1- Soil Analysis

Soil is the system which supplies plant with available nutrients through the root. Physical and Chemical analysis of the soil are carried out to indicate the efficiency of soil for supplying plants with nutrients in available forms as well as identification of the factors affecting this efficiency in the soil. Therefore, besides perfect sampling in the field, soil samples must be properly prepared and analyzed in order to reach the correct evaluation of the soil nutritional status.

Sample preparation:

In order to represent the soil characters and chemical contents of available nutrients, soil particles must be in the suitable size (expressed by diameter) and dryness conditions.

Principle:

The 2 mm sieved air-dry soil is suitable for determination of water-soluble constituents, exchangeable cations, PH, free lime and various other chemical extractions.

Equipments:

1- Wooden mortar or soil mill.

2- Plastic containers.

3- Sieve (suitable size, e.g. 2 mm).

Procedures:

1- As soon as samples are received, they are arranged, coded and listed in the sample registration book.

2- Every sample is spread out on a separate paper or polyethylene sheet (the container sac may be used). Sample number (code) must be immediately written on both the bearing material and on a label inserted in the sample.

3- The sample is left to air for dryness in a dust and fume-free location.

4- At air dryness, soil clods and aggregates are pulverized by means of wooden mortar and pestle or by a soil mill to reduce particle size so as to pass through 2-mm sieve.

5- Soil samples are screened through a 2 mm sieve.

6- After screening, soil is thoroughly mixed and put in a plastic pot till analysis.

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1.1 - Determination Of Mositure Content

Principle:

Hygroscopic water of air-dry soil is determined by heating in an oven at 105°C.

Apparatus:

1- Electric oven with thermostate.

2- Desiccators with siccative.

Procedure:

1- Weigh 5.00 g of air-dry soil < 2 mm into a perviously dried (at 105°C) and weighed weighing-dish with lid (a labelled aluminium dish).

2- Dry in an oven at 105°C with unfitted lid over-night.

3- Remove from oven, fit lid, cool in a desiccator for at least 30 minutes and reweigh. All weighings should be recorded to 3 decimal places.

Calculation:

1- % moisture = $\frac{Wet\ soil\ (g) - Dry\ soil\ (g)}{Dry\ soil\ (g)} \times 100$

2- Mositure Correction Factor = 100/(100-% moisture)

Result should be recorded to 3 decimal places .

1.2 -Particle Size Distribution

Individual soil particles vary widely in any soil type. Similarly, as these particles are cemented together, a variety of aggregate shapes and sizes occur. For standard particle size measurement, the soil fraction that passes a 2-mm sieve is considered. Laboratory procedures normally estimate percentage of sand (0.05 - 2.0 mm), silt (0.002 - 0.05 mm), and clay (<0.002 mm) fractions in soils. Particle size distribution is an important parameter in soil classification and has implications on soil water, aeration, and nutrient availability to plants.

As primary soil particles are usually cemented together by organic matter, this has to be removed by H_2O_2 treatment. However, if substantial amounts of $CaCO_3$ are present, actual percentages of sand, silt or clay can only be determined by prior dissolution of the $CaCO_3$. The two common procedures used for particle size analysis or mechanical analysis are the hydrometer method (Bouyoucos, 1962; Day, 1965; FAO, 1974) or the pipette-gravimetric method.

The hydrometer method of silt and clay measurement relies on the effect of particle size on the differential settling velocities within a water column. Theoretically, the particles are assumed to be spherical having a specific gravity of 2.65 g/cm^3. If all other factors are constant, then the settling velocity is proportional to the square of the radius of the particle (Stoke's Law). The settling velocity is also a function of liquid temperature, viscosity and specific gravity of the falling particle. In practice, therefore, we must know and make corrections for the temperature of the liquid. Greater temperatures result in reduced viscosity, due to liquid expansion and a more rapid descent of falling particles.

Apparatus

Soil dispersing stirrer: A high-speed electric stirrer with a cup receptacle.
Hydrometer with Bouyoucos scale in g/L (ASTM 152H).

Reagents

A. Dispersing Solution

Dissolve 40 g sodium hexametaphosphate [$(NaPO_3)_{13}$], and 10 g sodium carbonate (Na_2CO_3) in DI water, and bring to 1-L volume with DI water. This solution deteriorates with time and should not be kept for more than 1 to 2 weeks.

B. Amyl Alcohol

Procedure

1. Weigh 40 g air-dry soil (2-mm) into a 600-mL beaker.
2. Add 60-mL dispersing solution.
3. Cover the beaker with a watch-glass, and leave overnight.
4. Quantitatively transfer contents of the beaker to a soil-stirring cup, and fill the cup to about three-quarters with water.
5. Stir suspension at high speed for 3 minutes using the special stirrer. Shake the suspension overnight if no stirrer is available.
6. Rinse stirring paddle into a cup, and allow to stand for 1 minute.
7. Transfer suspension quantitatively into a 1-L calibrated cylinder (hydrom eter jar), and bring to volume with water.

A. Determination of Blank

- Dilute 60 mL dispersing solution to 1-L hydrometer jar with water.
- Mix well, and insert hydrometer, and take hydrometer reading, R_b.
- The blank reading must be re-determined for temperature changes of more than 2°C from 20°C.

B. Determination of Silt plus Clay

- Mix suspension in the hydrometer jar, using a special paddle carefully, withdraw the paddle, and immediately insert the hydrometer.
- Disperse any froth, if needed, with one drop of amyl alcohol, and take hydrometer reading 40 seconds after withdrawing the paddle. This gives reading, R_{sc}.

CALCULATIONS

Percentage Silt plus Clay in soil

$$\% \,[\text{Silt} + \text{Clay}]\,(w/w) = (R_{sc} - R_b) \times \frac{100}{\text{Oven-dry soil (g)}}$$

C. Determination of Clay

- ☐ Mix suspension in the hydrometer jar with paddle, withdraw the paddle, with great care, leaving the suspension undisturbed.
- ☐ After 4 hours, insert the hydrometer, and take hydrometer reading, R_C.

Percentage Clay in soil:

$$\% \text{ Clay (w/w)} = (R_c - R_b) \times \frac{100}{\text{Oven-dry soil (g)}}$$

Percentage Silt in soil:

$$\% \text{ Silt (w/w)} = [\% \text{ Silt + Clay (w/w)}] - [\% \text{ Clay (w/w)}]$$

D. Determination of Sand

- ☐ After taking readings required for clay and silt, pour suspension quantitatively through a 50-µm sieve.
- ☐ Wash sieve until water passing the sieve is clear.
- ☐ Transfer the sand quantitatively from sieve to a 50 mL beaker of known weight.
- ☐ Allow the sand in the beaker to settle, and decant excess water.
- ☐ Dry beaker with sand overnight at 105°C.
- ☐ Cool in a desiccator, and re-weigh beaker with sand.

Percentage Sand in soil:

$$\% \text{ Sand (w/w)} = \text{Sand weight} \times \frac{100}{\text{Oven-dry soil (g)}}$$

Where: Weight of sand follows from:

Sand weight(g) = [Beaker + Sand (g)] - [Beaker (g)]

Note:

1. If possible, all hydrometer jars should be placed in a water bath at constant temperature (20°C); in that case, temperature corrections are not needed.

2. For temperature correction, use a value of 0.4 for each degree temperature difference from 20°C. Add or subtract this factor if the temperature is more or less than 20°C, respectively.

3. All results of mechanical analysis should be expressed on the basis of oven-dry soil (24 hours drying at 105°C).

4. In the above procedure, carbonates and organic matter are not removed from the soil.

5. The Hydrometer method, as described in this section, cannot be applied to soils that contain free gypsum (gypsiferous soils). For gypsiferous soils, see Hesse (1971).

6. Sum of % silt and clay + % sand should be 100 %. The magnitude of deviation from 100 is an indication for the degree in accuracy.

Soil Texture

Once the percentage of sand, silt, and clay is measured, the soil may be assigned a textural class using the USDA textural triangle (Fig. 4). Within the textural triangle are various soil textures which depend on the relative proportions of the soil fractions.
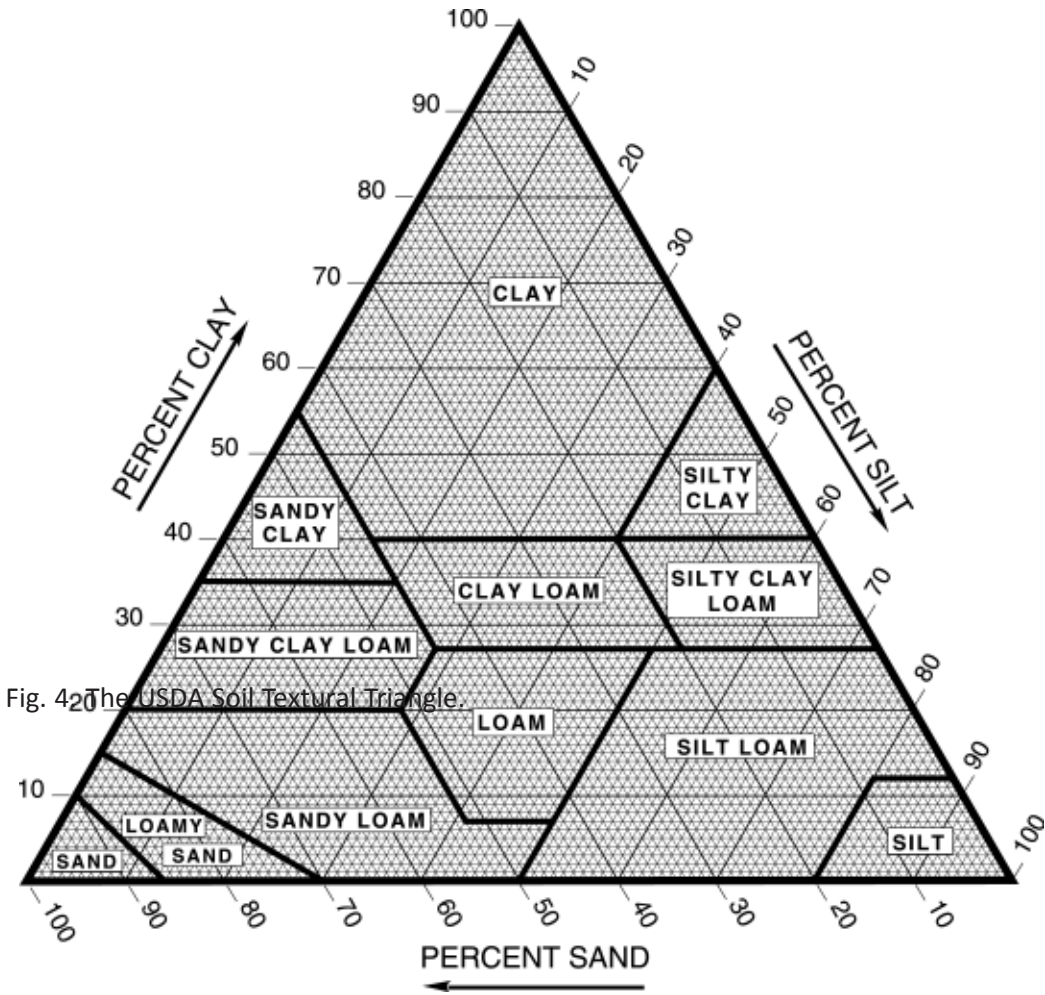
Fig. 4: The USDA Soil Textural Triangle.

1.3 - Field Capacity Moisture and Permanent Wilting Point

Principle

Soils are equilibrated with water at various tensions and moisture content is determined. The ability of soil to retain water depends on several factors, e.g., texture or particle-size distribution, organic matter content (due to its hydrophilic nature), nature of mineral colloids, and soil structure or arrangement of particles.

A. Low Range: moisture at O - lOO kpa (O - l bar) pressure

Apparatus

One-bar pressure plate extractor

One-bar ceramic plates.

Rubber rings (5-cm diameter, 1-cm height).

Compressed air source with a manifold, regulator, and gauge.

Balance.

Drying oven.

Disposable aluminum dishes or soil-moisture cans.

Desiccator.

Procedure

1. Submerge the ceramic plates in water for 24 hours to saturate.
2. Place plates on a workbench.
3. Place labeled rubber rings in order on the plate (each plate accommodates 12 samples).
4. Fill ring with 2-mm air-dry soil using a spatula (about 20 g sample). In order to avoid particle-size segregation, place entire soil sample into the ring.
5. Level, but don't pack, the sample in the ring.
6. Cover plate with water to wet sample from below. Add water between the rings until there is an excess of water (at least 3-mm deep) on the plate.
7. Cover samples with wax paper or a plastic sheet.
8. Allow samples to stand overnight.

9. The next morning, remove excess water from the plate with a syringe, disposable pipette, or siphon.

10. Place the triangular support in the extractor vessel on the bottom.

11. Install plate with samples in the lower-most position in the extractor. Then install the middle and top plates (the plastic spaces should be placed between plates).

12. Connect outflow tubes.

13. Close extractor and tighten, ensuring that the "O" ring is in place and all nuts are uniformly tightened. Apply desired pressure in the 0 - 100 kpa (0 - 1 bar) range. Build up the pressure in the vessel gradually.

14. Place a beaker to collect water from the outflow tubes.

15. Maintain pressure until no more water is being released (generally 18 - 20 hours, but for some soils 48 hours or even longer).

16. Release pressure from extractor (remove outflow tubes from water before turning instrument off).

17. Open extractor.

18. Without undue delay, transfer moist soil sample from ring with a wide-bladed spatula to a tarred dish. (It is not necessary to make a quantitative transfer of the entire soil.)

19. Immediately weigh wet sample (accuracy 0.01 g) and place in drying oven at 105 ◻C for 24 hours.

20. Place sample in desiccator, cool, and weigh.

CALCULATION

$$\% \text{ Moisture} = \frac{\text{Wet soil (g) - Dry soil (g)}}{\text{Dry soil (g)}} \times 100$$

B. High Range: moisture at lOO - l5OO kpa (O - l5 bar) pressure

Apparatus

Fifteen-bar ceramic plate extractor.
Fifteen-bar ceramic plates.
Rubber rings.
Balance.
Drying oven.
Weighing dishes (disposable aluminum dishes or tarred soil-moisture cans)
Burette.
Desiccator.

Procedure

1. Use 15-bar ceramic plates and follow Steps 1 - 12 of the previous method, applying 1 - 15 bar pressure (100 - 1500 kpa).

2. Place beaker to collect water from outflow tubes.

3. Leave overnight.

4. Connect outflow tube to burette partially filled with tap water.

5. Samples should stay in extractor until flow has ceased from all samples on plate and the soils have reached equilibrium (24 - 48 hours for most soil; however some fine textured and organic soils may needs up to 120 hours). No change in reading on burette would indicate that flow has stopped from all samples and equilibrium has been attained.

6. Disconnect burette to prevent backflow of tap water.

7. Release pressure from extractor.

8. Follow Steps 17 - 20 of the previous method.

CALCULATION

$$\% \text{ Moisture} = \frac{\text{Wet soil (g) - Dry soil (g)}}{\text{Dry soil (g)}} \times 100$$

Saturated Paste

The use of an extract from a saturated paste is advantageous for characterizing saline soils since it closely approximates salinity in relation to plant growth. One can also obtain soluble cations and anions by this method and estimate other important parameters such as Sodium Adsorption Ratio (SAR) which, in turn, predicts Exchangeable Sodium Percentage (ESP). Criteria for boron (B) toxicity tolerance by various plant species have been also developed for such an extract (Richards, 1954).

Thus, a saturation extract is routinely used where salinity is a concern. However, in dryland areas, which constitute major part of the CWANA region, it is seldom used. Nevertheless, with encroachment of supplementary irrigation in traditionally dry areas, increased use is likely to be made of saturation extracts in soil analysis.

Apparatus

Porcelain dishes.
Spatulas or mixing spoons.
Vacuum filtration system.

Procedure

1. Weigh 200 - 300 g air-dry soil (< 2-mm) into a porcelain dish.

2. Slowly add DI water, and mix with a spatula until the paste glistens and flows slightly as the porcelain dish is tipped; it should slide off the spatula without collection of any free water on the surface of the paste.

3. Allow the paste to stand for 1 hour, then re-check the criteria for saturation by adding more DI water or soil, as needed.

4. Leave the paste for 6 to 16 hours, and then filter with a vacuum filtration system using a Buchner funnel fitted with Whatman No. 42 filter paper.

5. Collect filtrate in a small bottle and keep it for subsequent measurements. If the initial filtrate is turbid, re-filter.

* The cations analyzed in saturation extracts are Ca^{++}, Mg^{++}, K^{+}, and Na^{+}, while the anions are SO_4^{--}, CO_3^{--}, HCO_3^{-}, and Cl^{-}. Boron in saturation extracts is often measured where its toxicity is suspected.

1.4 - SOIL REACTION (pH)

Principle:

The pH value of an aqueous solution is the negative logarithm of hydrogen concentration. Ion activity is measured by using a pH meter. Soil pH is determined in 1:2.5 soil/water extract.

Reagent:

Standard pH solutions with values 2, 4, 7, 9.

Apparatus:

1- pH meter.

2- Beaker 50 ml.

3- Glass rods.

4- Thermometer.

Procedure:

Extraction:

1- Add 25 ml distilled water to 10 g air-dried sample in a beaker 50 ml. Read the suspension temperature by thermometer.

2- Stirr at regular intervals for 20-30 minutes.

3- Wash the pH meter electrode with distilled water.

4- Open the contact switch, wait 5 minutes, adjust temperature knob to room temperature.

Calibration:

5- Rinse the electrode and adjust the pH dial with a standard pH solution followed by another acid or alkaline one.

Measurement:

6- Rinse the electrode with distilled water, then with the soil suspension after stirring.

7- Read the pH value of the soil suspension.

1.5 -ELECTRICAL CONDUCTIVITY (EC)

Electrical conductivity is commonly used for measuring the electrical resistance in the solution which indicates the total concentration of jonized constituents in solutions. It is closely related to the sum of the cations and anions in the suspension. Accordingly, it can be used for indicating the salinity in soil extracts. Electric conductivity can be expressed as millimhos/cm in 1:2.5 soil/water extract.

Apparatus:

1- Conductivity meter, immersion type with platinized platinium electrodes (direct indicating bridge).

2- Beakers 100 ml.

3- Thermometer.

4- Glass rodes.

5- Funnels & filter paper.

Reagents:

1- Potassium chloride solution 0.01 N : Dissolve 0.7456 g of dry potassium chloride in distilled water and make to 1 L at 25°C. This is a standerd reference solution, which at 25°C has an E.C. of 1411.8×10^{-6} (0.0014118) mhos/cm or 1.4118 m mhos/cm.

2- Calcium sulfate dihydrate saturated solution. At 25°C it has an E.C. of 2.2 mmhos/cm.

Procedures:

Extraction:

1- Put 10 g air-dry soil in 100 ml beaker, add 25 ml distilled water.

2- Stirr for 10 minutes, repeat stirring 4 times on 30 minutes intervals.

3- Measure the suspension temperature by thermometer.

Operation:

Before measuring, rise and fill the cell with reagent 1,

1- Set the temperature compensation dial.

2- Open the contact switch, wait for 5 minutes.

3- Balance the bridge with the main dial.

Calibration:

4- Measure the accuracy of the conductivity meter by using reagent 2, it should give an E.C.   2.2 mmhos/cm.

Measurement:

5- Rinse the electrode with the suspension solution.

6- Read the conductivity of the soil suspension.

Remarks:

1- mhos/cm = 1000 mmhos/cm.

mmhos/cm = 1000 micromhos/cm.

2- The electrode must be full of the reagent (till the mark before reading).

3- At switching the contact on, if the bridge will not balance, the conductivity of the extract may be below or over that of the scale limits. So either set on reading with micromhos instead of mmhos or dilute the extract.

4- The cell constant is calculated from the relation:

$$K = EC_{25} \times R_{25}$$

Where K = cell constant, EC_{25} = electrical conductivity at $25^{o}C$ and R_{25} = reading of cell resistance at $25^{o}C$.

Note: It is preferable to determine pH and EC in the same soil suspension 1 : 2.5.

1.6 - DETERMINATION OF CALCIUM CARBONATE

Amount of calcium carbonate in soil indicates one of the most important soil propertise. In this method it is determined volumetrically by measuring the CO_2 volume evolved from the reaction of hydrochloric acid with soil carbonate.

Reagents:

1- Commercial hydrochloric acid: 1 : 3.

2- $105^{o}C$. dried calcium carbonate.

3- Potassium bichromate 1 N.

Apparatus:

1- Calcimeter.

2- Thermometer.

Procedure:

Calibration:

1- Fill the graduated tubes with potassium bichromate and adjust the upper level in both tubes equally.

2- Put 0.10 gm oven-dried $CaCO_3$ in the vail and inserted in an upright position in the reaction flask containing 10 ml of HCL 1:3 after mixing 22.4 cm^3 CO_2 are evolved under the conditions of the standard temperature and pressure.

Measurement:

1- A weight of 1 g air dried soil sample is transferred into a plastic vial and inserted in an upright position in the reaction flask containing 10 ml of HCL 1 : 3.

2- Mix.

3- Record the difference of potassium bichromate volume, due to evolved CO_2 gas pressure   in the graduated upper tubes.

4- Record room temperature.

5- Correct the volume of CO_2 for the standard temperature and pressure. Then milligrames   of soil carbonates as $CaCO_3$ can be calculated.

Calculation:

$CaCO_3$ % = $\dfrac{volume\ of\ CO_2\ (cm^3) \times 273 \times 100 \times 100 \times 10}{1000 \times (273 + temperature) \times 224 \times 1}$

$CaCO_3$ % can be calculated directly using the attached table as follows:

% $CaCO_3$ = volume of CO_2 × Factor.

1.7 - SOIL ORGANIC MATTER:

WALKLEY-BLACK METHOD

Equipment:

1- 500- ml Erlenmeyer flasks.
2- 10 ml pipette.
3- 10 and 20 ml dispensers.
4- 50 ml burette.
5- Analytical balance.
6- Magnetic strirrer.
7- Incandescent lamp.

Reagents:

1- H_3PO_4 85%
2- H_2SO_4 concentrated (96%)

3- NaF, solid

4- Standard 1.00 $\underline{N}$ $K_2Cr_2O_7$: Dissolve 49.04 g of dried (105°C) $K_2Cr_2O_7$ in water and dilute to 1 litre.

5- 0.5 $\underline{N}$ Fe^{++} solution: Dissolve 196.1 g of Fe (NH_4)$_2(SO_4)_2$. 6 H_2O in 800 ml of water containing 20 ml of concentrated h_2so_4 and dilute to 1 litre. The Fe^{++} in this solution oxidizes slowly on exposure to air so it must be standardized against the dichromate daily.

6- Ferroin indicator: Dissolve 3.71 g of O-phenanthroline and 1.74 g of $FeSO_4$. $7H_2O$ in 250 ml of water.

Prcedure:

1- Weigh out 0.10 to 2.00 dried soil (< 60 mesh) and transfer to a 500 ml Erlenmeyer flask. The sample should contain 10 to 25 mg of organic C (17 to 43 mg organic matter). For a 1 g sample, this would be 1.2 to 4.3% organic matter. Use up to 2.0 g of sample for light colored soils and 0.1 g for organic soils.

2- Add 10 ml of 1 $\underline{N}K_2Cr_2O_7$ by means of a pipette.

3- Add 200 ml of concentrated H_2SO_4 by means of dispenser and swirl gently to mix. Avoid excessive swirling that would result in organic particles adhering to the sides of the flask out of the solution.

4- Allow to stand 30 minutes. The flasks should be placed on an asbestos sheet during this time to avoid rapid loss of heat.

5- Dilute the suspension with about 200 ml of water to provide a clearer suspension for viewing the endpoint.

6- Add 10 ml of 85% H_3PO_4, using a suitable dispenser, and 0.2 g of NaF, using the "calibrated spatula" technique. The H_3PO_4 and NaF are added to complex Fe^{3+}, which would interfere with the titration endpoint.

7- Add 10 drops of ferroin indicator. The indicator should be added just prior to titration to avoid deactivation of adsorption onto clay surfaces.

8- Titrate with 0.5 $\underline{N}$ Fe^{++} to a burgundy endpoint. The color of the solution at the beginning is yellow-orange to dark green, depending on the amount of the unreacted $Cr_2O_7^{=}$ remaining, which shifts to a turbid gray before the endpoint and then changes sharply to a wine red at the endpoint. Use of a magnetic stirrer with an incandescent light makes the endpiont easier to see in the turbid system.

(Fluorescent lighting gives a different endpoint color). If less than 5 ml of Fe^{++} solution was required to backtitrate the excess $Cr_2O_7^{=}$, there was insufficient $Cr_2O_7^{=}$ present, and the analysis should be repeated either by using a smaller sample size or doubling the amount of $K_2Cr_2O_7$ and H_2SO_4. Alternatively use a Pt electrode to determine the endpoint after step 5 above. This will eliminate uncertainty in deremining the endpoint by color change.

9- Run a reagent blank following the above procedure without soil. The reagent blank is used to standardize the Fe^{++} solution daily.

10- Calculate % C and % organic matter:

      a. % easily oxidizable organic C

$$C = \frac{(B-S) \times N \text{ of } Fe^{++}}{g \text{ of soil}} \times \frac{12}{4000} \times 100$$

      <u>Where</u>: B = ml of Fe^{++} solution used to titrate blank,

              S = ml of Fe^{++} solution used to titrate sample, and 12/ 4000 = milliequivalent weight of C in g.

To convert easily oxidizable organic C to total C, divide by 0.77 (or multiply by 1.30) or other experimentally determined correction factor.

      b. % organic matter = $\frac{\% C}{0.58}$ = %C × 1.72

1.8 - Total Nitrogen

Principle:

Organic and nitrate nitrogen is converted to ammonium sulphate and the ammonium is distilled into boric acid and titrated with HCL or H_2SO_4 using appropriate indicator.

Reagents:

1- Digestion mixutre:
      a. Potassuin sulphate K_2SO_4.
      b. Copper sulphate $CuSO_4$. $5H_2O$.
      c. Selenium.
      A,b,c are mixed with proportion of 10 : 1 : 0.5 respectively.
2- H_2SO_4 conc.
3- NaOH solution (40%).
4- H_3BO_3 solution (4%).

5- 0.01 N HCL

6- Tashiro's indicator (0.248 g methylene blue and 0.375 g methyl red dissolved in 300 ml ethyl alcohol absolute, Allen, 1953).

Apparatus:

1- Micro-kjeldahl distillation apparatus.
2- Erlenmeyer flasks 100 ml.
3- Kjeldahl digestion flasks (or conical flask) 500 ml.
4- Test tubes.
5- Automatic burettes.
6- Pipette 10 ml (one pulp).
7- Measuring flask 250 ml.

Procedure:

Digestion:

1- Weigh 5 g soil into digestion flask.
2- Add 5 g digestion mixture and 20 ml H_2SO_4 conc.
3- Put the flask on digestion board with electric heaters.
   Heat gradually; low at 10-30 minutes, then raise heating degree.
4- After the end of fuming, the digestion is continued for 1 hour after the solution had cleared with white colour of digestion mixture.
5- Transfer the sample to 250 ml volumetric flask, complete the volume with dist. Water.

Distillation:

6- Put 20 ml H_3BO_3 in Erlenmeyer flask and 4 drops of the indicator.
   Put the flask so that the lower tip of the glass receiver tube is below the boric acid surface.
7- Start running the cooling water in condenser
8- Start boiling the water in the boilers.
9- Put 25 ml of the sample in the funnel with dist. Water. Released ammonia is trapped in boric acid.

Titration:

10- Ammonia is titrated with HCL or H_2SO_4. At end point the green colour just disappears.

Calculation:

N % in soil = $\frac{(Sample\ titration - Blank) \times noramlity \times 14 \times dilution}{sample\ weight}$

$$= \frac{S - B \times \frac{14}{100} \times \frac{250}{50} \times \frac{1000}{5}}{10,000}$$

To catch NO_3 in soils the salicylic acid or phenolic sulfuric acid methods are necessary.

1.9 - Available Phosphorus

Many tests for determining the so-called " available" phosphorus in soils have beem in use for many years. The sodium bicarbonate method of Olsen et al. (1954) has given better correlation with field responses to phosphate fertilizers on both acid and alkaline soils than many of the other methods. For that reason, the details of this method are outlined here.

Extraction from soil:

Sodium Bicarbonate Method.

Principle:

This method extrants some of the exchangeable or surface absorbed phosphorus of soils, some of the calcium phosphates and other phosphates, and has given a good correlation with field responses. The soil is extracted with 0.5 M sodium bicarbonate and the phosphate extracted is determined colorimetrically.

Reagents:

Sodium bicarbonate, 0.5 N (pH 8.5):

1- Dissolve 42.0 g Na HCO_3 in about 900 ml of distilled water.
2- Adjust to pH 8.5 with Na OH (50%) and make to 1 L volume with distilled water.
3- Add mineral oil and avoid exposure to air.

Apparatus:

(1) Shaker **(2)**, Beakers **(3)**, Cylinder 100 ml., **(4)** funnels and filter paper.

Procedure:

1. 5 g air-dried soil (passed through 1-2 mm sieve) is suspended in 100 ml of Na HCO_3 extraction solution.
2. The suspension is shaken for a period of 30 minutes.
3. The solution is filtrated through a whatman 40 or other suitable filter paper.

Determination:

The concentration of phosphorus in the filtrate is determined by one of the following methods:

Micro-Vanadate-Molybdate method:

Principle:

This method is sensitive to 20-200/ug p/50 ml. The developed yellow colour is stable for 6 hours at least.

Reagents:

1. Solution (1): 25 g ammonium molybdate in 400 ml dist. H_2O.
   Solution (2): 1.25 g of ammonium metavandate in 300 ml of boiling dist. H_2O.
   Solution (2): is cooled and then 250 ml of concentrated HNO_3 is added and the solution is again cooled to room temperature.
   Finaly solution 1 is poured into solution 2 and the mixture is diluted 1 litre.
   The solutions are mixed at a ratio of 1:1:1.
2. Potassium dihydrogen phosphate (500 ppm P): Dissolve 2.197 grams dried KH_2PO_4 in 1 L distilled water. A dilute standard solution is prepared for making up a series of standards for the calibration curve, from 0.2 ppm to 2.5 ppm.

Apparatus:

Spectrophotometer

Procedure:

1- 1-35 ml of soil extract is transferred to a 50 ml volumetric flask.
2- 10 ml of the vanadate solution are added and make the volume to 50 ml with distilled water.
3- After 10 minutes read at 405 nm by using spectrophotometer.

Calculation:

P mg/100g = $\dfrac{Reading \times factor}{10} \times \dfrac{100}{dry\ weight\ at\ 105^oC}$

Potassium, Sodium and Magnesium:

1- **Extraction from soil:** by ammonium acetate method.

Principle:

Ammonium acetate is known to extract the soil exchangeable potassium among other cations. This is rapid procedure for extraction of potassium separately from other exchangeable metallic cations. Potassium adsorbed on the soil particles is exchanged with ammonium of the acetate solution, maximally at the latter's strength. Sodium and magnesium could be also extracted by the same reagent.

Apparatus:

Shaker, plastic bottle with caps, cylinder, beaker.

Reagents: Ammonium acetate 1 N:

To 700 – 800 ml distilled water, add 57 ml. conc. acetic acid + 68 ml of conc. ammonium hydroxide. Dilute to a volume of 1 liter and adjust to pH 7.0 by the addition of more ammonium hydroxide or acetic acid. The same solution can be also made by dissolving 77 g ammonium acetate in 1 liter dist. Water.

Procedures:

(1) Add 100 ml of ammonium acetate solution to 5 g air dried soil (passed through 1-2 mm sieve) in a plastic bottle. Place the bottles in the shaker.

(2) Shake for 1 hour.

(3) Filtrate through filter paper whatman 41 or S & S 512; M & N 280. Receive in a plastic container. Discard the first third of the filtrate.

2- Determination:

1.10 - Potassium and sodium:

Principles:

Potassium and sodium ions can be determined quantitatively when they are atomized from solution, led to burner and exited to spectral emission in a flame. Since the intensity of the light emitted by each element depends primarily on the concentration of its atoms in the flame at any given instant, a measurement of the light intensity produced by a given element makes possible the quantitative determination of that element.

Apparatus: Flame photometer.

---- Potassium chloride 1000 ppm:

Dissolve 1.9117 g dried KCl in distilled water and make to 1 L volume.

---- Sodium chloride 1000 ppm:

Dissolve 2.5422 g dried NaCl in distilled water and make to 1 L volume.

Standard curve solutions:

Prepare the following dilution 10, 20, 30, …….., 100 ppm from the standard 1000 ppm solution in solution of 1 N ammonium acetate pH 7.0.

Procedure:

(1) Pipet an aliquot of the solution to be analyzed into a 50 ml volumetric flask, complete with 1 N ammonium-acetate solution (pH 7).

(2) Determine the potassium concentration by use of the flame photometer and the appropriate calibration curve.

Terminating operations:

(1) Introduce distilled water for approximately 3 minutes to clean the intake and the atomizer/burner assembly.

(2) Close the valve of supplying gas.

(3) Push down button of gas selection and wait until the flame is extinguished.

(4) Switch off main switch.

(5) Place cover over the housing after allowing sufficient time for cooling off.

3- calculation:

(1) Concentration of K or Na in sample-extract can be calculated by slope calculation. For every concentration of the standard solution (10-100 ppm) the concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope.

(2) Content of K and Na in soil sample:
Content of K or Na as mg/100g soil (at 105 ^{o}C)

1.11 - Magnesium:

Magnesium can be determined by atomic adsorption.

Reagents:

Standard stock solution 1000 ppm. A dilute standard solution is prepared for making up a series of standards for the calibration curve, from 0.1 to 8 ppm.

1.12 - AVBAILABLE SOIL MICRONUTRIENTS

Content of available nutrients is relevant to soil supplying power, as the available form of the nutrient can be easily taken up by plants for indicating the nutritional status of soil, available micronutrients are extracted by diluted solutions of chelating compounds, such as EDTA, EDDHA or DTPA.

1. Extration by DTPA (method):

Principle:

The DTPA method has been adopted most frequently on alkaline, calcareous areas for most crops. It is successful for evaluating soil nutritional status and separation deficient from non-deficient soils. It also exhibits correlation with plant response to micronutrient fertilizers. Moreover, only this method can extract all the 4 elements (Fe, Mn, Zn, and Cu) in one extraction. Therefore, it is easier, simpler and more economical to follow in routine analysis.

Reagents:

DTPA solution (extracting solution):

- **(a)** 0.1 M triethanolamine (TEA) weigh 14.9 g.
- **(b)** 0.005 M diethylenetriamine penta acetic acid (DTPA); weigh 1.967 g.
- **(c)** 0.01 M calcium chloride $CaCl_2$, $2H_2O$, weighs 1.47 g.

Dissolve a + b + c in about 800 ml distilled water, adjust pH to 7.3 with diluted HCl 1:1 (it needs 10 ml). Complete to 1 liter with distilled H_2O.

Apparatus:

- **(1)** Shaker.
- **(2)** 80 ml cups with caps (plastic).
- **(3)** Funnels.
- **(4)** Filter paper whatman 42 or S & S 512 or M & N 280.
- **(5)** Automatic pipette 50 ml.

Procedures:

- **(1)** Weigh 20 g air-dry soil sample passed through 2 ml sieve. Transfer the sample to a cup.
- **(2)** Add 40 ml DTPA solution, fix the cup in the shaker.
- **(3)** Shake for 2 hours (100 stroke/min) at 25°C.
- **(4)** Carry out filtration into cups. Discard the first third of the filtrate.
    The rest is ready for determination of available micronutrients.

2. Determination of micronutrients by atomic absorption spectrophotometer (AAS):

Principle:

An atom is capable of absorbing the same wavelength of light normally emitted upon exitation. In addition, the transitional intensity of a given wavelength is affected by concentration and thickness of the absorbing medium. According to both principles, the electrically exited element-from a hollow cathode lamp causes a known spectral emission of the element. A part of the lamp beam emission is absorbed in the flame by the neutral atom of the same element in the flame atomized sample solution. Absorbance correlates with element concentration in the sample.

Reagents:

Standard stock solutions 1000 ppm of Fe, Mn, Zn, Cu (in ampule).

Apparatus:

Atomic absorption spectrophotometer.

Procedures:

Preparation of standard-reference solutions:

Fe: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 ppm.

Mn: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

Zn: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

Cu: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

Mg: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

Determination:

(1) Set the AAS in work according to operation instructions in the manual of AAS apparatus. Care must be taken that compressed air must be introduced before the fuel gas.
(2) After setting on flame, atomize the sample (which must be clear). The value is recorded on digits and printed on chart.
(3) Make a blank with extracting solution. Set apparatus to zero with the blank value for every element.

3. Calculation:

Using slope calculation: for every concentration of the standard solution (0.2 – 8 ppm) the concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope.

$$\text{Concentration in ppm} = \text{reading X dilution x factor X} \frac{100}{d.w.at\ 105\ ℃}$$

1.13 - Gypsum

Soils with variable contents of gypsum ($CaSO_4.2H_2O$) are common in many countries of the CWANA region, including Syria and Iraq. Gypsum is primarily a concern in irrigated areas and 1ess so in rainfed agriculture. Thus, its determination is of importance to some laboratories in the region.

The standard method for gypsum determination described here is that of Richards (1954) which involves precipitation with acetone. Modifications of that method and other procedures (Sayegh et al., 1978) are found in the FAO bulletin on gypsiferous soils (FAO, 1990).

Apparatus

Centrifuge, capable of 4000 rmp.
Conical centrifuge tubes (50 mL)
Conductivity cell and Wheatstone bridge.
Mechanica1 shaker.

Reagent

Acetone.

Procedure (Quantitative)

1. Weigh 10 to 20 g air-dry soil (medium to fine textured) into a 250-mL bottle, and add a measured volume of DI water sufficient to dissolve the gypsum present.
2. Stopper the bottle and shake by hand six times at 15-minute intervals or agitate for 15 minutes in a mechanical shaker.
3. Filter the extract through filter paper of medium porosity, and transfer a 20-

mL aliquot of filtered extract into a 50-mL conical centrifuge tube.

4. Add 20 mL acetone, mix well, and let stand until precipitate is flocculated. This usually requires 5 to 10 minutes.

5. Centrifuge at 4000 rpm for 3 minutes, decant supernatant liquid, invert tube, and drain on filter paper for 5 minutes.

6. Disperse precipitate and rinse tube wall with a stream of 10 mL blown from a pipette.

7. Again, centrifuge for 3 minutes, decant supernatant liquid, invert tube, and drain on filter paper for 5 minutes.

8. Add exactly 40 mL DI water to tube, stopper, and shake until the precipitate is completely dissolved. Measure electrical conductivity of solution, and correct conductivity reading to 25oC.

9. Determine gypsum concentration in the solution by reference to a graph showing the relationship between the concentration and EC constructed by means of the following data from the International Critical Tables (Richards, 1954, Fig. 2).

Gypsum Concentration	Electrical Conductivity (25oC)
----- meq L^{-1} ------	--------------- dS m^{-1} -------------
1.0	0.121
2.0	0.226
5.0	0.500
10.0	0.900
20.0	1.584
30.5	2.205

CALCULATIONS

For Gypsum in soil:

$CaSO_4.2H_2O$ in aliquot (meq) = $CaSO_4.2H_2O$ from conductivity reading (meq/L) $\times$
water used to dissolve precipitate (mL)/1000

Gypsum (meq/100 g) = 100 $\times$ $CaSO_4.2H_2O$ in aliquot (meq)/(soil water) ratio $\times$
(soil - water) extract used (mL)

Note

1. Sodium and potassium sulfates, when present in sufficiently high concentrations, are also precipitated by acetone. The maximum concentrations of sodium sulfate and of potassium that may be tolerated are 50 and 10 meq/L, respectively.

2. At a 1:5 (soil: water) ratio, water will dissolve approximately 15 meq gypsum per 100 g soil. If it is found that the gypsum content of the soil approaches 15 meq/100 g using a 1:5 (soil: water) extract, the determination should be repeated, using a diluted extract.

3. In some soils from the Euphrates Basin, gypsum may be well over 25%, in which case dilution's of 1:500 or 1:1000 (soil: water) ratio have to be used.

4. Qualitative test for gypsum should be made on all soils as a routine in order to save time later when analyzing for gypsum. Pipette 5 mL of the soil extract into a small centrifuge tube and add 5 mL acetone. Mix well, and allow to stand for 10 minutes if a flocculate white precipitate forms, the soil contains gypsum; if no precipitate forms, the soil is considered to have no gypsum.

1.14 - Soluble Calcium and Magnesium

Soluble Ca and Mg are obtained by extracting the soil by water and measurement of their concentrations in the extract by titration with EDTA (Richards, 1954). However, Ca and Mg in the extracts can also be measured by atomic absorption spectrophotometry.

Reagents

A.   Buffer Solution (NH$_4$Cl-NH$_4$OH)

Dissolve 67.5 g ammonium chloride in 570 mL concentrated ammonium hydroxide, and transfer the solution to a 1-L volumetric flask, let it cool, and bring to volume with DI water.

B.   Eriochrome Black Indicator

Dissolve 0.5 g eriochrome black with 4.5 g hydroxylamine hydrochloride in 100 mL ethyl alcohol (95%). Prepare a fresh batch every month.

C.   Ethylene Diaminetetraacetic Acid Solution (EDTA), ≈0.01 N

Dissolve 2 g ethylene diaminetetraacetic acid, and 0.05 g magnesium chloride (MgCl$_2$) in DI water, and bring to 1-L volume with DI water.

D.   Sodium Hydroxide Solution (NaOH), 2 N

Dissolve 80 g sodium hydroxide in about 800 mL DI water, transfer the solution to a 1-L volume, cool, and bring to volume with DI water.

E.   Ammonium Purpurate Indicator (C$_8$H$_8$N$_6$O$_6$)

Mix 0.5 g ammonium purpurate (Murexid) with 100 g potassium sulfate (K$_2$SO$_4$).

F.   Standard Stock Calcium Chloride Solution (CaCl$_2$.2H$_2$O), 0.01 N

Dissolve 0.5 g pure calcium carbonate (CaCO$_3$ dried for 3 hours at 100°C),

in 10 mL 3 N hydrochloric acid and bring to1-L volume with DI water. This can also be prepared by dissolving 0.735 g calcium chloride dehydrate ($CaCl_2.2H_2O$) in 1-L volume with DI water.

Procedure

A. Calcium

1. Pipette 10 - 20 mL soil saturation extract, having not more than 1.0 meq Ca, into a 250-mL Erlenmeyer flask.

2. Dilute to 20 - 30 mL with DI water, add 2 - 3 mL 2 N sodium hydroxide solution, and about 50 mg ammonium purpurate indicator.

3. Titrate with 0.01 N EDTA. The color change is from red to lavender or purple. Near the end point, EDTA should be added one drop every 10 seconds since the color change is not instantaneous.

4. Always run a blank containing all reagents but no soil, and treat it in exactly the same way as the samples; and subtract the blank titration reading from the readings for all samples.

B. Calcium plus Magnesium

1. Pipette 10 - 20 mL soil saturation extract into a 250-mL flask, dilute to 20 - 30 mL with DI water. Then add 3 - 5 mL buffer solution. And a few drops eriochrome black indicator.

2. Titrate with 0.01 N EDTA until the color changes from red to blue.

CALCULATIONS

$$Ca \text{ or } Ca + Mg \ (meq/L) = \frac{(V - B) \times N \times R \times 1000}{Wt}$$

$$Mg \ (meq/L) = Ca + Mg \ (meq/L) - Ca \ (meq/L$$

For Soluble Calcium or Magnesium in soil:

Where: V = Volume of EDTA titrated for the sample (mL)

            B = Blank titration volume (mL)

            R = Ratio between total volume of the extract and extract volume

                 used for titration.

            N = Normality of EDTA solution.

            Wt = Weight of air-dry soil (g)

Standardization of EDTA

- Pipette 10 mL 0.01 N calcium chloride solution, and treat it as in

  determining Ca and Ca+Mg procedure, respectively.

$$N_{EDTA} = \frac{10 \times N_{CaCl2}}{V_{EDTA}}$$

- Take the reading, and calculate EDTA normality:

Where: N_{EDTA} = Normality of EDTA solution.

            V_{EDTA} = Volume of EDTA solution used (mL)

            N_{CaCl2} = Normality of $CaCl_2$ solution

1.15 - Carbonate and Bicarbonate

Carbonate and bicarbonate are generally determined in soil saturation extract by titration with 0.01 N H_2SO_4 to pH 8.3 and 4.5, respectively (Richards, 1954).

Reagents

A. Methyl Orange Indicator [4-$NaOSO_2C_6H_4N:NC_6H_4$/-4-$N(CH_3)_2$],

  (F.W. 327.34), 0.1%

   Dissolve 0.1 g methyl orange indicator in 100 mL DI water.

B. Sulfuric Acid Solution (H_2SO_4), 0.01 N

- Dilute 28 mL concentrated sulfuric acid (98 %, sp.gr.1.84) in DI water, mix well, let it cool, and bring to 1-L volume with DI water. This solution contains 1 N H_2SO_4 solution.

- Then dilute 100 times (10 mL to 1-L volume) to obtain 0.01 N H_2SO_4 solution.

C. Phenolphthalein Indicator, 1%

   Dissolve 1 g phenolphthalein indicator in 100 mL ethanol.

Procedure

1. Pipette 10 - 15 mL soil saturation extract into a wide-mouthed porcelain crucible or a 150-mL Erlenmeyer flask.

2. Add 1 drop phenolphthalein indicator. If pink color develops, add 0.01 N sulfuric acid by a burette, drop by drop, until the color disappears.

3. Take the reading, y.

4. Continue the titration with 0.01 N sulfuric acid after adding 2 drops 0.1% methyl orange indicator until the color turns to orange.

5. Take the reading, t.

6. Always run two blanks containing all reagents but no soil, and treat them in exactly the same way as the samples. Subtract the blank titration reading from the readings for all samples.

CALCULATIONS

$$CO_3 \ (meq/L) = \frac{2y \times N \times R \times 1000}{Wt}$$

$$HCO_3 \ (meq/L) = \frac{(t - 2y) \times N \times R \times 1000}{Wt}$$

For Carbonate and Bicarbonate in soil:

Where: R   = Ratio between total volume of the extract and extract volume used for titration.

N   = Normality of H_2SO_4 solution.

Wt   = Weight of air-dry soil (g)

1.16 - Chloride

Soluble chloride is obtained in the saturation extract (as prepared for soluble Ca, Mg and anions), and its concentration in the extract is determined by silver nitrate titration (Richards, 1954).

Reagents

A. Potassium Chromate Solution (K_2CrO_4), 5% in water

• Dissolve 5 g potassium chromate in 50 mL DI water.

- Add dropwise 1 N silver nitrate ($AgNO_3$) until a slight permanent red precipitate is formed.

- Filter, and bring to 100-mL volume with DI water.

B. Silver Nitrate Solution ($AgNO_3$), 0.01 N

- Dry about 3 g silver nitrate in an oven at 105°C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle.

- Dissolve 1.696 g dried silver nitrate in DI water, and bring to 1-L volume with DI water.

C. Sodium Chloride Solution (NaCl), 0.01 N

Dissolve 0.585 g dried sodium chloride in DI water, and bring to 1-L volume with DI water.

Procedure

1. Pipette 5 - 10 mL soil saturation extract into a wide-mouth porcelain crucible or a 150-mL Erlenmeyer flask.

2. Add 4 drops potassium chromate solution.

3. Titrate against silver nitrate solution until a permanent reddish-brown color appears.

4. Always run two blanks containing all reagents but no soil, and treat them in exactly the same way as for the samples. Subtract the blank titration reading from the readings for all samples.

CALCULATION

$$Cl\ (meq/L) = \frac{(V - B) \times N \times R \times 1000}{Wt}$$

For Chloride in soil:

Where: V   = Volume of 0.01 N $AgNO_3$ titrated for the sample (mL).

          B   = Blank titration volume (mL)

          R   = Ratio between total volume of the extract and extract

                    volume used for titration.

          N   = Normality of $AgNO_3$ solution.

          Wt   = Weight of air-dry soil (g)

Standardization of $AgNO_3$

- Titrate 10 mL 0.01N of sodium chloride solution against 0.01 N silver

  nitrate solution after adding 4 drops potassium chromate solution until a

  permanent reddish-brown color appears.

$$N_{AgNO3} = \frac{10 \times N_{NaCl}}{V_{AgNO3}}$$

- Take the reading, and calculate $AgNO_3$ normality:

Where: N_{AgNO3} = Normality of $AgNO_3$ solution.

                V_{AgNO3} = Amount of $AgNO_3$ solution used (mL).

                N_{NaCl} = Normality of NaCl solution.

1-17   Cation Exchange Capacity

Apparatus

Flame photometer.
Mechanical shaker, reciprocating.
Centrifuge, capable of 3000 rmp.
Conical centrifuge tubes (50 mL)

Reagents

A.   Sodium Acetate Solution (NaOAc), I N

•   Dissolve 136 g sodium acetate trihydrate ($CH_3COONa.3H_2O$) in about 950 mL DI water, mix well, and 1et the mixture cool.

•   Adjust pH to 8.2 by adding more acetic acid or sodium hydroxide, and bring to 1-L volume with DI water.

B.   Ethanol (C_2H_5OH), 95%

C.   Ammonium Acetate Solution (NH_4OAc), IN

•   Add 57 mL concentrated acetic acid (CH_3COOH) to 800 mL DI water, then add 68 mL concentrated ammonium hydroxide (NH_4OH), mix well, and let the mixture cool.

•   Adjust to pH 7.0 by adding more acetic acid or ammonium hydroxide, and bring to 1-L vo1ume with DI water.

D.   Standard Stock Solution

•   Dry about 5 g sodium chloride (NaCl) in an oven at 105oC for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle.

•   Dissolve 2.5418 g dried sodium chloride in DI water, and bring to 1-L volume with DI water. This solution contains 1000 ppm Na (Stock Solution).

•   Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 2, 4, 6, 8, 10, 15, and 20 mL Stock Solutions to 100 mL final volume by adding 1 N ammonium acetate solution, and 25 mL LiCl (Diluted Stock Solution). These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm Na, with each containing the same concentration of LiCl (25 ppm).

Procedure

1. Weigh 4 g (for medium to fine textured) or 6 g (for coarse textured) air-dry soil into a 40-mL centrifuge tube, and add 33 mL 1 N sodium acetate trihydrate solution, stopper tube, and shake for 5 minutes.

2. Remove stopper from tube and centrifuge at 3000 rpm until supernatant liquid is clear. Decant the supernatant as completely as possible and discard.

3. Repeat with 33-mL portions 1 N sodium acetate trihydrate solution, a total of four times, discarding the supernatant liquid each time. Then add 33-mL 95% ethanol, stopper tube, and shake for 5 minutes, unstopper tube, and centrifuge until the supernatant is clear and decant.

4. Wash the sample with 33 mL portions 95% ethanol, a total of three times, discarding the supernatant liquid each time. The electrical conductivity (EC) of the supernatant liquid from the third washing should be less than 400 μS/cm.

5. Replace the adsorbed sodium (Na^+) from the sample by extraction with three 33-mL portions 1 N ammonium acetate solution. Each time shake for 5 minutes, and centrifuge until supernatant liquid is clear.

6. Decant the three supernatant liquids as completely as possible into a 100-mL volumetric flask, bring to volume with 1 N ammonium acetate solution, and mix well.

7. Run a series of suitable Na standards, and draw a calibration curve.

8. Measure the samples (soil extract) and take the emission readings by a Flame Photometer.

9. Calculate sodium (Na) concentration according to the calibration curve.

CALCULATION

For Cation Exchange Capacity in soil:

$$\text{CEC (meq/100 g)} = \text{meq/L Na (from calibration curve)} \times \frac{A}{Wt} \times \frac{100}{1000}$$

Where: A = Total volume of the extract (mL)
            Wt = Weight of the air-dry soi1 (g)

2- PLANT ANALYSIS

Sample Preparation for Analysis:

Plants were washed at first with running tap water to remove dust, then in 0.001 M HCL followed by 2 times in bidistilled water. Samples are air-dried for few hours, then dried in a ventilated oven at 70°C. After dryness samples are ground in a stainless-steel mill and passed through 0.5 mm sieve, then kept in polyethylene cups till analysis.

Macro and micronutrients determination:

The digestion of the plant samples is carried out by one of the following methods:

Perchloric acid digestion (wet digestion of plant material):
Principle:

Total content of P ,K ,Fe ,Zn ,Cu and Mn are determined in plant samples as sulphate using the wet digestion method with acid mixture (nitric: perchloric : sulphuric acid) at the ratio of (8: 1 : 1).

Reagent:

Nitric acid AR. Conc.
Perchloric acid AR. 60%.
Sulphuric acid AR. Conc.

Apparatus:

250 ml kjeldahl flask.
Whatman no. 42 filter paper.
Electric heater.
Funnels
Measuring flask 50 ml.

Procedure:

1- 1.25 g of ground plant material (oven dried 70oC) are weighed into a 250 ml digestion flask which has been previously washed with acid and distilled water.

2- 25 ml mixture of HNO_3, $HClO_4$ and H_2SO_4 at the ratio of 8:1:1 are added.

3- The sample are digested on electric heater until dense white fumes appear and finally the solution is clear and is about 5 ml.

4- Let the sample to cool and dilute it with distilled water and quantitatively transfer it into a 50 ml volumetric flask. The volume make up to mark with distilled water.

5- Filtration is carried out using filter paper Whatman No. 42.

6- The solution are store for P (spectrophotometer or colorimeter) and K (Flame photometer) Fe , Mn , Cu & Zn (atomic absorption spectrometry).

2.1 - Determination of Micronutrients by Atomic Absorption:

Apparatus:

Atomic absorption spectrophotometer.

Procedure:

1- preparation of standard-reference solutions:

Fe : 0.25   0.5   1   2   4   8   16 ppm

Mn : 0.25   0.5   1   2   4   8   16 ppm

Zn : 0.25   0.5   1   2   4   8   16 ppm

Cu : 0.25   0.5   1   2   4   8   16 ppm

Mg : 0.25   0.5   1   2   4   8   16 ppm

2. Determination:

  1. Set the AAS in work according to operation instruction in the manual of apparatus. Care must be taken that compressed air must be introduced before the fuel gas.

  2. After setting on flame, atomize the sample. The value is recorded on digits and printed   on chart.

 3. Make a blank with extracting solution. Set apparatus to zero with the blank value for every element.

3. Calculation:

Using slope calculation for every concentration of the standard solution (0.25 – 16 ppm). The concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope. Concentration in ppm:

$$= \text{reading} \times \text{dilution} \times \text{factor.}$$

2.2 - TOTAL NITROGEN DETERMINATION:

Principle:

Organic nitrogen is converted to ammonium sulphate by H_2SO_4. Ammonia is liberated from ammonium sulphate by NaOH or KOH. Ammonia is distilled into boric acid and titrated with N/100 HCI using Tashiro's indicator.

Reagents:

1. **Digestion mixture:**
   (a) K_2SO_4
   (b) $CuSO_4.5H_2O$
   (c) Selenium

   (a, b & c are mixed with the ratio of 10:1:0.5 respectively)

2. H_2SO_4 conc.
3. NaOH solution (40%)
4. H_3BO_3 solution (4%)
5. 0.01 N HCI.
6. Tashiro's indicator (0.248 gm methylene blue + 0.375 gm methyl red dissolved in 300 ml ethyl alcohol absolute) (Allen, 1953).

Apparatus:

1. Digestion flask (100 ml).
2. Micro-K jeldahl distillation apparatus.
3. Test tube.
4. Conical flask 100 ml.

5. Dispenser 10 ml.
6. Automatic burette 10 ml.

Procedure:

1. About 0.020 g to 0.030 g of finely dired plant powder (dried at 70°C) is weighed.
2. Sample is transferred to a microkjeidahl flask with 0.5 g of catalyst (potassium sulphate : copper sulphate and selenium at the ratio of 10 : 1 : 0.5 respectively).
3. 2 ml of nitrogen free concentrated H_2SO_4 is added and the sample is completely digested until a clear liquid and free from black residue is obtained.
4. The flask is cooled and its contents are diluted with ammonia free distilled H_2O and quantitatively transferred to a Markham distillation apparatus, then 15 ml of 40% NaOH solution is added through the stoppered funnel at the receiving tip of the condenser.
5. A strong current of steam is passed and distillation is carried out for 5 minutes.
6. The liberated ammonia is received in 10 ml of 4% boric acid solution (approximately 50 ml of distillate).
7. Two drops of Tashiro's indicator (0.248 methylene blue and 0.375 g methyl red dissolved in 300 ml ethyl alcohol absolute), is added to the solution in the receiving flask and then titration of the distillate is performed by using N/100 HCl.

Calculation:

Total nitrogen is calculated as mg per   g of dry matter in the sample by using the following equation:

$$\text{Titration-blank} \times 0.14 \times \frac{1}{weight\,(g)}$$

The figure 0.14 is a factor when N/100 HCl is used for titration as one milliliter of N/100 HCl = 0.14 mg of nitrogen.

2.3 - Determination Of Total Phosphorous:

Phosphorous is determined in the digested solution by one of the following method:

- Vanadate Molybdate Method:

Vanadate molybdate and orthophosphates react to give a yellow complex in acid solutions. The acid concentration must be above 0.2 N and not over 1.6 N, the final concentration of 0.5 N is

recommended. Five millilitres of 5 N nitric acid per 50 ml of final volume are sufficient to give optimum acidity . The colour develops rapidly but is usually read after 10 minutes to assure full strength.

Determination :

The concentration of phosphorus in the digested material is determined by microvanadate-molybdate-yellow method. This method is sensitive to 1-20 ppm of P. The developed yellow colour is stable for 6 hours at least .

Reagents :

(1) Solution 1:

25 g ammonium molybdate in 400 ml H_2O.

Solution 2:

1.25 gm ammonium metavanadate in 300 ml of boiling H_2O, then cooled and 250 ml concentrated HNO_3 is added, solution is again cooled to room temperature.

Finally, solution 1 is poured into solution 2 and diluted to 1 liter.

(2) Potassium dihydrogen phosphate (500 ppm P): dissolve 2.197 g dried KH_2PO_4 in 1 L distilled water .

A dilute standard solution is prepared for making up a series of standards (0.05 – 8 ppm) for the calibration curve .

Procedures :

(1) 5 ml of the digested solution are transferred to a 50 ml volumet flask.

(2) 10 ml of the vanadate solution are added and the volume made up to 50 ml with distilled H_2O.

(3) After 10 minutes read at wave length 436 nm against blank.

(4) Blank: colour reagent filled to 50 ml with distilled water.

Apparatus:

Spectrophotometer.

Calculations:

P % = $\dfrac{Reading \times dilution \times factor}{10,000}$

2.4 - Determination of total potassium and sodium:

Principles:

Potassium and sodium ions can be determined quantitatively when they are atomized from solution, to burner and exited to spectral emission in a flame. Since the intensity of the light emitted by each element depends primarily on the concentration of its atoms in the flame at any given instant, a measurement of the light intensity produced by a given element takes possible the quantitative determination of that element.

Apparatus:

Flame photometer.

Reagents:

Potassium chloride 1000 ppm: 1.9117 g dried KCl are dissolve in distilled water and make up to 1 L with distilled water.

Sodium chloride 1000 ppm: 2,5422 g dried NaCl are dissolve in distilled water and make up to 1 L with distilled water.

Standard curve solutions:

Prepare the following dilution 5, 10, 40, …. 80 ppm.

Procedure:

(1) 5 ml of the digested solution are transfer to 50 ml volumetric flask, complete to the mark with distilled water.

(2) Potassium and sodium concentration are determine by use of the flame photometer and the appropriate calibration curve.

Calculation:

K or Na % = $\dfrac{Reading \times dilution \times factor}{10,000}$

2.5 - DETERMINATION OF TOTAL MAGNESIUM

Apparatus:

Atomic absorption spectrophotometer.

Standard Curve Solution:

Prepare the following dilution for the calibration curve:

0.75, 0.5, 1, 2, 4, 8 and 16 ppm.

Procedure:

1. 0.5 ml of the digested solution are transferred to a 50 ml volumetric flask, complete the volume to the mark with distilled H_2O.
2. After setting on, flame, atomize the sample. The value is recorded on digits and printed on chart.

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Calculation:

Using slope calculation for every concentration of the standard solution (0.25 – 16 ppm). The concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope.

Concentration in percent = $\dfrac{Reading \times dilution \times\ factor}{10,000}$

3- Water analysis

The same methods of analysis are recommended as for analysis of water extracts from soils it's used for water analysis.

References

1- United States Salinity Laboratory Staff, (USDA) .Agriculture Handbook No.60. (1969): (Diagnosis and Improvement of Saline and Alkali Soils).

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