x 50 cm) and by quartering take enough quantity of soil to fill the box or plastic bottle bearing the required information on the lid and the under part. Make sure to put the label on top of the sample in the box or bottle before covering it.

4. For a number of micro analysis and total elemental analysis use of material < 0.5 mm is required. For this purpose, crush about 25 g of the fine earth (< 2mm fraction) in the mortar and pass it through a 0.5mm sieve. Store it in a small bottle with label.

5. Sample grinding and sieving is a dusty job; avoid contaminating the surroundings. Work in a well-aerated area, if possible in an area that is specially arranged for this purpose.

6. Clean the grinding machine after grinding every sample.

7. After analysis, the samples must be stored. Most samples have to be stored only for a year, but some samples which are to be used for later analysis, should be stored indefinitely. No sample should be thrown away without authorization from the Head of the Laboratory or the researcher concerned.

8. Keep samples of the same series together by year in order to facilitate ease of identification if needed later.

9. The fine fraction (< 0.5mm) can be discarded after analysis, and so is the gravel.

1-1.4. REFERENCES

FAO, Guidelines No. 70 for soil description. 3rd. ed. FAO, Rome, 1990


1-2. MOISTURE CONTENT

1-2.1. PRINCIPLE

Calculation of the results of soil analysis is done on the basis of "oven-dry" soil for comparison purposes. For this reason a sub-sample is oven dried at 105°C shortly before soil analysis; the loss in soil weight is supposed to be hygroscopic water, which is physically adsorbed in the pores and on the surface.

1-2.2. APPARATUS

- Moisture tins (aluminum dishes) or flasks with fitting lid.
- Drying oven
- Desiccator
- Analytical balance.

1-2.3 PROCEDURE

1. Weigh out precisely 5 g of air-dry soil in a clean, dry, pre-weighed and recorded moisture-free tin with 0.001 g accuracy (weight \( A = \) air-dry soil + tin weight).
2. Put the moisture tin with sample in an oven at 105°C overnight or for at least 6 hours, with the lid off the tin.
3. Remove the tin from oven, close with lid and put into a desiccator to cool off (for 30 minutes).
4. Remove the tin from desiccator and weigh once more.

1-2.4. CALCULATION

The moisture content in % by weight is obtained as follows:

\[
\text{Percent moisture (wt \%) = } \frac{(A - B) \times 100}{B - \text{weight of tin}}
\]

Where:
- \( A \) = weight of air-dry soil (5 g) + tin weight
- \( B \) = weight of oven-dry soil in grams + tin weight
The corresponding moisture correction factor (mef) for analytical results or the multiplication factor for the amount of sample to be weighed for analysis is:

\[
\text{Moisture Correction Factor} = \frac{100 + \% \text{ moisture}}{100}
\]

For example:
CEC = 50 meq/100 g air-dry soil
\% moisture = 5\%, on oven dry basis
CEC meq/100 g oven-dry soil = 50 × 1.05 = 52.5

Moisture correction factors for a range of moisture percentages is given in Appendix 1.

Notes:
1. For routine analysis moisture content of a given sample is determined only once and the same result is used for correction of moisture in all analysis to be performed on that given sample
2. For certain analysis, which requires testing of samples in their wet or moist conditions, simultaneous testing of moisture content for correction factor is needed.
1-3. PARTICLE-SIZE ANALYSIS

1-3.1 PRINCIPLE

The density of a soil suspension at a given depth becomes less as the particles settle. Its value at different times is related empirically to particle size, so that, by selection of times, a density reading can be a measure of either silt + clay or clay.

The density of the soil-water suspension is measured with a Bouyoucos hydrometer that is calibrated to read the density of the soil-water suspension in grams per liter (g/liter), or calibrated directly in percentages (usually 0-60), for a given time and temperature. These figures refer to the percentage of particles less than a definite size in a suspension containing 100 g of oven-dry soil per liter.

The technique recommended by Bouyoucos involves no pretreatment to remove organic matter or calcium carbonate, soils being simply dispersed with sodium hexametaphosphate. The results are then unavoidably approximate if the soils contain much organic or calcareous material. More reliable results are obtained by putting soils through the normal pre-treatment procedures before dispersion in sodium hexametaphosphate, particularly for soils containing high amount of organic matter and calcareous materials.

Bouyoucos advocates that the settling time for silt + clay and clay based on his method (without removal of organic matter or calcium carbonate) is comparable with the standard pipette method. The settling times are not derived strictly from Stoke's Law, but seem to give reasonable results in many cases. However recent observations indicate that where no pretreatments are used, readings for clay should be taken at the original time of 2 hours proposed by Bouyoucos. The hydrometer reading at 40 seconds and 2 hours correspond to particles of less than, 50 and 2 microns respectively, when no pretreatment is used.

The hydrometer method is more widely used than the pipette method. This is because of its simplicity, ease and rapidity to use, though it is less accurate than the pipette method. For most soil fertility evaluation studies this method is sufficient.
1-3.2. APPARATUS

- Weighing balance
- Multi-mixer (milk shaker)
- Graduated cylinder, 1000 ml
- Hydrometer (Standard Bouyoucos hydrometer, ASTM No. 152H graduated in g/liter or percent)
- Thermometer
- Stop watch
- Plunger
- Oven
- Desiccator
- Oscillatory shaker
- Sieve (50 microns and 250 microns).

1-3.3. REAGENTS

1. Dispersing agent: Dissolve 40 g of sodium hexametaphosphate (NaPO₃) and 10 g of sodium carbonate (Na₂CO₃) in distilled water in a 1 liter volumetric flask and make to volume with distilled water. Dry the chemicals in oven at 105°C and cool in a desiccator the day before use.

2. Amyl alcohol.

1-3.4. PROCEDURE

1-3.4.1. Dispersion

1. Weigh 50g soil (< 2 mm) "not sandy" or 100 g of dry-soil (< 2 mm) "sandy" into a 1 liter plastic bottle with a stopper.

2. Add 100 ml of dispersing reagent.

3. Shake for 3 hours on an end to end or oscillatory shaker.

4. Transfer the soil and the solution into the cup of a mixer or mechanical stirrer by washing the bottle very well and bring the volume to 500 ml in the cup.

5. Stir for 5 minutes.
1-3.4.2. Reading

1. Transfer the dispersed soil suspension to a hydrometer jar, washing out the stirrer cup and adjusting the volume in the jar to 1 liter with distilled water.
2. Mix with a plunger and take the temperature of the solution.
3. Add a drop of amyl alcohol if surface is covered with foam.
4. Include blank with distilled water and all the pretreatment received by the sample, but without soil.
5. Cover the top of the cylinder tightly with one palm of your hand or a rubber stopper. Shake by inverting the cylinder several times (first energetically so that all the material deposited at the bottom of the cylinder goes into suspension and then slowly) for 30 seconds.
6. Gently place the cylinder on top of a flat surface (bench) and immediately start the top-watch.
7. Immediately lower the hydrometer carefully into the suspension. To do this, slide the hydrometer slowly into the suspension until it floats. Prevent the hydrometer from oscillating. The first hydrometer reading is taken at 40 seconds after the cylinder is set down. This reading measures the percent of silt and clay (particles < 50 microns) in suspension.
8. Remove the hydrometer from the cylinder and measure the temperature of the suspension with a thermometer.
9. Take both hydrometer and temperature readings for the blank too.
10. Take the reading on the scale to the nearest 0.5 unit at the top of the meniscus.
11. After the first hydrometer reading at 40 seconds, let the cylinder stand for two hours and take the second reading for both the samples and the blank. Also take temperature readings. This second reading gives the percent of clay (particles < 2 microns) in suspension.

1-3.5. CALCULATIONS

Results are corrected to a temperature of 20°C before doing computations. For temperature readings above 20°C correction values are added to the hydrometer reading and for temperature readings below 20°C correction values are subtracted from the hydrometer reading. Temperature correction values are given in Table 1. Also correction for compensating for the added dispersing agents is made by subtracting 2 from the hydrometer reading.
Examples for calculations of both temperature and reagent compensation are given below:

**Given:**

1st Reading
- Hydrometer reading at 40 seconds = \( d_1 = 22 \)
- Temperature reading at 40 seconds = \( T_1 = 23^\circ C \)

2nd Reading
- Hydrometer reading after 2 hours = \( d_2 = 12 \)
- Temperature reading after 2 hours = \( T_2 = 19^\circ C \)

**Temperature correction (see Table 1)**
- For the temperature at 40 seconds \( (23^\circ C) = + 1 \)
- For the temperature after 2 hours \( (19^\circ C) = - 0.5 \)

**Salt correction = 2**

Subtract 2 from every hydrometer reading

\[
\text{Sand} = 100 - [(d_1 + 1 - 2) \times 100/50] \quad \text{(100/50 to convert sample wt to 100)} \\
100 - [(22 + 1 - 2) \times 2] \\
100 - [(23 - 2) \times 2] \\
100 - 42 \\
= 58\%
\]

\[
\text{Clay} = (d_2 - 0.5 - 2) \times 100/50 \\
(12 - 0.5 - 2) \times 2 \\
(12 - 2.5) \times 2 = 9.5 \times 2 \\
= 19\%
\]

\[
\text{Silt} = 100 - (\% \text{ sand} + \% \text{ clay}) \\
= 100 - (58\% + 19\%) = 23\%
\]

The sand content could also be determined by passing the whole suspension after the 2 hours hydrometer reading through a 300-mesh sieve to remove clay and silt and retaining the sand fraction. The sand retained is washed off any remaining silt, be dried in an oven at 105\(^\circ\)C and weighed. The weight is multiplied by 2 to convert it to percent (wait of sample taken is 50g). After the three readings have been taken, it remains to determine the 5 sand categories by sieving.
Sieving

Pore the contents of the cylinder on a 50 micron sieve and wash carefully under the tap while eliminating all particles less than 50 microns, till the wash water becomes almost clear.

1. Transfer the contents of the sieve into a beaker with jet of distilled water from a water wash bottle. Eliminate by decanting any floating organic debris with densities of less than 1 g/cm³.
2. Put in the oven and dry overnight at 105°C.
3. The following day cool in a desiccator and weigh
4. Separate the sands into the different particle sizes (very fine, fine, medium, Coarse and very coarse) using the sieves with the diameter range indicated in Table 2.
5. Collect the different fractions into 5 tared cans and weigh.

* Remember to make corrections for the blank (dispersing agent, which increases the specific gravity of the soil suspension) and for the temperature deviations below and/or above 20°C.

Table 1. Temperature correction values for hydrometer reading

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Correction value, g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>-2.0</td>
</tr>
<tr>
<td>16</td>
<td>-1.5</td>
</tr>
<tr>
<td>17</td>
<td>-1.0</td>
</tr>
<tr>
<td>18</td>
<td>-0.5</td>
</tr>
<tr>
<td>19</td>
<td>0.0</td>
</tr>
<tr>
<td>20</td>
<td>+0.5</td>
</tr>
<tr>
<td>21</td>
<td>+1</td>
</tr>
<tr>
<td>22</td>
<td>+1</td>
</tr>
<tr>
<td>23</td>
<td>+1.5</td>
</tr>
<tr>
<td>24</td>
<td>+2.0</td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Classes of soil particle fractions according to the USDA and the International Classification system

<table>
<thead>
<tr>
<th>USDA System</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separates</td>
<td></td>
</tr>
<tr>
<td>Very coarse sand</td>
<td>2.00 - 1.00</td>
</tr>
<tr>
<td>Coarse sand</td>
<td>1.00 - 0.50</td>
</tr>
<tr>
<td>Medium sand</td>
<td>0.50 - 0.25</td>
</tr>
<tr>
<td>Fine sand</td>
<td>0.25 - 0.10</td>
</tr>
<tr>
<td>Very fine sand</td>
<td>0.10 - 0.05</td>
</tr>
<tr>
<td>Silt</td>
<td>0.05 - 0.002</td>
</tr>
<tr>
<td>Clay</td>
<td>&lt; 0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>International System</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractions</td>
<td></td>
</tr>
<tr>
<td>Course sand</td>
<td>2.00 - 0.20</td>
</tr>
<tr>
<td>Fine sand</td>
<td>0.20 - 0.02</td>
</tr>
<tr>
<td>Silt</td>
<td>0.02 - 0.002</td>
</tr>
<tr>
<td>Clay</td>
<td>&lt; 0.002</td>
</tr>
</tbody>
</table>

1-3.6. REFERENCES


1-4. **SOIL pH**

1-4.1. **PRINCIPLE**

The pH value of a solution is the negative logarism of hydrogen ion concentration. Soil pH is measured potentiometrically in the supernatant suspension of a 1:1 or 1:2.5 soil: liquid mixture by using a pH meter. The liquid is either water (pH-H2O), a 1 M KCl solution (pH-KCl) or a 0.01 M CaCl2 solution (pH-CaCl2).

1-4.2. **APPARATUS**

- pH meter with glass-calomel combination electrode
- Automatic stirrer with timer (glass rods can also be used to stir mechanically).  
- 100 ml beakers 
- Analytical balance with 0.1 g precision 
- 50 ml graduated cylinders 
- Thermometer.

1-4.3. **REAGENTS**

- Standard buffer solutions with pH values of 4.00, 7.00 and 9.00 (or 10.00); Dilute standard analytical concentrate capsules according to instructions or prepare buffers as outlined below:

  - Buffer solution pH 4.00 (20°C): Dissolve 11.806g citric acid (C6H8O7) and 10.9468g disodium phosphate (Na2HPO4·2H2O) in pure distilled water and dilute to 1 liter. Or, dissolve 2.0423g of potassium hydrogen-phthalate (KHC8H4O4) in 200ml of distilled water.

  - Buffer solution pH 7.00 (20°C): Dissolve 3.3910g of potassium dihydrogen orthophosphate (KH2PO4) and 4.5g disodium phosphate (Na2HPO4·2H2O) in distilled water and dilute to 1 liter. Or, dissolve 0.68g of potassium dihydrogen phosphate (KH2PO4) and 0.89g of disodium hydrogen phosphate (Na2HPO4·2H2O) in 200ml distilled water. This solution has a pH of 6.88.
• Buffer solution pH 9.22: Dissolve 0.7627g of sodium tetraborate (Na₂B₄O₇, 10H₂O) in 200ml distilled water.

• KCl 1 M: Dissolve 74.6 g KCl in 1 liter volumetric flask and make to volume with distilled water.

• CaCl₂ 0.01 M: Dissolve 1.11 g CaCl₂ in 1 liter volumetric flask and make to volume with distilled water.

1-4.4. PROCEDURE

1-4.4.1. Checking the Electrode

• Check to see if the liquid level in the outer section of the electrode is sufficient.
• If not unclamp the electrode carefully, shift the rubber cover down so that the small opening comes free and fill with reference electrode filling solution.
• In the absence of reference electrode filling solution, use saturated potassium chloride solution.

1-4.4.2. Calibrating the pH Meter

a) The pH-meter is always left with power on and the selector switch on "check" or STAND-BY. The electrode and temperature sensors are kept in distilled water.

b) Remove the standard solutions from the refrigerator sufficiently in advance in order for them to take the ambient temperature.

c) If the pH-meter is not on, put it on at least ½ an hour before use.

d) Adjust the pH-meter for the measurement.

• Adjust the button for temperature regulation to the temperature of the solutions (normally equal to the room temperature).
• Put the function selector switch on the mode "pH" or on the "STAND BY" (STD BY) position in case where this position is provided on the instrument.

e) Remove the combination electrode (or the reference and glass electrodes) from the conservation solution (i.e. distilled water), rinse it with water and wipe with absorbent paper.
i) Plunge the electrode into the neutral pH buffer, put the function selection on the mode "pH" and regulate the needle (or the digital display) to the value of the buffer solution pH 7 with the aid of the calibration knob.

g) Return the function selector to the STAND BY position if this position exists.

h) Before effecting the following determination, rinse the electrode with a jet of water from a flash bottle and blot water droplets with tissue paper.

i) Adjust the pH-meter with the acid or basic buffer solution as a function of the soils to be analyzed (pH 4.0 buffer for acid soils and pH 9.22 buffer for alkaline soils).

j) Repeat operations (f) to (i) till you obtain an exact reading without having to modify the position of the adjustment buttons.

1-4.4.3. Measuring pH in Water Suspension

1. Weigh 10g air-dried < 2mm soil into 100 ml beakers.

2. Add 10 ml distilled water from a measuring cylinder for 1:1 soil/water suspension or 25 ml distilled water for 1:2.5 soil/water suspension.

3. Transfer the samples to an automatic stirrer, stir for 30 minutes and measure pH on the upper part of the suspension. (If you are using a glass rod for stirring, stir for 1 minute and allow the sample to equilibrate and measure pH after 1 hour on the upper part of the suspension at an accuracy of 0.1 unit).

1-4.4.4. Measuring pH in KCl Suspension

1. Weigh 10g air-dried 2mm soil into 100 ml beakers.

2. Add 10 ml 1 M KCl solution for 1:1 or 25 ml 1 M KCl solution for 1:2.5 soil/KCl suspension.

3. Transfer the samples to an automatic stirrer and stir for 30 minutes. Measure pH on the upper part of the suspension. If you are using a glass rod for stirring, stir for 1 minute and allow the sample to equilibrate and measure pH after 1 hour at an accuracy of 0.1 unit.

1-4.4.5. Measuring pH in CaCl₂ Suspension

1. Weigh 10.0 g air-dried 2mm soil into 100ml beakers.

2. Add 10 ml 0.01 M CaCl₂ solution for 1:1 or 25 ml 0.01 M CaCl₂ solution for 1:2.5 soil/CaCl₂ suspension.
3. Transfer the samples to an automatic stirrer and stir for 30 minutes. Measure pH on the upper part of the suspension. If you are using a glass rod for stirring, stir for 1 minute and allow the sample equilibrate and measure pH on the upper part of the suspension after 1 hour at an accuracy of 0.1 unit.

1-4.4.6. Reading the Soil pH in Water, KCl and CaCl₂ Suspensions

1. Stir each sample well, take out the glass rod, or remove the sample from the automatic stirrer, wait for about 1 minute for the soil particles to sediment and introduce the electrode (electrodes) into the suspension.

2. Wait 3-4 seconds before turning the selector knob from STAND BY to "pH" (or from "check" to "auto"). Wait for the reading to stabilize and record the readings.

   Note: The reading is considered stable when it does not change more than 0.1 units per 30 seconds (or 0.02 units per 5 secs). In calcareous soils stabilization may be difficult to achieve because of non-equilibrium conditions.

3. After reading, lift the electrodes unit, wash with distilled water (wiping with tissue paper is not very necessary).

4. Repeat this process until the whole batch is completed, checking the calibration of the pH-meter after every 10 samples. The procedure is entirely the same for water, 1 M potassium chloride and 0.01 M calcium chloride suspensions.

5. After taking reading for all samples, rinse the electrodes well, and lower them into a 100ml beaker containing distilled water.

1-4.5. REFERENCE


1-5. EXCHANGEABLE ACIDITY

1-5.1. PRINCIPLE

A neutral 1N potassium chloride solution is used to leach exchangeable hydrogen and aluminum ions from soil samples. The acidity brought into solution from various sources in the soil is measured by titration with a standard solution of an alkali, the amount of alkali used being equivalent to the sum of the hydrogen and aluminum ions (Exchangeable acidity) as:

1. \(\text{HCl} + \text{NaOH} \rightarrow \text{NaCl} + \text{H}_2\text{O}\)

\(\text{AlCl}_3 + 3\text{NaOH} \rightarrow 3\text{NaCl} + \text{Al(OH)}_3\)

When aluminum is complexed by sodium fluoride, an equivalent quantity of alkali is released, as:

2. \(\text{Al(OH)}_3 + 6\text{NaF} \rightarrow \text{Na}_3\text{AlF}_6 + 3\text{NaOH}\)

The exchangeable aluminum may then be measured by titrating the released alkali with standard acid.

1-5.2. APPARATUS

- Burettes
- Pipettes
- 250 ml erlenmeyer flasks
- Analytical balance (0.01 g accuracy)
- Volumetric flasks (100 ml)
- Filter paper - whatman no. 1.
1-5.3. RAGENTS

1. Potassium chloride solution, 1 M: Dissolve 74.6 g KCl in water and make to 1 liter with distilled water.
2. Hydrochloric acid, 1 M: Pipette 42 ml conc. HCl (d = 1.18; 37%) to a 500 ml volumetric flask containing about 400 ml of distilled water and make to volume with distilled water.
3. Hydrochloric acid solution, 0.02 M: Dilute 20 ml of 1 M HCl with distilled water to 1000 ml in a 1 liter volumetric flask and standardize with NaOH 0.02 M.
4. Sodium hydroxide solution, 1 M: Dissolve 20 g of NaOH in distilled water, cool and make to 500 ml in a 500 ml volumetric flask with distilled water.
5. NaOH solution, 0.02 M: Dilute 20 ml of NaOH 1 M with distilled water to 1000 ml in 1 liter volumetric flask and standardize with 0.05 N oxalic acid
6. Oxalic acid 0.05 M: Dissolve 6.3035 g oxalic acid (HOOC COOH.2H₂O) in distilled water in a 1 liter volumetric flask and make to volume with distilled water.
7. Sodium fluoride solution, 1 M: Dissolve 41.99 g of NaF in 800 ml distilled water and then dilute to 1 liter with distilled water. Filter the solution if turbid. Keep in polyethylene bottle.
8. Phenolphthalein indicator solution, 0.1%: Dissolve 0.1 g phenolphthalein in 100 ml ethanol 96%.

Note: Sodium hydroxide standard solutions have a limited life and need to be restandardized after storage.

1-5.4. PROCEDURE

1-5.4.1. Percolation

1. Transfer 10 g fine earth (accuracy 0.05 g) to a dry filter paper in a funnel placed in a 100 ml volumetric flask. Include two blanks and a reference sample.
2. Add 10 portions of 10 ml 1 M KCl solution with 15 - minute intervals so that the percolation takes about 2½ hours.
3. After the last portion has percolated, remove the funnel and fill the volumetric flask to the mark with 1 M KCl solution and homogenize.
1-5.4.2. Determination of Exchangeable Acidity

1. Pipette 25ml aliquot of percolate into a 250 ml erlenmeyer flask and add 5 drops of phenolphthalein solution.
2. Titrate with 0.02 M NaOH until the color turns just permanently pink (in practice: wait for 1 minute). Record the number of ml NaOH used.
3. Do similar titration with the blank too.

Note 1) Weakening of the pink color can be caused by the hydroxy-Al precipitate. This can be remedied by adding another drop of phenolphthalein

4. Save the solution for the determination of exchangeable Al.

1-5.4.3. Determination of Exchangeable Aluminum

1. Add few drops of 0.02 M HCl to make the titrated solution in the erlenmayer flask saved above colorless
2. Add 10 ml of 1 M NaF to the solution. If there is exchangeable Al, pink color appears.
3. Titrate with 0.02 M standardized HCl until the pink color disappears. Set aside while other samples are titrated and see if the end point is lasting long. If there is a considerable amount of Al the pink color returns. Continue titrating with HCl 0.02 M until the color disappears and does not reappear.

1-5.5. CALCULATIONS

\[
\text{Exchangeable acidity (meq/100 g Soil) = } (a-b) \times 4 \times M \times \frac{100 \times \text{mcf}}{s}
\]

Where:
- \(a\) = ml NaOH needed for percolate (sample)
- \(b\) = ml NaOH needed for blank
- \(M\) = molarity of NaOH solution
- \(s\) = air-dry weight of sample in g (10g)
- \(4\) = aliquot factor (100/25)
- \(\text{mcf}\) = moisture correction factor.
Exchangeable Al (meq/100 g Soil) = \( (a - b) \times M \times 4 \times 100 \times \text{mcf} \)
\[ \frac{s}{s} \]

Where:
- \( a \) = ml HCl needed for percolate
- \( b \) = ml HCl needed for blank
- \( M \) = molarity of HCl solution
- \( 4 \) = aliquot factor
- \( s \) = air-dry sample weight in g (10g)
- \( \text{mcf} \) = moisture correction factor.

Exchangeable H = Exchangeable acidity - Exchangeable Al

1-5.6. REFERENCE


1-6. ELECTRICAL CONDUCTIVITY

1-6.1. PRINCIPLE

Soluble salts are determined in an extract of known quantity of solids or liquids. The methods commonly used are the EC on the saturated paste or 1:1, 1:5 and 1:10 soil: water ratios. For the 1:1 extract one part of water is added for one part of soil and so on. For the saturated paste extract, water is added to a soil sample until a given mechanical property of the soil is attained: the liquid limit.

The electrical conductivity of an aqueous salt solution increases with increase in temperature (about 2% per °C). Hence, EC should be referenced to a standard temperature of 25°C by adjusting with temperature correction factors (see Appendix 2 for temperature correction factors).

Since conductivity depends on temperature, it is necessary to have a thermometer with a precision of 1/10 degree, in the recipient where the electrode is to be immersed.

1-6.2. APPARATUS

- Analytical balance (0.1 mg)
- Oven
- Conductivity meter
- Spatula
- 1000 and 500 ml volumetric flasks
- 250 ml beakers
- Desiccator
- Thermometer

1-6.3. REAGENTS

1. KCl 0.01 N solution: Dry about 10 g of KCl in the oven at 105°C overnight. The next day remove and cool in a desiccator. Weigh exactly 7.456 g of the dried KCl and dissolve in distilled water in a 1 liter volumetric flask, bring to volume and mix well.
2. KCl 0.02 N solution: Pipette 100 ml of the 0.1 N solution into a 500ml volumetric flask and bring to volume with distilled water.
3. KCl 0.01 N solution: Pipette 50 ml of the 0.1 N solution into a 500ml volumetric flask and bring to volume with distilled water.

1.6.4. PROCEDURE

Extraction

1. Weigh 10 g of soil sample into a 250ml beaker.
2. Add 50 ml of distilled water and shake on the automatic stirrer for 30 minutes (or use a glass rod to stir the mixture periodically for 30 minutes).

Calibration of Cell Constant

A correction coefficient C, called the cell constant, must be used in the formula to calculate the conductivity.

In general, this constant is given on the electrode by the manufacturer and is considered exact. It can occur that with time the cell or the conductivity meter itself may be subjected to small errors in the measurement of conductivity; in this case, the error has to be experimentally determined to take it into consideration in the calculations.

It is advisable to verify the cell constant periodically, given that they are subject to variations, either through absorption of chemicals from the solutions into which they are immersed, or through dehydration (see the user's manual for maintenance of the cell):

The cell constant is verified using solutions of known conductivity; in general in KCl 0.02N and 0.01N solutions.
Before measuring, rinse and fill the cell with KCl solution. Set the temperature compensation dial, open the contact switch, wait for 5 minutes and balance the bridge with the main dial.

- In a 50ml or 100 ml beaker, previously rinsed twice with the measuring solution, pour 30 or 40 ml (approximately) of the 0.02 N KCl solution.
- Rinse the cell of the conductivity meter, previously cleaned with distilled water with the 0.02 N KCl solution.
- Immerse the cell into the beaker containing the solution of 0.02 N KCl.
- Measure the conductivity.
- Measure and note the temperature of the 0.02 N KCl solution in the beaker with a thermometer.
- Repeat the same operation on the 0.01 N KCl solution.

**Remark**

- **Recalibrate the conductivity meter and the measuring cell after a change of range or after about 10 to 15 measurements.**
- **Apply the cell constant.**

**Measurement**

1. Measure the temperature of the extract and set temperature compensation at this temperature. (The reading is then automatically corrected to 25°C).
2. Fill cell with extract or insert dip cell into extract and read conductivity.
3. Rinse the cell with extract to be measured between measurements (If the extract is insufficient, rinse the cell with distilled water and dry with an air-jet).
4. It may be necessary to check the standard solutions and adjust the conductivity meter during a sample batch.

After all the samples have been determined wash the cell and place in clean distilled water.
Calculation of Cell Constant

- Where Ox. K. 50 and Ox. K. 100 are the conductivities of the 0.02 N and 0.01 N KCl solutions respectively, read in micro S cm\(^{-1}\); Os.50 and Os.100, the specific conductivities of the 0.02 N and 0.01 N KCl solution, respectively in micro S cm\(^{-1}\) as a function of temperature given in Annex 2.

- The cell constant, C is:

\[
C = \frac{\text{Os.50}}{\text{Ox.K.50}} \quad \text{or} \quad C = \frac{\text{Os.100}}{\text{Ox.K.100}}
\]

- Take an average of the two results obtained
- For example, the cell constant of an electrode used in the NSRC Laboratory in Addis Ababa can be calculated as follows at a temperature of 27°C.

\[
C = \frac{\text{Os.50}}{\text{Ox.K.50}} = \frac{2873 \, \text{micro S cm}^{-1}}{2660 \, \text{micro S cm}^{-1}} = 1.080
\]

Or

\[
C = \frac{\text{Os.100}}{\text{Ox.K.100}} = \frac{1468 \, \text{micro S cm}^{-1}}{1380 \, \text{micro S cm}^{-1}} = 1.064
\]

Mean of \( C = \frac{1.080 + 1.064}{2} = 1.072 \)

Example:

- Conductivity obtained at \( t_1 \)°C = S.C.

Where:

\[ S = \text{reading obtained for the sample at } t_1 \text{°C} \]

\[ C = \text{Cell constant cm}^{-1} = \frac{[\text{measured resistance}(R_m)]}{[\text{specific resistance}(R_S)]} \]

- Conductivity at 25°C = \( \frac{S \cdot C}{t_f} \)

Where:

\( f = \) conversion factor for adjusting \( t_0 \) to 25°C (Annex 2)

\( t_1 \)°C = room temperature when conductivity is measured.
Note:

1 mS = 1 mmho
1 cmhos/cm = 1000 mmhos/cm
1 dS/m = 1 mmhos/cm

1-6.8. REFERENCES


1-7. **CALCIUM CARBONATE**

1-7.1. **PRINCIPLE**
In the acid neutralization method, soil is treated with excess of standard HCl to decompose carbonates. The excess acid is back titrated with standard NaOH after filtration. From the amount of acid required to neutralize the carbonate, the calcium carbonate equivalent is calculated. The amount of calcium carbonate determined are generally higher because some of the acid is used up by exchangeable calcium and magnesium, and sodium carbonate if present and possibly also by reaction with primary minerals.

1-7.2. **APPARATUS**

- Hot plate
- Burettes
- 100 ml volumetric flasks
- Filtering funnel
- Filter paper whatman no. 42
- 250 ml erlenmeyer flasks.

1-7.3. **REAGENTS**

1. Hydrochloric acid 0.5 M standard solution: Add about 500 ml distilled water to a 1 l volumetric flask, slowly add 42 ml concentrated HCl. Homogenize and bring up to the 1 liter mark and then rehomogenize.
2. Sodium hydroxide solution, 0.1 M: Dissolve 4 g NaOH pellets in 1 liter of distilled water. Standardize by titrating immediately before use against 0.100 M standard HCl using phenolphthalein as indicator.
3. Hydrochloric acid, 0.1 M: Add about 500 ml distilled water to a 1 liter volumetric flask, slowly add 8.5 ml concentrated HCl. Homogenize and bring up to volume and then rehomogenize.
4. Phenolphthalein indicator solution, 0.1%: Dissolve 100mg phenolphthalein in 100ml ethanol 96%.
5. CaCO₃ powder
1-7.4. **PROCEDURE**

1. Weigh 5g of soil sample into a 250ml erlenmeyer flask. Include two blanks (no soil) and a reference sample or 500mg CaCO₃ powder.
2. Add 50 ml of 0.5 M HCl with a pipette.
3. Cover with a watch glass and let it stand for 1 hour, mixing the contents occasionally.
4. Boil the contents gently on the hot plate for 5 minutes starting from the time it begins boiling, cool and filter.
5. Wash the soil on the filter paper with two portions of 25 ml distilled water, and make up to 100 ml volume with distilled water; homogenize.
6. Pipette 10 ml of the filtered solution as well as the blank into erlenmeyer flasks.
7. Add 4 drops of phenolphthalein to both extract and blank and titrate with 0.1 M NaOH until the pink color persists for a few seconds.

1-7.5. **CALCULATION**

\[
\text{% CaCO₃ equivalent} = \frac{(a-b) \times M \times 50 \times \text{mef}}{s}
\]

Where:

- \(a\) = ml NaOH used for blank
- \(b\) = ml NaOH used for sample
- \(s\) = air-dry soil sample weight in gram
- \(M\) = molarity of the NaOH solution
- \(50\) = \(50 \times 0.001 \times 100/10 \times 100\% = 50\) (50 = equivalent weight of CaCO₃)
- \(\text{mef}\) = moisture correlation factor

**Note**

- *By this method, the calcium carbonate equivalent is somewhat overestimated as also non-carbonate components of the soil may react with the HCl. At very low carbonate contents (<1 %) the error could be relatively large. Since many other and more complicated methods cannot claim a high accuracy in this range either, the present method in most cases offers a good compromise between convenience of operation and accuracy.*
- *The analysis is not carried out on soils with a pH-H₂O < 6.7 as carbonate is then assumed to be absent.*
• Take 5 g soil if calcium carbonate values are up to about 20%; 2 g soil for up to 50% and 1 g soil for higher values.

1-7.6. REFERENCES


1-8. ORGANIC CARBON

1-8.1. PRINCIPLE

Soil organic matter is oxidized under standard conditions with potassium dichromate in sulfuric acid solution. A measured amount of $\text{K}_2\text{Cr}_2\text{O}_7$ is used in excess of that needed to destroy the organic matter and the excess is determined by titration with ferrous ammonium sulfate or ferrous sulfate solution, using diphenylamine indicator to detect the first appearance of unoxidized ferrous iron.

During the oxidation of $\text{K}_2\text{Cr}_2\text{O}_7$ with organic matter the $\text{Cr}_2\text{O}_7^{2-}$ reacts with carbon as follows:

$$2\text{Cr}_2\text{O}_7^{2-} + 3\text{C} + 16\text{H}^+ \rightarrow 4\text{Cr}^{3+} + 3\text{CO}_2 + 8\text{H}_2\text{O}$$

The excess $\text{Cr}_2\text{O}_7^{2-}$ is back titrated with standard $\text{Fe}^{3+}$ solution to determine the amount that has reacted.

Ferrous ion ($\text{Fe}^{2+}$) reacts with $\text{Cr}_2\text{O}_7^{2-}$ as follows:

$$6\text{Fe}^{2+} + \text{Cr}_2\text{O}_7^{2-} + 14\text{H}^+ \rightarrow 6\text{Fe}^{3+} + 7\text{H}_2\text{O} + 2\text{Cr}^{3+}$$

1-8.2. APPARATUS

- 500 ml Erlenmeyer flask
- 10 ml pipette
- 10 and 20 ml dispensers
- 50 ml burette
- Analytical balance
- Magnetic stirrer
- Incandescent lamp
1-8.3. REAGENTS

1. Potassium dichromate solution, 1 N: Dissolve 49.04 g $\text{K}_2\text{Cr}_2\text{O}_7$ (dried at 105°C) in distilled water in a 1 liter volumetric flask and make to volume with distilled water; store in a glass stoppered bottle.

2. Concentrated sulfuric acid (Sp. gr. 1.84) 98% (w/w).

3. Concentrated orthophosphoric acid ($\text{H}_3\text{PO}_4$) (Sp. gr. 1.75).

4. Barium diphenylamine sulphonate indicator, 0.16%: Dissolve 0.16 g of barium diphenylamine sulphonate in 100 ml of distilled water.

5. Ferrous sulphate solution 0.5 N: Dissolve 139 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 750 ml of water and add 20 ml conc. $\text{H}_2\text{SO}_4$. Transfer to a 1 liter volumetric flask and make to volume with distilled water.

6. If ferrous sulfate is not available use Mohr's salt ($\text{Fe(NH}_4)_2\text{(SO}_4)_6\cdot\text{H}_2\text{O}$): Dissolve 196.10 g of pure ferrous ammonium sulfate in 800 ml of distilled water in a 1 liter volumetric flask, add 20 ml conc. $\text{H}_2\text{SO}_4$ and make to volume with distilled water.

1-8.4. PROCEDURE FOR VISUAL END POINT TITRATION

1. Weigh 0.1 – 2 g air-dry soil (< 2 mm) and transfer to a 500 ml erlenmeyer flask. Use up to 2 g of sample for light colored soils and 0.1 g for organic soils. Include two blanks.

2. Add 10 ml 1 N $\text{K}_2\text{Cr}_2\text{O}_7$ solution with pipette to both samples and blank.

3. Carefully add 20 ml conc. $\text{H}_2\text{SO}_4$ with measuring cylinder in the fume cupboard and swirl the flask and allow to stand on an asbestos or cork pad for 30 minutes.

4. Then add 200 ml distilled water and allow it to cool.

5. Add 10 ml conc. orthophosphoric acid and just before titration, add 0.5 ml of barium diphenylamine sulphonate indicator.

6. Titrate both samples and blanks with 0.5 N ferrous sulfate solution until the color changes to purple or blue, then add ferrous sulfate solution drop by drop until the color flashes to green then continue to a light green end point.
Note 1: When potassium dichromate and \( H_2SO_4 \) are added to the sample, the color of the mixture should remain orange or pale orange. If the color becomes green, it indicates that all dichromate has been reduced. In such cases, repeat the procedure by reducing the sample input or adding more dichromate solution and more acid. Note the volume of dichromate used. The strength of ferrous ammonium sulfate solution changes on standing, because of oxidation of the ferrous ion by the atmospheric oxygen. Therefore, this solution has to be standardized every time before use. That is why the second blank is used.

1-8.5. CALCULATION

\[
\% C = \frac{N \times V_1 - V_2 \times 0.39 \times \text{mcf}}{S}
\]

Where:

\( N \) = normality of ferrous sulfate solution (from blank titration).

\[ \frac{(N \, K_2\text{Cr}_2\text{O}_7 \times V \, K_2\text{Cr}_2\text{O}_7)}{V \, \text{FeSO}_4} \]

\( V_1 \) = ml ferrous sulfate solution used for blank.

\( V_2 \) = ml ferrous sulfate solution used for sample.

\( S \) = weight of air-dry sample in gram

0.39 = \( 3 \times 10^{-3} \times 100 \% \times 1.3 \) (3 = equivalent weight of carbon).

\( \text{mcf} \) = moisture correction factor.

Note 2: In this method about 77 percent of the C is oxidized by potassium dichromate, so a correction factor of \( 100/77 = 1.3 \) is used in the calculation. The value of 77% is only approximation since the ineffectiveness of combustion varies with the type of organic matter present.
Soil organic matter contains 58% C. Conversion of % carbon to % organic matter is, therefore, done with the empirical factor of 1.724, which is obtained by dividing 100 by 58 (100/58).

\[
\text{% Organic matter} = 1.724 \times \text{% carbon}
\]

Organic matter content of a soil can also be roughly estimated from the total nitrogen content of soil by multiplying the percent total nitrogen by 20. This assumes a 5% N in the organic matter of a C:N ratio of 11:6 since the organic matter is conventionally assumed to contain 58% carbon. This estimation of the soil organic matter from the nitrogen content may be as accurate as from the carbon content.

\[
\text{% Organic matter} = \text{% Total Nitrogen} \times 20
\]

1-8.6. REFERENCES


1-9. NITROGEN

1-9.1. PRINCIPLE

The Kjeldahl procedure is based on the principle that the organic matter is oxidized by treating soil with concentrated sulfuric acid, nitrogen in the organic nitrogenous compounds being converted into ammonium sulfate during the oxidation. The acid traps NH₄⁺ ions in the soil, which are liberated by distilling with NaOH. The liberated NH₄⁺ is absorbed in boric acid and back titrated with standard H₂SO₄.

Potassium sulfate is added to raise the boiling point of the mixture during digestion and copper sulfate and selenium powder mixture is added as a catalyst. The procedure determines all soil nitrogen (including adsorbed NH₄⁺) except that in nitrate form.

1-9.2. APPARATUS

- Digestion block
- Semi-micro Kjeldahl distillation unit
- Burettes
- Pipettes
- Erlenmeyer flasks - 250 ml
- Magnetic stirrer
- Kjeldahl flasks-300 ml

1-9.3. REAGENTS

1. Concentrated sulfuric acid Sp. gr. 1.84 (96%).
2. Catalyst mixture: Mix by grinding in a mortar 100 parts Na₂SO₄ or K₂SO₄ with 10 parts of copper sulfate (CuSO₄.5H₂O) and 1 part of selenium powder; mix thoroughly.
3. Sodium hydroxide, 40%: Dissolve 400 g NaOH in about 800 ml of distilled water in a 1 l volumetric flask; Cool the solution with the flask stoppered to prevent absorption of atmospheric CO₂. Bring to volume with distilled water.
4. Boric acid solution, 2%: Dissolve 20 g of boric acid in 600 ml of distilled water in a 1 l volumetric flask and make to volume with distilled water.
5. Mixed indicator: Dissolve 0.5 g bromocresol green + 0.1 g methyl red in 100 ml of 95% ethanol.
6. Sulfuric acid solution 0.1 N: Pipette 2.82 ml of conc. H₂SO₄ (96%, d=1.84) in a 1 l volumetric flask and make to volume with distilled water. Standardize with 0.1 N NaOH.
7. Oxalic acid, 0.05 M: Dissolve 6.3035 g HOOCCHOH₂H₂O in distilled water in a 1 liter volumetric flask and make to volume with distilled water.
8. Phenolphthalein indicator, 0.1 %: Dissolve 100mg phenolphthalein in 100 ml 95% ethanol.
9. Sodium hydroxide, 0.1 N solution: Dissolve 4 g of NaOH in distilled water. Cool and make to 1 liter with distilled water. Standardize with oxalic acid.

1-9.4. PROCEDURE

1. Accurately weigh 1 g soil sample (< 0.5 mm sieve) and transfer into a digestion tube. For soils rich in organic matter (> 10% organic matter) weigh in 0.5 g. In each batch, include a reference sample and two blanks.
2. Add 2 g (½ spoon) of catalyst mixture and few carborundum boiling stones, mix well and rinse with a little water just enough to moisten the mixture.
3. Add 7 ml of conc. H₂SO₄ and mix by swirling.
4. Place the digestion tube stand with the samples beside the block digester and fit the exhaust manifold on top of it.
5. Place the tubes with rack and exhaust manifold on the digestion block, preheated in the fume-hood.
6. Digest for 3 hours or until the digest is white or pale yellow on a block digester preheated to 300°C.
7. Allow to cool, and cautiously add 50 ml of distilled water, and then cool again.
8. Transfer the acid digest quantitatively to the macro-kjeldahl flasks and rinse using distilled water.
9. Measure 20ml boric acid solution from a dispenser into a receiver erlenmeyer flask corresponding to the number of samples. Add to it 2 drops of indicator solution and place under the condenser. Take care that the end of the condenser is immersed in the boric acid solution to prevent any loss of ammonia.
10. Pour 75 ml of 40 percent NaOH carefully down the neck of the distillation flasks containing the digests and mix gently.

11. Fit the prepared 250 ml kjeldhal distillation flasks containing the digest to the corresponding holder, close it as soon as possible and start the distillation by heating the flasks containing the digests.

12. When the distillation is complete, i.e. when about 80 ml of distillate has been collected, remove the receiver flask. Continue with the next sample.

13. Add a stirrer bar and titrate the receiver flask solution from green to a pink end point with 0.1 N H₂SO₄. Record the reading of the burette. Transfer the magnet by means of a magnetic rod to the next flask to be titrated. Always standardize the acid to obtain the exact normality of the titrant.

1-9.5. CALCULATION

Let \( V \) be the volume of 0.1 N H₂SO₄ used after correcting for the blank.

\[
N \times V = \text{meq of N/g of soil}
\]
\[
N \times V \times 14 = \text{mg of N per g of soil}
\]
\[
N \times V \times 0.014 = \text{g of N per g of soil}
\]

\[
\% N = \frac{(a - b) \times N \times 0.014 \times 100 \times \text{mcf}}{s}
\]

Where:

- \( a \) = ml of H₂SO₄ required for titration of sample
- \( b \) = ml of H₂SO₄ required for titration of blank
- \( s \) = air-dry sample weight in grams
- \( N \) = Normality of H₂SO₄ (0.1 N)
- 0.014 = meq weight of nitrogen in g
- mcf = moisture correction factor
1-9.6. REFERENCES


1-10. AVAILABLE PHOSPHORUS

Two methods (Bray II and Olsen) are described for the determination of available phosphorus. The Bray method is suitable for acid soils and the Olsen method is used for both acid and nonacid soils.

1-10.1. PHOSPHORUS SOLUBLE IN DILUTE ACID-FLUORIDE
       (Extraction According to Bray II)

1-10.1.1. PRINCIPLE

When ammonium fluoride in acid solution is used to extract acid or neutral soils, it will remove some phosphate ions from "insoluble" phosphates of iron and aluminum and also dissolve a little calcium phosphate. Increase of acidity from 0.025 M (as with P-Bray I) to 0.10 M in Bray II tends to dissolve more calcium phosphate.

With the ammonium fluoride concentration of 0.03 M and an acidity of 0.10 M HCl the method is said to remove "adsorbed" and "acid soluble" Phosphate. Phosphate in the extract is determined photometrically. This method has been most successful on acid soils.

1-10.1.2. APPARATUS

- Extraction bottle or flask
- Funnel and Filter papers
- Whatman No. 42 filter paper or equivalent
- Spectrophotometer suitable for measurement in the 880-nm range
- Funnel racks
- Volumetric flasks and pipettes
- Analytical balance
- Desiccator
1. Hydrochloric acid 1 M: Dilute 41 ml of HCl (d = 1.19, 37%) with distilled water to 500 ml.
2. Ammonium fluoride 1 M: Dissolve 37 g NH₄F in 1 liter of distilled water.
3. Extracting Solution Bray II: Mix 100 ml of HCl 1 M with 30 ml of NH₄F 1 M, adjust the pH to 1.8 using special electrode and dilute to 1 liter with distilled water. Check the pH (Remark 2) and store in a polythene bottle.
4. Sulfuric Acid 1.85 M: Add 104 ml of H₂SO₄ (95-97%) to about 800 ml of distilled water and fill up with distilled water after cooling to 1 liter volumetric flask.
5. Boric Acid 0.5%: Dissolve 2.5 g of H₃BO₃ in a volumetric flask of 500 ml in about 400 ml of distilled water. Add 25 ml of sulfuric acid 1.85 M, cool and fill up cooling to the mark with distilled water.
6. Ammonium Molybdate Solution: Dissolve 6.0 g of (NH₄)₆Mo₇O₂₄.4H₂O in 500 ml of sulfuric acid 1.85 M. Store in the dark.
7. Potassium Antimony Tartrate (1000 ppm Sb): Dissolve 0.275 g of K₂SbO₄O₆.½H₂O in 100 ml of distilled water.
8. Mixed Reagent: In the sequence mentioned, bring together in a volumetric flask of 100 ml.
   - 50 ml of ammonium molybdate solution
   - 5 ml of potassium tartrate solution, and mix. Dissolve 0.553 g of ascorbic acid in the mixture and fill up to the mark with distilled water.
9. Standard Solution of 100 mg/l P: Dissolve 0.4394 g of KH₂PO₄ (dried at 110°C for 2 hrs and cooled in a desiccator) in distilled water in a volumetric flask of 1 liter and fill up to the mark with distilled water.
10. Standard Series: 0-1-2-3-4 ppm P: Pipette into volumetric flasks of 100 ml 0-1-2-3-4 ml of the 100 ppm P standard solution and fill to the mark with extracting solution Bray II.
1.0. PROCEDURE

1. Weigh 2.0g air-dry soil < 2 mm in a 50 ml bottle or flask with stoppers. Include one standard sample and two blanks with each series.

2. Add 20 ml of extracting solution Bray II. Shake exactly 1 minute by hand and filter directly after mixing through a whatman No. 42 filter paper. If the filtrate is turbid, filter again through the same filter.

3. Pipette 2 ml of standard series, samples and blanks. Add 8 ml of boric acid 0.5 % and mix. Add 2 ml of mixed reagent and mix.

4. Measure the absorbance with a 10mm diameter cuvette at 882nm after 30 minutes but within 12 hours.

5. The absorbance of the 4 ppm P standard is about 1.0.

1.0.5. CALCULATION

Plot a graph relating the absorbance to the amount of phosphate present in the standards. Read the P concentration in the samples against the absorbance on the graph.

\[
P (\text{ppm or mg/Kg soil}) = \frac{(a-b) \times 20 \times \text{mcf}}{S} = (a-b) \times 10 \times \text{mcf}
\]

Where:

\[ a = \text{ppm P or mg/L P in sample extract} \]
\[ b = \text{ppm P or mg/L P in blank} \]
\[ 10 = \text{extraction ratio (20/2)} \]
\[ S = \text{sample weight in g (2)} \]
\[ 20 = \text{ml of extracting solution} \]

Conversion factor P\(_2\)O\(_5\) = 2.29 x P

Remarks:

1. Boric acid is added to eliminate the interference of the fluoride ions, which have a slight depressive effect on the molybdate color development.

2. The pH of the extracting solution Bray II has to be 1.8. Check the pH every two weeks with the pH meter. Use the special electrode because of damage from the NH\(_4\)F.
REFERENCES


1-10.2. PHOSPHORUS SOLUBLE IN SODIUM BICARBONATE  
(Extraction according to Olsen et al.)

1-10.2.1. PRINCIPLE

The sample is extracted with a sodium bicarbonate solution at pH 8.5. Phosphate in the extract is determined colorimetrically after treating it with ammonium molybdate – sulfuric acid reagent with ascorbic acid as reducing agent. The high pH of the extracting solution renders the method suitable for calcareous, alkaline or neutral soils containing Ca-phosphates because the Ca concentration in solution is suppressed by precipitation as CaCO₃. As a result, the phosphate concentration in solution can increase.

This procedure can also be applied to acid soils as the relatively high pH of the carbonate buffer suppresses the solubility of Al and Fe and thus allows the phosphate concentration to increase.

1-10.2.2. APPARATUS

- Spectrophotometer suitable for measurement at 880 nm.
- Polythene shaking bottles 250ml
- Reciprocating shaking machine
- Analytical balance
- Funnel racks
- Funnel
- Whatman No. 42 filter paper (or equivalent)
- Volumetric flasks and pipettes as required for preparation of reagents, standard solutions and color development.
10.2.3. REAGENTS

1. Sodium bicarbonate solution, 0.5 M, pH 8.5 (extracting solution): Dissolve 42 g NaHCO₃ in water and make to 1 l. Adjust the pH to 8.5 by adding NaOH 1 M (4 g/100 ml). In case of overshooting of pH above 8.5, add some NaHCO₃ 0.5 M.

   Note: Check and re-adjust the pH after storage.

2. Sulfuric acid, 4 M: Slowly add 56 ml concentrated H₂SO₄ (96%, s.g. 1.84) to about 150 ml distilled water in a graduated beaker under constant stirring. After cooling, make to 250 ml with distilled water.

3. Ammonium molybdate solution, 4%: Dissolve 4 g of (NH₄)₆Mo₇O₂₄·4H₂O in water and make to 100 ml. Store in polythene or pyrex bottle.

4. Potassium antimony tartrate solution, 0.275% (1g/l Sb): Dissolve 0.275 g K₂SbO₃·H₂O₆ in water and make to 100 ml.

5. Ascorbic acid solution, 1.75 %: Dissolve 1.75 g ascorbic acid in water and make to 100 ml. Prepare fresh daily.

6. Mixed Reagent: Successively add with a measuring cylinder to a 500 ml polythene or pyrex bottle and homogenize after addition of each of the following:

   - 50 ml of 4 M H₂SO₄
   - 15 ml of NH₄ - molybdate solution
   - 30 ml of ascorbic acid solution
   - 200 ml of water.
   - 5 ml potassium antimony tartrate solution

Standard solutions (prepare fresh daily)

- Standard phosphate solution, 100 mg/l P: Dissolve 0.4394 g KH₂PO₄ (dried at 105°C for 2 hours in an oven) in distilled water in a 1 liter volumetric flask and make to volume with distilled water.
- Standard phosphate solution, 4 mg/l P: Pipette 10 ml of the 100 mg/l P standard solution into a 250 ml volumetric flask and make to volume with extracting solution.
- Standard series: Pipette into 100ml volumetric flasks, 0-10-20-30-40-50 ml of the 4mg/l P standard solution. Make to volume with extracting solution. The standard series is 0-0.4-0.8-1.2-1.6-2.0 mg/l P.
1-10.2.4. PROCEDURE

1. Weigh 5 g of <2 mm soil (accuracy 0.01 g) into a 250ml polythene shaking bottle. Include two blanks and a reference sample.
2. Shake for 30 minutes on a mechanical shaker.
3. Filter through a whatman no. 42 filter paper.
4. If filtrate is not clear, add 1 spoon P-free charcoal, shake again and filter.
5. Pipette in (short) test tubes 3 ml of the standard series, the blanks and the sample extracts.
6. Slowly add 3 ml of the mixed reagent by pipette and swirl (CO₂ evolution!).
7. Allow the solutions to stand for at least 1 hour for the blue color to develop to its maximum (see remark below).
8. Measure absorbance on spectrophotometer at 882 or 720 nm.

1-10.2.5. CALCULATION

Plot a calibration graph of absorbance against P concentration for the standard series. Read the concentration of the samples from the graph using the absorbance values recorded.

\[
P(\text{ppm or mg/Kg soil}) = (a-b) \times \frac{100}{s} \times \text{mcf} = (a-b) \times \frac{100}{s} \times \text{mcf}
\]

Where:
\[
\begin{align*}
a & = \text{mg/l P in sample extract} \\
b & = \text{mg/l P in blank} \\
s & = \text{sample weight in gram (5 g)} \\
\text{mcf} & = \text{Moisture correction factor} \\
100 & = \text{ml of extracting solution.}
\end{align*}
\]

Conversion factor \(P₂O₅ = 2.29 \times P\)

Remark: With the acid molybdate solution phosphate forms phospho-molybdenic acid which is reduced to phospho-molybdenic-blue with ascorbic acid. The antimony accelerates the development of the blue color and stabilizes it for up to 24 hours. With this method interference of Si is not to be expected. Should such interference still occur (blue colored zero standard) then repeat procedure using distilled water.
REFERENCES


1-11. AVAILABLE POTASSIUM

1-11.1. PRINCIPLE

This method is primarily used for determination of potassium in acid soils with cation exchange capacities of less than 20 meq/100g. Under this procedure, the sample is extracted with Morgan's solution and K in the extract is measured by flame photometer.

1-11.2. APPARATUS

- Extraction bottle 100 ml, with stoppers
- Mechanical reciprocating shake
- Filter funnel, 11 cm
- Whatman No. 1 filter paper (or equivalent)
- Funnel racks
- Flame photometer
- Analytical balance
- Volumetric flasks and pipettes as required for preparing reagents and standard solutions
- Erlenmeyer flasks, 100 ml.
- Desiccator

1-11.3. REAGENTS

1. Extraction Reagent: Dissolve 100g sodium acetate (CH₃COONa.3H₂O) in about 900 ml distilled water. Add 30ml glacial acetic acid (CH₃COOH), adjust the pH to 4.8, and dilute to 1 liter with distilled water.

2. Potassium Stock Solution, 1000mg/l K:
   - Dry about 3 g of potassium chloride (KCl) in an oven at 105°C for 2 hours and cool in a desiccator
   - Accurately weigh 1.91 g of potassium chloride into a 100ml beaker
   - Wash into a 1 liter volumetric flask, and make to mark with distilled water and mix well to dissolve.
1-11.4. PROCEDURE

1. Weigh 10 g of soil passing through 2mm sieve into 100 ml shaking bottle.
2. Add 50 ml of extracting solution and shake for 30 minutes at a minimum of 180 oscillations per minute.
3. Filter and collect the filtrate in a 100ml erlenmeyer flask.
4. Dilute extracts 5 times for sandy soils and 10 times for clay soils, using the extracting solution.

1-11.4.1. Measurement K by Flame Photometer

Standard Series

1. 100 mg/l K diluted standard series: Pipette 100 ml of the potassium stock solution, 1000 ppm K, into 1 liter volumetric flask and dilute to volume with distilled water.

2. Standard series working solution of 0-2-4-6-8-10 mg/l K: Pipette into 250ml volumetric flasks, respectively 0-5-10-15-20-25 ml of the diluted 100 mg/l standard solution. Dilute to volume with Morgan's extracting solution.

Measurement of K

- Aspirate the standard series working solutions in to the flame and establish the transmittance curve. In a similar manner, aspirate the samples and record transmittance. If dilution is necessary the Morgan extracting solution should be used as diluent.
1-11.5.  CALCULATIONS

**Curve method:** Establish a graph with the mg/l K on the Y axis and the % transmittance on the X axis. Read off the sample concentrations from the standard curve.

\[
\text{Available K (mg/kg soil)} = \frac{(a-b) \times 50 \times 1000 \times df \times mcf}{1000} \times \frac{s}{s} = 50 \times df \times \frac{a-b \times mcf}{s}
\]

Where:

- \( a \) = concentration of K, measured in the sample (mg/l)
- \( b \) = concentration of K, measured in the blank (mg/l)
- \( s \) = air-dry soil sample weight in g (10 g)
- \( mcf \) = moisture correction factor
- \( df \) = dilution factor
- \( 50 \) = ml of extractant used/sample

**Factor Method**

\[
\text{Available K (mg/kg soil)} = \frac{(a-b) \times Z \times 50 \times 1000 \times df \times mcf}{1000} \times \frac{s}{s} = \frac{(a-b) \times 50 \times Z \times df \times mcf}{s}
\]

Where:

- \( a \) = concentration of K, measured in the sample (mg/l)
- \( b \) = concentration of K, measured in the blank (mg/l)
- \( s \) = air-dry soil sample weight in g (10g)
- \( mcf \) = moisture correction factor
- \( df \) = dilution factor
- \( 50 \) = ml of extractant used/sample
- \( Z \) = Standard reading for K \( \frac{\%T}{2+4+6+8+10} = 30 = 0.10 \)
  \[\frac{(\%T)}{20+40+60+80+100} = \frac{30}{300}\]
REFERENCES


1-12. CATION EXCHANGE CAPACITY and EXCHANGEABLE BASES

1-12.1. PRINCIPLE

The sum total of the exchangeable cations that a soil can adsorb is called the cation exchange capacity of the soil (CEC). Cation exchange capacity is an important parameter of soil because it gives an indication of the type of clay mineral present in the soil and its capacity to retain nutrients against leaching. It is determined by measuring the total amount of a given cation needed to replace all the cation from a soil exchange site and it is expressed in centimoles per 100g soil (cmol/100g soil). The operation therefore, calls for the preparation of a saturated sample, followed generally by an extraction of the saturation cations adsorbed on the exchange complex and measuring its amount.

The determination of CEC is very delicate since it varies with pH. In effect the OH and H modify the ionic dissociations and especially the OH ions tied to the surfaces. This is why CEC is always determined at a known pH value. The commonly used reagents are: neutral 1 N ammonium acetate, alkaline sodium acetate (pH 8.2) and 0.5 N alkaline barium chloride and 0.2 N triethanolamine solution (BTA, pH 8.2). The ammonium acetate method is suitable for slightly acid to neutral soils. The sodium acetate method is used for calcareous soils and the barium chloride triethanolamine method is employed for estimating CEC in acid soils as well as for the estimation of exchangeable hydrogen. This method gives good estimate of CEC for soils with permanenent charge, but the CEC of soils with pH dependent charge are over estimated by this method.
Phases involved in CEC determination

- The exchangeable cations (Ca\(^+\), Mg\(^+\), Na\(^+\), K\(^+\), and Al\(^{3+}\)) and H\(^+\) of the adsorption complex are displaced with a mono-ionic solution.
- The adsorption complex is saturated with this solution (generally buffered at a given pH, for example ammonium acetate, at pH 7 or sodium acetate at pH 8.2).
- The moist soil with the saturating solution therefore contains more than the saturating cation associated with the adsorption complex or to the colloidal surfaces. The excess saturating cation not adsorbed on the exchange complex is eliminated by washing with alcohol.
- The saturating cation held on the exchange complex is then displaced with another cation, and the displaced cation measured. If for example sodium acetate is used, the sodium in the percolate measured by flame photometer is a measure of CEC. In the ammonium acetate method, the saturating ammonium displaced by neutral salt is measured by distillation to determine the CEC.

1-12.2. CEC BY AMMONIUM ACETATE METHOD

1-12.2.1. Displacement of Cations

Apparatus

- Glass funnels
- Flame photometer
- Atomic absorption spectrophotometer
- Kjeldahl distillation unit
- Analytical balance
- Filter paper, whatman No. 1 or equivalent
- Pipettes
- Volumetric flasks (100 ml, 250 ml, 1000ml)
- 150 ml beakers
- Hot plate.
Reagent

1. Ethanol 96%
2. Nessler reagent: Dissolve 100g HgI₂ and 70g KI in small amount of distilled water and add this mixture slowly and by stirring to a cool solution of 160g NaOH in 500 ml distilled water. Dilute to 1 liter, store in a rubber stoppered, borosilicate glass-bottle and away from sunlight to maintain reagent stability.
3. Ammonium acetate solution, 1 M: Dissolve 385g NH₄Ac in distilled water in a 5 liter beaker. Adjust the pH to 7 with ammonia or acetic acid and make to 1 liter in a volumetric flask. If solid NH₄Ac is not available prepare by mixing acetic acid and ammonia as follows: to 900 ml of distilled water in a 5 liter flask add 285 ml concentrated acetic acid and 340 ml conc. Ammonium hydroxide. Make to volume of 5 liters and adjust the pH to 7 with ammonia or 1 M acetic acid.
4. Sodium chloride, 10% solution: Dissolve 100 g of NaCl in 1000 ml of distilled water in a 1 liter volumetric flask.
5. Acetic acid, 1 M: Dissolve 60.05 gram acetic acid (CH₃.COOH) in 1 liter volumetric flask containing 500 ml distilled water and make to volume with distilled water.

Note: If desired, acetate solutions may be adjusted to pH 8.2 (for CEC pH 8.2 of calcareous soils).

Procedure

1. Weigh 5 g of soil into a 250 ml beaker, add 100 ml of ammonium acetate 1 M pH 7.0 solution. Stir instantly with a stirring rod and allow to stand overnight while taking the precaution to cover the beakers with watch glasses.
2. The next day transfer the soaked samples onto filter funnels placed on 250 ml volumetric flasks and wash the remaining soil with about 50 ml of 1 M ammonium acetate pH 7.0 into the funnel while making sure that each filtration is complete before adding the next.
3. Wash two times, each with additional 25 ml aliquots of 1 M ammonium acetate to a volume of ± 200 ml, remove the volumetric flasks and bring up to volume with distilled water.
Keep this solution for the determination of exchangeable bases.

If the determination of the bases is not immediate, the samples should be preserved in the refrigerator, preferably in plastic flasks (to avoid contamination from Na⁺ from the glass flasks).

4. Place under filter funnels 250ml plastic bottles and wash the soil on the filter with 25 ml ethanol. Repeat this process 2 to 3 times. Take some drops of the filtrate into Nessler tubes and add 3 drops of Nessler's reagent. If a yellow precipitate is deposited at the bottom of the tube, continue to wash the soil with ethanol. Test for precipitation with Nessler reagent, repeating the procedure until no precipitate formed.

5. Saturate the soil with Na by washing with successive 20 ml of sodium chloride (NaCl 10%), collect the filtrate in 250ml plastic bottles. Do a total of 5-20 ml successive washes to give a total of 100 ml. Include a blank. This filtrate is used for CEC determination by distillation.

1-12.2.2. Distillation of Ammonium

Principle

After percolation of the sample with ammonium acetate, the sample is washed free of the excess salt with ethanol, the adsorbed ammonium is displaced by percolation with sodium chloride. The displaced ammonium is distilled and the evolved ammonia determined.

Generally, this determination is done immediately following the determination of exchangeable bases using the same sample and percolation funnel set up.

Apparatus

- Distillation assembly (Kjeldahl)
- Burettes.
Reagents

1. Sodium hydroxide 1 N: Dissolve 40 g of NaOH in 1 liter of distilled water.
2. Sulfuric acid 0.20 N: Quantitatively transfer 5.6 ml of conc. H₂SO₄ (96%, sp.g. 1.84) into 500 ml of distilled water contained in a 1 liter volumetric flask. Make to volume with distilled water.
3. Sodium hydroxide 0.1 N: Dissolve 4 g of NaOH in 1 liter of distilled water or pipette 100 ml of the 1 N NaOH solution into a 1 liter volumetric flask and bring to volume with distilled water. Standardize using oxalic acid or potassium hydrogen phthalate.
4. Methyl red indicator: Dissolve 1 g of methyl red in 100 ml of ethanol.

Procedure

1. Transfer the leachate (NaCl percolate) in the plastic bottles from the displacement process, to a 500ml Kjeldahl flask and wash the plastic bottles with 25 ml of distilled water, with small amounts at a time.
2. Arrange distillate receiver by pouring 15 ml of 0.20 N H₂SO₄ to a 250ml erlenmayer flask and dip the distillation tip in the sulfuric acid solution.
3. Add 10 ml of 1N NaOH solution to the Kjeldahl flask and connect immediately to the distillation apparatus.
4. Distill about 75 ml of the NaCl percolate over the 15 ml 0.20 N H₂SO₄ in the 250 ml erlenmeyer flask.
5. Remove erlenmeyer from distiller, rinse condenser tip and titrate the distillate with 0.1 N NaOH using methyl red indicator until color changes from purple to yellow.
Calculation

\[
\text{CEC (meq/100 g soil)} = \frac{(a-b) \times N \times 100 \times \text{mcf}}{S}
\]

Where:

\[a = \text{ml of 0.1 N NaOH required for titration of sample}\]
\[b = \text{ml of 0.1 N NaOH required for titration of blank}\]
\[S = \text{air-dry sample weight in gram (5 g)}\]
\[N = \text{normality of the NaOH (0.1N)}\]
\[\text{mcf} = \text{moisture correction factor.}\]

1-12.2.3. ‘Measurement of Exchangeable Bases

Principle

Exchangeable Ca and Mg in the ammonium acetate leachate are measured by atomic absorption spectrophotometry (AAS) or by the EDTA titrimetric method and exchangeable K and Na by flame photometer. For measurement Ca and Mg by AAS, La (5000 mg/l or 0.5 %) is introduced to prevent formation of refractory compounds of Ca and Mg in the flame. For Na and K measurement by AAS, Cs (1000 mg/l or 0.1 %) is introduced to overcome ionization interference.

Reagents

1. Ammonium acetate solution, 1 M: Dissolve 385g NH₄Ac in distilled water in a 5 liters beaker. Adjust the pH to 7 with ammonia or acetic acid and make to 1 liter in a volumetric flask. If solid NH₄Ac is not available prepare by mixing acetic acid and ammonia as follows: to 900 ml of distilled water in a 5 liter flask add 285 ml concentrated acetic acid and 340 ml conc. Ammonium hydroxide. Make to volume of 5 liter and adjust the pH to 7 with ammonia or 1 M acetic acid.

2. Lanthanum solution, 1.0 %: Dissolve 26.6 g of Lanthanum chloride, LaCl₃.7H₂O in some distilled water and make up to the 1 liter mark in a volumetric flask with distilled water.

3. Nitric acid solution, 1 N: Pipette 68.2 ml conc. HNO₃, 65% into a 1 liter volumetric flask containing about 500 ml of distilled water and bring up to volume with distilled water.
4. Calcium stock solution 1000 mg/l: Dry 1.5 g of calcium carbonate (CaCO₃) at 105°C for 2 hours in the oven and cool in a desiccator.
   - Accurately weigh 1.249 g CaCO₃ in a 100 ml beaker and dissolve in 1 N HNO₃.
   - Wash into a 500ml volumetric flask with 1 N HNO₃ and bring up to volume with 1 N HNO₃.
5. Lanthanum solution 0.55 %: Dilute 550 ml of the 1 % La suppressant solution to 1 liter with distilled water.
6. Magnesium stock solution, 1000 mg/l: Dissolve 5.07 g of magnesium sulfate (MgSO₄·7H₂O) in 500 ml of 1 N HNO₃.
7. Sodium stock solution, 1000 mg/l:
   - Dry 3 g of NaCl at 105°C in an oven for 2 hours. Cool in a desiccator.
   - Weigh accurately 2.542 g NaCl into a 100ml beaker.
   - Wash into a 1 liter volumetric flask and make to mark with distilled water, shake well to dissolve.
8. Potassium stock solution, 1000 mg/l:
   - Dry about 3 g of potassium chloride (KCl) in an oven at 105°C for 2 hours.
   - Accurately weigh 1.91 g of potassium chloride into a 100 ml beaker.
   - Wash into a 1 liter volumetric flask, and make to mark with distilled water and mix well to dissolve.

1-12.2.3.1. Exchangeable Ca and Mg by AAS

Procedure

a) Exchangeable Ca and Mg

Standard Series

1. Ca 250 mg/l: Pipette 50 ml of 1000 mg/l Ca stock solution into a 200 ml volumetric flask, make to volume with distilled water.
2. Mg 25 mg/l: Pipette 25 ml of 1000 mg/l Mg stock solution of into a 1 liter volumetric flask, make to volume with distilled water.
3. Of the 250 mg/l Ca solution and the 25ml/l Mg solution pipette a series of 0-5-10-15-20-25 ml into 250ml volumetric flasks respectively.
4. To each flask add 25 ml NH₄Ac 1 M solution and 125 ml 1% La solution. Make to volume with distilled water. The standard series are then: 0-5-10-15-20-25 mg/l Ca and 0-0.5-1.0-1.5-2.0-2.5 mg/l Mg.

**Measurement**

- Pipette 1 ml of the original ammonium acetate leachate and 9 ml of the 0.55% La suppressant solution into a test tube, homogenize and measure Ca and Mg in the solution by AAS at wavelengths of 422.7 and 285.2 nm, respectively.
- Pipette 0.5 ml of the ammonium acetate leachates into test tubes.
- Add 4.5 ml of distilled water.
- Add 5 ml of 0.5% La suppressant solution mixed and homogenized on test tube shaker.
- Measure Ca and Mg by aspirating into atomic absorption spectrophotometer (AAS) after setting the respective cathode and calibrating the instrument.
- The wavelengths for measurement are 422.7 and 285.2 nm for Ca and Mg, respectively.

**1-12.2.3.2. Exchangeable K and Na by Flame-photometer**

**Procedure**

**Standard Series**

1. Combined potassium and sodium standard solution: Pipette into a 100 ml volumetric flask:
   - 10 ml of 1000 mg/l K
   - 15 ml of 1000 mg/l Na
   - Fill up to the mark with distilled water. The final concentrations are:
     - 100 mg/l K
     - 200 mg/l Na
2. Combined potassium and sodium working standards:
   - Pipette 0, 2, 4, 6, 8 and 10 ml of the combined standard solution of K and Na above into 100ml volumetric flasks respectively.
   - Bring up to volume with 1 M ammonium acetate solution. These solutions contain the following concentrations:

<table>
<thead>
<tr>
<th>Standard Series</th>
<th>0</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>---------------</td>
<td>mg/l</td>
<td>---------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Sodium</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>16</td>
<td>20</td>
</tr>
</tbody>
</table>

Where S1, S2, S3, S4 and S5 are standards 1, 2, 3, 4 and 5, respectively.

**Measurement**

- Aspirate the original ammonium acetate leachate and the standards into the flame and measure the transmittance of K and Na at wavelengths of 768 and 598nm, respectively. If sample measurements are out of range, dilute with the original extractant (1N ammonium acetate).
Calculations of Exchangeable Bases

\[
\text{Exch. Ca (cmol (+)/kg soil)} = \frac{(a-b) \times 20 \times 250 \times \text{mcf}}{10 \times 20.04 \times s} \\
\text{Exch. Mg (cmol(+)/kg soil)} = \frac{(a-b) \times 20 \times 250 \times \text{mcf}}{10 \times 12.15 \times s} \\
\text{Exch. K (cmol(+)/kg soil)} = \frac{(a-b) \times 250 \times \text{mcf}}{10 \times 39.10 \times s} \\
\text{Exch. Na (cmol(+)/kg soil)} = \frac{(a-b) \times 250 \times \text{mcf}}{10 \times 23.00 \times s} \\
\text{Base saturation(%) } = \frac{\text{Exch. (Ca+Mg+K+Na)}}{\text{CEC}} \times 100
\]

Where:
\( a \) = mg/l Ca, Mg, K, or Na in the diluted ammonium acetate leachate
\( 20 \) = the value 20 in the numerator for Ca and Mg represent the dilution factor.
\( b \) = mg/l Ca, Mg, K or Na in the
\( s \) = air-dry weight in gram
\( \text{mcf} \) = moisture correction factor.

The values 20.04, 12.15, 39.10 and 23.00 in the denominator represent the equivalent weights of Ca, Mg, K and Na respectively.

10 = The value 10 in the denominator represents the factor to convert from mg/l to cmol/kg
250 = The value 250 in the numerator is the total volume of the percolate; change this value accordingly if a greater volume was used.

1-12.2.3.3. Determination of Ca and Mg by EDTA Titration

Principle

The method is based on the fact that Ca and Mg and a number of other ions form stable complexes with EDTA salt. Sn, Cu, Zn, Fe and Mn may interfere in the determination if present in appreciable amounts. Their interference is prevented by the use of KCN and hydroxyamine hydrochloride solution. Addition of this solution removes interference of Mn, and addition of KCN removes interference of Cu, Co, and Ni.
Determination of Ca + Mg

In the determination of calcium plus magnesium the ion EDTA forms soluble complexes with the Ca$^{++}$ and Mg$^{++}$ ions in the solution, at an optimal pH of 10.0; in this state these ions can be removed from the solution without being precipitated. At the same pH, the dye Eriochrome Black T assumes a greenish (turquoise) blue color in the absence of Ca$^{++}$ and Mg$^{++}$ ions, but forms red compounds with Ca and Mg, which are less stable than the complexes of EDTA-Ca and EDTA-Mg.

To a solution containing Ca and Mg ions a buffer solution is added to bring the pH to about 10 and then Eriochrome Black T is added to obtain the red color. Then in adding slowly the EDTA, the Ca and Mg ions are liberated gradually from the complexes that they had formed with Eriochrome Black T to form more stable complexes with EDTA. When all the ions in question are transferred, the red color of the Eriochrome Black T complexes gives place to the turquoise blue color of the dye itself.

Schematically:

1. Ca$^{++}$ + Mg$^{++}$ at pH 10 + Eriochrome → EBT-Ca and EBT-Mg
   (Slightly stable compounds)
   (red color)

2. EBT-Ca + EBT-Mg + EDTA
   (Red color) → EDTA-Ca + EDTA-Mg
   (Turquoise blue color)

Apparatus
- Hot plate
- Timer
- Erlenmeyer flasks
- Burettes
- Pipettes.
Reagents

1. Methyl orange indicators, 1%: Dissolve 1 g of methyl orange in 100 ml of ethanol
2. Hydrochloric acid 0.10 N: Pipette 8.3 ml of conc. HCl 37% into a 1 liter volumetric flask containing 500 ml of distilled water and make to volume with distilled water.
3. Potassium cyanide 2% solution: Dissolve 2 g of KCN in 100 ml of distilled water in a 100 ml volumetric flask (KCN is a deadly poison).
4. Ammonium chloride – ammonium hydroxide buffer: Dissolve 67.5 g of ammonium chloride in about 400 ml of distilled water. Add 570 ml of concentrated NH₄OH and dilute to 1 liter with distilled water.
5. Eriochrome Black T: (1 g EBT + 100 g NaCl ground and mixed thoroughly).
6. Ethylenediamine tetra acetate solution, EDTA approx 0.02 N: Dissolve 4.0 g of disodium EDTA (EDTA-Na₂) in distilled water and dilute to 1 liter with distilled water. Before carrying out this determination standardize EDTA against standard calcium or preferably magnesium solution. Titrate 20 ml of standard 0.02 N magnesium chloride or 0.02 N calcium carbonate with 0.02 N EDTA. Calculate the exact normality of EDTA and use for subsequent calculations.

Procedure

1. Pipette 10 ml of the ammonium acetate soil extract obtained from the cation exchange capacity and exchangeable bases extraction into a 250 ml erlenmeyer flask and add 40 ml of distilled water to bring the volume up to 50 ml.
2. Add 2 to 3 drops of methyl orange and 0.1 N HCl until the color turns orange and then add 0.2 ml excess 0.1 N HCl.
3. Boil the content for 3-minutes and allow the solution to cool to 60°C.
4. Add 2 ml KCN solution (from a burette) and the buffer solution to bring up to about pH 10.
5. Then add a pinch of Eriochrome Black T and NaCl mixture.
6. Titrate with 0.02 N EDTA-disodium salt to a pure turquoise blue without any trace of red.
Calculations:

\[ \text{Ca + Mg (meq/100 g)} = \frac{\text{N \cdot T \cdot V \cdot 100 \cdot mcf}}{\text{A \cdot S}} \]

Where:

- **N** = Normality of EDTA (0.02 N)
- **T** = Volume of 0.02 N EDTA-disodium salt used
- **V** = Total volume of extract (250 ml)
- **A** = Aliquot of sample taken for titration (10 ml)
- **S** = Air-dry weight of sample (g)
- **mcf** = Moisture correction factor
- **100** = to convert to 100g soil bases

**Determination of Ca**

**Principle**

Similarly Ca alone may be determined by direct titration with EDTA. If a solution containing Ca + Mg ions is made strongly alkaline (pH about 12), Mg is selectively precipitated as magnesium hydroxide, although when magnesium is present in small quantities no evidence of a precipitate is seen. At the same pH, the dye HHSNN or Patton and Reeder's indicator forms a red compound with calcium ions but is not affected by magnesium present as magnesium hydroxide. The color of HHSNN in alkaline solutions in the absence of Ca is turquoise blue and also the HHSNN-Ca complex is less stable than EDTA-Ca complex. Therefore when the soil extract is made strongly alkaline and treated with HHSNN, a red color develops by reaction of the dye with calcium ions. If EDTA is then slowly added, the calcium ions are slowly transferred from the dye complex to the more stable EDTA complex, until when all calcium ions have been transferred, the liquid acquires a pure turquoise blue color.

**Apparatus**

- 250 ml erlenmeyer flasks
- Burettes
- Pipettes.
Reagents

1. Methyl orange indicator solution 1 %. Dissolve 1 g of methyl orange in 100 ml of ethanol in a 100 ml volumetric flask.

2. Hydrochloric acid 0.1 N: Pipette 8.3 ml of conc. HCl, 37 %, into a 1 liter volumetric flask containing 500 ml of distilled water and make to volume with distilled water.

3. Sodium hydroxide, 1 N solution: Dissolve 40 g of NaOH in 1 liter of distilled water in a 1 l volumetric flask.

4. Potassium cyanide 2 % solution: Dissolve 2 g KCN in 100 ml of distilled water in a 100 ml volumetric flask.

5. HHSNN (Paton and Reeder's reagent): 1 g HHSNN + 100 g NaCl ground and mixed thoroughly.

6. EDTA-ditosodium salt 0.02 N: Dissolve 4.0 g of EDTA-\(\text{Na}_2\) in distilled water and dilute to 1 liter with distilled water. Standardize the EDTA as in Ca + Mg above.

Procedure

1. Transfer 10 ml of the ammonium acetate soil extract, used for the determination of CEC and exchangeable bases, to a 250 ml erlenmeyer flask and add 40 ml distilled water to bring the volume to 50 ml.

2. Add 2 to 3 drops of methyl orange and 0.1 N HCl until the color turns orange and then add 0.2 ml HCl in excess.

3. Boil the contents for 2 to 3 minutes and then cool to about 60°C.

4. Add 10 ml of freshly prepared 1 N NaOH, 2 ml of KCN solution and a pinch of HHSNN.

5. Titrate with 0.02 N EDTA-\(\text{Na}_2\) solution to a pure turquoise blue without any trace of red.
Calculations

\[
\text{Ca (meq/100 g)} = \frac{N \times T \times V \times 100 \times \text{mcf}}{A \times S}
\]

Where:
- \(N\) = Normality of EDTA (0.02 N)
- \(T\) = Volume of EDTA used
- \(V\) = Total volume of extract used (250 ml)
- \(A\) = Aliquot of sample taken for titration (10 ml)
- \(S\) = Air-dry weight of sample (g)
- \(\text{mcf}\) = Moisture correction factor
- \(100\) = To covert to 100 g soil bases

\[
\text{Mg (meq/100 g)} = \text{Ca} + \text{Mg (meq/100 g)} - \text{Ca(meq/100 g)}
\]

REMARKS:

Application of the described method to calcareous (and gypsiferous) soils leads to erroneous results. Dissolution of carbonates interferes particularly with the determination of exchangeable Ca (over-estimation) but to only a limited extent with that of the CEC. Results can be improved to some extent by raising the pH of both acetate buffer solutions to 8.2 where the solubility of calcium (and magnesium) carbonate is reduced. This can also be achieved by using acetate buffer (pH 7)/ethanol mixtures (e.g 1:1). Since in neither case the solubility is reduced to zero the results remain unreliable.

The base saturation of carbonates and gypsiferous soils may safely be considered to be 100%.
1-12.3. EFFECTIVE CATION EXCHANGE CAPACITY (ECEC)

The CEC determined by ammonium acetate at pH 7 does not really represent what is happening in the soil. The value of CEC is dependent on soil pH. If one desires to know the real state of the exchange complex of acid soils (weathered soils), it is advisable to measure the CEC at the pH of the soil. Because, raising the pH of acid soils to 7, as in the case of measurement by the ammonium acetate method, artificial condition is created in the soil, which favor the development of pH dependent charges, resulting in higher CEC.

A manner of determining the CEC in a more realistic fashion is to sum up the amounts of the different exchangeable cations (in meq.) present on the exchange complex: Ca++, Mg++, Na+, K+, H+ and Al+++.

The CEC so determined is called effective CEC or ECEC.

1. The "Effective CEC" or ECEC is obtained by:

\[
\text{Effective CEC (ECEC)} = \text{exchangeable bases} + \text{exchangeable acidity}
\]

1-12.4. REFERENCES


1-13. AVAILABLE MICRONUTRIENTS

1-13.1. PRINCIPLE

Chelating agents, such as EDTA and DTPA are increasingly being used for the extraction of available micronutrients in soils. One of the most commonly used methods is that of Lindsay and Norvell. Both potentially available quantities and mobile reserves of Fe, Mn, Cu and Zn can be determined by this method using DTPA as an extractant. The amount of these nutrients in the extracts is determined by atomic absorption spectrophotometer in comparison with standards at 248.3 nm, 279.5 nm, 324.7 nm and 213.9 nm wavelength for Fe, Mn, Cu and Zn, respectively. Lanthanum is added to prevent condensed phase interference.

1-13.2. APPARATUS

- Extraction flask, 100 ml polyethylene conical flasks
- Mechanical reciprocating shaker, 180 oscillations per minute
- Filter funnel, 11 cm
- Whatman No. 42 ashless filter paper (or equivalent 11 cm)
- Atomic absorption spectrophotometer
- pH meter
- Analytical balance
- Volumetric flasks, pipettes and microburettes as required for preparation of reagents and standard solutions.

1-13.3. REAGENTS

1. DTPA extracting solution: Dissolve 1.96g DTPA (diethylenetriaminepentaacetic acid), 14.92g triethanolamine (TEA) and 1.47 g calcium chloride (CaCl₂·2H₂O) in about 950 ml with distilled water in 1 liter volumetric flask with distilled water. Adjust the pH to 7.30 (± 0.05) with HCl (1:1) and bring to volume with distilled water. The final concentration will be 0.005 M DTPA, 0.1 M TEA, and 0.01 M CaCl₂. (Note: The DTPA reagent should be the acid form). If solid DTPA and TEA is not available, use 1.57 ml and 13.3 ml solution, respectively, to prepare a liter solution.
2. Conc. HCl s.g 1.18 (about 37% as HCl)

3. Lanthanum Solution, 0.1%: Dissolve 2.66 g of lanthanum chloride, LaCl₃·7H₂O in some distilled water and make up to the 1 liter mark in a volumetric flask with distilled water.

4. Hydrochloric Acid, 1 N: Add 82.6 ml of concentrated HCl (37%, s.g 1.18) to about 500 ml distilled water, shake, let cool and fill up to 1 liter with distilled water.

5. Nitric acid 5M: Add 347ml of concentrated HNO₃ (s.g 1.4) to about 500 ml of distilled water in a 1 liter volumetric flask, shake well and let cool, and fill up to mark with distilled water.

6. Iron Stock Solution, 1000 mg/l Fe: Dissolve 7.022g of ferrous (II) ammonium sulfate hexahydrate [Fe(NH₄)₂(SO₄)₂·6H₂O] in 1000 ml of 1 N HCl.

7. Manganese Stock Solution, 1000 mg/l Mn: Dissolve 3.076 g of MnSO₄·H₂O in 1000 ml of 1 N HCl.

8. Copper Stock Solution, 1000 mg/l Cu: Dissolve 3.929 g CuSO₄·5H₂O in 1 liter of 1 N HCl.

9. Zinc stock solution, 1000 mg/l Zn: Dissolve 4.398 of zinc sulfate heptahydrate, ZnSO₄·7H₂O in about 500 ml of distilled water in a 1000 ml volumetric flask. Add 10 ml of 5 M nitric acid and bring up to volume with distilled water.

10. Combined Standard Solution of Fe, Mn, Cu and Zn:

   Pipette into a 100ml volumetric flask
   *
   * 10 ml of 1000 mg/l Fe;
   * 10 ml of 1000 mg/l Mn;
   * 5 ml of 1000 mg/l Zn;
   * 5 ml of 1000 mg/l Cu;

   Fill up to the mark with 1 N HCl. The final concentrations are:
   *
   * 100 mg/l Fe; 100 mg/l Mn; 50 mg/l Zn, and 50 mg/l Cu.

11. Combined Microelement Standard Working Solutions: Pipette 0-0.5-1-2-4 ml of the combined standard solution above into 100 ml volumetric flasks and fill up to the mark with DTPA extractant. These solutions contain the following concentrations:

<table>
<thead>
<tr>
<th>Micro Element</th>
<th>Blank</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Mn</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Zn</td>
<td>0</td>
<td>0.25</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cu</td>
<td>0</td>
<td>0.25</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Where S1, S2, S3, S4 are the standards 1, 2, 3, 4, respectively.
1-13.4. PROCEDURE

1. Weigh out 20.0g air-dry soil < 2 mm in a 100ml polythene bottle. Include one standard sample and two blanks with each series.
2. Add 40.0 ml DTPA extractant.
3. Shake for 2 hr minutes lengthwise in horizontal position in a reciprocal shaker with a shaking speed of 150 rpm at 20°C.
4. Filter and collect filtrate.
5. Pipette 10.0 ml of the sample extracts, the blank extracts and the working standard solutions of Fe, Mn, Cu and Zn above into test tubes.
6. Add 1 ml of 0.1 % lanthanum solution and homogenize.
7. Establish the concentration curve for the working standard solutions containing the lanthanum solution for each of the elements (Fe, Mn, Cu and Zn) by aspirating into the air-acetylene flame and measuring the absorbance or concentration at the following wavelengths:

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Slit width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Zn</td>
<td>219.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>

8. Nebulize the sample extracts and blanks containing the lanthanum chloride solution and record the reading of each of the elements (Fe, Mn, Cu, and Zn)

1-13.5. CALCULATION

\[
\text{Fe, Mn, Cu, Zn (mg/Kg soil)} = (a-b) \times 40 \times \text{mef} / S
\]

Where:
- \(a\) = Concentration of (Fe, Mn, Cu or Zn) in the sample extract, (mg/l)
- \(b\) = concentration of (Fe, Mn, Cu or Zn) in the blank extract, (mg/l)
- 40 = volume of extractant used per sample-ml
- \(S\) = weight of air-dry soil g (20 g)
- \(\text{mef}\) = moisture correction factor
PART II

PLANT ANALYSIS PROCEDURES
2. PLANT ANALYSIS

2-1. PRINCIPLES AND PRACTICES

The concentration of a nutrient within a plant is an integral value of all factors affecting it. Analysis of plants normally deal with all essential nutrients, toxic or beneficial ones, except carbon, hydrogen and oxygen.

2-2. PLANT SAMPLING

Sampling is the first important step in plant analysis. There are large variations in nutrient concentrations in the different parts of some plants. Therefore, careful selection of the appropriate part is important in sampling. Confining sampling to a particular organ also increases the accuracy of estimation. The general rule for most plants is to sample the upper, recently matured leaves, just prior to the beginning of the reproductive stage. When nutrient disorders are suspected, sampling may be done at the time when the symptoms are observed. Old and new leaves on the same plant have different mineral compositions. In Sample collection, dead plants or stressed ones should be avoided.

2-3. SAMPLE PREPARATION

The following are four major steps involved in the preparation of plant samples for laboratory analysis:

- Cleaning to remove surface contaminants such as dust and spray residues.
- Drying to stop enzymatic reactions and to facilitate grinding.
- Grinding to reduce the material to fineness.
- Drying the fine material to constant weight on which analytical figures are to be based.

2-3.1. Cleaning (decontamination)

Wash with 0.1 to 0.3 % detergent solution, followed by rinsing in distilled water to remove dust and other contaminants. Washing should be quick to avoid danger of leaking of some nutrients like K and Ca.
2-3.2. Drying

- After washing, samples should be dried as rapidly as possible to minimize chemical and biological changes.
- If drying is unduly delayed, considerable loss in dry weight may occur due to respiration.
- Two requirements in sample drying are sufficiently high temperature to destroy the enzymes responsible for the decomposition process and an optimum temperature for moisture removal without appreciable thermal decomposition (60 to 70°C for 24 hours).

2-3.3. Grinding

Fine grinding (200 mesh) with materials free of contaminants, especially from micronutrients.

2-4. LABORATORY ANALYSIS

Most plant tissue analysis procedures involve ashing of tissues to destroy the organic components in order to obtain a solution of inorganic ions. This may be achieved by dry ashing or wet digestion. The methods used for the determination of nutrient elements in filtrates from ash solutions or digests are fundamentally the same as for soil extracts. The techniques used are volumetric, spectrophotometric (colorimetric), potentiometric and photometric (flame and atomic absorption).

2-4.1. Dry Ashing

Principle

Dry ashing is the decomposition of plant tissues using high temperature. It is not recommended for plant materials high in silicon content. In this procedure, the plant material is calcinated in a muffle furnace, dissolved in 20% nitric acid and filtered for the determination of nutrient elements. In the filtrate P is determined by spectrophotometer, K and Na by flame photometer and Ca, Mg and micronutrients by atomic absorption.
Apparatus

- Porcelain crucibles, aluminum dish, oven, heating plate, etc.
- Muffle furnace
- Analytical balance
- Flasks and filter-paper
- Atomic absorption, flame photometer and spectrophotometer

Reagents

20% HNO₃ solution: In a 1000 ml volumetric flask containing 300 ml of distilled water, add 285 ml of concentrated HNO₃ and bring to volume.

Procedure

1. Dry about 1.0g of ground plant sample in aluminum dish over night at 105°C in an oven.
2. Cool in a desiccator
3. Weigh exactly 0.5g of dried sample into a porcelain crucible and put it in a muffle furnace
4. Switch on the furnace and watch the temperature rise to 450°C, regulate and set the furnace at this temperature.
5. Ash for 4 hours at this temperature, switch off the furnace and allow the sample to cool inside the closed furnace overnight.
6. Transfer the ashed material into 200 ml erlenmayer flask with 20 ml 20 % HNO₃.
7. Heat the acid treated sample with content on hot-plate to boiling. Maintain at this temperature for 30 minutes with periodic steering by glass rod, and allow to cool.
8. Filter the sample through a no. 1 filter paper into a 100ml volumetric flask.
9. Wash contents until 90 ml of the filtrate is collected, and bring to volume with distilled water.
10. This solution can be used for the determination of P, K, Na, Ca Mg, and the micronutrient such as Fe, Mn, Zn and Cu.
2.4.2. Wet digestion

Principle
Wet ashing involves the decomposition of plant tissues using various combinations of HNO₃, H₂SO₄ and HClO₄ for the determination of Ca, Mg, K, Na, P, total-N and micronutrients.

The dry matter is treated on a hot plate with concentrated acid and small additions of H₂O₂. This technique takes longer time, but is easier to perform on a larger scale. Nitrogen determination, which requires a separate destruction when the samples are dry ashed, can be determined from the same digest in the case of wet digestion.

Apparatus

- Digestion block
- Digestion tubes
- Distillation unit

Reagents

1. Sulfuric acid
2. Hydrogen peroxide (30 %)
3. Selenium powder
4. Salicilic acid
5. Sulfuric acid - selenium mixture: dissolve 3.5 g of selenium powder in 1 lt of H₂SO₄, by heating to about 300°C, while covering with watch-glass until color changes to light-yellow (requires 3 - 4 hrs).
6. Digestion mixture: dissolve 7.2 g of salicilic acid in 100 ml H₂SO₄, selenium mixture (this mixture should not be stored for more than 48 hrs).
7. Mixed indicator: mix 0.5 g bromocresol green + 0.1 g methyl red. Dissolve in 100 ml of 95 % ethanol and adjust to pH 4.5 with NaOH or HCl as necessary.
8. Sodium hydroxide, 40%: Dissolve 400 g NaOH in about 800 ml of distilled water in a 1 liter volumetric flask. Cool the solution with the flask stoppered to prevent absorption of atmospheric CO₂. Bring to volume with distilled water.
9. Boric acid solution, 2%: Dissolve 20 g of boric acid in 600 ml of distilled water in a 1 liter volumetric flask and make to volume with distilled water.
10. Phenolphthalein indicator, 0.1 %: Dissolve 100 mg phenolphthalein in 100 ml 95% ethanol.
11. Sodium hydroxide, 0.1 N solution: Dissolve 4 g of NaOH in distilled water. Cool and make to 1 liter with distilled water. Standardize with oxalic acid.
12. Sulfuric acid 0.1 N solution: Pipette 2.82 ml of concentrated H₂SO₄ (96%, s.g. 1.84) in a 1 liter volumetric and make to volume with distilled water. Standardize with 0.1 NaOH.

Procedure

1. Weigh 0.3 g of dried plant material and transfer to a digestion tube.
2. Add 2.5 ml of the digestion mixture and swirl carefully to moisten the plant material.
3. Let stand for at least 2 hrs. Prepare also blank.
4. Place the tube in a heating block and heat at 100°C for at least 2 hrs.
5. Remove the tube from the block, allow to cool and add successively three 1 ml of hydrogen peroxide and mix thoroughly.
6. Place the tube again in the preheated block and heat at 300°C until the digest turns to colorless or light-yellow (about 2 hrs).
7. Remove the tube from the block and cool to room temperature.
8. Add 48.3 ml of distilled water and mix.
9. Let it stand over-night, mix again, filter on a 100 ml volumetric flask, bring to volume with distilled water and store in plastic bottles for the determination of nutrient elements.

2-4.3. Determination of Nutrient Elements

2-4.3.1. Total Nitrogen

Total N is determined by distillation of an aliquote from the digest with NaOH, collecting the distillate in boric acid and titrating with 0.01 N HCl to the end point of the mixed indicator.
Principle

The Kjeldahl procedure is based on the principle that by treating plant material with concentrated sulfuric acid it is oxidized and nitrogen in the plant material is being converted into ammonium sulfate during the oxidation. The ammonia liberated in the distillation process with NaOH are trapped by the acid. The ammonia is adsorbed in the form of \( \text{NH}_4^+ \) ion in boric acid and back titrated with standard \( \text{H}_2\text{SO}_4 \).

Apparatus

- Burettes
- Pipettes
- Erlenmeyer flasks - 250 ml
- Magnetic stirrer
- Kjeldahl flasks 300ml

Procedure

1. Transfer the acid digest quantitatively to the macro-kjeldahl flasks respectively using distilled water.
2. Measure 20 ml boric acid solution from a dispenser into a receiver erlenmeyer flask corresponding to the number of samples. Add to it 2 drops of indicator solution and place under the condenser. Take care that the end of the condenser is immersed in the boric acid solution to prevent any loss of ammonia.
3. Pour 75 ml of 40 percent NaOH carefully down the neck of the distillation flasks containing the digests and mix gently.
4. Fit the prepared 250 ml kjeldhal distillation flasks containing the digest to the corresponding holder, close it as soon as possible and start the distillation by heating the flasks containing the digests.
5. When the distillation is complete, i.e. when about 80 ml of distillate have been collected, remove the receiver flask. Continue with the next sample.
6. Add a stirrer bar and titrate the receiver flask solution from green to a pink end point with 0.1 N \( \text{H}_2\text{SO}_4 \). Record the reading of the burette. Transfer the magnet by means of a magnetic rod to the next flask to be titrated. Always standardize the acid to obtain the exact normality of the titrant.
Calculations

Let V and N be the volume and normality of 0.1 N H₂SO₄, used after correcting for the blank, respectively

\[ N \times V = \text{meq of } N/\text{g of soil} \]
\[ N \times V \times 14 = \text{mg of } N \text{ per g of soil} \]
\[ N \times V \times 0.014 = \text{g of } N \text{ per g of soil} \]

\[ \% N = \frac{(a - b) \times N \times 0.014 \times 100 \times \text{mcf}}{s} \]

Where
\[ a = \text{ml of } H₂SO₄ \text{ required for titration of sample} \]
\[ b = \text{ml of } H₂SO₄ \text{ required for titration of blank} \]
\[ s = \text{air-dry sample weight in grams} \]
\[ N = \text{Normality of } H₂SO₄ \text{ (0.1 N)} \]
\[ 0.014 = \text{meq weight of nitrogen in g} \]
\[ \text{mcf} = \text{moisture correction factor} \]

2-4.3.2. Determination of Phosphorus

Principle

Determination of phosphorus is carried out on the digest aliquote obtained through calcination or wet digestion. The phosphorus in the solution is determined colorimetrically by using molybdate and metavanadate for color development. The reading is made at 460nm wavelength.

Apparatus
- Spectrophotometer
- 1000 ml volumetric flasks
- Volumetric flasks
- Volumetric pipettes
Reagents

1. Dissolve 20 g of ammonium molybdate in 25 ml distilled water in a 1 liter volumetric flask (solution I).

2. Dissolve 1.25 g of ammonium metavanadate (NH₄VO₃) in 300 ml of distilled water in a 1 litre beaker. Heat if it does not dissolve; cool and add 425 ml of concentrated perchloric acid (solution II).

3. Add solution II on solution I and bring up to volume with distilled water.

4. Phosphorus stock solution 500 ppm:
   * Dry about 3 g potassium dihydrogen phosphate (KH₂PO₄) in an oven at 105°C for 2 hours. Cool in a desiccator.
   * Accurately weigh 2.197 g KH₂PO₄ into a 100 ml beaker.
   * Wash into a 1 liter volumetric flask. Add about 800 ml of distilled water, shake and dissolve. Make to volume, and shake well. Store in an amber bottle.

5. 100 ppm P solution: Dilute 50 ml of the 500 ppm P solution to 250 ml in a 250 volumetric flask with distilled water.

6. 10 ppm P solution: Dilute 50 ml of the 100 ppm P solution to 500 ml in a 500 ml volumetric flask with distilled water.

7. Phosphorus working standards of 0, 1, 2, 3, 4 and 5 ppm P: pipette 0, 5, 10, 15, 20 and 25 ml of the 10 ppm P solution into 50 ml volumetric flasks.

Procedure

1. Pipette 5 ml of aliquot from the sample digest of the dry ashing or wet digestion into a 50 ml volumetric flask.

2. Add 10 ml of the molybdate and vanadate solutions to the samples and the standards and bring up to volume with distilled water.

3. Wait 10 minutes for the color to develop and read on the spectrophotometer at a wavelength of 460 nm. Use the blank to set zero absorbance or 100% Transmittance.
Calculations

Prepare a curve on graph paper, with absorbance on the X-axis and concentration on the Y-axis. Plot the standards and read off the concentrations of the samples in ppm from the graph.

\[
P \text{ ppm} = \frac{C \times V1 \times V2 \times \text{mcf}}{\text{S.A}}
\]

Where:
- \( C \) = P concentration in sample digest read from the curve, ppm
- \( V1 \) = Volume of the digest (100 ml)
- \( V2 \) = Volume of the dilution (50 ml)
- \( S \) = Weight of the plant material calcinated in g (1)
- \( A \) = Aliquot (5 ml).
- \( \text{mcf} \) = Moisture correction factor.

2-4.3.3. Determination of Ca and Mg

Principle

The determination of Ca and Mg by atomic absorption spectrophotometer is carried out on the digest aliquot obtained through calcination or wet digestion. The sample is nebulized into an air-acetylene flame, where it is vaporized, the Ca and Mg compounds are atomized and the Ca and Mg atoms thus formed absorb radiation from the hollow-cathode lamps. The absorbance is measured for Ca and Mg at wavelengths of 422.7 nm and 285.2 nm respectively. An excess of lanthanum is added to avoid the formation of refractory compounds that would depress the signal.

Apparatus

- Atomic absorption spectrophotometer
- Volumetric flasks (100, 500 and 1000 ml)
- Pipettes.
Reagents

1. 20% Nitric acid solution: In a 1000 ml volumetric flask containing 400 ml of distilled water add 290 ml of conc. HNO₃ (69%) and bring up to the 1 liter mark with distilled water.

2. 4% Nitric acid solution: In a 1000 ml volumetric flask containing about 400 ml of distilled water add 58 ml of conc. HNO₃ (69%) and bring up to volume with distilled water.

3. Nitric acid solution, 1 N: Pipette 65.2 ml of conc. HNO₃ (63%, d = 1.40) into a 1 liter volumetric flask containing about 400 ml of distilled water and make to volume with distilled water.

4. Lanthanum solution, 0.1%: Dissolve 2.66 g of Lanthanum Chloride LaCl₃·7H₂O in some distilled water and make up to the 1 liter mark in a volumetric flask with distilled water.

5. Standard Solutions:

- Calcium stock solution 1000 ppm: Dry 1.5 g of calcium carbonate (CaCO₃) at 105°C for 2 hours in the oven and cool in a desiccator. Accurately weigh 1.249 g CaCO₃ in a 100ml beaker and dissolve in 1N HNO₃. Wash into a 500ml volumetric flask with 1 N HNO₃ and bring up to volume with 1 N HNO₃.

- Magnesium stock solution 1000 ppm: Dissolve 5.07 g of Magnesium sulfate (MgSO₄·7H₂O) in 500 ml of 1 N HNO₃.

- Combined standard solution of Ca and Mg:
  
Pipette into a volumetric flask of 100 ml:
  * 10 ml of 1000 ppm Ca.
  * 1 ml of 1000 ppm Mg.
  * Fill up to the mark with 1 N HNO₃.

  The final concentrations are 100 ppm Ca and 10 ppm Mg.

- Combined calcium and magnesium working standards:
  
Pipette 0, 2.5, 5 and 10 ml of the combined standard solution of Ca and Mg above into 100-ml volumetric flasks:
  * Add 1 ml of 20% HNO₃ to each flask.
  * Add 50 ml of 0.1% Lanthanum solution.
  * Bring up to volume with distilled water.
This series should be prepared fresh daily. These solutions contain the following concentrations.

<table>
<thead>
<tr>
<th>Macro Element</th>
<th>S0</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0</td>
<td>2.5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0</td>
<td>0.25</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Where S0, S1, S2, and S3 are standards 0, 1, 2 and 3, respectively.

Procedure

For the determination of calcium and magnesium dilute the digests with 0.1% Lanthanum solution which depresses or reduces the interference from other elements.

Pipette:
- 1 ml digest from calcinated or wet digest in 10 ml test tubes.
- Add 5 ml 0.1% Lanthanum solution.
- Add 4ml distilled water and homogenize.

Establish the concentration curve for the standards by nebulizing each of the standard series solution into the air-acetylene flame and measuring the absorbance at a wavelength of 422.7nm and 285.2nm for Ca and Mg, respectively.

Calculations

\[
\text{Ca, Mg (ppm)} = \frac{(a - b) \times V \times df \times mef}{s}
\]

Where:
- \(a\) = Concentration sample reading from AAS (ppm)
- \(b\) = Blank reading (ppm)
- \(V\) = Final volume of digest (100 ml)
- \(mef\) = Moisture correction factor
- \(s\) = Weight of sample
- \(df\) = Dilution factor (10)
2-4.3.4. Determination of K and Na

Principle

The K and Na in the sample digest (calcination or wet digestion) are determined by flame photometer. The sample is nebulized into the flame where it is vaporized; potassium and sodium compounds are atomized and the potassium and sodium atoms thus formed are measured at wavelength of 766.5 and 589.0 nm, respectively.

Apparatus

- Flame photometer.
- Pipettes.
- Volumetric flasks as required.

Reagents

1. 20% Nitric acid solution: In a 1000 ml volumetric flask containing 400 ml of distilled water add 290 ml of conc. HNO₃ (69%) and bring up to the 1 liter mark with distilled water.
2. 4% Nitric acid solution: In a 1000 ml volumetric flask containing about 400 ml of distilled water add 58 ml of conc. HNO₃ (69%) and bring up to volume with distilled water.
3. Sodium stock solution 1000 ppm:
   * Dry 3 grams of NaCl at 105°C in an oven for 2 hours. Cool in a desiccator.
   * Weigh accurately 2.542 g NaCl into a 100-ml beaker.
   * Wash into a 1 liter volumetric flask and make to mark with distilled water, shake well to dissolve.
4. Potassium Stock Solution 1000 ppm:
   * Dry about 3 g of Potassium Chloride (KCl) in an oven at 105°C for 2 hours.
   * Accurately weigh 1.91 g of potassium chloride into a 100 ml beaker.

   * Wash into a 1 liter volumetric flask, make to mark with distilled water and mix well to dissolve.
5. Combine Potassium and Sodium Standard Solution:
   Pipette into a volumetric flask of 100 ml:
   * 10 ml of 1000 ppm K
   * 15 ml of 1000 ppm Na
   * Fill up to the mark with distilled water. The final concentrations are
     100 ppm K and 150 ppm Na.

6. Combined Potassium and Sodium Working Standards:
   - Pipette 0, 2, 4, 6, 8 and 10 ml of the combined standard solution of
     potassium and sodium above into 100 ml volumetric flasks.
   - Add 10 ml of 20% HNO₃ to each flask.
   - Bring up to volume with distilled water. These solutions contain the
     following concentrations:

     | Macro Element | 0 | S1 | S2 | S3 | S4 | S5 |
     |---------------|---|----|----|----|----|----|
     | Potassium     | 0 | 2  | 4  | 6  | 8  | 10 |
     | Sodium        | 0 | 3  | 6  | 9  | 12 | 15 |

     Where S1, S2, S3, S4 and S5 are standards 1, 2, 3, 4 and 5, respectively.

     For the determination of sodium aspirate the standard working solutions and the diluted
     digests directly to the flame photometer. For the determination of potassium dilute the
     extract with 4% HNO₃ before reading.
Calculations

Establish a graph with the ppm on the Y-axis and %T on the X-axis. Read the sample concentration from the standard curve.

\[
K, \ Na \ (ppm) = \frac{(a-b) \times V \times df \times mcf}{s}
\]

Where:
- \(a\) = Concentration reading (K, Na) for sample from curve (ppm)
- \(b\) = Concentration reading for (K, Na) for blank from curve, ppm
- \(V\) = Volume of the digest adjusted to the mark (100 ml)
- \(df\) = Dilution factor
- \(s\) = Sample weight, g.
- \(mcf\) = Moisture correction factor

2-4.3.5. Determination of Micronutrients (Fe, Mn, Zn and Cu)

Principle

These determinations are carried out on the digest obtained either through calcination or wet digestion. The sample is nebulized into an air-acetylene flame, where it is vaporized, the Fe, Mn, Cu and Zn compounds are atomized and the Fe, Mn, Cu and Zn atoms thus formed absorb radiation from the hollow-cathode lamps. The absorbance is measured at 248.3 nm, 279.5 nm, 324.7 nm and 213.9 nm for Fe, Mn, Cu and Zn, respectively. Lanthanum is added to prevent condensed phase interference.

Apparatus

- Atomic absorption spectrophotometry.
- Volumetric flasks (50, 100, 500, 1000 ml).
- Pipettes.
Reagents

1. 20% Nitric acid solution: In a 1000 ml volumetric flask containing 400 ml of distilled water add 290 ml of conc. \( \text{HNO}_3 \) (d = 1.40) and bring up the 1 litre mark with distilled water.

2. 4% Nitric acid solution: In a 1000 ml volumetric flask containing about 400 ml of distilled water add 58 ml of conc. \( \text{HNO}_3 \) (69%) and bring up to volume with distilled water.

3. Nitric acid solution 1 N. Pipette 65.2 ml of conc. \( \text{HNO}_3 \) (d = 1.40) into a 1 liter volumetric flask containing about 400 ml of distilled water and make to volume with distilled water.

4. Lanthanum solution, 0.1%: Dissolve 2.66 of lanthanum chloride, \( \text{LaCl}_3.7\text{H}_2\text{O} \) in some distilled water and make up to the 1 liter mark in a volumetric flask with distilled water.

5. Iron stock solution 1000 ppm. Dissolve 7.022 g (NH4)2- Fe(SO4)2.6H2O in 1000 ml of 1 N \( \text{HNO}_3 \).

6. Manganese stock solution 1000 ppm. Dissolve 3.076 g MnSO4.5H2O in 1000 ml of 1 N \( \text{HNO}_3 \).

7. Zinc stock solution 1000 ppm. Dissolve 4.398 g ZnSO4.7H2O in 1 liter of 1 N \( \text{HNO}_3 \).

8. Copper stock solution 1000 ppm. Dissolve 3.929 g CuSO4.5H2O in 1 liter of 1 N \( \text{HNO}_3 \).

9. Combined standard solution of Fe, Mn, Cu and Zn
   Pipette into a volumetric flask of 100 ml:
   * 10 ml of 1000 ppm Fe,
   * 10 ml of 1000 ppm Mn,
   * 5 ml of 1000 ppm Zn,
   * 5 ml of 1000 ppm Cu,
   Fill up to the mark with 1 N \( \text{HNO}_3 \). The final concentrations are:
   * 100 ppm Fe
   * 100 ppm Mn
   * 50 ppm Zn
   * 50 ppm Cu.
10. Combined Micro-element standard working solutions

* Pipette 0, 0.5, 1, 2, and 4 ml of the combined standard solution above into 100 ml volumetric flasks and fill up to the mark with 4% HNO₃. These solutions contain the following concentrations:

<table>
<thead>
<tr>
<th>Macro Element</th>
<th>S1 (ppm)</th>
<th>S2 (ppm)</th>
<th>S3 (ppm)</th>
<th>S4 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (Fe)</td>
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<td>Copper (Cu)</td>
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<td>2.0</td>
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</tbody>
</table>

Where S1, S2, S3, and S4 are standard 1, 2, 3, and 4 respectively.

Procedure

1. Pipette 10.0 ml of the sample digests and the blank digests from, calcination or wet digestion, and combined micro-element standard working solutions of Fe, Mn, Cu, and Zn above into test tubes.
2. Add 1 ml of 0.1% lanthanum solution and homogenize.
3. Establish the concentration curve for the standard series solutions containing the lanthanum solution for each of the elements (Fe, Mn, Cu and Zn) by aspirating into the air-acetylene flame and measuring the absorbance at the following wavelengths:

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (mm)</th>
<th>Slit width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (Fe)</td>
<td>248.3</td>
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</tr>
<tr>
<td>Manganese (Mn)</td>
<td>279.5</td>
<td>0.2</td>
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<tr>
<td>Copper (Cu)</td>
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</tr>
<tr>
<td>Zinc (Zn)</td>
<td>213.9</td>
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</tr>
</tbody>
</table>

Nebulize the sample digests and blanks containing the lanthanum chloride solution and record the readings of each of the elements (Fe, Mn, Cu and Zn).
Calculations

\[
\text{Fe, Mn, Cu, Zn (ppm)} = \frac{(a-b) \times V \times \text{mcf}}{s}
\]

Where:

- \(a\) = Concentration of (Fe, Mn, Cu, Zn) in plant digest from AAS reading (ppm)
- \(b\) = Concentration of (Fe, Mn, Cu, Zn) in blank digest from AAS reading (ppm)
- \(V\) = Total volume of digest (100 ml)
- \(s\) = Weight of sample (g)
- \(\text{mcf}\) = Moisture correction factor
PART III

APPENDICES
## APPENDIX 1: MOISTURE CORRECTION FACTOR CALCULATED FROM % MOISTURE:

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<th>Factor</th>
<th>Moisture</th>
<th>Factor</th>
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### Appendix 2. Factors for Conversions of Electrical Conductivities to 25°C Temperature \( F = \frac{(E_{25})}{E} \)

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Appendix 4. The Textural Triangle

In using the diagram, the points corresponding to the percentage of silt and clay in the soil under consideration are located on the silt and clay lines on the diagram, respectively. Lines are then projected inward, parallel in the first case to the clay side of the triangle and in the second case parallel to the sand side. The name of the compartment in which the two lines intersect is the class name of the soil in question.
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<td>Cu</td>
<td>29</td>
<td>63.546</td>
<td>Rhenium</td>
<td>Re</td>
<td>75</td>
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</tr>
<tr>
<td>Curium</td>
<td>Cm</td>
<td>96/</td>
<td>247*</td>
<td>Rhodium</td>
<td>Rh</td>
<td>45</td>
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<tr>
<td>Dysprosium</td>
<td>Dy</td>
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<td>Es</td>
<td>99</td>
<td>252*</td>
<td>Ruthenium</td>
<td>Ru</td>
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<td>101.07</td>
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<td>Erbium</td>
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<td>167.26</td>
<td>Samarium</td>
<td>Sm</td>
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<td>63</td>
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<td>Scandium</td>
<td>Se</td>
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<tr>
<td>Ferrum</td>
<td>Fm</td>
<td>100</td>
<td>257*</td>
<td>Selenium</td>
<td>Se</td>
<td>34</td>
<td>78.96</td>
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<td>Fluorine</td>
<td>F</td>
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<td>18.9984</td>
<td>Silicon</td>
<td>Si</td>
<td>14</td>
<td>28.08555</td>
</tr>
<tr>
<td>Francium</td>
<td>Fr</td>
<td>87</td>
<td>223*</td>
<td>Silver</td>
<td>Ag</td>
<td>47</td>
<td>107.868</td>
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<tr>
<td>Gadolinium</td>
<td>Gd</td>
<td>64/</td>
<td>157.25</td>
<td>Sodium</td>
<td>Na</td>
<td>11</td>
<td>22.9898</td>
</tr>
<tr>
<td>Gallium</td>
<td>Ga</td>
<td>31</td>
<td>69.72</td>
<td>Strontium</td>
<td>Sr</td>
<td>38</td>
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<tr>
<td>Element</td>
<td>symbol</td>
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<td>symbol</td>
<td>Atomic number</td>
<td>Atomic weight</td>
</tr>
<tr>
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<td>---------------</td>
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<td>---------------</td>
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<tr>
<td>Germanium</td>
<td>Ge</td>
<td>32</td>
<td>72.59</td>
<td>Sulphur</td>
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<td>32.06</td>
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<td>180.9479</td>
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<td>Hf</td>
<td>72</td>
<td>178.49</td>
<td>Technetium</td>
<td>Tc</td>
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<td>98*</td>
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<tr>
<td>Helium</td>
<td>He</td>
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<td>4.0026</td>
<td>Tellurium</td>
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<td>127.60</td>
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<tr>
<td>Holmium</td>
<td>Ho</td>
<td>67</td>
<td>164.9384</td>
<td>Terbium</td>
<td>Tb</td>
<td>63</td>
<td>158.9254</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>H</td>
<td>1</td>
<td>1.0079</td>
<td>Thallium</td>
<td>Tl</td>
<td>81</td>
<td>204.383</td>
</tr>
<tr>
<td>Indium</td>
<td>In</td>
<td>49</td>
<td>114.82</td>
<td>Thorium</td>
<td>Th</td>
<td>90</td>
<td>232.0381</td>
</tr>
<tr>
<td>Iodine</td>
<td>I</td>
<td>53</td>
<td>126.9045</td>
<td>Thallium</td>
<td>Tl</td>
<td>69</td>
<td>168.9342</td>
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<td>Iridium</td>
<td>Ir</td>
<td>77</td>
<td>192.22</td>
<td>Tin</td>
<td>Sn</td>
<td>50</td>
<td>118.69</td>
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<tr>
<td>Iron</td>
<td>Fe</td>
<td>26</td>
<td>55.847</td>
<td>Titanium</td>
<td>Ti</td>
<td>22</td>
<td>47.88</td>
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<tr>
<td>Krypton</td>
<td>Kr</td>
<td>36</td>
<td>83.80</td>
<td>Tungsten</td>
<td>W</td>
<td>74</td>
<td>183.85</td>
</tr>
<tr>
<td>Lanthanum</td>
<td>La</td>
<td>57</td>
<td>138.9055</td>
<td>Uranium</td>
<td>U</td>
<td>92</td>
<td>238.0289</td>
</tr>
<tr>
<td>Lawrencium</td>
<td>Lr</td>
<td>103</td>
<td>260*</td>
<td>Vanadium</td>
<td>V</td>
<td>23</td>
<td>50.9415</td>
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<tr>
<td>Lead</td>
<td>Pb</td>
<td>82</td>
<td>207.2</td>
<td>Xenon</td>
<td>Xe</td>
<td>54</td>
<td>131.29</td>
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<tr>
<td>Lithium</td>
<td>Li</td>
<td>3</td>
<td>6.941</td>
<td>Ytterbium</td>
<td>Yb</td>
<td>70</td>
<td>173.04</td>
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<td>Lutetium</td>
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<td>71</td>
<td>174.967</td>
<td>Yttrium</td>
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<td>39</td>
<td>88.9059</td>
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<tr>
<td>Magnesium</td>
<td>Mg</td>
<td>12</td>
<td>24.305</td>
<td>Zinc</td>
<td>Zn</td>
<td>30</td>
<td>65.38</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
<td>25</td>
<td>54.9380</td>
<td>Zirconium</td>
<td>Zr</td>
<td>40</td>
<td>91.22</td>
</tr>
</tbody>
</table>

Value Marked * indicate mass numbers of most stable known isotope.
APPENDIX 6: TABLE OF CONVERSION FROM NON-S.I. TO INTERNATIONAL SYSTEM UNITS (S.I)

<table>
<thead>
<tr>
<th>Non-S.I</th>
<th>S.I Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mmhos/cm</td>
<td>1 dS/m or 1 mS/cm</td>
</tr>
<tr>
<td>CEC meq/100 g</td>
<td>cmol (+) Kg⁻¹</td>
</tr>
<tr>
<td>Exchangeable Cations</td>
<td>cmol.(+) Kg⁻¹ Ca²⁺</td>
</tr>
<tr>
<td>e.g Ca meq/100 g</td>
<td>micro-g cm⁻³ (for solutions)</td>
</tr>
<tr>
<td>ppm</td>
<td>micro-g g⁻¹ (for solids)</td>
</tr>
<tr>
<td>ppm</td>
<td>mg Kg⁻¹ (for solids)</td>
</tr>
<tr>
<td>ppm</td>
<td>cm³</td>
</tr>
<tr>
<td>ml = cm³</td>
<td>dm³</td>
</tr>
<tr>
<td>litre (l)</td>
<td>mgdm⁻³</td>
</tr>
<tr>
<td>mg/l</td>
<td>gdm⁻³</td>
</tr>
<tr>
<td>g/l</td>
<td>Mgm⁻³</td>
</tr>
<tr>
<td>g/cm³</td>
<td>1micrometer</td>
</tr>
<tr>
<td>1 □ (micron)</td>
<td>10.1 kPa (0.1 KNm⁻²)</td>
</tr>
<tr>
<td>(1/10 bar or 1/10 atmosphere)</td>
<td>33 kPa (33 KNm⁻²)</td>
</tr>
<tr>
<td>1/3 bar or 1/3 atmosphere</td>
<td>1520 kPa (1520 KNm⁻²)</td>
</tr>
<tr>
<td>15 bar or 15 atmosphere</td>
<td></td>
</tr>
</tbody>
</table>

Other relations

ppm by weight = \( \frac{mg/l}{\text{specific gravity}} \)

% by weight = \( \frac{mg/l}{10.000 \times \text{specific gravity}} \)
### APPENDIX 7: CONVERSION FACTORS FOR SOME SI UNITS TO NON-SI UNITS

<table>
<thead>
<tr>
<th>TO CONVERT</th>
<th>INTO</th>
<th>MULTIPLY BY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kilometer, km (10^3 m)</td>
<td>mile, mi</td>
<td>0.621</td>
</tr>
<tr>
<td>meter, m</td>
<td>yard, yd</td>
<td>1.094</td>
</tr>
<tr>
<td>meter, m</td>
<td>foot, ft</td>
<td>3.28</td>
</tr>
<tr>
<td>micrometer, um (10^-6 m)</td>
<td>micron, u</td>
<td>1.0</td>
</tr>
<tr>
<td>millimeter, mm (10^-3 m)</td>
<td>inch, in</td>
<td>3.94 x 10^2</td>
</tr>
<tr>
<td>nanometer, nm (10^-9 m)</td>
<td>Angstrom, Å</td>
<td>10</td>
</tr>
<tr>
<td><strong>Area</strong></td>
<td>acre</td>
<td>2.47</td>
</tr>
<tr>
<td>square kilometer, km² (10^4 m²)</td>
<td>acre</td>
<td>2.47</td>
</tr>
<tr>
<td>square kilometer, km² (10^5 m²)</td>
<td>square mile, mi²</td>
<td>0.386</td>
</tr>
<tr>
<td>square meter, m²</td>
<td>acre</td>
<td>2.47 x 10^4</td>
</tr>
<tr>
<td>square meter, m²</td>
<td>square foot, ft²</td>
<td>10.76</td>
</tr>
<tr>
<td>square millimeter, mm² (10^-3 m)</td>
<td>2 square inch, in²</td>
<td>1.55 x 10^-2</td>
</tr>
<tr>
<td>volume cubic meter, m³</td>
<td>acre-inch</td>
<td>9.73 x 10^3</td>
</tr>
<tr>
<td>cubic meter, m³</td>
<td>cubic foot, ft³</td>
<td>35.3</td>
</tr>
<tr>
<td>cubic meter, m³</td>
<td>cubic inch, in³</td>
<td>6.10 x 10^4</td>
</tr>
<tr>
<td>litre, L (10^-3 m³)</td>
<td>bushel, bu</td>
<td>2.84 x 10^2</td>
</tr>
<tr>
<td>litre, L (10^-3 m³)</td>
<td>quart (liquid) qt</td>
<td>1.057</td>
</tr>
<tr>
<td>litre, L (10^-3 m³)</td>
<td>cubic foot, ft³</td>
<td>3.53 x 10^2</td>
</tr>
<tr>
<td>litre, L (10^-3 m³)</td>
<td>gallon</td>
<td>0.265</td>
</tr>
<tr>
<td>litre, L (10^-3 m³)</td>
<td>ounce (fluid), oz</td>
<td>33.78</td>
</tr>
<tr>
<td>litre, L (10^-3 m³)</td>
<td>pint (fluid), pt</td>
<td>2.11</td>
</tr>
<tr>
<td>Mass gram, g (10^-3 Kg)</td>
<td>pound</td>
<td>2.20 x 10^-3</td>
</tr>
<tr>
<td>gram, g (10^-3 Kg)</td>
<td>ounce (avdp), oz</td>
<td>3.52 x 10^2</td>
</tr>
<tr>
<td>kilogram, kg</td>
<td>pound</td>
<td>2.205</td>
</tr>
<tr>
<td>kilogram, kg</td>
<td>quintal (metric), q</td>
<td>0.01</td>
</tr>
<tr>
<td>kilogram, mg</td>
<td>ton (2000 lb), ton</td>
<td>1.10 x 10^-3</td>
</tr>
<tr>
<td>megagram, Mg (tonne)</td>
<td>ton (U.S.), ton</td>
<td>1.102</td>
</tr>
<tr>
<td>tonne, t</td>
<td>ton (U.S.), ton</td>
<td>1.102</td>
</tr>
</tbody>
</table>

### Yield and Rate

<table>
<thead>
<tr>
<th>TO CONVERT</th>
<th>INTO</th>
<th>MULTIPLY BY</th>
</tr>
</thead>
<tbody>
<tr>
<td>kilogram per hectare</td>
<td>pound per acre⁻¹</td>
<td>0.893</td>
</tr>
<tr>
<td>kilogram per cubic meter</td>
<td>pound per bushel, 7.77 x 10⁻²</td>
<td></td>
</tr>
<tr>
<td>kilogram per hectare,</td>
<td>bushel per acre, 60 lb</td>
<td>1.49 x 10⁻²</td>
</tr>
<tr>
<td>kilogram per hectare</td>
<td>bushel per acre, 56 lb</td>
<td>1.59 x 10⁻²</td>
</tr>
</tbody>
</table>
APPENDIX 8: GOOD LABORATORY PRACTICE

It is very important that an acceptable level of efficiency be maintained in the laboratory. Efficiency generally means the accuracy and speed with which data is generated. Proper safety precautions and care of laboratory equipment are important for sustained production of qualitative and quantitative laboratory results. To safeguard this instrument of production some of the steps necessary for its attainment are listed below.

* The need for proper handling and care of the equipment and laboratory material e.g. after working in any area in the laboratory make sure the area is cleaned up after use.

* Balances can easily lose their sensitivity or get corroded with improper handling. Always make sure the balances are cleaned up before and after use. Avoid water, soil or chemical spills on the balances and surrounding area and clean up immediately in case of accidental spills.

* Always make sure no pockets of water, acids, bases or extraneous material remain on the bench tops. Accidental spills should be cleaned up immediately with sponge and water to avoid corrosion; do not wait for the next person to use the area to clean it or that the cleaning staff should do it. Be a good example.

* Chemical spills on hot plates and digestion equipment should be cleaned up immediately after use to avoid caking and subsequent corrosion.

* Always replace the covers or stoppers on bottles containing chemicals after weighing or pipetting; place covers with the screwing side up on the bench.

* Fumehood and chimney should be washed as regularly as practical after digestion while bearing in mind that condensed perchloric acid could start a fire or explosion.

* After pipetting acids and bases always stand the pipettes in a beaker or pipette holder and not place them on the bench top.
* Make sure any flask or glassware you have any chemical in is labeled with the name of the chemical possibly its concentration.
* Signal any abnormalities you might find in the laboratory to the Head of laboratory, this will make it possible for corrective measures to be taken.
* Place any material sitting out of place in its rightful position.
* Signal any equipment that is broken down to the head of laboratory; do not attempt to repair it yourself.
* Use clean and dry glassware for analysis. Dry glasswares on the racks and ovens provided, and not on bench tops except where absorbant paper is available.
* Always arrange materials in your area of work neatly.
* Keep an exercise book for recording broken glassware (pipettes, flasks, etc.). Please always make sure you register any materials that may be broken accidentally or that you find broken. This will enable the laboratory keep tap of broken glassware and make arrangements for replacement.

A) **STANDARDIZATION OF 0.1 N HCl OR 0.05 N H₂SO₄**

**Principle:**

These acids are standardized by titration of sodium carbonate with methyl red-bromocresol green as an indicator.

Approximately 0.1 N solution of HCl is obtained by measuring 9 ml of conc. HCl (37%) with a burette into a 1 liter volumetric flask containing 800 ml of distilled water and brought upto the 1 liter mark with distilled water.

Approximately 0.05 N H₂SO₄ is obtained by measuring 1.5 ml conc. H₂SO₄ (96%) with a burette into a 1 liter volumetric flask containing 800 ml of distilled water and brought upto the 1 liter mark with distilled water.
Reagents:

Mixed methyl red-bromocresol green indicator

i. Weigh 0.100 g of methyl red

ii. Weigh 0.150 g of bromocresol green

iii. Dissolve the above two reagents in a little ethanol (96%) and then bring to 100 ml volumetric flask mark with ethanol.

- Sodium Carbonate
  Dry 1.0 g of sodium carbonate in a muffle furnace at 285°C for 2 hours.

Procedure

- Weigh exactly 200 mg of the dried sodium carbonate in a 300 ml erlenmeyer flask. This has to be done rapidly to avoid Na₂CO₃ absorbing water because it is hygroscopic.

- Add 75 ml of distilled water, dissolve the sodium carbonate and add 3 drops of the mixed indicator.

- Titrate with the acid until the color changes from green via grey to rose.

- Then boil until the CO₂ is driven out of the solution. Cool. Titrate further until the color has become rose again.

Calculation

The titer (N) of the acid is:

\[
\text{HCl (t)} = \frac{\text{weight of Na}_2\text{CO}_3 \text{ in mg} \times 1}{\text{Volume of acid used} \quad \text{Equiv wt. Na}_2\text{CO}_3 \quad (52.97)}
\]

\[
\text{H}_2\text{SO}_4(t) = \frac{\text{Weight of Na}_2\text{CO}_3 \text{ in mg} \times 1}{\text{Volume of acid used} \quad \text{Equiv. wt. Na}_2\text{CO}_3 \quad (52.97)}
\]

Remarks:
The color change before boiling stage should be reached 0.2-0.3 ml before the equivalence point. After boiling, the consumption of acid should not exceed 0.3 ml, otherwise repeat the determination.
8) STANDARDIZATION OF 0.1N NaOH OR 0.1N KOH WITH POTASSIUM HYDROGEN PHTALATE (KHC₈H₄O₄).

Principle:

The base is standardized by titration of the oxalic acid with methyl red as an indicator. Approximately 0.1 N NaOH can be obtained by rapidly weighing 4.0 g of the NaOH crystals in an erlenmeyer flask. The base is dissolved in water and quantitatively transferred to a 1 liter volumetric flask and bring up to the mark with distilled water.

Approximately 0.1 N KOH can be obtained by rapidly weighing 5.69 g of KOH crystals in an erlenmeyer flask. Dissolve in distilled water and transfer quantitatively into a 1 liter volumetric flask and brought up to the mark with distilled water.

Reagents:

- Phenolphthalein indicator: Dissolve 100 mg of phenolphthalein in 96% ethanol in a 100 ml volumetric flask.
- Potassium hydrogen phthalate Dry 2.5 g of potassium hydrogen phthalate at 120°C in the oven for 2 hours.

Procedure:

- Weigh 600 mg of potassium hydrogen phthalate in a 250 ml erlenmeyer flask.
- Add 75 ml of boiled distilled water to dissolve the reagent and add 3 drops of phenolphthalein.
- Titrate with the base until you obtain a red end point.
- Register the quantity of base used for the titration.
- Repeat the procedure 2 to 3 times and find the mean of the number of mls of base used in the titration.
Calculations:

The titer of NaOH = \( \frac{\text{Weight of phthalate in (mg)} \times 1}{\text{Equiv. wt. phthalate \ V}} \)

where \( V = \text{volume of NaOH used} \) = \( \frac{600 \text{ mg}}{204.22 \times V} \)

The titer of KOH = \( \frac{\text{weight of phthalate in mg} \times 1}{\text{volume of KOH used \ (Equiv. wt of Phthalate)}} \)

C) STANDARDIZATION OF 0.1 N AgNO₃ WITH SODIUM CHLORIDE

Principle:

A known amount of NaCl is titrated with an AgNO₃ solution, so that silver chloride precipitates. After the equivalence point, any excess of AgNO₃ forms a red precipitate of silver chromate with the indicator.

Reagents

- Silver nitrate (AgNO₃) 0.1 N: Dissolve 17.0 g of AgNO₃ in 1 liter of distilled water.
- Potassium chromate solution. Dissolve 5 g of K₂CrO₄ in 100 ml of distilled water.
- Sodium chloride (NaCl): Dry about 500 mg of sodium chloride in a muffle furnace at 200°C for 24 hours.
- Calcium carbonate. CaCO₃ (analytical grade).

Procedure:

- First perform a blank determination in a 250 ml beaker that contains 140 ml of water, 4 ml of potassium chromate solution and 0.5 g of CaCO₃.
- Titrate with the silver nitrate solution until the suspension shows a weak, but distinct, red color which persists even with energetic stirring. Keep this suspension for future comparison.
- Then weigh out precisely about 200 mg of NaCl.
- Transfer it to a 250 ml beaker, and add 100 ml of water and 4 ml of potassium chromate solution.
- Titrate carefully, while stirring, until the red color which appears with every drop of AgNO₃ fades away slowly. Then titrate dropwise until the solution shows the same shade of red as the blank.

**Calculation:**

The titer (N) of the AgNO₃ solution is:

\[
t = \frac{1}{\text{Equiv. (58.443) of NaCl}} \times \frac{w}{a - b}
\]

Where:

- \(t\) = titer (Normality) of the silver nitrate solution.
- \(w\) = weight of NaCl, in mg
- \(a\) = volume of AgNO₃ added to the analyte solution, in ml
- \(b\) = volume of AgNO₃ added to the blank solution, in ml
- 58.443 = weight of NaCl

**D) STANDARDIZATION OF 0.1 N EDTA WITH CALCIUM CARBONATE CaCO₃**

**Principle:**

An EDTA solution is standardized by titration of CaCO₃ at pH 10 with Eriochrome Black-T as an indicator.

**Reagents**

- Hydrochloric acid (HCl) 1N: Add 83 ml of concentrated hydrochloric acid (36%) to about 400 ml water and make up to 1 liter.
- Buffer solution, pH 10. Dissolve 54 g of ammonium chloride, NH₄Cl, and 29 g of Mg-EDTA, in 350 ml of concentrated aqueous ammonia (25%) and dilute with water to 1000 ml.
- EDTA solution 0.1N: Dissolve 18.6 g of disodium ethylenediamine tetra acetate, Na₂EDTA₂H₂O in 1 litre of water.
Indicator solution. Eriochrome Black T solution: Dissolve 0.5 g Eriochrome Black-T in 100 ml of ethanol 96%. Add 4.5 g of hydroxylamine HCl and stir on a magnetic stirrer for at least 30 minutes.

Procedure:

- Weigh out precisely 150 mg of CaCO₃ previously dried at 105°C for 2 hours.
- Dissolve it in a small excess of 1N HCl (about 3 ml). Dilute to about 100 ml with distilled water and boil for some minutes.
- Check whether all CaCO₃ has dissolved; if not, add 0.5 ml of 1N HCl extra and boil again.
- Then add to the still hot liquid 10 ml of the buffer solution and 0.4 ml of the indicator solution.
- Titrate from red to a blue end point with EDTA solution. Perform also a blank determination.

Calculation:

The titer (N) of EDTA solution is:

\[
t = \frac{1}{\text{Equiv wt of CaCO}_3} \times \frac{w}{a-b} (50.02)
\]

in which =

- \(t\) = titer (Normality) of the EDTA solution
- \(w\) = weight of CaCO₃ in mg
- \(a\) = volume of EDTA solution used for the analyte, in ml
- \(b\) = volume of EDTA solution used for the blank, in ml

Remarks:

- Store the standardized EDTA solution in polythene bottles
- The addition of Mg-EDTA to the buffer solution provides a sharper color change at the end point when using Eriochrome Black T.
### APPENDIX. 9. COMMON INDICATOR SOLUTIONS

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Satisfactory concentration (%)</th>
<th>ml of 0.01N NaOH required to convert 0.1 g of indicator to monosodium salt</th>
<th>Solvent</th>
<th>pH range</th>
<th>Color change from – to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymol blue</td>
<td>0.04</td>
<td>21.5</td>
<td>Water</td>
<td>1.2-2.8</td>
<td>Red-yellow</td>
</tr>
<tr>
<td>Methyl orange</td>
<td>0.10</td>
<td>-</td>
<td>Water</td>
<td>-</td>
<td>Red-orange</td>
</tr>
<tr>
<td>Brom phenol blue</td>
<td>0.04</td>
<td>14.9</td>
<td>Water</td>
<td>3.0-4.6</td>
<td>Yellow-blue</td>
</tr>
<tr>
<td>Brom cresol green</td>
<td>0.04</td>
<td>14.3</td>
<td>Water</td>
<td>3.8-5.4</td>
<td>Yellow-blue</td>
</tr>
<tr>
<td>Chlor phenol red</td>
<td>0.04</td>
<td>23.6</td>
<td>Water</td>
<td>4.8-6.4</td>
<td>Yellow-red</td>
</tr>
<tr>
<td>Brom cresol purple</td>
<td>0.04</td>
<td>19.5</td>
<td>Water</td>
<td>5.2-6.8</td>
<td>Yellow-red</td>
</tr>
<tr>
<td>Brom thymol blue</td>
<td>0.04</td>
<td>18.5</td>
<td>Water</td>
<td>5.2-6.8</td>
<td>Yellow-purple</td>
</tr>
<tr>
<td>Brom thymol blue</td>
<td>0.04</td>
<td>16.0</td>
<td>Water</td>
<td>6.0-7.6</td>
<td>Yellow-blue</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.04</td>
<td>26.2</td>
<td>Water</td>
<td>6.8-8.4</td>
<td>Yellow-red</td>
</tr>
<tr>
<td>Cresol red</td>
<td>0.04</td>
<td>21.5</td>
<td>Water</td>
<td>7.2-8.8</td>
<td>Yellow-red</td>
</tr>
<tr>
<td>Thymol blue</td>
<td>0.04</td>
<td>21.5</td>
<td>Water</td>
<td>8.0-9.6</td>
<td>Yellow-blue</td>
</tr>
</tbody>
</table>
## APPENDIX 10. CONCENTRATION, NORMALITY AND AMOUNTS OF CONCENTRATED ACIDS

AND BASES TO MAKE 1 LITER OF NORMAL SOLUTION

<table>
<thead>
<tr>
<th>Acid or base</th>
<th>Per cent</th>
<th>Specific gravity $^{20\degree C}$</th>
<th>Grams per liter</th>
<th>Approximate normality</th>
<th>Milliliters to make 1 liter of approximately 1N solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>99.0</td>
<td>1.0524</td>
<td>1042.0</td>
<td>17.45</td>
<td>58</td>
</tr>
<tr>
<td>Ammonium hydoxide</td>
<td>28.33</td>
<td>0.9000</td>
<td>255.0</td>
<td>15.0</td>
<td>67</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>38.0</td>
<td>1.1885</td>
<td>451.6</td>
<td>12.4</td>
<td>81</td>
</tr>
<tr>
<td>Hydrofluoric acid</td>
<td>50.</td>
<td>1.158</td>
<td>577.5</td>
<td>28.8</td>
<td>35</td>
</tr>
<tr>
<td>Nitric acid*</td>
<td>72.0</td>
<td>1.4218</td>
<td>1024.0</td>
<td>16.2</td>
<td>62</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>85.0</td>
<td>1.689</td>
<td>1436.0</td>
<td>44.0</td>
<td>23</td>
</tr>
<tr>
<td>Perchloric acid</td>
<td>70.0</td>
<td>1.664</td>
<td>1165.0</td>
<td>11.6</td>
<td>86</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>50.0</td>
<td>1.5253</td>
<td>762.7</td>
<td>19.0</td>
<td>53</td>
</tr>
<tr>
<td>Sulfuric acid</td>
<td>95.0</td>
<td>1.8337</td>
<td>1742.0</td>
<td>35.5</td>
<td>28</td>
</tr>
</tbody>
</table>
APPENDIX 11: CLEANING MIXTURES AND LABORATORY PREQUOTIONS

11.1. Cleanliness of Vessels

Cleanliness of Vessels is of enormous importance in quantitative analysis. A vessel can be called clean if no contamination of any kind can be seen even by very careful examination, and if water runs down its walls smoothly without leaving any drops. Drops are formed if a glass surface is greasy; the presence of grease is most undesirable, as precipitates formed in chemical reactions adhere very closely to the grease layer and are very difficult to transfer to the filter. In addition volume measurement becomes in accurate. To avoid all this inconvenience the following cleaning steps have to be followed:

- Wash the vessels with hot water and rub thoroughly inside out with brushes.
- Repeat this procedure with soap or soda solution and then wash out with tap water.
- If the vessel is not clean after this treatment and drops remain on its inner walls, wash out with "Chromic mixture" (which is a mixture of potassium dichromate and concentrated sulfuric acid). The chromic mixture is poured into the vessel so that its inside walls are wetted thoroughly. The chromic mixture should then be returned to its container, it can be used repeatedly.
- After the vessel has been treated with chromic mixture it should be thoroughly washed with tap water and finally rinsed with a small amount of distilled water.
- Dry the cleaned vessels in an oven.

11.2. Cleansing Mixtures

"Chromo-sulfuric acid" cleansing mixture is the most widely used cleansing agent. It is essentially a dichromate-sulfuric acid mixture used for cleaning glass vessels. This cleaning agent has powerful oxidizing and solvent properties. The mixture can be prepared following one of the following methods:

Five gram of sodium-dichromate is dissolved in 5 ml of water in a 25 ml beaker. Then 100 ml of concentrated sulfuric acid is added slowly with constant stirring. The temperature will rise to 70-80. The mixture is allowed to cool to about 40°C and then transferred to a dry, glass-stoppered bottle.
• About 100 ml of concentrated sulfuric acid in a 250 ml pyrex beaker, is cautiously heated to about 100°C and 3 grams of sodium or potassium dichromate is gradually added with stirring and the stirring continued for several minutes to prevent the resulting chromic acid from caking. The mixture is allowed to cool to about 40°C and be transferred to a dry, glass-stoppered bottle. The chromic acid mixture prepared by shaking excess of sodium dichromate or finely-powered potassium dichromate with concentrated sulfuric acid under laboratory room temperature is not as efficient as that prepared by method (1) or (2), but could be used for cleaning glassware for volumetric analysis. The chromic acid cleansing mixture has powerful oxidizing and solvent properties, its exhaustion is readily recognized by the change in color from reddish-brown to green.

Before using the chromic acid mixture for cleaning, the vessel should be rinsed with water to remove organic matter and particular reducing agents as much as possible. After draining away as much of the water as practicable, a quantity of the cleaning mixture is introduced into the vessel, the soiled surface thoroughly wetted with the mixture, and the main quantity of the cleaning mixture returned to the stock bottle. After standing for a short time with occasional rotation of the vessel to spread the liquid over the internal surface, the vessel is thoroughly rinsed successively with tap and distilled water. If a black solid, probably consisting largely of carbon produced by overheating the contents of the apparatus, remains after the above treatment, it is recommended that a small volume of the reagent be introduced into the flask and the latter gently and evenly heated with a free flame until the acid commences to fume. Under this condition, most carbonaceous matters are oxidized.

Instead of chromic mixture laboratory ware can also be washed with a mixture of equal volumes of approximately 0.1N solutions of KMnO₄, alcoholic solutions of KOH or NaOH.

11.3. Laboratory accidents and first aid

• In case of accidents always call or notify the supervisor as soon as possible.

• A first aid box or cupboard should be kept in a readily accessible position in the laboratory and should contain the following articles clearly labeled:
  ▪ Bandage (several sized) gauze, lint cotton wool, adhesive plaster "elasto plast" or equivalent, and a sling.
  ▪ Delicate forceps, needles, thread, scissors, and safety pins.
  ▪ Fine glass dropper.
  ▪ Two eye glasses.

• Vaseline, castor oil, olive oil, sal volatile, boracic acid powder sodium bicarbonate powder, chloramine-T powder, sulpha-pyridine powder butesin picrate ointment. Acriflavine jelly or emulsion (e.g. "Tannafax, Burnol")
- Tannic acid jelly (e.g. "Tannafax.")
- One fireproof blanket - this is best stored in a special container just outside the first aid cupboard.
- Bottles containing
  - One per cent. acetic acid.
  - One per cent. boric acid.
  - Saturated sodium bicarbonate solution.
  - One per cent. sodium bicarbonate solution.
  - Rectified spirit.
  - Glycerin.
  - Light petroleum, b.p. 80-100°C
  - A disinfectant, e.g. "dettol" or "T.C.P."
  - A laboratory emergency chart, "Which should be hung in prominent position near the first aid box, is obtainable from the Fisher Scientific Company."

11.4. Burns

Burns are caused by dry heat (e.g. flames, hot objects, etc.). For slight burns in which the skin is not broken, apply tannic acid jelly ("Tannafax"), acriflavine jelly ("burnol") or butesin picate ointment (butesin is n-butyl p-aminobenzoate).

**Acids on the skin:** Wash immediately and thoroughly with a liberal quantity of water, then with saturated sodium bicarbonate solution, and finally with water. For a serious acid burn, follow this by applying a disinfectant, drying the skin and covering with acriflavine jelly.

**Alkalis on the skin:** Wash immediately with a large volume of water then with 1 percent, acetic acid, and finally with water. For a serious burn, follow this treatment by applying a disinfectant, drying the skin and covering with acriflavine jelly.

**Bromine on the skin:** Wash the affected part immediately with a liberal supply of light petroleum, b.p. 80-100 and then rub glycerin well into the skin. After a little time remove the superficial glycerin and apply acriflavine jelly or butesion picrate ointment.
**Phosphorus on the skin**: Wash well with cold water and treat with 1 percent silver nitrate solution.

**Methyl Sulfate on the skin**: Wash immediately and liberally with concentrated ammonium solution and then rub gently with wads of cotton wool soaked in concentrated ammonium solution.

**Organic substances on the skin**: Wash freely with rectified spirit, then with soap and warm water.

**11.5. Cuts**

If the cut is only a minor one, allow it to bleed for a few seconds see that no glass remains and apply a disinfectant (rectified spirit), "Dettol" 1 percent. Aqueous chloramine-T solution, or sulphapyridine powder and a bandage.

For serious cuts, send for a doctor at once. Meanwhile wash with a disinfectant and endeavor to check bleeding by applying pressure immediately above the cut. Continuous pressure should be maintained for more than five minutes.

**11.6. Eye Accidents**

In all cases the patient should see a doctor. If the accident appears serious, medical aid should be summoned immediately while first aid is applied.

**Acid in the eye**: If the acid is dilute, wash the eye repeatedly with 1 percent sodium bicarbonate solution in the eye cup. If the acid is concentrated, first wash the eye with a large amount of water and then continue washing with the bicarbonate solution.

**Caustic alkali in the eye**: proceed as for acid in the eye but wash with 1 percent boric acid solution in place of bicarbonate solution.

**Glass in the eye**: remove loose glass very gently with forceps or by washing with water in an eye bath. Call for a doctor immediately, soreness which may follow minor accidents to the eye may be relieve by placing 1 drop of castor oil in the corner of the eye.
11.7. Fires

**Burning clothing:** prevents the person from running and fanning the flames. Make the victim lie down on the floor or throw him (her) down if necessary and wrap fireproof blanket firmly around the ignited clothes until the fire is extinguished.

**Burning reagents:** Turn out all gas burners and switch off all electric hot plates in the vicinity; remove everything which may ignite. The control of the fire depends upon its size and kind.

A small fire (for example, liquid in a beaker of flask, or an oil bath) may usually be extinguished by covering the opening of the vessel with a clean damp cloth or duster: the fire usually dies out from lack of air. For large fires, dry sand many be employed. Buckets of dry sand should be distributed round the laboratory and should be strictly reserved for this purpose. Most fires on the laboratory bench can be smothered by the liberal use of sand. Sand, once employed for this purpose should always be thrown away afterwards as it may contain appreciable quantities of inflammable, non-volatile substances (e.g., nitrobenzene).

Although sand is usually very effective for extinguishing fires, it has the disadvantage that the compound or reaction mixture is usually lost and any glass apparatus around which the fire centers may be broken under the weight of the sand. Alternatively small fires may be extinguished with carbon tetrachloride under high pressure of carbon dioxide (as contained for example in the commercial autelex extinguisher). The mixture is directed on the fire and the "blanketing" effect of the carbon dioxide and heavy carbon tetrachloride vapor will soon put out the fire. If must be noted particularly that:-

a) Carbon tetrachloride should not be used if sodium or potassium is present, as violent explosions may occur.

b) The laboratory must be ventilated immediately after the fire is extinguished, in order to disperse the highly poisonous phosgene vapors which is always formed.

It is usually better to use a fire extinguisher charged with carbon dioxide under pressure; this produces a spray of solid carbon dioxide upon releasing the pressure intermittently and is effective for extinguishing most fires in the laboratory.

For burning oil (or organic solvents), do not use water, as it will only spread the fire: a mixture of sand and sodium bicarbonate is very effective.
11.8. Poisons

Solid or liquid poisons

a) If in the mouth and not swallowed spit out at once and wash repeatedly with water.

b) If swallowed, call a doctor immediately, in the meantime:

- If the poison is an acid (including oxalic acid), dilute by drinking much water, followed by lime-water or milk of magnesia. Milk may then be given but no emetics.

- If the poison is caustic alkalis, dilute by drinking much water followed by vinegar, lemon or orange juice, or solution of lactic acid or citric acid. Milk may then be given but no emetics.

- If the poison is salt of heavy a metal, milk or white of an egg should be taken.

- If the poison is arsenic or mercuric compound, give an emetic immediately, e.g., one teaspoonful of mustard, or one tablespoonful of salt or zinc sulphate, in a tumbler of warm water.

11.9. Gas Suffocation

Remove the victim to the open air, and loosen clothing at neck. To counter chlorine or bromine fumes if inhaled in only small amounts, inhale ammonium vapor or gargle with sodium bicarbonate solution. Afterwards the patient should suck eucalyptus pastilles, or drained warm dilute peppermint or cinnamon essence, to soothe the throat and lungs. If breath has stopped, apply artificial respiration.