6. SOIL NUTRIENTS, SODIUM AND ANION ANALYSIS

6.1 Nitrogen

In view of high nitrogen (N) requirements of crops and the low levels of available N in virtually all type of soils, it is the most important nutrient element in agriculture. Monitoring N fertilizer dynamics in soils is also important from the environmental perspective.

Nitrogen in soils occurs in many forms, both organic and inorganic. The former fraction, composed mostly of plant and microbial remains, is variable in composition. It can be substantial in actual and relative amounts in soils of temperate regions. With increasing aridity, however organic and total soil N tend to decrease.

The inorganic phase of soil N is composed of ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) forms. Environmental (temperature and moisture) and management (fertilization, cropping, etc.) factors influence its dynamic relationship with the organic fractions, and also within the inorganic forms (see N cycle in Fig. 5).

The NH_4-N and NO_3-N forms are routinely measured in soil laboratories, as they reflect the extent of mineralization, and are the forms of N taken up by plants. In the CWANA region, nitrate-N content in soils has proven to be a good index for predicting N fertilizer need of crops. The organic-N fraction is a measure of the soil reserve of N or its capacity to release N for crop needs through mineralization. Thus, methods of N analysis vary depending on the N fractions or forms of interest.

Total soil N (mainly organic) is generally measured after wet digestion using the well-known Kjeldahl procedure. Total inorganic N (NH_4^+, NO_3^+, NO_2^-) is usually determined by distillation of 2 M KCl soil extract. And after distillation, NO_3-N can be determined by a procedure involving chromotropic acid.

6.1.1 Kjeldahl Nitrogen

This procedure involves digestion and distillation. The soil is digested in concentrated H_2SO_4 with a catalyst mixture to raise the boiling temperature and to promote the conversion from organic-N to ammonium-N. Ammonium-N from the digest is obtained by steam distillation, using excess NaOH to raise the pH. The distillate is collected in saturated H_3BO_3; and then titrated with dilute H_2SO_4 to pH 5.0 (Bremner and Mulvaney, 1982).

The method determines ammonium-N, most of the organic-N forms, and a variable fraction of nitrate-N in soil. For most soils, the Kjeldahl procedure is a good estimate of total soil N content.

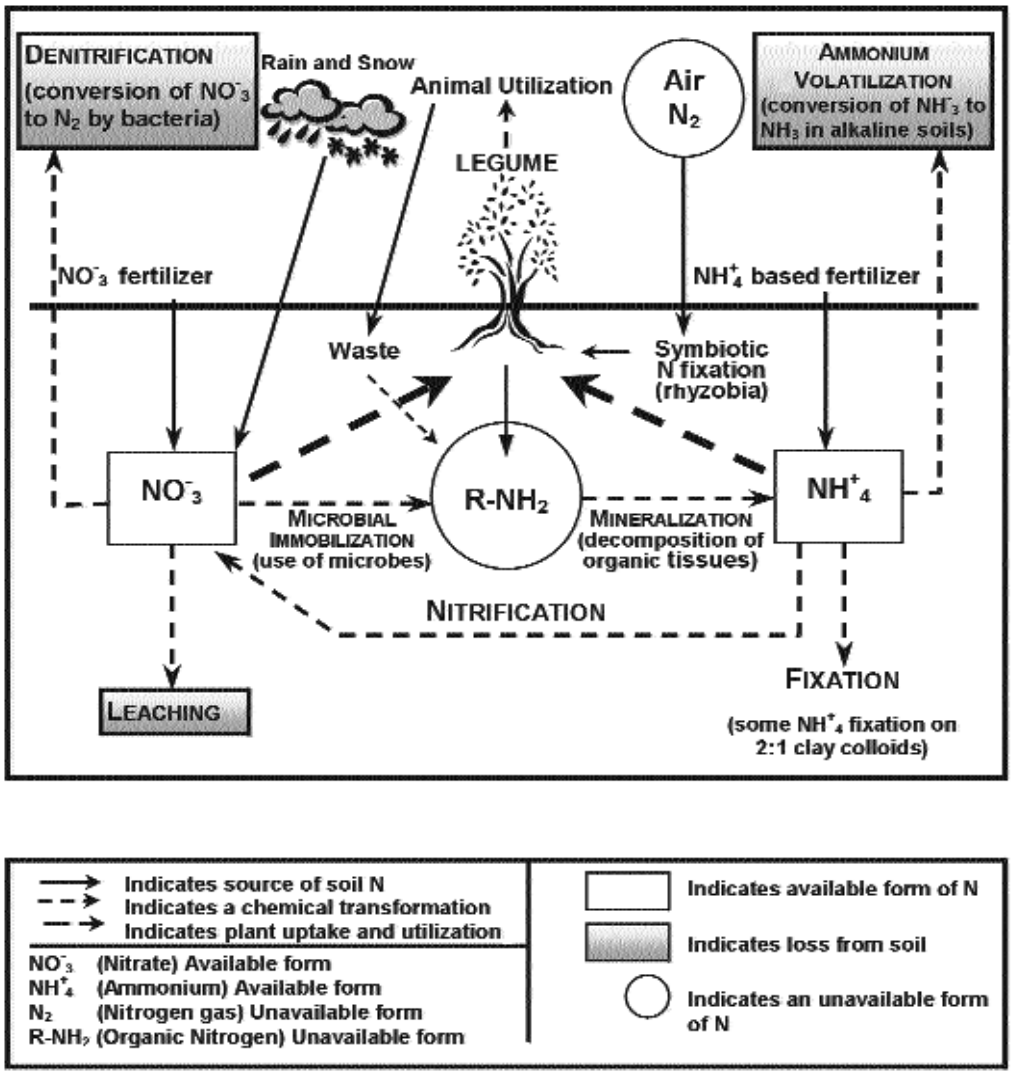

Fig. 5. The Nitrogen Cycle (Hach Company, 1992).

If desired, nitrate-N can be included through the reduced iron or salicylic acid modifications of the Kjeldahl procedure (see following section).

Apparatus

Block-digester.
Distillation unit.
Automatic titrator connected to a pH-meter.
Vortex tube stirrer.

Reagents

A. Catalyst Mixture (K_2SO_4 - $CuSO_4.5H_2O$ - Se), 100:10:1 w/ w ratio

Grind reagent-grade chemicals separately and mix. If caked, grind the mixture with a porcelain pestle and mortar to pass a 60-mesh screen (0.250 mm), taking care not to breath Se dust or allow Se to come in contact with skin.

B. Sulfuric Acid (H_2SO_4), concentrated (98 %, sp. gr. 1.84)

C. Sodium Hydroxide Solution (NaOH), 10 N

Dissolve 400 g sodium hydroxide in DI water, transfer to a 1-L volumetric heavy walled Pyrex flask, let it cool, and bring to volume with DI water.

D. Boric Acid Solution (H_3BO_3), saturated

- Add 500 g boric acid to a 5-L volumetric flask.
- Add 3 L DI water, and swirl vigorously.
- Leave overnight.
- There should always be solid H_3BO_3 on the bottom of the flask.

E. Tris Solution [hydroxymethyl aminomethane] ($C_4H_{11}NO_3$), 0.01 N

- Dry reagent-grade Tris in an oven at 80°C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 1.2114 g Tris in DI water, transfer to a 1-L volumetric flask, and bring to volume with DI water.

F. Sulfuric Acid Solution (H_2SO_4), 0.01 N

- Take about 600 - 800 mL DI water in a 1-L volumetric flask, add 28 mL concentrated sulfuric acid, mix well, let it cool, and bring to 1-L volume with DI water. This is 1 N H_2SO_4 solution.
- Then dilute 100 times (10 mL to 1-L volumetric flask) to obtain a 0.01 N H_2SO_4 solution.

G. Standard Stock Solution

- Dry reagent-grade ammonium sulfate $(NH_4)_2SO_4$ in an oven at 100°C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 5.6605 g dried ammonium sulfate in DI water, and bring to 1-L volume with DI water. This solution contains 1.2 g NH_4-N per Liter (Stock Solution).

Procedure

A. Digestion

1. Weigh 1 g air-dry soil (0.15 mm) into a 100-mL calibrated digestion tube.

2. Add about 5.0 - 5.5 g catalyst mixture, a few pumice boiling granules, 15 mL concentrated sulfuric acid (in the fume hood), and swirl carefully. Place a glass funnel in the neck of the tube, then place tubes in the rack, and leave overnight.

3. Place the tubes rack in the block-digester, and slowly increase temperature setting to about 370°C. The H_2SO_4 should condense about half-way up the tube neck; and when solution clears, continue heating for about 3 hours.

4. Lift the tubes rack out of the block-digester, carefully place on a rack holder, and let tubes cool to room temperature.

5. Slowly add about 15 mL DI water to the tubes, cool, and bring to volume with DI water. If tube contents are solidified and do not dissolve, heat the tubes again until the precipitate (gypsum) dissolves. Then cool with tap water.

6. Each batch of samples for digestion should contain at least one reagent blank (no soil), and one chemical standard (no soil, 1 mL of the Stock Solution).

B. Distillation

7. Before starting a batch for distillation, calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the 0.01 N H_2SO_4 in the Auto-Titrator by titrating three separate 10-mL aliquots of the primary standard, 0.01 N Tris solution, to pH 5.0. The titrations should agree within 0.03 mL; if not, titrate further aliquots until agreement is found.

   H_2SO_4 normality is:

$$N_{H_2SO_4} = \frac{10 \times N_{Tris}}{V_{H_2SO_4}} \quad \text{.....(23)}$$

8. Carry out distillations as follows (see diagram of the distillation unit in Fig. 6):
   - Dispense 1 mL saturated boric acid solution and 1 mL DI water into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
   - Pipette 10 mL aliquot into a 100-mL distillation flask, and add 10 mL 10 N sodium hydroxide solution.
   - Immediately attach the flask to the distillation unit with a clamp, start distillation, and continue for 3 minutes. Lower the dish to allow distillate to drain freely into the dish.
   - After 4 minutes when about 35 mL distillate is collected, turn off the steam supply, and wash tip of the condenser into the evaporating dish with a small amount of DI water.
   - Titrate the distillate to pH 5.0 with standardized 0.01 N H_2SO_4 using the Auto-Titrator.
   - After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish.
   - Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100-mL

empty distillation flask to distillation unit. Place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds.

- Each distillation should contain at least two standards and two blanks (reagent blanks).

CALCULATIONS

Percentage recovery of Ammonium-N standard:

$$\% \text{ Recovery} = \frac{(V - B) \times N \times 14.01 \times 100}{C \times D} \quad \ldots\ldots\ldots\ (24)$$

Percentage Nitrogen in soil:

$$\% N = \frac{(V - B) \times N \times R \times 14.01 \times 100}{Wt \times 1000} \quad \ldots\ldots\ldots\ldots\ (25)$$

Where:
V = Volume of 0.01 N H_2SO_4 titrated for the sample (mL)
B = Digested blank titration volume (mL)
N = Normality of H_2SO_4 solution.
14.01 = Atomic weight of N.
R = Ratio between total volume of the digest and the digest volume used for distillation.
Wt = Weight of air-dry soil (g)
C = Volume of NH_4-N standard solution (mL)
D = Concentration of NH_4-N standard solution (µg/mL)

Note

1. The block-digester may be insulated with an asbestos shield to obtain a more uniform temperature distribution.
2 Add 3 mL concentrated H_2SO_4 to DI water in the round bottom flask in

the heating mantle to trap any NH_3 present. Also, add Teflon Boiling Chips to ensure smooth boiling.

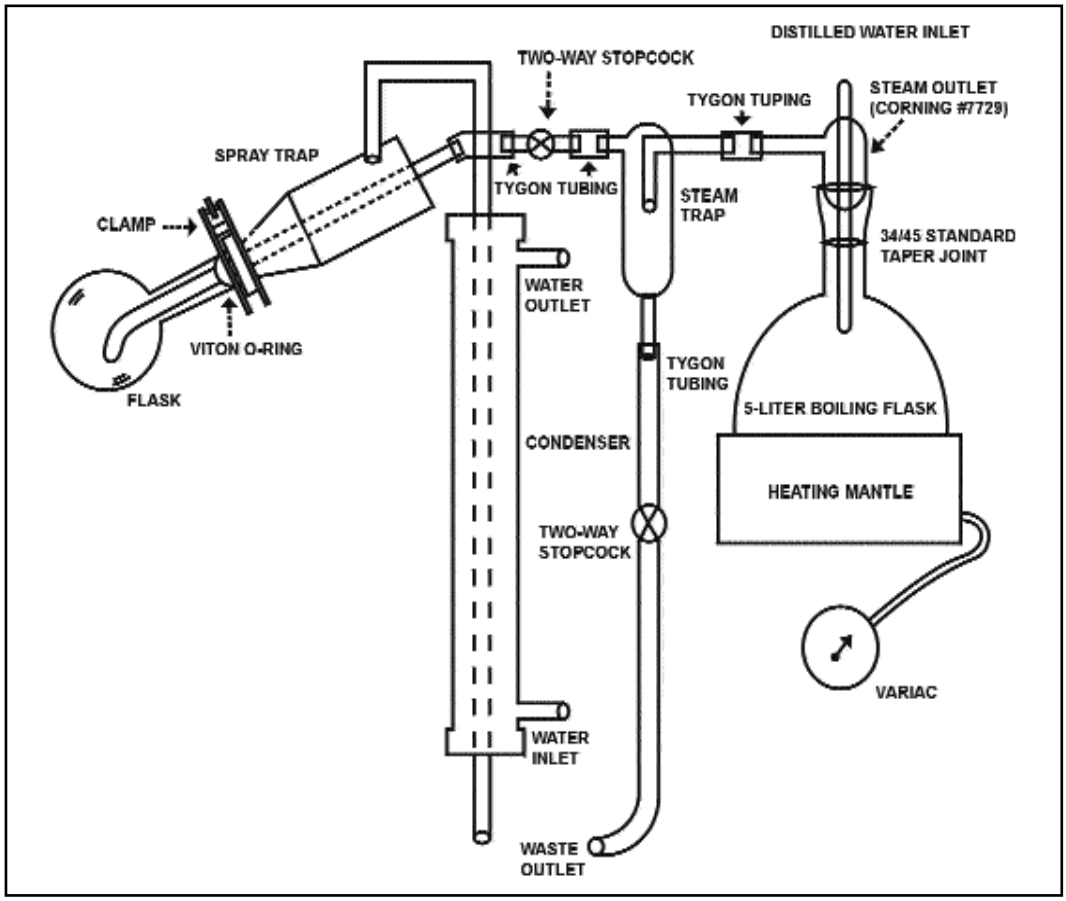

Fig. 6. Diagram of a Distillation Unit.

6.1.2 Total Nitrogen

The difference between Kjeldahl-N and total-N in soil is normally very small, due mainly to the presence of nitrate-N in the total-N determination. In the following procedure, NO_3-N fraction (present in the soil) is reduced and subsequently included in the distillation (Bremner and Mulvaney, 1982; Buresh et al., 1982).

Reagents

A. Sulfuric Acid (H_2SO_4), concentrated (98 %, sp. gr. 1.84)

B. Potassium Permanganate Solution ($KMnO_4$)

Dissolve 50 g potassium permanganate in DI water, and bring to 1-L volume. Store the solution in an amber bottle.

C. Sulfuric Acid Solution (H_2SO_4), 50% v/v ratio

Slowly add 1-L concentrated sulfuric acid with continuous stirring, to 1-L DI water already placed in a 4-L flask.

D. Reduced Iron

Grind in a ball mill and sieve to remove any material that does not pass a 0.15-mm sieve (<150 mesh).

E. N-Octyl Alcohol Solution

F. Catalyst Mixture

Prepare as in Kjeldahl-N.

G. Ethylene Diaminetetraacetic Acid, Disodium Salt (EDTA), M.W. = 372.2

Store in a desiccator.

H. Sodium Hydroxide Solution (NaOH), 10 N

Prepare as in Kjeldahl-N.

I. Boric Acid Solution (H_3BO_3), saturated

Prepare as in Kjeldahl-N.

J. Tris

Prepare as in Kjeldahl-N.

K. Sulfuric Acid Solution (H_2SO_4), 0.01 N

Prepare as in Kjeldahl-N.

Procedure

A. Digestion

1. Mix and spread the finely ground soil sample (0.15-mm) in a thin layer on a sheet of paper, until it looks uniform.
2. Take a representative soil sample, which contains about 3 to 8 mg N, by withdrawing 10 small portions from the soil sample, e.g., 10 g.
3. Weigh the sample to 0.01 g and place into a 250-mL calibrated digestion tube.
4. At the same time, take a soil sample for moisture determination (105°C).
5. Add 10 mL DI water to each tube and swirl thoroughly to wet the soil. Allow wet soil to stand for 30 minutes.
6. Prepare a blank digest, weigh 0.1 g EDTA standard digest (accurately weighed to 0.1 mg) with each batch.
7. Add 10 mL potassium permanganate solution, swirl well, allow to stand for 30 seconds, then hold the digestion tube at 45° angle and slowly add 20 mL 50% sulfuric acid in a manner which washes down material adhering to the tube neck.
8. Allow to stand for 15 minutes then swirl.

   Important: Do not swirl digestion tube immediately after adding acid because this may result in excessive frothing.

9. Add 2 drops N-octyl alcohol solution.
10. Add a few pumice boiling granules to the blank, EDTA, and sample digest tubes.
11. Add 2.5 g reduced iron through a long-stem funnel and immediately place a 5-cm (internal diameter) glass funnel (with stem removed) in the tube neck, and swirl.

12. Excessive frothing at this stage may be halted by pouring 5 mL DI water through the 5-cm glass funnel; do not swirl.
13. Allow the tubes to stand overnight.
14. Pre-digest the samples by placing them on the cold block and heating at 100°C for 1 hour. The block digester comes to 100°C within 15 minutes; therefore, total time on the block digester will be approximately 1 hour and 15 minutes.
15. Samples should be swirled at 45 minutes.
16. Remove tubes from the block-digester, and cool. Rapid cooling may be affected in tap water.
17. Leave overnight.
18. Add about 5 g catalyst mixture through a long stem funnel. Then add 25 mL concentrated sulfuric acid to each tube, and swirl (more acid may be required if larger amount of soil is used).
19. Place the tubes back on the block-digester pre-heated to 100°C, increase the block temperature setting to 240°C, and remove the funnels.
20. Arrange funnels systematically/ in an order so that they may afterwards be placed into the same digestion tube. It takes 40 minutes to reach 240°C.
21. Continue boiling off the water for 1 hour after reaching 240°C.
22. After the water has been removed, replace the funnels and raise the temperature to 380°C.
23. Set the timer on the block-digester, and digest for 4 hours at this temperature.
24. Remove the tubes from the block-digester, add about 50 mL DI water, and mix using a vortex mixture. If any solid precipitate remains in the tubes, break it up with a glass rod.
25. After cooling, add DI water to the 250-mL mark.

B. Distillation

1. Prior to distillation, shake the digestion tube to mix thoroughly its contents, and then immediately pipette 50 mL into a 250-mL distillation flask.
2. Acid digests are distilled with excess NaOH. The quantity of 10 N NaOH required for soil digestion is 25 mL and 50 mL for distillation of 50 mL and 100-mL aliquot, respectively.

3. Carry out distillations as follows:

- Dispense 1 mL saturated boric acid solution and 1 mL DI water into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with thetiptouching the solution surface.
- Carefully dispense appropriate volume of 10 N NaOH down the side of the flask, while holding the distillation flask containing the digest at a 50° angle.
- Immediately attach the flask to the distillation unit with a clamp, start distillation, and continue for 3 minutes. Lower the dish to allow distillate to drain freely into the dish.
- After 4 minutes, when about 35 mL distillate is collected, turn off the steam supply, and wash tip of the condenser into the evaporating dish with a small amount of DI water.

  Important: The first appearance of distillate will be delayed when large aliquots are used. The distillation time should always be 4 minutes from the first appearance of distillate flow.
- Titrate the distillate to pH 5.0 with standardized 0.01 N H_2SO_4 using the Auto-Titrator.
- After finishing titration, the Teflon-coated magnetic stirring bar, the burette tip and the combined electrode are washed into the dish.
- Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100-mL empty distillation flask to distillation unit, and place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds.
- Each distillation should contain at least two standards and two blanks (reagent blanks).

CALCULATIONS

Percentage recovery of EDTA standard:

$$\% \text{ Recovery} = \frac{(V - B) \times N \times R \times 186.1 \times 100}{Wt_1 \times 1000} \quad \ldots\ldots\ldots\ldots \quad (26)$$

Percentage Nitrogen in soil:

$$\% \text{ N} = \frac{(V - B) \times N \times R \times 14.01 \times 100}{Wt_2 \times 1000} \quad \ldots\ldots\ldots\ldots\ldots \quad (27)$$

Where:

V	=	Volume of 0.01 N H_2SO_4 titrated for the sample (mL)
B	=	Digested blank titration volume (mL)
N	=	Normality of H_2SO_4 solution.
14.01	=	Atomic weight of N.
R	=	Ratio between total volume of the digest and the digest volume used for distillation.
Wt_1	=	Weight of EDTA (g)
Wt_2	=	Weight of air-dry soil (g)
186.1	=	Equivalent weight of the EDTA.

6.1.3 Mineral Nitrogen

Nitrogen is absorbed by plant roots in two forms, ammonium-N and nitrate-N. Ammonium ions are produced in soils through breakdown of organic matter or manures. Nitrate ions are the final form of N breakdown/reactions, but it can also be supplied to soil by fertilizers.

Available-N can be lost from the soil in several ways; i.e., volatilization, anaerobic de-nitrification, and leaching. Normally, NH_4^+ does not leach from soil because the positive charge is attracted and "held" by the negative (-) charge present on the surface of clay and humus particles. However, when NH_4^+ is transformed to NO_3^-, the positive (+) charge is lost and the soil no longer attracts the available N. Water percolating through a soil profile may leach and deplete the mobile NO_3^- from the upper layers to the lower layers, and even into the groundwater if leaching is excessive. Excessive nitrate leaching is most likely in over-fertilized fields.

Nitrate in groundwater is a major environmental and public health concern. High nitrate levels in drinking water (>10 ppm) are linked with health problems (i.e., methemoglobinemia) resulting in "blue" babies.

Mineral-N is determined using 2 M KCl as the extracting solution in a 1:5 (soil: water) ratio. Ammonium (NH_4^+) and nitrate (NO_3^-) plus nitrite (NO_2^-) are determined by steam distillation of ammonia (NH_3), using heavy MgO for NH_4^+ and Devarda's Alloy for NO_3^- (Bremner and Keeney, 1965). The distillate is collected in saturated H_3BO_3 and titrated to pH 5.0 with dilute H_2SO_4. This method determines dissolved and adsorbed forms of NH_4^+, NO_3^- and NO_2^- in soils. The sum determined by this method is referred to as Mineral-N (Keeney and Nelson, 1982; Buresh, et al., 1982).

Reagents

A. Potassium Chloride Solution (KCl), 2 M

Dissolve 150 g reagent-grade potassium chloride in DI water, and bring to 1-L volume with DI water.

B. Magnesium Oxide (MgO), powder

Heat heavy magnesium oxide in a muffle furnace at 600 - 700°C for 2 hours, and cool in a desiccator containing KOH pellets, and store in a tightly stoppered bottle.

C. Devarda's Alloy (50 Cu: 45 Al: 5 Zn)

Ball-mill reagent-grade Devarda's Alloy until the product will pass a 100-mesh sieve (0.150-mm) and at least 75% will pass a 300-mesh sieve (0.050-mm).

D. Boric Acid Solution (H_3BO_3), saturated

Prepare as in Kjeldahl-N.

E. Tris Solution (hydroxymethyl aminomethane) ($C_4H_{11}NO_3$), 0.01 N

Prepare as in Kjeldahl-N.

G. Sulfuric Acid Solution (H_2SO_4), 0.01 N

Prepare as in Kjeldahl-N

H. Standard Stock Solution

- Dry reagent-grade ammonium sulfate [$(NH_4)_2SO_4$], and potassium nitrate (KNO_3) in an oven at 100°C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 5.6605 g ammonium sulfate and 8.6624 g potassium nitrate in DI water, and transfer to a 1-L volumetric flask, mix well, and bring to volume with DI water. This solution contains 1.2 g NH_4-N, and 1.2 g NO_3-N per Liter (Stock Solution).
- Prepare a Standard Solution from the Stock Solution as follows: Dilute 50 mL Stock Solution to 1-L volume by adding 2 M potassium chloride solution (Diluted Stock Solution).
- A 20-mL aliquot of Diluted Stock Solution contains 1.2 mg NH_4-N and 1.2 mg NO_3-N.

Procedure

1. Weigh 30 g air-dry soil (2 mm) into a 250-mL Erlenmeyer flask, and add 150 mL 2 M potassium chloride solution (1:5 soil: solution ratio).
2. Stopper flasks, shake for 1 hour on an orbital shaker at 200 - 300 rpm, and filter suspensions using Whatman No. 42 filter paper.
3. Calibrate pH-meter, and standardize the 0.01 N H_2SO_4 in the AutoTitrator, as done for Kjeldahl-N.
4. Before starting distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 - 8 mL distillate per minute.
5. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22°C.
6. Carry out distillations as follows:

    - Dispense 1 mL saturated boric acid solution and 1 mL DI water into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
    - Pipette 20 mL aliquot of the clear supernatant into a 100-mL distillation flask.
    - To determine NH_4-N in solution, add 0.2 g heavy magnesium oxide with a calibrated spoon to the distillation flask.
    - Immediately attach the flask to the distillation unit with a clamp, start distillation, and continue for 3 minutes. Lower the dish to allow distillate to drain freely into the dish.
    - After 4 minutes, when about 35 mL distillate is collected, turn off the steam supply, and wash tip of the condenser into the evaporating dish with a small amount of DI water.
    - Titrate the distillate to pH 5.0 with standardized 0.01 N H_2SO_4 using the Auto-Titrator.
    - After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish.

- To determine NO_3-N (plus NO_2-N) in the same extract, add 0.2 g Devarda's alloy with a calibrated spoon to the same distillation flask.
- Attach flask to distillation unit with a clamp, and start distilling. Further proceed as for ammonium-N.
- Between different samples, steam out the distillations. Disconnect distillation flasks containing the KCl extracts, and attach a 100-mL empty distillation flask to distillation unit, and place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds. Steaming-out is done only between different samples, not between distillation for ammonium (MgO) and nitrate (Devarda's alloy) in the same sample.
- Each distillation should contain at least two standards and two blanks, i.e., 2 M KCl extracts with no soil added (reagent blanks).

CALCULATIONS

For Ammonium-N in air-dry soil:

$$NH_4 - N\ (ppm) = \frac{(V - B) \times N \times R \times 14.01 \times 1000}{Wt} \quad \ldots\ldots\ldots (28)$$

For Ammonium-N in oven-dry soil:

$$NH_4 - N\ (ppm) = \frac{(V - B) \times N \times R \times 14.01 \times 1000}{Wt - \theta} \quad \ldots\ldots\ldots (29)$$

Where:
- V = Volume of 0.01 N H_2SO_4 titrated for the sample (mL)
- B = Blank titration volume (mL)
- N = Normality of H_2SO_4 solution.
- 14.01 = Atomic weight of N.
- R = Ratio between total volume of the extract and the extract volume used for distillation.
- Wt = Weight of air-dry soil (30 g)
- θ = Weight of water (g) per 30 g air-dry soil.

Note

1. The concentration of NO_3-N (ppm) is calculated in the same manner as for NH_4-N, except that the Devarda's Alloy blank has to be inserted in the formula.
2. In some laboratories, a 1:3 (soil: solution) extract is used for Mineral-N determination. For soils in northwest Syria, a 1:5 extract gives a higher recovery of NH_4-N than a 1:3 extract.
3. For determination of NO_3-N in calcareous soils, we recommend using de-ionized water as the extracting solution, because carbonates dissolve in the KCl solution and some CO_2 may be collected in the H_3BO_3 during

distillation. This causes a negative interference with NO_3-N determination in KCl extract.

4. If possible, mineral-N should be determined in field-moist soil, immediately after sampling. However, analytical results should be expressed on an oven-dry soil basis. If the analysis cannot be done immediately after sampling, soil samples may be kept in a freezer.

5. If soil samples are air-dried, mineralization/nitrification may occur because of change in moisture and temperature conditions. For soils in north-west Syria, mineral-N content in air-dry and field-moist soils was found to be quite similar, suggesting that biological-N transformations do not occur to a significant extent in these soil samples.

6. Often there is confusion about the relationship between NO_3 and NO_3-N. The nitrate ion is a combination of one nitrogen atom and 3 oxygen atoms. The total mass of NO_3 is 14 + 48 = 62. So, in 62 g NO_3 contains. 14 g N and 48 g oxygen (O).

This relationship can be expressed in two ways, either as 62 g NO_3^- or as 14 g NO_3^--N. Both expressions are correct. Since 62/14 = 4.43, one can convert NO_3^- measurement to actual N concentration. For example, 10 ppm NO_3-N can be expressed as 10 × 4.43 or 44.3 ppm NO_3. Both values indicate the same concentration,in two different formats.

6.1.4 Nitrate-Nitrogen

Nitrate-N is measured by a spectrophotometric method (using chromotropic acid). Chromotropic acid spectrophotometric method is quite rapid, used originally for water and later for soils (Sims and Jackson, 1971; Hadjidemetriou, 1982). It is an alternate for NO_3-N determination by the distillation method. A close relationship exists between NO_3-N determined by chromotropic acid and distillation method.

Apparatus

Spectrophotometer or colorimeter, 430-nm wavelength.
Mechanical shaker, reciprocating.
Standard laboratory glassware: Beakers, volumetric flasks, pipettes, and funnels.

Reagents

A. Copper Sulfate Solution ($CuSO_4.5H_2O$), 0.02 N

Dissolve 4.9936 g copper sulfate in DI water, and dilute to 2-L volume with DI water.

B. Chromotropic Acid Solution ($C_{10}H_6Na_2O_8S_2.2H_2O$), 0.1 %

Dissolve 0.368 g chromotropic acid in 200 mL concentrated sulfuric acid. Keep solution in a dark bottle for two weeks.

C. Sulfuric Acid (H_2SO_4), concentrated

D. Standard Stock Solution

- Dissolve 3.6092 g potassium nitrate (dried at 100°C for 2 hours) in 500 mL 0.02 N copper sulfate solution (Stock Solution).
- Dilute 10 mL Stock Solution to 200 mL final volume by adding 0.02 N copper sulfate solution. This solution contains 50 ppm NO_3-N (Diluted Stock Solution).
- Prepare a series of Standard Solutions from the Dilute Stock Solution as follows: Dilute 1, 2, 3, 4, 5, 6 and 7 mL Diluted Stock Solution to 100 mL final volume of each by adding 0.02 N copper sulfate solution.

These solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ppm NO_3-N, respectively.

Procedure

1. Weigh 10 g air-dry soil (2-mm) into an Erlenmeyer flask, and add 50 mL 0.02 N copper sulfate solution.
2. Shake for 15 minutes and filter through a double Whatman No. 42 filter paper.
3. Pipette 3 mL filtrate into a 50-mL conical flask, and put flask in cold water for a few minutes.
4. Add 1 mL 0.1% chromotropic acid solution, drop by drop, directly in the solution without mixing, and again put in cold water for few minutes to cool.
5. Mix solution, and add 6 mL concentrated sulfuric acid on the flask wall without mixing.
6. After adding acid in all samples, swirl flask and leave to cool at room temperature; color (yellow) develops after 45 minutes.
7. Prepare a standard curve as follows:
   - Pipette 3 mL of each standard (0.5 - 3.5 ppm), and proceed as for the samples.
   - Also make a blank with 3 mL 0.02 N $CuSO_4.5H_2O$ solution, and proceed as for the samples.
   - Read the absorbance of blank, standards, and samples after 45 minutes at 430-nm wavelength.
8. Prepare a calibration curve for standards, plotting absorbance against the respective NO_3-N concentrations.
9. Read NO_3-N concentration in the unknown samples from the calibration curve.

CALCULATION

For Nitrate-N in soil:

$$NO_3 - N\ (ppm) = ppm\ NO_3 - N\ (from\ calibration\ curve) \times \frac{A}{V} \times \frac{10}{Wt} \quad ...(30)$$

Where: A = Total Volume of the extract (mL)
V = Volume of extract used for measurement (3 mL)
Wt = Weight of air-dry soil (g)

Note

1. Where soils contain >1 ppm NO_3-N, add 0.1 mL sulphamic acid (0.2% w/v in 0.1 N H_2SO_4) to 3-mL sample solution.
2. If filter paper gives purple solutions, it must be washed with distilled water and dried before use.

6.1.5 Microbial Biomass Nitrogen and Carbon

Microbial biomass is determined by the fumigation/incubation technique in which a fresh soil sample is subjected to chloroform fumigation, which causes cell walls to lyse and denature, the cellular contents are extractable in 0.5 M K_2SO_4. This is not a measure of soil microbial activity because no differentiation is made between quiescent and active organisms, or between different classes of microorganisms.

Care must be exercised when comparing soils from different locations as microbial biomass fluctuates greatly within a single soil in response to litter inputs, moisture availability and temperature. If different agricultural soils are being compared at a single time, the fresh soils should be at or near moisture holding capacity. If soils from different ecosystems are being compared, samples should be collected toward the middle of the wet and dry seasons. The following procedure is based on that of Anderson and Ingram (1993), and taken from Okalebo et al. (1993).

Apparatus

Block-digester, and calibrated digestion tubes.
Distillation unit.
Automatic titrator connected to a pH meter.
Vortex tube stirrer.
Desiccator.
Mechanical shaker, orbital.
Standard laboratory glassware: Beakers, volumetric flask, pipettes, and funnels.

Reagents

A. Chloroform Solution ($CHCl_3$), alcohol-free

Wash chloroform with 5% concentrated sulfuric acid in a separation funnel, separate the acid and then rinse repeatedly (8 - 12 times) in DI water. Store in a dark bottle.

B. Potassium Sulfate Solution (K_2SO_4), 0.5 M

Dissolve 87.13 g potassium sulfate in DI water, and bring to 1-L volume with DI water.

C. Copper Sulfate Solution ($CuSO_4.5H_2O$), 0.2 M

Dissolve 49.94 g copper sulfate in DI water, and bring to 1-L volume with DI water.

D. Potassium Dichromate Solution ($K_2Cr_2O_7$), 0.4 N

Dissolve 19.616 g potassium dichromate in DI water, and bring to 1-L volume with DI water.

E. Ferrous Ammonium Sulfate Solution [Fe $(NH_4)_2(SO_4)_2.6H_2O$], 0.2 N

Dissolve 78.4 g ferrous ammonium sulfate in DI water, add 5 mL concentrated sulfuric acid, mix well, and bring to 1-L volume in DI water.

F. 1.10-Phenanthroline Indicator

Dissolve 14.85 g 1.10-phenanthroline indicator, and 6.95 g ferrous sulfate ($FeSO_4.7H_2O$) in DI water, and bring to 1-L volume in DI water.

G. Sulfuric-Orthophosphoric Acid Mixture (H_2SO_4: H_3PO_4), 2:1 concentrated

Add 1000 mL concentrated sulfuric acid to 500 mL concentrated orthophosphoric acid.

Procedure

1. Weigh duplicate 30 g fresh soil samples into a 100-mL beaker. Conduct a moisture determination on soil sub-samples so that the results can be expressed on an oven-dry-weight basis.
2. Place the beakers into the two desiccators. Place a 100-mL beaker containing 50 mL chloroform into the center of the desiccator. Adding pumice boiling granules to the chloroform assists in rapid volatilization of the chloroform.

   The second desiccator contains non-fumigated control samples, which apart from fumigation-evacuation are to be handled in the same fashion. Close the lids of the desiccators, paying particular attention that the sealant is uniformly distributed (Fig. 7).
3. Apply vacuum to the fumigated treatment until the chloroform is rapidly boiling.
4. Close the desiccator and store under darkened conditions for 72 hours at room temperature.

5. Evacuate the fumigated treatment using a vacuum pump repeatedly (8 - 12 times).

   Important : Remember that the chloroform is being trapped by the oil in the vacuum pump; so the oil must be changed more often than normal. Alternatively, chloroform can be trapped by a cooling finger to prevent contamination of the vacuum oil. It is not necessary to evacuate the control desiccator.

6. Open the desiccators, and transfer the fumigated/nonfumigate soil samples to 250-mL Erlenmeyer flasks. Add 100 mL 0.5 M potassium sulfate solution and shake on an orbital shaker for 1 hour.

7 To obtain a clear extract, filter the soil suspensions using Whatman No. 42 filter paper or a centrifuge.

1. Determination of Nitrogen

A. Digestion

1. Pipette 50 mL of the filtrate into a 250-mL calibrated digestion tube, and add 1 mL 0.2 M copper sulfate solution.
2. Add 10 mL concentrated sulfuric acid, and a few pumice boiling granules.
3. Place the tubes rack in the block-digester and increase the temperature setting to 150°C to remove extra water.
4. Increase the temperature slowly to reach to 380°C, and digest for 3 hours.
5. Carefully lift the tubes rack out of the block-digester, let tubes cool to room temperature, and bring to volume with DI water.
6. Each batch of samples for digestion should contain at least one blank (no soil), and one EDTA standard (0.1g EDTA accurately weighed to 0.1 mg).

B. Distillation

Distillate the samples and analyze for N, as described in total-N (50 mL digest, and 15 mL 10 N NaOH).

CALCULATIONS

For Biomass Nitrogen in soil:

$$\text{Biomass N (ppm)} = (V - B) \times N \times 14.01 \times \frac{100 + \theta}{Wt} \times \frac{250}{V_1} \times \frac{1000}{V_2} \quad \ldots\ldots (31)$$

$$\text{Microbial Biomass N} = (N_{fumigated} - N_{control}) \quad \ldots\ldots\ldots\ldots\ldots\ldots (32)$$

Where: V = Volume of 0.01 N H_2SO_4 titrated for the sample (mL)
B = Digested blank titration volume (mL)
N = Normality of H_2SO_4 solution.
Wt = Weight of fresh soil (g)
V_1 = Aliquot of soil digest measured (mL)
V_2 = Aliquot of distillate measured (mL)
14.01 = Atomic weight of N.
θ = Weight of water (g) per 30 g fresh soil.

2. Determination of Carbon

A. Digestion

1. Pipette 8 mL of the filtrate into a 100-mL calibrated digestion tube, and add 2 mL 0.4 N potassium dichromate solution.
2. Add 0.07 g mercury (II) oxide (HgO), 15 mL (2:1) sulfuric: orthophosphoric acid mixture, and a few pumice boiling granules.
3. Place the tubes rack in the block-digester, increase temperatures setting to 150°C and digest for 30 minutes.
4. Carefully lift the tubes rack out of the block-digester, let tubes cool to room temperature, and transfer the digested sample with 25 mL DI water into a 250-mL Erlenmeyer flask.

B. Titration

Add 2 - 3 drops 1.10-phenalthroline indicator, and then titrate with 0.2 N ferrous ammonium sulfate solution, until the color changes from bluish-green to reddish-brown.

CALCULATION

For Biomass Carbon in soil

$$\text{Biomass C (ppm)} = (V - B) \times N \times 0.003 \times \frac{100 + \theta}{Wt} \times \frac{1000}{V} \times 1000 \quad \text{... (33)}$$

$$\text{Microbial Biomass C} = (C_{fumigated} - C_{control}) \quad \text{..... (34)}$$

Where: V = Volume of 0.2 N $[Fe(NH_4)_2(SO_4)_2.6H_2O]$ titrated for the sample (mL)

B = Digested blank titration volume (mL)

N = Normality of $[Fe(NH_4)_2(SO_4)_2.\ 6H_2O]$ solution.

0.003= 3×10^{-3}, where 3 is equivalent weight of C.

Wt = Weight of fresh soil (g)

V = Aliquot used for soil digest measured (mL)

θ = Weight of water (g) per 30 g fresh soil.

Note

Some authors suggest that empirically derived correction factors should be applied to these results. These factors may be obtained by conducting the fumigation/extraction procedure on inert soils containing a known quantity of microbial biomass (e.g., mushrooms or washed bacterial cells). Vance et al. (1987) advocate a factor of 2.64 for microbial biomass, while Brooks et al. (1985) recommend a factor of 1.46 for biomass N.

If these factors are applied, this should be clearly indicated when reporting the results. Because of the large variation in soil microbial (and micro-faunal) populations in soils, it is suggested that these factors may not be applied.

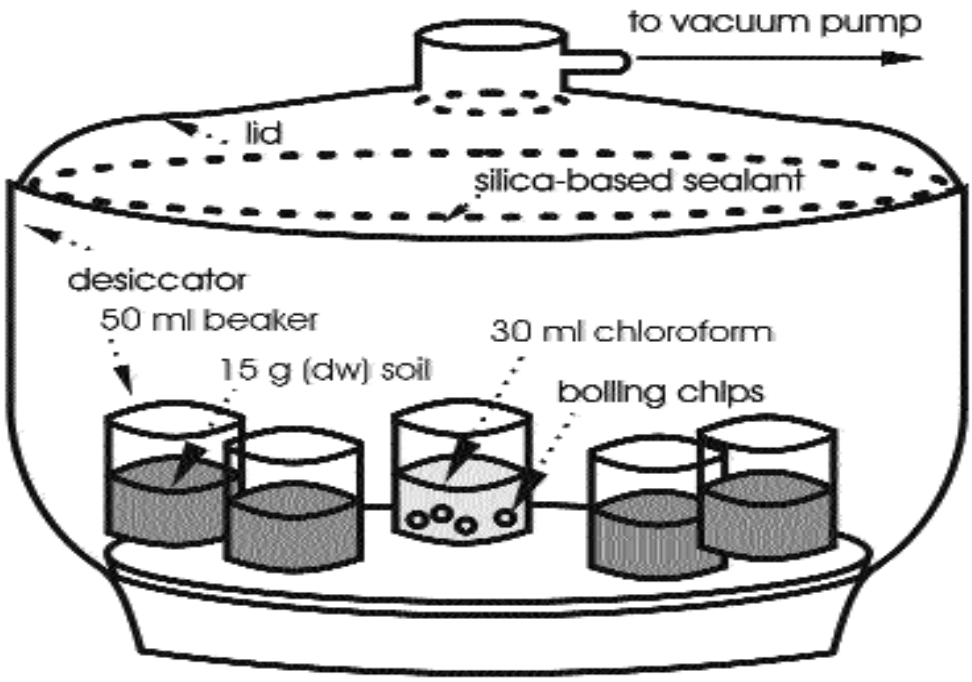

Fig. 7. Experimental Apparatus and Sample Arrangement in the Fumigation Procedure (Okalebo et al., 1993)

6.2 Phosphorus

6.2.1 Extractable Phosphorus

Because of its significance as a major nutrient, coupled with the fact that it is widely deficient in alkaline-calcareous soils, phosphorus (P) is measured in virtually all soil laboratories of the CWANA regions. Compared to N and most other nutrients, soil tests for P are generally fairly reliable in predicting the need for P fertilizer for growing field crops. Since P compounds in soils are highly variable and are related to soil type or parent material, several extractants are used worldwide for evaluating soil fertility. Few, if any of these procedures, are satisfactory for all soil types. Even a good test must be well correlated with crop P uptake and must be calibrated to crop response to fertilizer application in field situations.

A soil tests for routine use should be simple, quick, easy to execute, and inexpensive. The sodium bicarbonate procedure of Olsen et al. (1954) meets these criteria and is generally accepted as a suitable index of P "availability" for alkaline soils, where the solubility of calcium phosphate is increased because of the precipitation of Ca^{++} as $CaCO_3$. Field research has confirmed its usefulness in the CWANA region since the region's soils are mainly calcareous (Ryan and Matar, 1990; 1992). Consequently, this soil test has been adapted for routine use almost in all laboratories of the region.

The original sodium bicarbonate method, developed and described by Olsen et al. (1954), involved the use of carbon black in the extraction reagent to eliminate the color (because of soil organic matter) in the extract. The procedure was, however, modified later, eliminating the use of carbon black (Murphy and Riley, 1962; Watanabe and Olsen, 1965; Olsen and Sommers, 1982). In the modified method, a single solution reagent containing ammonium molybdate, ascorbic acid and a small amount of antimony is used, for color development in the soil extracts.

Apparatus

Spectrophotometer or colorimeter, 882-nm wavelength.

Mechanical shaker, reciprocating.

Extraction bottle, 250 mL with stopper.

Standard laboratory glassware: Beakers, volumetric flasks, pipettes, funnels.

Reagents

A. Sodium Hydroxide Solution (NaOH), 5 N

Dissolve 200 g sodium hydroxide in DI water, and transfer the solution to a 1-L volumetric heavy walled Pyrex flask, let it cool, and bring to volume with DI water.

B. Sodium Bicarbonate Solution ($NaHCO_3$), 0.5 M

Dissolve 42 g sodium bicarbonate in about 900 mL DI water, adjust to pH 8.5 with 5 N NaOH solution. Bring to 1-L volume with DI water. Keep the bottle closed and do not store over one month in a glass container; or use polyethylene container for periods more than one month.

C. Sulfuric Acid Solution (H_2SO_4), 5 N

Dilute 148 mL concentrated sulfuric acid (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume with DI water.

D. p-nitrophenol Indicator, 0.25 % w/v

E. Standard Stock Solution

- Dry about 2.5 g potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C for 1 hour, cool in desiccator, and store in a tightly stoppered bottle.
- Dissolve 2.197 g dried potassium dihydrogen phosphate in DI water, and bring to 1-L volume with DI water. This solution contains 500 ppm P (Stock Solution).
- Dilute 50 mL Stock Solution to 250 mL final volume by adding DI water. This solution contains 100 ppm P (Diluted Stock Solution).
- Prepare a series of Standard Solutions from the Diluted Stock Solution as follows: Dilute 5, 10, 15, 20 and 25 mL Diluted Stock Solution to 500 mL volume. These solutions contain 1, 2, 3, 4, and 5 ppm P, respectively.

F. Reagent A

- Dissolve 12 g ammonium heptamolybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250

mL DI water.

- Dissolve 0.2908 g antimony potassium tartrate ($KSbO.C_4H_4O_6$) in 100 mL DI water.
- Add both dissolved reagents to a 2-L volumetric flask, and add 1-L 5 N H_2SO_4 (148 mL concentrated H_2SO_4 per liter) to the mixture.

  Mix thoroughly, and dilute to 2-L volume with DI water. Store in a Pyrex bottle in a dark, cool place.

G. Reagent B

Dissolve 1.056 g L-Ascorbic acid ($C_6H_8O_6$) in 200 mL Reagent A, and mix. This reagent should be prepared as required because it does not keep for more than 24 hours.

Procedure

1. Weigh 5 g air-dry soil (2-mm) into a 250-mL Erlenmeyer flask; add 100 mL 0.5 M sodium bicarbonate solution.
2. Close the flask with a rubber stopper, and shake for 30 minutes on a shaker at 200 - 300 rpm. Include one flask containing all chemicals but no soil (Blank).
3. Filter the solution through a Whatman No. 40 filter paper, and pipette 10 mL clear filtrate into a 50-mL volumetric flask.
4. Acidify with 5 N sulfuric acid to pH 5.0. This can be done by taking 10 mL 0.5 M $NaHCO_3$ solution and determining the amount of acid required to bring the solution pH to 5.0, using P-nitrophenol indicator (color change is from yellow to colorless). Then add the required acid to all the unknowns. Adding 1 mL 5 N H_2SO_4 is adequate to acidify each 10 mL $NaHCO_3$ extract.

   Important: Do not swirl flasks immediately after adding 1 mL 5 N H_2SO_4 because this may results is excessive frothing.

5. Add DI water to about 40-mL volume, add 8 mL Reagent B, and bring to 50-mL volume.

6. Prepare a standard curve as follows:
   - Pipette 2 mL of each standard (1 - 5 ppm), and proceed as for the samples.
   - Also make a blank with 10 mL 0.5 M $NaHCO_3$ solution, and proceed as for the samples.
   - Read the absorbance of blank, standards, and samples after 10 minutes at 882 nm wavelength.
7. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
8. Read P concentration in the unknown samples from the calibration curve.

CALCULATION

$$\text{Extractable P (ppm)} = \text{ppm P (from calibration curve)} \times \frac{A}{Wt} \times \frac{50}{V} \quad \ldots (35)$$

For Extractable Phosphorus in soil:

Where: A = Total volume of the extract (mL)
Wt = Weight of air-dry soil (g)
V = Volume of extract used for measurement (mL)

Note

1. The unit ppm (parts per million) is commonly used in soil and plant analysis. One ppm is exactly equal to 1 mg/L if the specific weight of the solution is exactly 1 kg/L. For dilute standard solutions in distilled water, 1 ppm is approximately equal to 1 mg/L at room temperature.
2. The amount of P extracted from a soil depends on pre-treatment of samples, shaking frequency and time, and on temperature during extraction. Therefore, sample treatment and the conditions during extraction should be standardized.

3. If the sample solutions are too dark-colored for measurement against the highest standard, a smaller soil extract aliquot should be taken, and the calculation modified accordingly. Once the blue color has developed, the solution cannot be diluted.
4. Glassware used in P analysis should not be washed with detergents containing P (and remember that most detergents do contain P).
5. As glass tube density may vary, it is best to use the same tube (cuvette) for each absorbance reading on a spectrophotometer.
6. If AB-DTPA test (described at 6.10.2) is used for evaluating micronutrient status of the soil, then P can also be determined in the same extract. The beauty of this 'universal' test for alkaline soils is that macro - (NO_3-N, P, K)

and micronutrients (Zn, Fe, Mn, Cu) can be determined in a single extract.

6.2.2 Total Phosphorus

The "plant-available P" fraction is normally a small proportion of total P. Total P measurement involves digestion of a soil sample with a strong acid and the dissolution of all insoluble inorganic minerals and organic P forms. This measurement is usually employed only for soil genesis or mineralogical studies (Olsen and Sommers, 1982).

Apparatus

Spectrophotometer or colorimeter, 410-nm wavelength.

Block-digester.

Standard laboratory glassware: Beakers, volumetric flasks, pipettes, and funnels.

Vortex tube stirrer.

Reagents

A. Perchloric Acid ($HClO_4$), 60%

B. Ammonium Heptamolybdate-Ammonium Vanadate in Nitric Acid

- Dissolve 22.5 g ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in 400 mL DI water (a).
- Dissolve 1.25 g ammonium metavanadate (NH_4VO_3) in 300 mL hot DI water (b).
- Add (b) to (a) in a 1-L volumetric flask, and let the mixture cool to room temperature.
- Slowly add 250 mL concentrated nitric acid (HNO_3) to the mixture, cool the solution to room temperature, and dilute to 1-L volume with DI water.

C. Standard Stock Solution

- Dry about 2.5 g potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C for 1 hour, cool in a desiccator, and store in a tightly stoppered

bottle.

- Dissolve 0.4393 g dried potassium dihydrogen phosphate in DI water, and bring to 1-L volume with DI water. This solution contains 100 ppm P (Stock Solution).
- Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 1, 2, 3, 4, and 5 mL Stock Solution to 50-mL final volume by adding DI water. These solutions contain 2, 4, 6, 8, and 10 ppm P, respectively.

Procedure

A. Digestion

1. Weigh 2 g air-dry soil (0.15-mm) into a 250-mL calibrated digestion tube.
2. Add 30 mL 60% perchloric acid, and a few pumice-boiling granules. Mix well.
3. Place the tubes rack in the block-digester and gently heat to about 100°C.
4. Slowly increase the block-digester temperature to 180°C and digest the samples until dense white fumes of acid appear. Use a little extra perchloric acid to wash down the sides of the digestion tube as necessary.
5. Continue heating at the boiling temperature for 15 - 20 minutes longer. At this stage the insoluble material becomes like white sand. The total digestion with perchloric acid usually requires about 40 minutes.
6. Cool the mixture, and add DI water to obtain a volume of 250 mL, and mix the contents, and filter through Whatman No. 1 filter paper.

Note

If the soil samples are high in organic matter content, add 20 mL concentrated HNO_3 before step 2 and cautiously heat to oxidize organic matter.

B. Measurement

1. Pipette 5 mL of the sample digest into a 50 - mL volumetric flask.
2. Add 10 mL ammonium-vanadomolybdate reagent, and dilute to volume

with DI water.

3. Prepare a standard curve as follows:
   - Pipette 5 mL of each standard (2 - 10 ppm), and proceed as for the samples.
   - Also make a blank with 10 mL ammonium-vanadomolybdate reagent, and proceed as for the samples.
   - Read the absorbance of blank, standards, and samples after 10 minutes at 410-nm wavelength.
4. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
5. Read P concentration in the unknown samples from the calibration curve.

CALCULATION

$$\text{Total P (ppm)} = \text{ppm P (from calibration curve)} \times \frac{A}{Wt} \times \frac{50}{V} \quad .. (36)$$

For Total Phosphorus in soil:

Where: A = Total volume of the digest (mL)
Wt= Weight of air-dry soil (g)
V = Volume of digest used for measurement (mL)

6.2.3 Organic Phosphorus

Organic P content in soils, by the Ignition Method, is estimated by igniting the soil at 550°C. Simultaneously, inorganic P in the soil is estimated by extracting with 1 N sulfuric acid. Later, the organic P content in the soil is calculated by subtracting P in the unignited sample from P in the ignited sample.

Apparatus

Spectrophotometer or colorimeter, 882-nm wavelength.

Muffle furnace, for igniting soils at 550°C.

Mechanical shaker.

Centrifuge, capable of 1500 rmp.

Standard laboratory glassware: Porcelain crucibles, volumetric flasks, pipettes.

Reagents

A. Sulfuric Acid (H_2SO_4), 1 N

Dilute 30 mL concentrated sulfuric acid (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume with DI water.

B. Sodium Hydroxide Solution(NaOH), 5 N

Prepare as for Extractable Phosphorus in soils.

C. p-Nitrophenol, 0.25 % (w/v)

D. Reagent A

Prepare as for Extractable Phosphorus in soils.

E. Reagent B

Prepare as for Extractable Phosphorus in soils.

F. Standard Stock Solution

Prepare as for Extractable Phosphorus in soils.

Procedure

A. Digestion:

1. Weigh 1 g air-dry soil (0.15-mm) into a porcelain crucible.
2. Place the porcelain crucible in a cool muffle furnace, and slowly raise the temperature to 550°C over a period of 1 to 2 hours.
3. Maintain the temperature at 550°C for 1 hour, allow the crucible to cool, and transfer the ignited soil to a 100-mL polypropylene centrifuge tube.
4. In a separate 100-mL polypropylene centrifuge tube, weigh 1 g sample of unignited soil.
5. Add 50 mL 1 N sulfuric acid to both samples, and place the tubes on a shaker for 16 hours, then centrifuge the samples at 1500 rmp for 15 minutes (if the extract is not clear, filtration may be needed using acid-resistant filter paper).

B. Measurement

1. Pipette 2 mL clear filtrate into a 50-mL volumetric flask.
2. Add 5 drops 0.25 % p-nitrophenol solution, and neutralize with 5 N sodium hydroxide (yellow color).
3. Dilute to about 40 mL with DI water, and add 8 mL Reagent B, and bring to volume with DI water.
4. Prepare a standard curve as follows:
   - Pipette 2 mL of each standard (2 - 10 ppm), and proceed as for the samples.
   - Also make a blank with 2 mL 1 N H_2SO_4 solution, and proceed as for the samples.
   - Read the absorbance of blank, standards, and samples after 15 minutes at 882-nm wavelength.
5. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
6. Read P concentration in the unknown samples from the calibration curve.

CALCULATION

$$\text{Organic P (ppm)} = (\text{Ignited P - Unignited P})\ \text{ppm (from calibration curve)} \times \frac{A}{Wt} \times \frac{50}{V} \quad \text{.....(37)}$$

For organic Phosphorus in soil:
Where: A = Total volume of the digest (mL)
Wt= Weight of air-dry soil (g)

V = Volume of digest used for measurement (mL)

6.3 Potassium

Along with N and P, potassium (K) is also of vital importance in crop production. Most soils contain relatively large amounts of total K (1 - 2%) as components of relatively insoluble minerals, however, only a small fraction (about 1%) is present in a form available to plants, i.e., water-soluble and exchangeable K.

Highly weathered acid soils (of tropical regions) are more frequently deficient in plant available K, whereas soils of arid and semi-arid areas tend to be well supplied with K. Thus, soils of the CWANA region are generally adequate in K; a possible exception is sandy soils and irrigated soils grown to high K-requiring crops, e.g., sugarbeet and potatoes.

Nevertheless, extractable-K, or exchangeable plus water-soluble K, is often considered the plant-available fraction and is routinely measured in the region's laboratories. Water-soluble K tends to be a large proportion of the extractable K fraction in drier-region soils.

Where levels of extractable-K values are less than 100 to 150 ppm; K deficiency is likely and fertilization is required to maximize crop production with irrigation or high K requiring crops, the critical level should be even higher.

6.3.1 Extractable Potassium

This fraction of soil K is the sum of water-soluble and exchangeable K. The method uses a neutral salt solution to replace the cations present on the soil exchange complex; therefore, the cation concentration determined by this method are referred to as "exchangeable" for non-calcareous soils. For calcareous soils, the cations are referred to as "exchangeable plus soluble" (Richards, 1954).

Apparatus

Flame photometer with accessories.
Centrifuge, capable of 3000 rmp.
Mechanical shaker, reciprocating.

Reagents

A. Ammonium Acetate Solution (NH_4OAc), 1 N

- Add 57 mL concentrated acetic acid (CH_3COOH) to 800 mL DI water, and 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 volume with DI water.

B. Standard Stock Solution

- Dry about 3 g potassium chloride (KCl) in an oven at 120°C for 1 - 2 hours and cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 1.907 g dried potassium chloride in DI water, and bring to 1-L volume with DI water. This solution contains 1000 ppm K (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 Solution to 100-mL final volume of each by adding DI water or 1 N ammonium acetate solution. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm K, respectively.

Note

Standard solutions for measuring soluble-K should be prepared in DI water, but for measuring extractable-K the standards should be made in ammonium acetate solution.

Procedure

1. Weigh 5 g air-dry soil (< 2-mm) into a 50-mL centrifuge tube, add 33 mL ammonium acetate solution, and shake for 5 minutes on a shaker. The tubes should be stoppered with a clean rubber or polyethylene stopper, but not corks, which may introduce errors.
2. Centrifuge until the supernatant liquid is clear and collect the extract in a 100-mL volumetric flask through a filter paper to exclude any soil particles. Repeat this process two more times and collect the extract each time.
3. Dilute the combined ammonium acetate extracts to 100 mL with 1 N ammonium acetate solution.
4. Run a series of suitable potassium standards, and draw a calibration curve.
5. Measure the samples (soil extracts), and take the emission readings on a Flame Photometer at 767-nm wavelength.

6. Calculate potassium (K) concentrations according to the calibration curve.

CALCULATION

$$\text{Extractable K (ppm)} = \text{ppm K (from calibration curve)} \times \frac{A}{Wt} \quad \text{.... (38)}$$

For Extractable Potassium in soil:

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

6.3.2 Soluble Potassium

This fraction is a measure of the amount of K extracted from the soil by water.

Procedure

1. Weigh 5 g air-dry soil (<2 mm) into a 250-mL Erlenmeyer flask, add 100 mL DI water, and shake for 1 hour.
2. Filter and measure soluble-K on a Flame Photometer.

CALCULATION

$$\text{Soluble K (ppm)} = \text{ppm K (from calibration curve)} \times \frac{A}{Wt} \quad \text{... (39)}$$

For Soluble Potassium in soil:

6.3.3 Exchangeable Potassium

Exchangeable K, or that held on the exchange sites or surfaces of clay minerals, is normally the dominant portion of total extractable K. It can be deduced by difference.

Exchangeable K (ppm) = Extractable K (ppm) - Soluble (ppm) ..... (40)

For Exchangeable Potassium in soil:

Note

1. Exchangeable sodium (Na), calcium (Ca) and magnesium (Mg) can be measured in the same way as derived for exchangeable potassium (K). Extractable-Na, Ca, and Mg are measured in the ammonium acetate extract and soluble Na, Ca, and Mg in the water extract. The difference will represent exchangeable Na, Ca, and Mg.
2. A range of 20 to 200 ppm of Na standards may be prepared in ammonium acetate solution for extractable Na and in de-ionized water for soluble Na.
3. After extraction, the filtrate containing K, Mg, Ca and Na should not be stored for longer than 24 hours unless it is refrigerated or treated to prevent bacterial growth.
4. Soils can be stored in an air-dry condition for several months without any

effect on the exchangeable K, Na, Ca, and Mg content.

6.4 Sodium

Sodium (Na) can be extracted with ammonium acetate solution in the same way as K, while soluble Na can be obtained in a water extract obtained from a saturated paste as for EC. Subsequently, Na in the extract can be determined by flame photometry. Certain elements, including Na, have the property that, when their salts are introduced into a flame, they emit light with a wavelength (color) specific to the element and of intensity proportional to the concentration (Richards, 1954). This is especially true for Na emitting a sparkling yellowish-red color.

Reagents

A. Lithium Chloride (LiCl), 1000 ppm

- Dissolve 6.109 g dry lithium chloride in DI water, and bring to 1-L volume This solution contains 1000 ppm LiCl (Stock Solution).
- Dilute 100 mL Stock Solution to 1-L. This solution contains 100 ppm LiCl (Diluted Stock Solution).

B. Standard Stock Solution

- Dry about 5 g sodium chloride (NaCl) in an oven at 110°C 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 final 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 Solution to 100 mL final volume by adding DI water or 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).

Note

Standard Solutions for measuring soluble Na should be prepared in DI water, but

for measuring extractable Na the standards should be made in ammonium acetate solution.

Procedure

1. Operate Flame Photometer according to the instructions provided for the equipment.
2. Run a series of suitable sodium standards, and draw a calibration curve.
3. Measure Na^+ in the samples (soil extracts) by taking the emission readings on the flame photometer at 589-nm wavelength.
4. Calculate sodium (Na) concentrations by inferring to the calibration curve.

CALCULATIONS

$$\text{Na (meq/L)} = \text{meq/L Na (from calibration curve)} \times \frac{A}{Wt} \quad \text{..... (41)}$$

$$\text{Na (ppm)} = \text{meq/L Na (from calibration curve)} \times \frac{A}{Wt} \times 23 \quad \text{... (42)}$$

For Extractable or Soluble Sodium in soil:

Where: A = Total volume of the extract (mL)

Wt = Weight of air-dry soil (g)
23 = Atomic weight of Na.

6.5 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_4Cl-NH_4OH)

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 ($C_8H_8N_6O_6$)

Mix 0.5 g ammonium purpurate (Murexid) with 100 g potassium sulfate (K_2SO_4).

F. Standard Stock Calcium Chloride Solution ($CaCl_2.2H_2O$), 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 dihydrate

($CaCl_2.2H_2O$) 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

$$\text{Ca or Ca + Mg (meq/L)} = - \frac{(V - B) \times N \times R \times 1000}{Wt} \quad \text{..... (43)}$$

$$\text{Mg (meq/L)} = \text{Ca + Mg (meq/L)} - \text{Ca (meq/L)} \quad \text{..... (44)}$$

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.

$$N_{EDTA} = \frac{10 \times N_{CaCl_2}}{V_{EDTA}} \quad \text{..... (45)}$$

- Take the reading, and calculate EDTA normality:

Where: N_{EDTA} = Normality of EDTA solution.
V_{EDTA} = Volume of EDTA solution used (mL)
N_{CaCl_2} = Normality of $CaCl_2$ solution

Note

1. Normality with Ca determination usually is 3 to 5% higher than with Ca + Mg.
2. If there is not enough saturation extract, a soil-water suspension (1:5 ratio) can be prepared. Shake for 30 minutes, filter, and use the filtrate for analysis.
3. If an Atomic Absorption Spectrophotometer is used, a small aliquot of the

saturation extract is sufficient to determine Ca and Mg.

6.6 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

$$CO_3\ (meq/L) = \frac{2y \times N \times R \times 1000}{Wt} \quad \text{..... (46)}$$

$$HCO_3\ (meq/L) = \frac{(t - 2y) \times N \times R \times 1000}{Wt} \quad \text{..... (47)}$$

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)

6.7 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

$$Cl\ (meq/L) = \frac{(V - B) \times N \times R \times 1000}{Wt} \quad \text{..... (48)}$$

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.

$$N_{AgNO_3} = \frac{10 \times N_{NaCl}}{V_{AgNO_3}} \quad \text{..... (49)}$$

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

Where: N_{AgNO_3} = Normality of $AgNO_3$ solution.
N_{AgNO_3} = Amount of $AgNO_3$ solution used (mL)

N_{NaCl} = Normality of NaCl solution.

6.8 Sulfate

6.8.1 Turbidimetric Method

The commonly used method for sulfur (S) determination in alkaline soils is the extraction of SO_4-S with 0.15% $CaCl_2.2H_2O$ (Williams and Steinbergs, 1959) and measurement of SO_4-S concentration in the extracts by a turbidimetric procedure using barium chloride (Verma, 1977).

A critical range of 10 - 13 mg/kg $CaCl_2$-extractable SO_4-S has commonly been reported for cereal (e.g., wheat, maize) and oilseed (e.g., mustard) crops (Tandon, 1991).

Apparatus

Mechanical shaker, reciprocal
Spectrophotometer or colorimeter, 470-nm wavelength

Reagents

A. Calcium Chloride Dihydrate Solution ($CaCl_2.2H_2O$), 0.15%

Dissolve 1.5 g calcium chloride dihydrate in about 700 mL DI water, and bring to 1-L volumetric flask with DI water.

B. Hydrochloric Acid Solution (HCl), 6 M

Dilute 496.8 mL concentrated hydrochloric acid (37%, sp. gr. 1.19) in DI water, mix well, let it cool, and bring to 1-L volume with DI water.

C. Barium Chloride ($BaCl_2.2H_2O$), crystal

D. Sorbitol, 70% aqueous solution

E. Standard Stock Solution

- Dissolve 0.5434 g potassium sulfate (K_2SO_4) in DI water, and bring to 1-L volume with DI water. This solution contains 100 ppm SO_4-S (Stock Solution).
- Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 5, 10, 20, 30, 40, and 50 mL Stock Solution to 100-mL final

volume by adding 0.15% calcium chloride dihydrate solution. These standards contain 5,10, 20, 30, 40 and 50 ppm SO_4-S, respectively.

Procedure

A. Extraction

1. Weigh 5 g air-dry soil (2-mm) into a 150-mL Erlenmeyer flask.

2. Add 25 mL 0.15% calcium chloride dihydrate solution (don't use a rubber stopper, or wrap the rubber stopper in thin polyethylene. Errors result from gradual oxidation of sulfur compounds present in the stopper).

3. Shake for 30 minutes on a reciprocal shaker (180+ oscillations per minute).

4. Filter the suspension through Whatman No. 42 filter paper. This procedure yields almost colorless extracts.

B. Measurement of SO_4-S

1. Pipette 10-mL aliquot of the extract into a 50-mL test tube, or a smaller aliquot diluted to 10 mL with DI water.

2. Add 1 mL 6 M hydrochloric acid solution followed by 5 mL 70% sorbitol solution from a pipette with an enlarged jet. Finally, add about 1 g barium chloride crystals (using a measuring spoon).

3. Shake vigorously (on a test tube shaker for 30 seconds) to dissolve the barium chloride and obtain a homogeneous suspension.

4. Prepare a standard curve as follows:
   - Pipette 10 mL of each standard (0 - 50 ppm), and proceed as for the samples.
   - Also make a blank with 10 mL 0.15% calcium chloride dihydrate solution, and proceed as for the samples.
   - Read the absorbance (turbidity) of the blank, standards, and samples at 470-nm wavelength.

5. Prepare a calibration curve for standards, plotting absorbance against the respective SO_4-S concentrations.

6. Read SO_4-S concentration in the unknown samples from the calibration curve.

CALCULATION

$$SO_4 - S\ (ppm) = ppm\ SO_4 - S\ (from\ calibration\ curve) \times \frac{A}{Wt} \quad ... (50)$$

For Turbidimetric of Sulfate in soil:

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

Note

1. Do not let the standards and unknowns (soil extracts) stand for longer than 2 - 3 minutes, otherwise re-shake the suspension before spectrophotometric reading.

2. Allow approximately the same time to standards and unknowns between shaking and turbidimetric reading.

6.8.2 Precipitation Method

Sulfate in water is determined normally by barium sulfate precipitation (Richards, 1954).

Apparatus

Mechanical shaker, reciprocating.
Muffle furnace.

Reagents

A. Methyl Orange Indicator [4-$NaOSO_2C_6H_4N$: NC_6H_4 /-4-N $(CH_3)_2$], 0.1 %

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

B. Hydrochloric Acid Solution (HCl), 1:1

Mix equal portions of concentrated hydrochloric acid with DI water.

C. Barium Chloride Solution ($BaCl_2.2H_2O$), 1 N

Dissolve 122 g barium chloride in DI water, and bring to 1-L volume with DI water.

Procedure

1. Put an aliquot of soil extract containing 0.05 to 0.5 meq SO_4-S into a 250-mL Pyrex beaker and dilute to 50 mL.
2. Add 1 mL 1:1 hydrochloric acid solution and 2 - 3 drops methyl orange; if the color does not turn pink, add some more 1:1 hydrochloric acid.
3. Put beakers on a hotplate, heat to boiling, then add 10 mL 1 N barium chloride solution in excess to precipitate SO_4 as barium sulfate.
4. Boil for 5 to 10 minutes, cover with a watchglass, and leave to cool.
5. Filter solution through ashless filter paper, collect the barium sulfate precipitate on the filter paper, and then wash it several times with warm DI water until no trace of chloride remains. The presence of chloride in the filtrate can be checked by $AgNO_3$ solution.

6. After washing, place filter paper with precipitate into a pre-weighed and dried porcelain crucible and put in an oven at 105°C for 1 hour to dry.

7. Transfer crucible to a muffle furnace heated to 550°C, and leave to dry ash for 2 - 3 hours.
8. Take crucible out of the muffle furnace, and place in a desiccator to cool, weigh crucible on an analytical balance, and take the reading, t.

$$SO_4\text{-S (meq/L)} = \frac{t - b}{V} \times 8583.7 \quad \text{..... (51)}$$

CALCULATION

For precipitate of Sulfate in soil:

Where: t = Weight of crucible + $BaSO_4$ precipitate (g)
b = Weight of empty crucible (g)
V = Volume of extract used for measurement (mL)

6.9 Boron

6.9.1 Hot-Water Method

The hot-water extraction procedure was introduced by Berger and Truog (1939), and was modified by later researchers. It is still the most popular method for measuring "available" soil B or the fraction of B related to plant growth in alkaline soils. Boron in soil extracts is measured colorimetrically using azomethine-H (Bingham, 1982).
Where soil B levels are less than 0.5 ppm, deficiency is likely to occur for most crops. However, where levels are greater than about 5 ppm, toxicity may occur.

Apparatus

Erlenmeyer flasks 50 mL (Pyrex), pre-treated with concentrated HCl for one week.

Spectrophotometer or colorimeter, 420-nm wavelength.

Polypropylene test tubes, 10-mL capacity.

Reagents

A. Buffer Solution

Dissolve 250 g ammonium acetate (NH_4OAc), and 15 g ethylenediamine-tetraacetic acid, disodium salt (EDTA disodium) in 400 mL DI water. Slowly add 125 mL glacial acetic acid (CH_3COOH), and mix well.

B. Activated Charcoal (Boron-free)

This is prepared by giving repeated washings (8 - 9 times) of DI water (boiling charcoal with water in 1:5 ratio), and subsequent filtering. Boron in the filtrate is checked by azomethine-H color development. Continue washing until it is B-free.

C. Azomethine-H Solution ($C_{17}H_{12}NNa\ O_8S_2$)

Dissolve 0.45 g azomethine-H in 100 mL 1% L-ascorbic acid solution. Fresh reagent should be prepared weekly and stored in a refrigerator.

D. Standard Stock Solution

- Dissolve 0.114 g boric acid (H_3BO_3) in DI water, and bring to 1-L volume with DI water. This solution contains 20 ppm B (Stock Solution).
- Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mL Stock Solution to 100 mL final volume by adding DI water. These solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm, respectively.

Procedure

A. Extraction

1. Weigh 10 g air-dry soil (2-mm) into a 50-mL Erlenmeyer flask (Pyrex), pre-treated with concentrated HCl for one week.
2. Add about 0.2 g activated charcoal (B-free).
3. Add 20 mL DI water.
4. Boil on a hot plate for 5 minutes with flask covered by a watch glass.
5. Filter the suspension immediately through Whatman No. 40 filter paper. Filtrate is ready for B determination.

B. Measurement

1. Pipette 1 mL aliquot of the extract into a 10-mL polypropylene tube.
2. Add 2 mL buffer solution.
3. Add 2 mL azomethine-H solution, and mix well.
4. Prepare a standard curve as follows:
   - Pipette 1 mL of each standard (0.5 - 3.0 ppm), and proceed as for the samples.

- Also make a blank with 1 mL DI water, and proceed as for the samples.
- Read the absorbance of blank, standards, and samples after 30 minutes at 420-nm wavelength.

5. Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations.
6. Read B concentration in the unknown samples from the calibration curve.

$$B\ (ppm) = ppm\ B\ (from\ calibration\ curve) \times \frac{A}{Wt} \quad \text{..... (52)}$$

CALCULATION

For Extractable Boron in soil:

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

Note

Use of glassware should be minimal; and always use concentrated HCl-treated glassware (soaking for a week) where absolutely essential.

6.9.2 Dilute Hydrochloric Acid Method

Though the hot-water extraction method (HWE) is quite popular for predicting B availability in alkaline soils, the procedure is tedious and prone to error (because of difficulty in maintaining uniform boiling time). In an effort of having a convenient substitute, researchers (Kausar et al., 1990; Rashid et al., 1994; Rashid et al., 1997) have found that the dilute HCl method of Ponnamperuma et al. (1981), originally designed for acid soils, is equally effective in diagnosing B deficiency in alkaline and calcareous soils. The HCl method is simple, economical, and more efficient.

Reagents

A. Buffer Solution

Prepare as for hot-water extractable B.

B. Azomethine-H Solution ($C_{17}H_{12}NNa\ O_8S_2$)

Prepare as for hot-water extractable B.

C. Activated Charcoal (Boron-free)

Prepare as for hot-water extractable B.

D. Standard Stock Solution

Prepare as for hot-water extractable B.

E. Hydrochloric Acid (HCl), 0.05 N

Dilute 4.14 mL concentrated hydrochloric acid (37%, sp. gr. 1.19) in DI water, mix well, and bring to 1-L volume with DI water.

Procedure

A. Extraction

1. Weigh 10 g air-dry soil (2-mm) into a polypropylene tube.

2. Add about 0.2 g activated charcoal (B-free)
3. Add 20 mL 0.05 N Hydrochloric acid solution.
4. Shake for 5 minutes, and then filter.

B. Measurement (by Azomethine-H Method)

Same as in hot-water extractable B in soils.

$$B\ (ppm) = ppm\ B\ (from\ calibration\ curve) \times \frac{A}{Wt} \quad \text{..... (53)}$$

CALCULATION

For Boron in soil:

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

6.10 Micronutrient Cations (Iron, Zinc, Manganese and Copper)

Though required by plants in much smaller amounts than the major plant nutrients (like N, P, K), micronutrients are, nevertheless, equally essential for crop growth. Solubility of micronutrient cations decreases with an increase in soil pH. As most soils of the CWANA region are alkaline, micronutrient deficiencies are becoming more frequent and widespread in crops particularly, with increased intensification of cropping.

The DTPA test of Lindsay and Norvell (1978) is commonly used for evaluating fertility status with respect to micronutrient cations, i.e., Fe, Zn, Mn, and Cu. However, the universal soil test for alkaline soils (i.e., AB-DTPA described in Section 6.10.2) is equally effective for determining micronutrient cations in alkaline soils. Deficiencies of Mo, Cl, Ni and Co are not known to occur in alkaline soils.

6.10.1 DTPA Method

Apparatus

Mechanical shaker, reciprocal
Atomic absorption spectrophotometer

Reagents

A. DTPA Extraction Solution

- Weigh 1.97 g diethylene triamine pentaacetic acid (DTPA), and 1.1 g calcium chloride ($CaCl_2$) or [(1.47 g calcium chloride dihydrate ($CaCl_2.2H_2O$)] into a beaker. Dissolve with DI water and then transfer to a 1-L volumetric flask.
- Into another beaker, weigh 14.92 g (or add 13.38 mL) Triethanolamine (TEA), transfer with DI water into the 1-L volume, and make up to about 900 mL with DI water.

- Adjust the pH to exactly 7.3 with 6N hydrochloric acid (HCl), and make to 1-L volume with DI water. The final extractant solution is 0.005 M DTPA, 0.1 M TEA, 0.1 M $CaCl_2$.

B. Standard Stock Solutions

Prepare a series of Standard Solutions for micronutrients in DTPA extraction solution:

Fe: 0, 1, 2, 3, 4, 5 ppm; Zn: 0, 0.2, 0.4, 0.6, 0.8, 1.0 ppm;
Cu: 0, 1, 2, 3, 4 ppm; Mn: 0, 1.0, 1.5, 2.0, 2.5 ppm.

Procedure

1. Weigh 10 g air-dry soil (2-mm) into a 125-mL Erlenmeyer flask.
2. Add 20 mL extraction solution. Shake for 2 hours on a reciprocal shaker.
3. Filter the suspension through a Whatman No. 42 filter paper.
4. Measure Zn, Fe, Cu, and Mn directly in the filtrate by an Atomic Absorption Spectrophotometer.

Note

Follow the operating procedure for the Atomic Absorption Spectrophotometer using appropriate lamp for each element.

$$\text{Fe, Cu, or Mn (ppm)} = (\text{ppm in extract - blank}) \times \frac{A}{Wt} \quad \ldots\ldots\ldots (54)$$

CALCULATION

For Extractable Micronutrient cations in soil:
Where: A = Total volume of the extract (mL)
Wt = Weight of air-dry soil (g)

Note

1. The theoretical basis for the DTPA extraction is the equilibrium of the metals

in the soil with the chelating agent. The pH of 7.3 enables DTPA to extract Fe and other metals.

2. The DTPA reagent should be of the acid form (not a disodium salt).

6.10.2 Ammonium Bicarbonate-DTPA Method

The AB-DTPA is a multi-element soil test for alkaline soils developed by Soltanpour and Schwab (1977), and later modified by Soltanpour and Workman (1979) to omit the use of carbon black. The extracting solution is 1 M in the ammonium bicarbonate (NH_4HCO_3), and 0.005 M DTPA adjusted to pH 7.6, NO_3-N, P, and K can also be determined in the same extract.

This method is highly correlated with sodium bicarbonate method for P, ammonium acetate method for K, and DTPA method for Zn, Fe, Mn and Cu. Its range and sensitivity are the same as that of the DTPA test, sodium bicarbonate test, and ammonium acetate test for micronutrients, P, and K, respectively.

Apparatus

Atomic absorption spectrophotometer.
Spectrophotometer suitable for measurement at 880 and 420-nm wavelength.
Accurate automatic dilutor.
Flame photometer.
Mechanical shaker, reciprocal.

Reagents

A. Extracting Solution

A 0.005 M DTPA solution is obtained by adding 1.97 g DTPA to 800 mL DI water. Approximately 2 mL 1:1 ammonium hydroxide (NH_4OH) is added to facilitate dissolution and to prevent effervescence when bicarbonate is added.

When most of the DTPA is dissolved, 79.06 g ammonium bicarbonate (NH_4HCO_3) is added and stirred gently until dissolved. The pH is adjusted to 7.6 with ammonium hydroxide. The solution is diluted to 1-L volume with DI water, and is either used immediately or stored under mineral oil.

B. Mixed Reagent for Phosphorus

Dissolve 12 g ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}$.$4H_2O$] in 250 mL DI water. Dissolve 0.2908 g antimony potassium tartarate [KSbO $C_4H_4O_6$.

½H_2O] in 1-L 5 N sulfuric acid (148 mL concentrated H_2SO_4 per liter), mix the two solutions together thoroughly, and make to 2-L volume with DI water. Store in a Pyrex bottle in a dark, cool place.

C. Color Developing Solution for Phosphorus

Add 0.739 g L-ascorbic acid to 140 mL mixed reagent for P. This solution should be prepared as required, as it does not keep for more than 24 hours.

D. Hydrazine Sulfate Stock Solution ($H_2N_2H_2.H_2SO_4$)

Dissolve 27 g hydrazine sulfate (F.W. 130.12) in 750 mL DI water, make up the volume to 1-L volume, and mix well.

Prepare hydrazine sulfate working solution by diluting 22.5 mL stock solution to 1-L volume with DI water. This solution remains stable for 6 months.

E. Copper Sulfate Stock Solution ($CuSO_4.5H_2O$)

Dissolve 3.9 g copper sulfate pentahydrate (F.W. 249.68) in 800 mL DI water, make up to 1-L volume, and mix well.

Prepare copper sulfate working solution by diluting 6.25 mL of the stock solution to 1-L volume with DI water.

F. Sodium Hydroxide Stock Solution (NaOH), 1.5 N

Dissolve 60 g sodium hydroxide (F.W.40.0) in 500 mL DI water, cool, and bring to 1-L volume with DI water.

Prepare sodium hydroxide working solution (0.3 N) by diluting 200 mL stock solution to 1-L volume with DI water.

G. Color Developing Solution for Nitrate-Nitrogen

Add 5 g sulfanilamide (F.W. 172.21), and 0.25 g N- (1-naphthyl)-ethylenediamine dihydrochloride to 300 mL DI water. Slowly add 50 mL 85% orthophosphoric acid (H_3PO_4) with stirring, and bring the volume to 500 mL.

This reagent should be prepared as required, as it cannot be used after appearance of pink color.

H. Standard Stock Solutions

Nitrate-N:

Prepare working standards containing 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ppm NO_3-N.

Phosphorus:

Prepare working standards containing 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ppm P.

Potassium:

Prepare working standards containing 0, 5, and 10 ppm K.

Micronutrients:

Prepare working standards for:

- Fe: 0, 1.0, 2.0, 3.0, 4.0, 5.0 ppm.
- Cu: 0, 1.0, 2.0, 3.0, 4.0 ppm.
- Mn: 0, 1.0, 1.5, 2.0, 2.5 ppm.
- Zn: 0, 0.2, 0.4, 0.6, 0.8, 1.0 ppm.

Procedure

1. Extraction Method

   Weigh 10 g air-dry soil (2-mm) into a 125 mL conical flask. Add 20 mL extracting solution, and shake on a reciprocal shaker for 15 minutes at 180 cycles/minute with flasks kept open. The extracts are then filtered through Whatman No. 42 filter paper.

2. Nitrate-N

   Transfer 1 mL of the soil extract to 25 mL test tube, add 3 mL copper sulfate working solution, add 2 mL hydrazine sulfate working solution, and 3 mL sodium hydroxide working solution. Mix and heat in a water bath (38°C) for 20 minutes Remove from water bath, add 3 mL color-developing reagent for NO_3^--N, mix, and let stand at room temperature for 20 minutes. Read

absorbance at 540-nm wavelength on a Spectrophotometer (Kamphake et al., 1967).

The standards are developed the same way as described above; and a standard calibration curve is obtained using absorbance values for standards.

3. Phosphorus

Dilute 1 mL aliquot of the soil extract to 10 mL with DI water. Add 2.5 mL color developing reagent carefully to prevent loss of sample due to excessive foaming. Stir, let stand for 30 minutes, and measure color intensity at 880-nm wavelength using a Spectrophotometer.

The standards are developed the same way as described above; and a standard calibration curve is obtained using absorbance values for standards.

4. Potassium

The potassium in soil extracts is determined directly either by a Flame Photometer, or by an Atomic Absorption Spectrophotometer using potassium hollow cathode lamp.

The standard solutions are made in the extracting solution.

5. Micronutrients

Zinc, Fe, Cu, and Mn are determined by Atomic Absorption Spectrophotometer. The standard solutions of these metals are made in the extracting solution.

$$NO_3 - N \text{ (ppm)} = NO_3 - N \text{ (ppm in extract)} \times \text{Dilution Factor} \quad \text{..... (55)}$$

CALCULATIONS

$$P \text{ (ppm)} = P \text{ (ppm in extract)} \times \text{Dilution Factor} \quad \text{..... (56)}$$

For Nitrate-N in soil:

$$K\ (ppm) = K\ (ppm\ in\ extract) \times Dilution\ Factor \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (57)$$

For Phosphorus in soil:

$$Zn, Fe, Mn, Cu\ (ppm) = Zn, Fe, Mn, Cu\ (ppm\ in\ extract) \times Dilution\ Factor \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (58)$$

For Potassium in soil:

For Micronutrient in soil:

Note