

## 7. PLANT ANALYSIS

The concentration of nutrients in plant tissues can be measured in a plant extract obtained from fresh plant material, (i.e., tissue analysis), as well as in whole dried plant material. The former test is qualitative and is appropriate only for quick measurements on a growing crop.

Total plant analysis is quantitative in nature and is more reliable and useful. Generalized ranges of deficiency, adequacy, and excess of nutrient-concentrations in cereal crops are given in the Appendix 11. Of prime concern are forms of N, as well as P, B, and micronutrient cations.

More detailed interpretative guidelines for plant analysis data are available in Reuter and Robinson (1986, 1997) and Jones et al. (1991).

## 7.1 Nitrogen

One common plant analysis is that of nitrogen (N) by Kjeldahl method. However, wet ashing with  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$  is also used for eliminating the use of selenium in the former method (Van Schouwenberg and Walinge, 1973).

### 7.1.1 Kjeldahl Nitrogen

#### Apparatus

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

#### Reagents

The chemicals used here are the same as for soil Kjeldahl-N.

- A. Catalyst Mixture ( $\text{K}_2\text{SO}_4\text{-Se}$ ), 100: 1 w/ w ratio
- B. Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ), concentrated
- C. Ethylene Diaminetetraacetic Acid Disodium Salt (EDTA), M.W. = 372.2
- D. Sodium Hydroxide Solution ( $\text{NaOH}$ ), 10 N
- E. Boric Acid Solution ( $\text{H}_3\text{BO}_3$ ), saturated
- F. Sulfuric Acid Solution ( $\text{H}_2\text{SO}_4$ ), 0.01 N
- G. Standard Stock Solution: 1.2 g  $\text{NH}_4^+\text{-N}$  per L

## Procedure

### A. Digestion

1. Mix and spread finely ground (Cyclone mill) plant sample in a thin layer on a sheet of paper until it looks uniform.
2. Select representative sub-samples of about 1 g by taking at least 10 small portions from all parts of the sample with a spatula, and put them into a plastic vial.
3. Dry the sub-sample at 60°C in an oven (overnight), and then cool in a desiccator.
4. Weigh 0.25 g (grain) or 0.50 g (straw) of dry plant material, and transfer quantitatively into a 100-mL digestion tube.
5. Add a few pumice boiling granules, and add about 3 g catalyst mixture using a calibrated spoon.
6. Add 10 mL concentrated sulfuric acid using a dispenser, and stir with Vortex tube stirrer until mixed well.
7. Place tubes in a block-digester set at 100°C for 20 minutes, and remove the tubes to wash down any material adhering to the neck of the tube with the same concentrated sulfuric acid. Thoroughly agitate the tube contents, and then place the tubes back on the block-digester set at 380°C for 2 hours after clearing.
8. After digestion is complete, remove tubes, cool, and bring to 100-mL volume with DI water.
9. Each batch of samples for digestion should contain at least one reagent blank (no plant), and one chemical standard (weigh 0.1 g EDTA standard digest), and one standard plant sample (internal reference).

### B. Distillation

1. Set distillation and titration apparatus as for soil Kjeldahl-N, and steam out the apparatus for at least 10 minutes.
2. Prior to distillation, shake the digestion tube to thoroughly mix its contents. And pipette 10 mL aliquot into a 100-mL distillation flask.
3. Carefully add 10 mL 10 N sodium hydroxide solution, and immediately connect the flask to distillation unit and begin distillation.
4. Collect about 35 mL distillate in the collecting dish.

5. Remove distillation flask and connect an empty 100-mL distillation flask to the distillation unit. Drain water from the condenser jacket and steam out apparatus for 90 seconds before connecting the next sample.
6. Titrate the distillate to pH 5.0 with standardized 0.01 N H<sub>2</sub>SO<sub>4</sub> using the Auto-Titrator; record titration volume of acid.
7. Each batch of distillations should include a distillation of 10 mL ammonium-N standard with 0.2 g MgO and 10 mL DI water with 0.2 g MgO. Recovery of ammonium-N standards should be at least 98%. Recovery of EDTA, corrected for reagent blank, should be at least 97%.

### CALCULATIONS

Percentage recovery of Ammonium-N standard:

$$\% \text{ Recovery} = \frac{(V - B) \times N \times 14.01 \times 100}{C \times D} \dots\dots\dots (59)$$

Where: V = Volume of 0.01 N H<sub>2</sub>SO<sub>4</sub> titrated for the sample (mL).  
 B = Distillate blank titration volume (mL)  
 N = Normality of H<sub>2</sub>SO<sub>4</sub> solution.  
 C = Volume of NH<sub>4</sub>-N standard solution (mL)  
 D = Concentration of NH<sub>4</sub>-N standard solution (µg/mL)  
 14.01= Atomic weight of N.

Percentage recovery of EDTA standard:

$$\% \text{ Recovery} = \frac{(V - B_1) \times N \times R \times 186.1 \times 100}{Wt_1 \times 1000} \dots\dots\dots (60)$$

Percentage Nitrogen in plant:

$$\% N = \frac{(V - B_1) \times N \times R \times 14.01 \times 100}{Wt_2 \times 1000} \dots\dots\dots (61)$$

- Where: R = Ratio between total digest volume and distillation volume.  
B<sub>1</sub> = Digested blank titration volume (mL)  
Wt<sub>1</sub> = Weight of EDTA (g)  
Wt<sub>2</sub> = Weight of dry plant (g)  
186.1 = Equivalent weight of EDTA.

### 7.1.2 Nitrate-Nitrogen

The routine use of heavy metals as catalysts is not environmentally sound. From this standpoint, treatment of plant material with a mixture of  $\text{H}_2\text{O}_2$ - $\text{H}_2\text{SO}_4$  in the absence of metal catalysts has been proposed as an alternative digestion procedure for replacing Kjeldahl-N determination in soils and plants (McGill and Figueiredo, 1993).

#### Reagents

A. Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ), concentrated

B. Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ), 30%

#### Procedure

1. Weigh 0.5 g dry plant material into a 100-mL digestion tube.
2. Add 3-4 pumice boiling granules, and then add 5 mL concentrated sulfuric acid, and mix
3. Keep overnight.
4. Heat on a block-digester at a moderate temperature i.e., 100 - 150°C.
5. Swirl to restrict foaming. If foaming enters the neck of the digestion tube, add 2 mL 30 % hydrogen peroxide.
6. Heat the tubes for 30 - 60 minutes on the block-digester.
7. Cool the tubes, then add 2 mL hydrogen peroxide.
8. Raise the temperature of the block-digester to 280°C.
9. Heat the tubes for 10 minutes at 280°C.
10. Cool, then add 2 mL hydrogen peroxide, and heat for 10 minutes.
11. Repeat 9 and 10 until solution remains clear after 10 minutes of heating.
12. Cool, and make up to the 100-mL volume with DI water.

#### Measurement

Nitrogen content can be measured in this digest by the Distillation Method. Also, measurement of phosphorus can be done Colorimetrically, after filtering the digest through Whatman No. 1 or 5 filter paper, as described by Murphy and Riley (1962). The results for both N and P are highly correlated with the standard Kjeldahl digestion method.

### 7.1.3 Total Nitrogen

This method is based on digestion of plant material in a sulfuric-salicylic acid mixture (Buresh et al., 1982).

#### Reagents

- A. Sulfuric-Salicylic Acid Mixture (concentrated  $\text{H}_2\text{SO}_4$  containing 2.5 % w/v salicylic acid)  
Dissolve 62.5g reagent-grade salicylic acid ( $\text{C}_7\text{H}_6\text{O}_3$ ) in 2.5-L concentrated sulfuric acid.
- B. Catalyst Mixture ( $\text{K}_2\text{SO}_4\text{-Se}$ ), 100:1 w/w ratio.
- C. Sodium Thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ), crystal
- D. Ethylene Diaminetetraacetic Acid Disodium Salt (EDTA), M.W. = 372.2

#### Procedure

- A. Digestion
  1. Mix and spread finely ground plant sample in a thin layer, on a sheet of paper or plastic until the sample looks uniform.
  2. Take a representative sub-sample of about 1 g by systematically withdrawing at least 10 small portions from all parts of the sample with a spatula, and put them into a plastic vial.
  3. Dry the sub-sample at  $60^\circ\text{C}$  in an oven (overnight), and then cool in a desiccator.
  4. Weigh 0.25 g (grain) or 0.50 g (straw) dry plant material, and then transfer quantitatively into a dry 250-mL digestion tube.
  5. Add 20 mL sulfuric-salicylic acid mixture while rotating the tube to wash down any sample adhering to the neck of the tube, and allow to stand 2 hours or longer with occasional swirling.
  6. Add 2.5 g sodium thiosulfate through a long-stemmed funnel to the contents of the tube and swirl gently a few times, and allow to stand overnight.

7. Add 4 g catalyst mixture, and 3-4 pumice boiling granules, and place tubes on the block-digester pre-heated to 400°C.
8. Place a small glass funnel in the mouth of the tubes to ensure efficient refluxing of the digestion mixture and prevent loss of H<sub>2</sub>SO<sub>4</sub>, and proceed with the digestion until the mixture clears.
9. Remove the tubes from the block-digester and allow them to cool for about 20 minutes. Then wash down any material adhering to the neck of the tube with a minimum quantity of DI water.
10. Thoroughly agitate the tube contents, place tubes back on the block-digester, and digest for 2 hours after clearing. No particulate material should remain in the tube after digestion.
11. After the digestion is finished, allow the digest to cool, and add water slowly shaking until the liquid level is about 2 cm below the graduation mark.
12. Allow tube to cool to room temperature, and add DI water to bring the volume to the 250 mL mark.
13. Each batch of samples for digestion should contain at least one reagent blank (no plant), and one chemical standard (weigh 0.1 g EDTA standard digest), and one standard plant sample (internal reference)

#### B. Distillation

The reagents needed for distillation are the same as for soil Kjeldahl-N.

1. Set distillation and titration apparatus as for soil Kjeldahl-N, and steam out the apparatus for at least 10 minutes.
2. Prior to distillation, shake the digestion tube to thoroughly mix its contents, and pipette an aliquot in a 300-mL distillation flask.
3. Carefully add 7 mL or 15 mL 10 N sodium hydroxide solution for 25 mL or 50 mL aliquot, respectively, and immediately connect flask to distillation unit and begin distillation.
4. Collect about 35 mL distillate in the collecting dish.
5. Remove distillation flask and connect an empty 100-mL distillation flask to the distillation unit. Drain water from the condenser jacket and steam out apparatus for 90 seconds before connecting the next sample.

6. The distillate is then titrated to pH 5.0 with standardized 0.01 N H<sub>2</sub>SO<sub>4</sub> using the Auto-Titrator; record titration volume of acid.
7. Each batch of distillations should contain at least two standards and two blanks (reagent blanks). Recovery of EDTA, corrected for reagent blank, should be at least 97%.

### CALCULATIONS

Percentage recovery of EDTA standard

$$\% \text{ Recovery} = \frac{(V - B) \times N \times R \times 186.1 \times 100}{Wt_1 \times 1000} \dots\dots\dots (62)$$

Percentage Nitrogen in plant:

$$\% \text{ N} = \frac{(V - B) \times N \times R \times 14.01 \times 100}{Wt_2 \times 1000} \dots\dots\dots (63)$$

- Where: V = Volume of 0.01 N H<sub>2</sub>SO<sub>4</sub> titrated for the sample (mL).  
 B = Digested blank titration volume (mL)  
 N = Normality of H<sub>2</sub>SO<sub>4</sub> solution.  
 14.01= Atomic weight of N.  
 R = Ratio between total digest volume and distillation volume.  
 Wt<sub>1</sub> = Weight of EDTA (g)  
 Wt<sub>2</sub> = Weight of dry plant (g)  
 186.1= Equivalent weight of the EDTA.

## 7.2 Phosphorus

Total P in plant material can be determined either by wet digestion procedure (detailed in Section 7.1.1) or by dry-ashing procedure (given in Section 7.3). Both methods are satisfactory. However, dry ashing is a simpler, easier, non-hazardous and economical option. Later, P content in the digests or dissolved ash aliquots are measured colorimetrically.

### Apparatus

Spectrophotometer or colorimeter, 410-nm wavelength.  
Block-digester.  
Vortex tube stirrer.

### Reagents

#### A. Ammonium Heptamolybdate-Ammonium Vanadate in Nitric Acid

- Dissolve 22.5 g ammonium heptamolybdate  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$  in 400 mL DI water (a).
- Dissolve 1.25 g ammonium metavanadate  $(\text{NH}_4\text{VO}_3)$  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  $(\text{HNO}_3)$  to the mixture, cool the solution to room temperature, and bring to 1-L volume with DI water.

#### B. Standard Stock Solution

- Dry about 2.5 g potassium dihydrogen phosphate  $(\text{KH}_2\text{PO}_4)$  in an oven at  $105^\circ\text{C}$  for 1 hour cool in desiccator, and store in a tightly stoppered bottle.
- Dissolve 0.2197 g dried potassium dihydrogen phosphate in DI water, and bring to 1-L volume with DI water. This solution contains 50 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 100-mL final volume by adding DI water. These solutions contain 0.5, 1.0, 1.5, 2.0, and 2.5 ppm P, respectively.

## Procedure

### A. Wet-Digestion Procedure

- Digest the plant material (as described for Kjeldahl-N in plants in Section 7.1.1).
- Filter plant digest with Whatman No.1 filter paper, and collect filtrate in a small bottle.

or as an alternative procedure

### Dry-Ashing Procedure

- Dry ash the plant material (as described for Macro- and Micronutrients by dry ashing, in Section 7.3).
- Dissolve the ash in 2 N HCl (as described in Section 7.3).

### B. Measurement

1. Pipette 10 mL of the digest filtrate or aliquot of the dissolved ash (depending on the procedure used) into a 100-mL volumetric flask, add 10 mL ammonium-vanadomolybdate reagent, and dilute the solution to volume with DI water.
2. Prepare a standard curve as follows:
  - Pipette 1, 2, 3, 4, and 5 mL standard stock solution, 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 the blank, standards, and samples after 30 minutes at 410-nm wavelength.
3. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
4. Read P concentration in the unknown samples from the calibration curve.

## CALCULATION

Percentage Total Phosphorus in plant

$$\% P = \text{ppm P (from calibration curve)} \times \frac{R}{W_t} \times \frac{100}{10000} \dots (64)$$

Where: R = Ratio between total volume of the digest/aliquot and the digest  
/aliquot volume used for measurement

W<sub>t</sub> = Weight of dry plant (g)

Note

The plant digest by the hydrogen peroxide and sulfuric acid can also be used for phosphorus measurement in plants.

### 7.3 Macro- and Micro-nutrients by Dry Ashing

Plant analysis by dry ashing is simple, non-hazardous and less expensive, compared with  $\text{HNO}_3\text{-HClO}_4$  wet digestion. Dry ashing is appropriate for analyzing P, K, Ca, Mg, and Na. Micronutrient cations (Fe, Zn, Cu, and Mn) can also be analyzed by dry ashing, but only in plant tissues containing low silica contents (like legumes).

The  $\text{HNO}_3\text{-HClO}_4$  wet digestion is required for full recovery of micronutrient cations in high-silica plant tissues (like wheat, barley, rice, and sugarcane, etc.) (Procedure in Section 7.5). In dry ashing for B, use of glassware should be avoided (use specific procedure for B analysis in plant tissue, given in Section 7.4).

#### Apparatus

Spectrophotometer or colorimeter, 410-nm wavelength.

Flame photometer.

Atomic absorption spectrophotometer.

Porcelain crucibles or Pyrex glass beakers (30 - 50 mL capacity).

#### Reagent

Hydrochloric Acid (HCl), 2N

Dilute 165.6 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.

#### Procedure

The procedure is that of Chapman and Pratt (1961) with slight modifications.

1. Weigh 0.5 - 1.0 g portions of ground plant material in a 30 - 50 mL porcelain crucibles or Pyrex glass beakers.
2. Place porcelain crucibles into a cool muffle furnace, and increase temperature gradually to 550°C.
3. Continue ashing for 5 hours after attaining 550°C.
4. Shut off the muffle furnace and open the door cautiously for rapid cooling.
5. When cool, take out the porcelain crucibles carefully.
6. Dissolve the cooled ash in 5-mL portions 2 N hydrochloric acid (HCl) and mix with a plastic rod.

7. After 15 - 20 minutes, make up the volume (usually to 50 mL) using DI water.
8. Mix thoroughly, allow to stand for about 30 minutes, and use the supernatant or filter through Whatman No. 42 filter paper, discarding the first portions of the filtrates.
9. Analyze the aliquots for P by Colorimetry (by Ammonium Vanadate-Ammonium Molybdate yellow color method), for K and Na by Flame Photometry, and for Ca, Mg, Zn, Cu, Fe, and Mn by Atomic Absorption Spectroscopy.

#### Note

For Ca and Mg measurement, the final dilution should contain 1% w/v lanthanum (La) and the determinations should be against standards and blank containing similar La concentration to overcome anionic interference.

## 7.4 Boron

Boron in plant samples is measured by dry ashing (Chapman and Pratt, 1961) and subsequent measurement of B by colorimetry using Azomethine-H (Bingham, 1982).

### Apparatus

- Porcelain crucibles
- Spectrophotometer or colorimeter, 420-nm wavelength.
- Polypropylene test tubes, 10 mL capacity.

### Reagents

A. Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ), 0.36 N

B. Buffer Solution

Same as for B analysis in soil.

C. Azomethine-H

Same as for B analysis in soil.

D. Standard Stock Solution

Same as for B analysis in soil.

### Procedure

A. Dry Ashing

1. Weigh 1 g dry, ground plant material in porcelain crucible.
2. Ignite in a muffle furnace by slowly raising the temperature to 550°C.
3. Continue ashing for 6 hours after attaining 550°C.
4. Wet the ash with five drops DI water, and then add 10 mL 0.36 N sulfuric acid solution into the porcelain crucibles.
5. Let stand at room temperature for 1 hour, stirring occasionally with a plastic rod to break up ash.
6. Filter through Whatman No.1 filter paper into a 50-mL polypropylene volumetric flask and bring to volume. Filtrate is ready for B determination.

B. Measurement

Same as in hot water extractable B in soils.

CALCULATION

For Boron in plant:

$$B \text{ (ppm)} = \text{ppm B (from calibration curve)} \times \frac{A}{W_t} \dots\dots\dots (65)$$

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

Wt = Weight of dry plant (g)

## 7.5 Micronutrient Analysis by Wet Digestion

Full recovery of micronutrient cations (Zn, Fe, Mn, Cu) in high-silica containing plant tissues (like wheat, barley, rice, sugarcane, etc.) is not possible by dry ashing procedure. Therefore, this kind of plant materials should be wet-digested using  $\text{HNO}_3\text{-HClO}_4$ . The digestion procedure is adapted from Rashid (1986). Many other elements (like P, K, Ca, Mg, Na) can also be determined in the same digest.

### Apparatus

Block-digester.  
Vortex tube stirrer.  
Atomic absorption spectrophotometer.  
Flame photometer.

### Reagent

Nitric Acid-Perchloric Acid ( $\text{HNO}_3\text{-HClO}_4$ ), 2:1 ratio

To 1 L concentrated nitric acid add 500 mL concentrated perchloric acid.

### Procedure

#### A. Digestion

1. Weigh 1 g dry plant material, and then transfer quantitatively into a 100-mL Pyrex digestion tube.
2. Add 10 mL 2:1 nitric-perchloric acid mixture, and allow to stand overnight or until the vigorous reaction phase is over.
3. Place small, short-stemmed funnels in the mouth of the tubes to reflux acid.
4. After the preliminary digestion, place the tubes in a cold block-digester, and then raise temperature to  $150^\circ\text{C}$  for 1 hour.
5. Place the U-shaped glass rods under each funnel to permit exit of volatile vapors.
6. Increase temperature slowly until all traces of nitric acid disappear, and then remove U shaped glass rods.
7. Raise temperature to  $235^\circ\text{C}$ .
8. Note time, when dense white fumes of perchloric acid appear in the tubes, and continue digestion for 30 minutes more.

9. Lift the tubes rack out of the block-digester, allow to cool a few minutes, and add a few drops DI water carefully through the funnel.
10. After vapors condense, add DI water in small increments for washing down walls of tubes and funnels.
11. Bring to volume with DI water. Mix the solution of each tube and then leave undisturbed for a few hours.
12. Each batch of samples for digestion should contain at least one reagent blank (no plant material).

#### B. Measurement

Decant the supernatant liquid and analyze Zn, Fe, Mn, Cu, Ca, and Mg in the aliquots by Atomic Absorption Spectrophotometry. Determine K and Na by Flame Photometry.

#### CALCULATIONS

For Micronutrient Cations in plant:

$$\text{Zn, Fe, Cu or Mn (ppm)} = (\text{ppm in extract} - \text{blank}) \times \frac{A}{W_t} \dots (66)$$

For Alkaline Earth Cations in plant:

$$\text{Ca, Mg, Na or K (ppm)} = (\text{ppm in extract} - \text{blank}) \times \frac{A}{W_t} \dots (67)$$

Where: A = Total volume of the extract (mL)  
 W = Weight of dry plant (g)

## 7.6 Ferrous Analysis in Fresh Plant Tissue

As total iron content in plant tissue does not indicate Fe nutritional status of plants, determination of ferrous iron ( $\text{Fe}^{++}$ ) in fresh tissue by o-phenanthroline extraction (Katyal and Sharma, 1980) is needed for the purpose. Then, ferrous content in the extracts can be measured by colorimetry or atomic absorption spectrophotometry.

### Ferrous Extraction with o-phenanthroline

#### Apparatus

Spectrophotometer or colorimeter, 510-nm wavelength.  
Atomic absorption spectrophotometer.

#### Reagents

A. Extraction Solution ( $\text{C}_{12}\text{H}_8\text{N}_2$ ), 1.5% in HCl-buffer with pH 3.0

Add 15 g 1 - 10 o-phenanthroline to about 850 mL DI water. Dropwise, add 1 N hydrochloric acid by continuously stirring solution until last traces of 1 - 10 o-phenanthroline are solubilized. Final pH of the solution will be around 3.0. Make volume to 1-L volume with DI water.

B. Standard Stock Solution

Prepare working solution standards of iron containing 0, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm  $\text{Fe}^{++}$  in the extraction solution.

#### Procedure

A. Extraction

1. Use carefully washed fresh plant tissues for ferrous analysis.
2. Weigh 2 g fresh (chopped with a stainless scissors) plant material into a 50-mL Erlenmeyer flask.
3. Add 20 mL extraction solution, and stir gently to ensure that all the plant tissue is completely dipped in the solution.
4. Close the flask using parafilm, and allow to stand for about 16 hours at room temperature.
5. Filter the contents through Whatman No. 1 filter paper.

## B. Measurement

1. Ferrous content in the filtrate is determined by a Colorimeter at 510-nm wavelength or by an Atomic Absorption Spectrophotometer. Standards for Fe are run along with the plant extracts.
2. Ferrous content in plant tissue is expressed on oven dry weight basis, after determining moisture content in a sub-sample of fresh plant tissue.

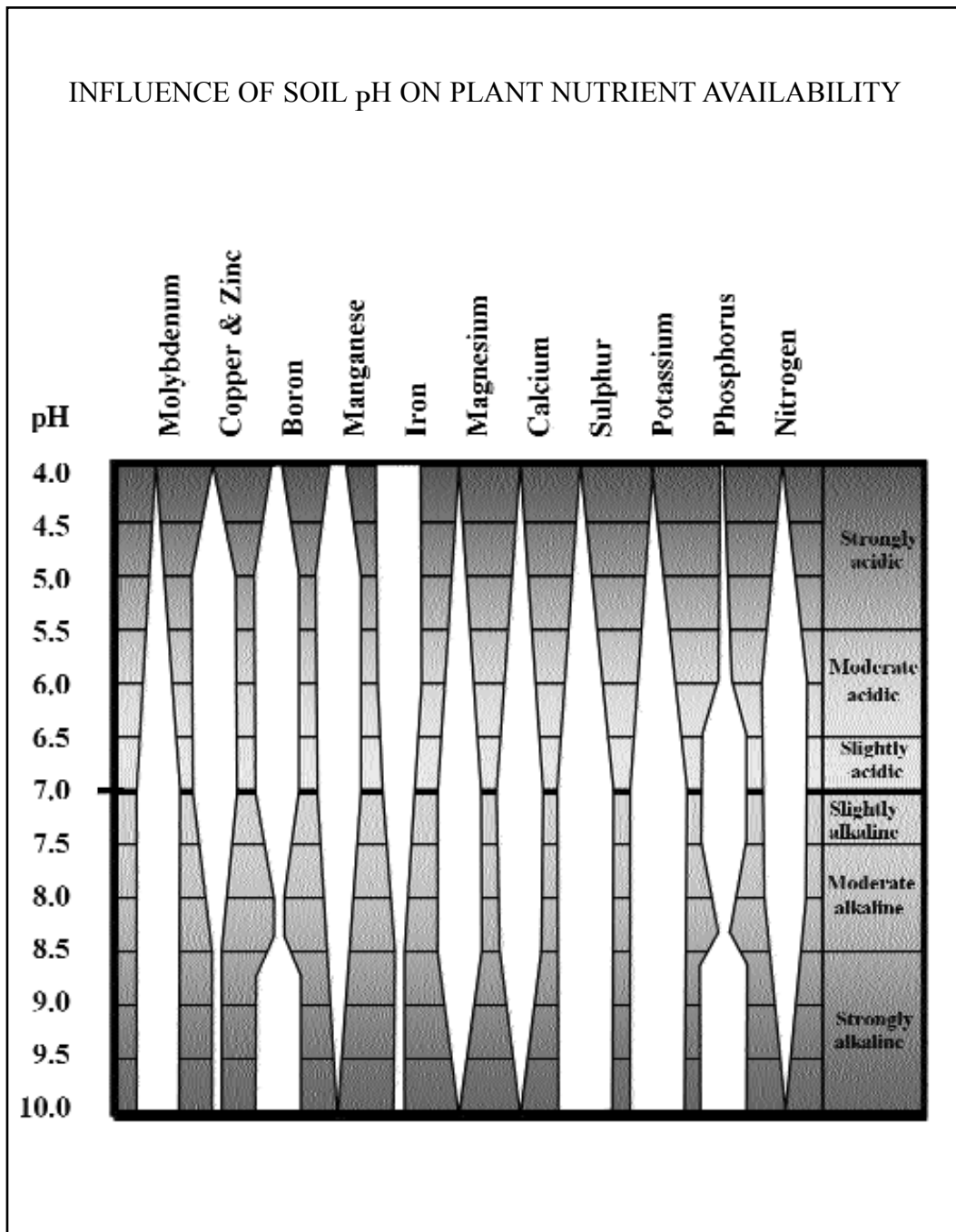
### CALCULATION

For Ferrous Iron in fresh plant tissue:

$$\text{Fe}^{++}(\text{ppm}) = \text{ppm Fe}^{++} \text{ (from calibration curve)} \times \frac{A}{Wt} \dots\dots\dots (68)$$

Where: A = Total volume of the extract (mL).  
Wt = Weight of oven-dry plant material (g)

Box 1



## Box 2

### SOIL-PLANT ANALYSIS BY AUTOMATED EQUIPMENT

With the advancement of technology, now many soil and plant analyses can be carried out much faster using automated laboratory equipments, such as an autoanalyzer and inductively coupled plasma (ICP) spectrometer.

An autoanalyzer is an improved version of a colorimeter, but because of its automated mechanism it can carry out colorimetric determinations continuously at a much faster rate than a spectrophotometer. Prepared samples (soil extracts, plant digests, etc.) loaded on an automatic sample changer (e.g., batches of 50 each) are read for color intensity continuously, after automatic reagent additions for color development. Autoanalyzers are generally used for determining  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , P, etc.; in fact, more than one measurement can be carried out simultaneously depending on the number of channels on the available instrument.

ICP is highly automated equipment used for simultaneous measurement of a number of elements in soil extracts, plant digests, etc. It works on the principle of emission spectroscopy, but the instrument can simultaneously measure 20-40 (or more) elements in the aliquot within seconds. Soil-plant analysis laboratories which have an ICP, would generally use it for determining a wide range of macro- and micronutrients and heavy metals, etc.

While automated laboratory equipment has obvious advantages over traditional ones, there are disadvantages or drawbacks associated with it. For example, cost of an ICP is extremely high, prohibitive in many cases, it needs a stable electric supply and an effective air-conditioning system; its supplies (like argon gas) as well as repair and maintenance (spares and technician cost), are very expensive. Additionally, an adequately qualified scientist is needed to operate and look after the instrument.

Therefore, one should opt for such type of sophisticated and expensive equipment only and only if there is adequate justification. If a laboratory has enough throughput to justify the investment and maintenance costs, and repair and maintenance are not a handicap, then it is worthwhile to have such automated laboratory equipment. In resource-poor and handicapped situations, however, the same automation can prove to be a curse. Therefore, managers of soil-plant analysis laboratories in the CWANA region should consider all the pros and cons before choosing such type sophistication.

### Box 3

#### SOIL AND WATER ANALYSIS FOR ENVIRONMENTAL POLLUTION

Soil and or water pollution can occur because of heavy metal-contaminated sewage sludge, land mining, excessive rates of N fertilizer application, and other activities. While the main focus of Soil-Plant Analysis Laboratories remains production agriculture, many laboratories in the CWANA region are quite alert to the increasing concern about environmental pollution. The most common environmental concerns relating to soil and water are those of pollution from excess nitrate, heavy metals, and toxic organic compounds. With increased sophistication in methodologies and equipment, any reasonably equipped laboratory can, in fact, deal with both agricultural and environmental aspects. Some generalized procedural guidelines regarding soil and water analysis for environmental pollution are summarized below.

##### Soil Analysis for Heavy Metals

Common concerns about soil pollution relate to excessive contents of heavy metals like lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), selenium (Se), etc. While analysis for total heavy metal contents is a tedious task, easier soil test methods can provide reliable indices for heavy metal content of soils and their availability to plants. However, the method used should be suitable for the soil type and the metal being analyzed.

For alkaline soils of the CWANA region, for example, the DTPA test of Lindsay and Norvell (1978) provides reliable index values for evaluating plant availability of heavy metals. Similarly, the AB-DTPA test (Soltanpour and Workman, 1979) is equally effective for screening soils contaminated with heavy metals. However, appropriate criteria should be used in interpreting the laboratory data.

##### Water Analysis for Nitrate, Hardness, Heavy Metals

Water analysis for soluble constituents is perhaps the simplest work to do for any Soil-Plant Analysis Laboratory, simply because no dissolution or extraction of ions or metals is involved; measurements are made directly. Moreover, procedures for various parameters are for soils and plants. For example, nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) content in water is measured exactly the same way as in soil extracts (Section 6.1.4).

Water hardness, commonly expressed as milligrams of calcium carbonate equivalent per liter, can also be measured adopting the respective procedures as described for soluble calcium (Ca) (Section 6.5) and soluble carbonate (Section 6.6) in soils. No additional equipment or reagents are required.

Interpretation of laboratory data, however, must be made using relevant criteria. Some possible sources of information for the purpose are WHO (1996), National Research Council (1977), Sonneborn et al. (1983), and Wigle et al. (1986).

## Box 4

### IRRIGATION WATER QUALITY

The concentration and composition of dissolved salts in any water determine its quality for irrigation. Mostly the concerns with irrigation water quality relate to possibility of high salt concentration, sodium hazard, carbonate and bicarbonate hazard, or toxic ions (e.g., boron or chloride). The analyses required for determining water quality include EC, soluble anions and cations. As all of these determinations are more or less a routine matter for any soil-plant analysis laboratory, all laboratories in the CWANA region can perform analyses for evaluating its quality for irrigation purposes. The EC of irrigation waters is usually expressed in units of deciSiemens per meter ( $\text{dS m}^{-1}$ ) at  $25^{\circ}\text{C}$ .

### CALCULATIONS

$$\text{Sodium Adsorption Ratio (SAR)} = \frac{\text{Na}^+}{\frac{\text{p}(\text{Ca}^{++} + \text{Mg}^{++})}{2}} \dots\dots\dots (69)$$

Where:  $\text{Na}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  represent the concentrations in meq/L of the respective ions in water (or solution).

$$\text{Residual Sodium Carbonate (RSC)} = (\text{CO}_3^{--} + \text{HCO}_3^-) - (\text{Ca}^{++} + \text{Mg}^{++}) \dots\dots\dots (70)$$

Where: The anion and cation concentrations in water/solution are in meq/L.

Thereafter, water quality can be determined by interpreting the data using the following guidelines:

<u>Quality</u>	<u>EC</u> <u>(dS/m)</u>	<u>Sodium Adsorption</u> <u>Ratio</u>	<u>Residual Sodium</u> <u>Carbonate (meq/L)</u>
Suitable	<1.5	<7.5	<2.0
Marginal	1.5 - 2.7	7.5 - 15	2.0 - 4.0
Unsuitable	>2.7	>15	>4.0

Source: Muhammed (1996).

Boron concentration in irrigation water is considered safe only up to 0.7 ppm, while sodium and chloride concentrations of less than 70 and 140 ppm, respectively, are considered safe (Muhammed, 1996).