6. Plant Sampling and Processing

The effects of time of sampling, variety or hybrid and environmental factors, such as soil moisture, temperature, and light quality and intensity can significantly affect the relationship between nutrient concentration and plant response. Therefore, it is important that they be aware of the necessity of proper sampling. Otherwise, analyses that they are asked to perform on plant samples may end up to be meaningless and a waste of time. The analytical procedures described here are derived from well established reference materials in the literature, e.g., Walsh and Beaton, 1973; Westerman, 1990; Reuter and Robinson, 1986: Sparks et al., 1996).

6.1. Field Processing

Preparation for Sampling

Preparation of a field trip for plant sampling has to be planned in advance. Always contact the people who will accompany you to the field for the necessary preparations, as follows:

- Plant samples must be put in labeled, perforated plastic bags or paper bags.
- Tags and markers are required.
- The bags should be examined for cleanliness as well as for strength.
- Plant samples can be transported to the laboratory in cardboard boxes.
- All information about samples is recorded; each sample is given a laboratory number.
- Clean tray or a clean cloth for collecting the plant and sub-sampling.
- Sketch your field. Diagram it the way you sampled it. Be sure the sampled areas are labeled the same as sample containers. (This is so you have a record of which recommendations apply to which areas – do not rely on your memory).
- Fill out the information sheets, writing clearly with a copying pen. The more information you can provide with each sample, the better your recommendation will be.

Where to Take Sample?

All plant samples taken from abnormal areas should be taken from *just inside* of the abnormal area. A separate plant analysis history must be completed for each sample taken as follow:

Uniform Fields

Where plant growth is uniform over the entire area, one composite sample is taken from at least 10 widely scattered areas in the field. One plant sample is necessary. One soil sample is recommended.

Non-uniform Fields

In areas where crop growth or appearance of one area differs from the rest of the field, plant analysis can often determine the cause of these differences and indicate the best method to correct the problem. Sample when abnormalities are discovered. Two plant and two soil samples are required. This includes collecting soil and plant samples from the normal area.

Sampling Time

- The recommended time to sample usually occurs just prior to the beginning of the reproductive stage for many plants. However, sampling earlier or even later than the specified time may be recommended for specific plants or circumstances.
- Sample plants that are showing a suspected nutrient deficiency symptom at the time or shortly after the visual symptoms appear.

Amount of Plant Material

All plant analyses require at least a rounded double handful of plant tissue.

What to Sample

- Leaves are most commonly chosen: recently matured ones are taken but new and old growth is generally avoided. However, young emerging leaves are sampled for diagnosing iron (Fe) chlorosis by determining ferrous (Fe) content of fresh leaves (Katyal and Sharma, 1980) and B content in certain crops (Bell, 1997). Damaged or diseased leaves are excluded, and plants should not be sampled when the crop is under moisture or temperature stress.
- Petioles are selected for certain crops, e.g., cotton, sugar beet.
- Seeds are rarely used for analysis, except for assessing of B toxicity, Zn and P deficiency in certain grain crops. In some cases, e.g., cereals, the entire above-ground young plants are sampled.
- Avoid any type of contamination at all stages. Plant samples should never be kept in the store along with fertilizer materials and detergents. Contamination is likely when the plant samples are spread out to dry near stored fertilizers or on floor where fertilizers were stored previously.
- Sampling procedures for important dryland crops of the WANA region are given in **Appendix 10**.

What Not to Sample

- Do not include diseased or dead plant material in a sample.
- Do not sample or include plants or leaf tissue that have been damaged by insects or mechanically injured in a sample. When whole plants are sampled, remove the roots and wash the upper portion to remove soil particles.
- Do not sample plants that have been stressed extensively by cold, heat, moisture deficiency, or by excess moisture. Examine both the below-ground as well as the above ground-ground portion of the plant. The presence of nematodes or roots damaged by other insects or diseases should preclude the need to sample.

Shipment of the Plant Material Sample

- Avoid decomposition during transport to the laboratory, which makes them useless for analysis purposes. Therefore, samples should be taken to the laboratory as quickly as possible.
- A history form goes in the small envelope, which is then placed inside the large envelope containing the dried sample.

6.2. Laboratory Processing

Sample preparation is critical in obtaining accurate analytical data and reliable interpretation of plant analysis results. Proven procedures must be followed during handling in the laboratory, decontamination, drying, grinding and mixing, and storage. Such preparatory procedures enhance the accuracy and reliability of the analytical results.

Handling in the laboratory

- As soon as the plant samples are received at the plant preparation laboratory, they should be checked with the accompanying information list. Information regarding samples should be entered in a register and each sample be given a laboratory number.
- Keep plant samples refrigerated until cleaning. Take care that fermentation does not occur.

Decontamination

Decontamination procedures involving washing and rinsing should only be used for fresh, fully-turgid plant samples. After decontamination, samples should be dried immediately to stabilize the tissue and stop enzymatic reactions.

A. Reagents and Apparatus

- Deionized water
- 0.1 to 0.3 % detergent solution (non-phosphate)
- Medium-stiff nylon bristle brush
- Plastic containers suitable for washing and rinsing tissue samples

B. Cleaning processing

- A preliminary dry-wiping can be done if the plant sample is very dirty.
- If the plant samples are too dirty and a dry-wiping is not possible, washing through the nylon bag can be done.
- The samples must be properly cleaned, but no part of it should be under water for more than a few seconds.
- Cleaning plant tissue to remove dust, pesticide and fertilizer residues, normally by washing the plants with DI water or with 0.1 – 0.3 % P-free detergent (like HCl 1%), followed by DI water.
- Rinse each portion of the plant sample into a bath of DI water, into which it is plunged, agitated, and immediately withdrawn. Change the water and repeat the rinsing. Dry by shaking vigorously by hand.
- Plant samples for soluble element determination may not be washed, particularly for long periods. However, **plant samples for total Fe analysis must be washed**.
- Excessive washing is worse than no decontamination since soluble elements, including B, K, and N, are likely to leach from the tissue.
- The wash and rinse periods should be as short as possible to avoid danger of N, B, K, and Cl leaching from the tissue.

Drying

Water is removed from plant tissue to stop enzymatic reactions and to stabilize the sample. Enzymes present in plant tissue become inactive at temperatures above 70 ^{o}C. As a result, air-drying may not stabilize samples and prevent enzymatic decomposition. Samples should, therefore, be properly dried as soon as possible after taking the sample. Some technical guidelines are as follows:

- The plant sample material should be evenly and thinly spread in a container.
- Place containers in well-ventilated drying oven.
- If samples absorb significant amounts of moisture during grinding, additional drying may be required prior to weighing for analysis.
- **Drying time required will vary**. Dry to constant weight.
- The original condition and sample size will affect drying time.
- The drying temperature should not exceed 70 ^{o}C, because higher temperatures may cause volatilization loss.
- Drying at temperatures less than 70 ^{o}C may not remove all combined water and may result in poor homogenization and incorrect analytical results.
- Drying temperatures above 70 ^{o}C may result in thermal decomposition and reduce dry weight.
- A drying time of 24 hours may be sufficient in normal conditions.
- Drying times longer than 24 hours may be required depending on the type and number of plant samples in the dryer.
- Quick drying of a limited number of samples can be done using a microwave oven and the drying process is closely monitored.

Grinding and Mixing

Plant tissue samples are reduced to 0.5 to 1.0 mm particle size to ensure homogeneity and to facilitate organic matter destruction.

A. Apparatus

- Standard mills equipped with 20, 40, and 60-mesh screens and stainless steel contact points.
- Tecator Cyclotec sample mill (standard equipped with a 1-mm sieve) or equivalent high-speed grinder.
- Medium bristle brush.
- Vacuum system.

B. Procedure

- After drying, samples should be ground to pass a 1.0-mm screen (20 mesh) using the appropriate Wiley mill. A 20-mesh sieve is adequate if the sample aliquot to be assayed is >0.5 g. However, if the sample aliquot to be assayed is less than 0.5 g, a 40-mesh screen should be utilized.
- After grinding, the sample should be thoroughly mixed and a 5 to 8 g aliquot withdrawn for analyses and storage.

Notes

- Using a brush or vacuum system, clean the grinding apparatus after each sample.
- Uniform grinding and mixing are critical in obtaining accurate analytical results.

- Exercise care when grinding very small samples or plant material that is pubescent, deliquescent, or that has a fibrous texture. These samples are difficult to grind in Wiley mills and the operator should allow sufficient time for the sample to pass through the screen to ensure homogeneity. In these instances, Cyclotec or equivalent high-speed grinders are preferable.
- Most mechanical mills contribute some contamination of the sample with one or more elements. The extent of contamination depends on condition of the mill and exposure time
- Use stainless steel for cutting and sieving surfaces to minimize contamination.
- Routine maintenance should be made on mills to ensure optimum operating conditions.
- Cutting knives or blades should be maintained in sharp condition and in adjustment.
- Avoid cross-contamination from one sample to the next by cleaning the sample mill thoroughly with a dry brush or by using dry air under pressure.
- If the plant sample is big enough, the mill can be rinsed with the material to be grinded.
- When sampling mixed stands particularly forages and pastures, separate plant species. Similarly, the sample should be of only leaves or petioles or whole tops and not mixtures.

Storage

After grinding and mixing (homogenization), samples should be stored in conditions that minimize deterioration and maintain sample integrity for weighing and follow-up analytical work.

Apparatus

- Airtight plastic storage containers
- Storage cabinet located in cool, dark, and moisture-free environment
- Refrigerator

Procedure

- After grinding and homogenization, a representative sub-sample is taken from the ground plant material for analyses and storage, which should be placed in a container that can be securely sealed.
- Containers should then be placed in a cool, dry place for storage.
- For long-term storage, ground samples should be thoroughly dried, sealed, and placed under refrigerated conditions (4 ^{o}C) until the required analysis can be completed.
- The dried and milled samples should be stored in a cool and dry place in flasks with tight stoppers or in sealed polyethylene bags, protected against direct sunlight.
- During storage, the plant material may attract moisture so that the drying procedure must be repeated just before weighing out a sample for analysis.
- Dry the sample 1 hour on 70 ^{o}C before analysis.

Technical Remarks

1. If samples are placed in a cool (4 ^{o}C), dark, dry environment, storage life is indefinite.
2. Small manila envelopes can also be used for sample storage, but care must be taken to prevent absorption of moisture. Collect the ground sample in the envelope and immediately place in a desiccator cabinet or desiccator to minimize moisture absorption.

7. Plant Analysis

After soil testing, plant analysis is critical to improving crop nutrition and yield. **From the nutritional standpoint, plant analysis is based on the principle that the concentration of a nutrient within the plant is an integral value of all the factors that have interacted to affect it**. The principles and procedures used for plant analyses have evolved over many years and changed as knowledge increased about each element that is essential for a plant to complete its life cycle. As such, the use of plant analyses has become an integral part of most agronomic research and a tool for crop consultants and fertilizer dealers to monitor production fields.

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), Munson and Nelson (1990) and Jones et al. (1991).

Effective Uses for Plant Analyses

Plant analysis is an effective management strategy for a sustainable soil fertility program because it provides a direct measure of nutrient concentrations and balance within the plant. Therefore, the effective uses for plant analysis are as follow:

- Confirm a diagnosis made from visible symptoms
- Identify “hidden hunger” where no symptoms are apparent
- Locate soil areas where deficiencies of one or more nutrients occur
- Determine whether applied nutrients have entered the plant
- Indicate among various nutrients
- Study the internal functioning of nutrients in plants
- Suggest additional tests or studies in identifying a crop production problem.

7.1. Moisture Factor

Weighing of oven-dried samples is, however, cumbersome (involves continuous oven-drying and use of desiccators, and is still prone to error), as plant material may absorb moisture during the weighing process, particularly if the relative humidity is high in the laboratory. To get around this difficulty, use moisture factor instead. ***The moisture factor*** *for each batch of samples can be calculated as follows: by oven-drying only a few sub-samples from the lot (say about 5 from a batch of 100 – 200 samples).*

$$\boldsymbol{Moisture\ Factor} = \frac{\boldsymbol{Weight\ of\ air-\ dry\ sample\ (g)}}{\boldsymbol{Weight\ of\ oven-\ dry\ sample\ (g)}}$$

Thereafter, air-dry samples are weighed, considering the moisture factor.

7.2. Nitrogen

Plants need a wide range of proteins to grow, develop and mature*. The main body of protein is amino acids and nitrogen (N) is the major component of amino acids. Nitrogen is also present in chlorophyll. Soil micro-organisms feed on soil N during breakdown of organic materials. Nitrogen improves quality of leafy vegetables. It promotes rapid growth and if the supply is out of balance with other nutrients flowering and fruiting may be delayed.*

The N content of plant dry matter *generally ranges from 1 to 5 %. However occasionally it may be either lower or higher than this range. The common plant analysis is that of N by Kjeldahl method. However, wet ashing with H_2SO_4 and H_2O_2 is also used for eliminating the standard use of selenium (Se) in the former method.*

7.2.1. Kjeldahl Nitrogen (with Catalyst)

Apparatus

Block-digester

Distillation unit

Automatic titrator connected to a pH-meter

Vortex tube stirrer

Reagents

A. Catalyst Mixture (K_2SO_4-Se), 100:1 w/ w ratio

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

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

C. Sodium Hydroxide Solution (NaOH), 10 *N*

Dissolve 400 g *NaOH* in DI water, transfer to a 1-L 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 H_3BO_3 to a 5-L flask.
- Add 3 L DI water, and swirl vigorously.
- Leave overnight.

Note

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 the solution to a 1-L flask, and bring to volume with DI water.
-

F. Sulfuric Acid Solution (H_2SO_4), 0.01 *N*

- Add 28 mL concentrated H_2SO_4 to about 600 – 800 mL DI water in a 1-L flask, mix well, let it cool, and bring to volume. This solution contains 1 *N* H_2SO_4 solution (*Stock Solution)*.
- Pipette 10 mL *Stock Solution* to 1-L flask, and bring to volume with DI water. This solution contains 0.01 *N* H_2SO_4.
-

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 $(NH_4)_2SO_4$ in DI water, and bring to 1-L volume with DI water. This solution contains 1.2 g NH_4-N/L (*Stock Solution*).

Procedure

A. Pre-treatment

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.

B. Digestion

1. Weigh 0.25 g (grain) or 0.50 g (straw) of dry plant material, and transfer quantitatively into a 100-mL digestion tube.
2. Add a few **pumice boiling granules**, and add about 3 g **catalyst mixtures** using a calibrated spoon.
3. Add 10 mL **concentrated H_2SO_4** using a dispenser, and stir with Vortex tube stirrer until mixed well.
4. 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 H_2SO_4**.
5. Thoroughly agitate the tube contents, and then place the tubes back on the block-digester set at 380 °C for 2 hours after clearing.
6. After digestion is complete, remove tubes, cool, and bring to 100-mL volume with DI water.
7. 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).

C. Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. 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. The **H_2SO_4** normality is:

$$N_{H_2SO_4} = \frac{10 \times N_{Tris}}{V_{H_2SO_4}}$$

3 Carry out distillations as follows:

- Dispense 1 mL **saturated H_3BO_3** 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.
- Carefully add 10 mL **10 *N* NaOH** solution, and immediately connect the flask to the distillation unit and begin distillation.
- Collect about 35 mL distillate in the collecting dish.
- 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.
- Titrate the distillate to pH 5.0 with standardized **0.01 *N* H_2SO_4** using an **Auto-Titrator**; record titration volume of acid.
- Each distillation should contain at least two standards (pipette 10 mL digested solution), two blanks (pipette 10 mL digested solution). Recovery of N should be at least 96 %.

Notes: 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

$$\textbf{Recovery}\ (\%) = \frac{(\boldsymbol{V - B}) \times \boldsymbol{N} \times \mathbf{14.01} \times \mathbf{100}}{\boldsymbol{V_1} \times \boldsymbol{C}}$$

$$\textbf{Recovery of EDTA}\ (\%) = \frac{(\boldsymbol{V - B_1}) \times \boldsymbol{N} \times \boldsymbol{V_2} \times \mathbf{186.1} \times \mathbf{100}}{\boldsymbol{Wt_1} \times \boldsymbol{V_3} \times \mathbf{1000}}$$

$$\boldsymbol{N}\ (\%) = \frac{(\boldsymbol{V - B_1}) \times \boldsymbol{N} \times \boldsymbol{V_2} \times \mathbf{14.01} \times \mathbf{100}}{\boldsymbol{Wt_2} \times \boldsymbol{V_3} \times \mathbf{1000}}$$

Where:

V = Volume of 0.01 *N* H_2SO_4 titrated for the sample (mL)

V_2 = Tolat volume of the plant digest (mL)

V_3 = Volume of plant digest used for distillation (mL)

B = Distillate blank titration volume (mL)

B_1 = Digested blank titration volume (mL)

Wt_1 = Weight of EDTA (g)

V_1 = Volume of NH_4-N standard solution (mL)

Wt_2 = Weight of dry plant (g)

N = Normality of H_2SO_4 solution

C = Concentration of NH_4-N standard solution (µg/mL)

14.01 = Atomic weight of N

186.1 = Atomic weight of EDTA

Technical Remarks

1. Samples should contain about 1 mg of N (but no more than 5 mg). The sensitivity of the procedure depends upon a number of factors, including sample weight, strength of the acid, and accuracy of titration.
2. If the ratio of acid to salt is low at the end of the digestion step, a significant amount of NH_3 may be volatilized during the digestion process. Other situations which may cause N loss during digestion and should be avoided are: localized heating in the digestion flask (temperatures above 410°C), and the use of 30% hydrogen peroxide (H_2O_2) as an oxidant.
3. The time required for digestion is affected by the catalyst, temperature, and type of plant tissue. It is important to allow an equal length of additional time after the sample clears. As much as 10% of the organic N may not yet be converted to NH_4 at clearing.
4. **Homogeneity of the sample is important for precision**. For best results, dried tissue should pass a 40-mesh sieve. In cases where sample size is less than 0.25 g, special care should be taken to insure sample homogeneity.
5. Digested samples may be stored for several days, provided samples are covered and placed in a cool area.
6. Samples distilled into the H_3BO_3 solution should be titrated within a short time to avoid absorption of atmospheric CO_2.

7.2.2. Kjeldahl Nitrogen (Without Catalyst)

*The routine use of **heavy metals as catalysts can be harmful environmentally.** Therefore, treatment of plant material with a mixture of H_2O_2-H_2SO_4 in the absence of metal catalysts is proposed **as an alternative digestion procedure for replacing the standard Kjeldahl-N** determination in soils and plants.*

Reagents

Sulfuric Acid (H_2SO_4), concentrated

Hydrogen Peroxide (H_2O_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 H_2SO_4,** mix well.
3. Keep overnight.
4. Heat on a block-digester at a moderate temperature 100 – 150 °C.
5. Swirl to restrict foaming. If foaming enters the neck of the digestion tube, add 2 mL **30 % H_2O_2**.
6. Heat the tubes for 30 – 60 minutes on the block-digester.
7. Cool the tubes, and then add 2 mL **30 % H_2O_2**.
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 **30 % H_2O_2**, and heat for 10 minutes.
11. Repeat Steps 9 and 10 until solution remains clear after 10 minutes of heating.
12. Cool, and make up to 100-mL volume with DI water.

Measurement

1. Nitrogen content can be measured in this digest by the **Distillation Method (Kjeldahl unit)**.
2. Phosphorus can be done **colorimetrically** as be used for P measurement in plants, after filtering the digest through Whatman No. 1 or No. 5 filter paper.

Note

The results for both N and P are highly correlated with the standard Kjeldahl digestion method.

7.2.3. Total Nitrogen (Sulfuric-Salicylic Acid Mixture)

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 H_2SO_4 containing 2.5 % w/v salicylic acid)
Dissolve 62.5g reagent-grade *salicylic acid* ($C_7H_6O_3$) in 2.5 L *concentrated H_2SO_4*.

B. Catalyst Mixture (K_2SO_4 - Se), 100:1 w/w ratio
Grind reagent-grade chemicals separately and mix. If caked, grind the mixture in 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.

C. Sodium Thiosulfate ($Na_2S_2O_3.5H_2O$), crystal

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

Procedure

A. Pre-treatment

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 °C in an oven (overnight), and then cool in a desiccator.

B. Digestion

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_2SO_4, 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***).

C. Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. 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. The **H_2SO_4** normality is:

$$N_{H_2SO_4} = \frac{10 \times N_{Tris}}{V_{H_2SO_4}}$$

3. Carry out distillations as follows:
   - Dispense 1 mL **saturated H_3BO_3** 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.
   - Prior to distillation, shake the digestion tube to thoroughly mix its contents, and pipette an aliquot in a 300-mL distillation flask.
   - Carefully add 7 mL **10 *N* NaOH** solution for 25-mL aliquot (or 15 mL **10 *N* NaOH** solution for 50-mL aliquot), and immediately connect flask to distillation unit and begin distillation.
   - Collect about 35-mL distillate in the collecting dish.
   - 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.
   - The distillate is then titrated to pH 5.0 with standardized **0.01 *N* H_2SO_4** using an **Auto-Titrator**; record titration volume of acid.
   - Each distillation should contain at least two standards and two blanks (reagent blanks). Recovery of EDTA, corrected for reagent blank, should be at least 97%.

Notes: 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.

Appendix 3 (Contd….)

Fluorine	F	9	18.9984	Silicon	Si	14	28.086
Francium	Fr	87	233*	Silver	Ag	47	107.87
Gadolinium	Gd	64	157.25	Sodium	Na	11	22.9898
Gallium	Ga	31	69.72	Strontium	Sr	38	87.62
Germanium	Ge	32	72.59	Sulfur	S	16	32.064
Gold	Au	79	196.967	Tantalum	Ta	73	180.948
Hafnium	Hf	72	178.49	Technetium	Tc	43	99*
Helium	He	2	4.0026	Tellurium	Te	52	127.6
Holmium	Ho	67	164.93	Terbium	Tb	65	158.925
Hydrogen	H	1	1.0079	Thallium	TI	81	204.37
Indium	In	49	114.82	Thorium	Th	90	232.038
Iodine	I	53	126.904	Thulium	Tm	69	168.934
Iridium	Ir	77	192.2	Tin	Sn	50	118.69
Iron	Fe	26	55.847	Titanium	Ti	22	47.9
Krypton	Kr	36	83.8	Tungsten	W	74	183.85
Lanthanum	La	57	138.91	Uranium	U	92	238.03
Lawrencium	Lr	103	257*	Vanadium	V	23	50.9412
Lead	Pb	82	207.19	Xenon	Xe	54	131.3
Lithium	Li	3	6.94	Ytterbium	Yb	70	173.04
Lutetium	Lu	71	174.97	Yttrium	Y	39	88.906
Magnesium	Mg	12	24.312	Zinc	Zn	30	65.37
Manganese	Mn	25	54.938	Zirconium	Zr	40	91.22
Mendelevium	Md	101	258*				

Appendix 4. Solution Concentrations

System Name	Abbreviation	Definition
Molar	M	gram-molecular weight (mole
Molal	M	of solute) per liter of solution gram-molecular weight (mole
		of solute) per kilogram of solvent
Formal	F	gram-formula weight of solute
Normal	N	per liter of solution gram-equivalent weight of solute per liter of solution
Weight per volume, percent	w/v, %	number of grams of solute × 100 per volume of solvent (mL).
Volume percent	Volume % or v/v %	Volume of solute × 100 per volume of solution.
Weight percent	wt % or w/w %	Weight of solute × 100 weight of solution.
Parts per million	ppm	milligrams of solute or milligrams per liter of solution kilogram
Parts per billion	ppb	micrograms of solute or micrograms per liter (kilogram) of solution.

Appendix 5. Some Useful Relationships

1 g = 1000 mg = 1,000,000 μg	ppm = μg/g (solid per liquid)
1 μg = 0.001 mg = 0.000001 g	ppm = μL/L (liquid per liquid)
1 L = 1000 mL	ppm × 2 = 1lbs/A
1 mL = 0.001 L	ppm × 10^{-4} = %
ppm = μg/mL (solid per liquid)	1% = 1 gm/100 ml
ppm = mg/L (solid per liquid)	1% = 10,000 ppm

Appendix 6. Concentration Normality, Amount of Concentrated Acids, and Bases to Make of 1 N Solution (1-L)

Acid or Base	**Chemical Properties**				**Solution**
	Specific	**Percent**	**Grams**	**Approximate**	**Needed**[1]
	Gravity	**by Weight**	**per Liter**	**Normality (*N*)**	**(mL)**
Acetic acid	1.05	99.0	1042.0	17.45	58
Ammonium	0.90	28.3	255.0 (NH_3)	15.0	67
hydroxide					
Hydrochloric acid	1.19	38.0	451.6	12.4	81
Hydrofluoric acid	1.16	50.0	577.5	28.8	35
Nitric acid	1.42	72.0	1024.0	16.2	62
Phosphoric acid	1.69	85.0	1436.0	44.0	23
Perchloric acid	1.66	70.0	1165.0	11.6	86
Sodium hydroxide	1.53	50.0	762.7	19.0	53
Sulfuric acid	1.84	96.0	1742.0	35.5	28

[1]To make up 1-L of 1 *N*

Appendix 7. Soil pH Levels and Associated Conditions

Soil pH	Indications	Associated Conditions
< 5.5	Soil is deficient in Ca and /or Mg, and should be limed	Poor crop growth due to low cation exchange capacity and possible Al^{3+} toxicity. Expect P deficiency.
5.5 – 6.5	Soil is lime-free, should be closely monitored.	Satisfactory for most crops
6.5 – 7.5	Ideal range for crop production.	Soil cation exchange capacity is near 100% base saturation.
7.5 – 8.4	Free lime ($CaCO_3$) exists in soil.	Usually excellent filtration and percolation of water due high Ca content on clays. Both P and micronutrients are less available.
>8.4	Invariably indicates sodic soil.	Poor physical conditions. Infiltration and percolation of soil water is slow. Possible root deterioration and organic matter dissolution.

Source: Hach Company, USA (1992).

Appendix 8. Summarized Soil Test Methods for Fertility Evaluation

Parameter	**Olsen P**	**AB-DTPA**	**NH_4OAc**	**DTPA**	**Hot water**
Property/		NO_3-N, P, K,	K, Mg, Na, Ca	Zn, Cu, Fe, Mn	B
Nutrient(s)		Zn, Cu, Fe, Mn			
Sample size (g)	2.5	10	5	10	10
Volume-extractant (mL)	50	20	25	20	20
Extracting solution	0.5 M $NaHCO_3$	1 M $NH_4HCO_3^+$	1 N NH_4Oac	0.005 M $DTPA^+$	H_2O
	at pH 8.5	0.005 M DTPA	pH 7.0	0.01 M TEA^+	
		(pH7.6)		0.01 M $CaCl_2$ (pH7.3)	
Shake/boil (minutes.)	30	15	5	120	5
Shaking action and speed: All use reciprocating, 180+ oscillations/minutes., except for B					
Extraction method	Colorimetry, at 880nm (Molybdenum blue)	P: Colorimetry, K: Flame emission Zn, Cu, Fe, Mn: AAS	K& Na: Flame emission Mg & Ca: AAS	AAS	Colorimetry, at 430 nm (Azomethine-H)
Soil nutrient conc.,	P, 2 – 200	P, 2 – 100; K, 5 – 750;	K, 50 – 1000;	Zn, 0.5 – 20	B, 1 – 10
no dilution (ppm)		Zn, 0.5 – 35	Ca, 500 – 2000;		
			Mg, 50 – 500;		
			Na, 10 – 250		
Primary reference	Olsen *et al.* (1954)	Soltanpour & Schawb (1977)	Schollenberger& Simon (1945)	Lindsay & Norvell (1978)	Berger & Truog (1939)

Source: Soil and Plant Analysis Council (1992). AAS = atomic absorption spectrophotometer.

Appendix 9. Generalized Guidelines for Interpretation of Soil Analysis Data

Nutrient /Organic Matter	Soil Test	Low	Marginal	Adequate
		---------- % ----------		
Organic matter	Walkley- Black	<0.86%	0.86 – 1.29%	>1.29
		----------ppm----------		
Nitrate	AB-DTPA	<11	11 – 20	>20
Phosphate	$NaHCO_3$	<8	8 – 15	>15
	AB-DTPA	<4	4 – 7	>7
Potassium	NH_4OAc	<100	100-150	>150
	AB-DTPA	<60	60 – 120	>120
Zinc	DTPA	<0.5	0.5 – 1.0	>1.0
	AB-DTPA	<1.0	1.0 – 1.5	>1.5
Copper	DTPA	<0.2	0.2 – 0.5	>0.5
	AB-DTPA	<0.2		>0.5
Iron	DTPA	<4.5		>4.5
	AB-DTPA	<2.0	2.1 – 4.0	>4.0
Manganese	DTPA	<1.0	1.0 – 2.0	>2.0
	AB-DTPA	<1.8		>1.8
Boron	Hot water	<0.5	0.5 – 1.0	>1.0
	HCl	<0.45	0.45 – 1.0	>1.0

DTPA= diethylene triamine pentoacetric acid. AB = ammonium bicarbonate.

$NaHCO_3$ = Sodium bicarbonate.

Sources: FAO (1980); Soltanpour (1985); Ludwick (1995); Martens and Lindsay (1990); Johnson and Fixen (1990); Soil and Plant Analysis Council (1992); Matar *et al.* (1988).

Appendix 10. Suggested Plant Tissue Sampling Procedures for Selected Dryland Crops1

Growth Stage	Plant Part to Sample	Plants Sampled
	Wheat and Barley	
Seeding stage (< 30 cm tall)	All the aboveground portion	50 – 100
Before head emergence	Flag leaf	25 – 50
	Corn	
Seedling stage (< 30 cm tall)	The entire aboveground shoot. The entire	20 – 30
Prior to tasselling	leaf fully developed below the whorl	15
From tasselling to silking	The entire leaf at the ear node (or	
	immediately above or below it)	15 – 25
	Sorghum	
Prior to or at heading	Second or 3rd leaf from top of plant	15 – 25
	Soybean or other Beans	
Seedling stage (<30 cm tall)	All the above ground portion	20 – 30
Prior to or during	Two or three fully developed	20 – 30
initial flowering	Leaves at the top of the plant	
	Peanut	
Maximum tillering	Recently matured leaflets	25
	Alfalfa, Clover and other Legumes	
Prior to or at 1/10th	Mature leaf blades taken about	40 – 50
bloom stage	One-third of the way down the plant	
	Food Legumes including Chickpea and Lentil	
Vegetative growth stage	Whole shoots	40 – 50
Bloom initiation	Recently matured leaf	50 – 200

[1]When specific guidelines are unknown; the general *rule of the thumb* is to sample *upper mature* leaves at flower imitation.

Sources: Jones *et al.* (1971, 1991); Reuter and Robinson (1986); Tandon (1993).

Appendix 11. Generalized Interpretation of Nutrient Concentrations in Cereal Plant Tissues Sampled at Boot Stage (Feekes Stage 10.1)

	Nutrient Concentration in Dry Tissue			
Nutrient	**Deficient**	**Low**	**Sufficient**	**High**
	----------%----------			
Nitrogen (winter grains)	<1.25	1.25 – 1.74	1.75 – 3.00	>3.00
(spring grains)	<1.50	1.50 – 1.99	2.00 – 3.00	>3.00
	<0.15	0.15 – 0.19	0.20 – 0.50	>0.50
Phosphorus	<1.25	1.25 – 1.49	1.50 – 3.00	>3.00
Potassium		<0.20	0.20 – 0.50	>0.50
Calcium (wheat, oats)		<0.30	0.30 – 1.20	>1.20
(barley)		<0.15	0.15 – 0.50	>0.50
Magnesium Sulfur		<0.15	0.15 – 0.40	>0.40
	----------ppm----------			
Manganese	<5	5 – 24	25 – 100	>100
Zinc		<15	15 – 70	> 70
Copper		<5	5 – 25	> 25

Source: Walsh and Beaton (1973).

Appendix 12. Classification Criteria for Salt-Affected Soils

Soil	ECe[1]	Exchangeable Sodium Percentage (ESP)	Sodium Adsorption Ratio (SAR)
	--dS/m--		
Normal	<4	<15	<15
Saline	=4	<15	<15
Sodic	<4	>15	=15
Saline-Sodic	=4	>15	=15

[1]EC in saturated paste extract

Source: Bohn *et al.* (1985).

Appendix 13. Soil Salinity Classification

Soil Texture	Degree of Salinity (Electrical Conductivity)[1]				
	None	Slight	Moderate	Strong	Very Strong
	---------- dS/m ----------				
Coarse sand to sandy loam	<1.2	1.2 – 2.4	2.5 – 4.4	4.5 – 8.9	>9.0
Loamy fine sand to loam	<1.3	1.3 – 2.4	2.5 – 4.7	4.8 – 9.4	>9.5
Silt loam to clay loam	<1.4	1.4 – 2.5	2.6 – 5.0	5.1 – 10.0	>10.1
Silty clay loam to clay	<1.5	1.5 – 2.8	2.9 – 5.7	5.8 – 11.4	>11.5

[1]EC in 1:1 soil/water suspension

Source: Hach Company (1992).

Appendix 14. Relative Salt-Tolerance Limits of Crops

ECe[1]	Crop	ECe[1]	Crop
-dS/m-		-dS/m-	
	FORGE CROPS		
22.0	Kallar grass (*Leptochloa fusca*)	14.4	Sudan grass (*Sorghum sudanense*)
15.0	Bermuda grass (*Cynodon dactylon*)	9.0	Alfalfa (*Medicago sativa*)
13.5	Barley, hay (*Hordeum vulgare*)	10.3	Berseem (*Trifolium alexandrium*)
14.0	Mustard (*Brassia campestris, Glauca group*)	7.0	Cowpea (*Vigna unguiculata*)
	FIELD CROPS		
18.0	Barley, grain (*Hordeum vulgare*)	9.0	Sesbania (*Sesbania aculeata*)
15.0	Sugar beet (*Beta vulgaris*)	10.0	Sugarcane (*Saccharum officinarum*)
16.0	Cotton (*Gossypium hirsutum*)	8.0	Rice, paddy (*Oryza sativa*)
12.0	Safflower (*Carthamus tinctorius*)	6.0	Maize (*Zea mays*)
14.0	Sunflower (*Helianthus annuus*)	6.5	Flax (*Linum usitatissimum*)
13.0	Wheat (*Triticum aestivum*)	9.1	Cowpea (*Vigna unguiculata*)
10.0	Sorghum (*Sorghum bicolor*)	4.9	Groundnut (*Arachis hypogaea*)
8.0	Soybean (*Glycine max*)		
	VEGETABLE CROPS		
9.6	Beet, garden (*Beta vulgaris*)	5.0	Lettuce (*Lactuca sativa*)
8.0	Spinach (*Spinacia oleracea*)	5.0	Bell pepper (*Capsicum annuum*)
8.0	Tomato (*Lycopersicon esculentum*)	4.0	Onion (*Allium cepa*)
7.0	Cabbage (*Brassica oleracea*)	4.5	Carrot (*Daucus carota*)
6.0	Cauliflower (*Brassica oleracea*)	3.5	French or green bean (*Phaseolus vulgaris*)
6.0	Potato (*Solanum tuberosum*)	5.0	Radish (*Raphanus sativus*)
6.0	Sweet corn (maize) (*Zea mays*)	6.3	Cucumber (*Cucumis sativus*)
6.0	Sweet potato (*Ipomoea batatas*)	6.5	Turnip (*Brassica rapa*)
	FRUIT CROPS		
18.0	Date (*Phoenix dactylifera*)	8.4	Olive (*Olea europaea*)
6.7	Grape (*Vitis spp.*)	4.8	Lemon (*Citrus limon*)

4.9	Grapefruit (*Citrus paradisi*)	4.8	apple (*Malus sylvestris*)
4.8	Orange (*Citrus sinensis*)	4.8	Pear (*Prunus communis*)
4.1	Peach (*Prunus persica*)	2.5	Strawberry (*Fragaria spp*.)
3.7	Apricot (*Prunus armeniaca*)	8.4	Pomegranate (*Punica granatum*)
4.3	Plum and prune (*Prunus domestica*)	4.8	Walnut (*Juglans regia*)
4.1	Almond (*Prunus dulcis*)		

[1]EC corresponding to or causing 50% crop yields reduction.

Source: California Fertilizer Association (1980), Ayers and Westcot (1985).

Appendix 15. Relative Tolerance of Plant Species to Boron Toxicity

Crop species	Threshold Concentration -- mol B/m^3 --	Crop Species	Threshold Concentration --mol B/m^3--
SENSITIVE CROPS			
Lemon (*Citrus limon*)	0.028	Pea (*Pisum sativum*)	
Lima bean (*Phaseolus lunatus*)		Carrot (*Daucus carota*)	0.093
Blackberry (*Rubus sp.*)		Potato (*Solanum tuberosum*)	
Avocado (*Persea americana*)		Cucumber (*Cucumis sativus*)	
Orange (*Citrus sinensis*)		Lettuce (*Lactuca sativa*)	
Grapefruit (*Citrus paradise*)		Cabbage (*Brassica oleracea*)	
Apricot (*Prunus armeniaca*)		Celery (*Cepium graveolens*)	
Peach (*Prunus persica*)		Turnip (*Brassica rapa*)	
Cherry (*Prunus avium*)		Barley (*Hordeum vulgare*)	
Plum (*Prunus domestica*)		Corn (*Zea mays*)	
Persimmon (*Diosysos kaki*)		Artichoke (*Cynara scolymus*)	
Fig (*Ficus carica*)		Radish (*Raphanus sativus*)	
Grape (*Vitis vinifera*)		Tobacco (*Nicotiana tabacum*)	
Walnut (*Juglans regia*)		Sweet clover (*Melilotus indica*)	
Pecon (*Carya illinoensis*)		Squash (*Cucurbita pepo*)	
Cowpea (*Vigna sinensis*)		Muskmelon (*Cucumis melo*)	
Onion (*Allium cepa*)		**Tolerant Crops**	
Garlic (*Allium sativum*)		Sorghum (*Sorghum bicolor*)	
Sweet potato (*Ipomoea batatas*)		Alfalfa (*Medicago sativa*)	
Wheat (*Triticum aestvium*)		Purple vetch (*Vicia benghlensis*)	
Mung bean (*Phaseolus aureux*)	0.046	Oat (*Avena vulgare*)	0.19
Strawberry (*Fragaria* sp.)	0.074	Parsley (*Petroselium crispum*)	0.37
Kindney bean (*Phaseolus vulgaris*)		Red beet (*Beta vulgaris*)	
SEMI -TOLERANT CROPS		Tomato (*Lycopersicum esculentum*)	

Sesame (*Sesamum indicum*)		Sugarbeet (*Beta vulgaris*)	0.56
Red pepper (*Capsicum annum*)		Cotton (*Gossypium hirsutum*)	0.56 – 0.93
	0.093	Asparagus (*Asparagus officinalis*)	0.93 – 1.39

Source: Keren and Bingham (1985).

Appendix 16. Mesh Sizes of Standard Wire Sieves

Sieve Opening	**Standard Mesh Number**		
(mm)	**US**	**British**	**French**
2.00	10	8	34
1.00	18	16	31
0.500	35	30	28
0.420	40	36	–
0.250	60	60	25
0.210	70	72	–
0.149	100	–	–
0.125	120	120	22
0.063	230	240	19
0.053	270	300	–

Appendix 17. Equivalent Weights

Symbol/Formula	Equivalent Weight		Common Name
	----g----		
Ions			
Ca^{++}	20.04		Calcium ion
Mg^{++}	12.16		Magnesium ion
Na^{+}	23.00		Sodium ion
K^{+}	39.10		Potassium ion
Cl^{-}	35.46		Chloride ion
SO_4^{--}	48.03		Sulfate ion
CO_3^{--}	30.00		Carbonate ion
HCO_3^{-}	61.01		Bicarbonate ion
PO_4^{---}	31.65		Phosphate ion
NO_3^{-}	62.01		Nitrate ion
Salts			
$CaCl_2$	55.50		Calcium chloride
$CaSO_4$	68.07		Calcium sulfate
$CaSO_4 \cdot 2H_2O$	86.09		Gypsum
$CaCO_3$	50.04		Calcium carbonate
$MgCl_2$	47.62		Magnesium chloride
$MgSO_4$	60.19		Magnesium sulfate
$MgCO_3$	42.16		Magnesium carbonate
NaCl	58.45		Sodium chloride
Na_2SO_4	71.03		Sodium sulfate
Na_2CO_3	53.00		Sodium carbonate
$NaHCO_3$	84.01		Sodium bicarbonate
KCl	74.56		Potassium chloride
K_2SO_4	87.13		Potassium sulfate
K_2CO_3	69.10		Potassium carbonate
$KHCO_3$	100.11		Potassium bicarbonate
Chemical Amendments			
S	16.03		Sulfur
H_2SO_4	49.04		Sulfuric acid
$Al_2(SO_4)_3 \cdot 18H_2O$	111.07		Aluminum sulfate
$FeSO_4 \cdot 7H_2O$	139.01		Iron sulfate (ferrous)

Conversion of milliequivalents per liter to parts per million:

ppm = milliequivalents/liter × equivalent weight.

Appendix 18. Preservation Methods and Holding Times for Water Samples

Determination	Container	Preservation	Maximum storage
Acidity	P, G(B)	Refrigerate	24 h
Alkalinity	P, G	Refrigerate	24 h
BOD	P, G	Refrigerate	6 h
Boron	P	$HN0_3$ to pH <2	28 d
Bromide	P, G	None required	28 d
Carbon, organic, total	G (B)	Analyze immediately; or refrigerate and add HCl, H_3PO_4, or H_2SO_4 to pH <2	7 d
Carbon dioxide	P, G	Analyze immediately	0.25 h
COD	P, G	Analyze as soon as possible, or add H_2SO_4 to pH <2; refrigerate	7d
Chloride	P, G	None required	N.S.
Color	P, G	Refrigerate	48 h
Conductance	P, G	Refrigerate	28 d
Hardness	P, G	Add $HN0_3$ or H_2SO_4 to pH <2	6 months
Metals, general	P(A), G(A)	Filter immediately, add HNO_3 to pH <2	6 months
Chromium VI	P(A), G(A)	Refrigerate	24 h
Mercury	P(A), G(A)	Add $HN0_3$ to pH <2, 4°C,	28 d
Ammonia	P, G	Analyze as soon as possible or add H_2SO_4 to pH <2, refrigerate	7 d
Nitrate	P, G	Analyze as soon as possible; refrigerate	24 h
Nitrate + nitrite	P, G	Add H_2SO_4 to pH <2, refrigerate	1-2 d
Organic, Kjeldahl-N	P, G	Refrigerate, add H_2SO_4 to pH <2	7d
Odor	G	Analyze as soon as possible; refrigerate	6 h
Dissolved Oxygen	G, BOD	Analyze immediately	0.25h
pH	P, G	Analyze immediately	0.25h
Phosphate	G (A)	Refrigerate	48h
Phosphorus total	P, G	Add H_2SO_4 to pH <2, Refrigerate	28d
Salinity	G	Use wax seal	6 month
Solids	P, G	Refrigerate	7d
Sulphide	P, G	Refrigerate; add 4 drops 2N zinc acetate/100ml; add NaOH to pH>9	28 d
Temperature	P, G	Analyze immediately	0.25h
Turbidity	P, G	Refrigerate in dark	24h

*For determinations not listed, use glass or plastic containers; preferably refrigerate during storage and analyze as soon as possible.

P = plastic (polyethylene or equivalent); G = glass; G (A) or P (A) = rinsed with 1 + 1 HNO3; G (B) = glass, borosilicate; G(S) = glass, rinsed with organic solvents or baked

Refrigerate = storage at 4°C ± 2°C; in the dark

N.S. = not stated in cited reference; stat = no storage allowed; analyze immediately.

Appendix 19. Relationships between EC (saturation extract basis), and leaching fraction under conventional irrigation management

Water class	Electrical conductivity	Salt concentration	Type of water
	dS/m	mg/L	
Non-saline	< 0.7	< 500	Drinking and irrigation water
Slightly saline	0.7 - 2	500 -1500	Irrigation water
Moderately saline	2 -10	1500 - 7000	Primary drainage water and groundwater
Highly saline	10 -25	7000 - 15000	Secondary drainage water and groundwater
Very highly saline	25 - 45	15000 - 35000	Very saline groundwater
Brine	> 45	> 45000	Seawater

About the authors

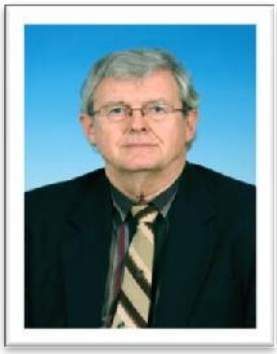
George Estefan

George Estefan is manager of the Soil, Plant and Water Analysis Laboratory at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. He has been with ICARDA since 1987 and has played a pivotal role in the establishment, development, and modernizing of the Laboratory facilities. He was co-author of the Second Edition of the Soil and Plant Analysis Manual and has led the effort to produce a third edition of the Manual in order to accommodate innovations in the area of soil, plant and water analysis. He has also been associated with the publications of articles in refereed journals related to laboratory management, technical training, and quality control. George has a B.Sc. in Agriculture from Aleppo University (1986) followed by a Diploma in soil science in 1990.

Rolf Sommer

Rolf Sommer is Senior Soil Scientist at the International Center for Tropical Agriculture, CIAT, in Kenya. Until the end of 2012 he was Soil Fertility Specialist at ICARDA, and responsible for scientific supervision of ICARDA's Soil, Plant, and Water Analysis Laboratory. He has a M.Sc. in Biology and a Ph.D. in agronomy. Previously he was Senior Fellow at the Center for Development Research, Department of Ecology and Resource Management at the University of Bonn, Germany, and Post-Doc at the International Center for Maize and Wheat Improvement, CIMMYT in Mexico. Besides being active in soil fertility research, he works in the field of agro-ecosystem modeling, and has published numerous research articles, book chapters' conference proceedings and online publications.

John Ryan



John Ryan was Soil Fertility Specialist at ICARDA from 1992-2004,and Soil Science Consultant from 2004 to 2012. He previously served as Professor of Agronomy at the University of Nebraska, based in Morocco, and as Professor of Soil Science at the American University of Beirut, Lebanon, and prior to that at the University of Arizona, Tucson, Arizona. He has published extensively in the area of soil fertility, plant nutrition, laboratory management, and soil science education. He has received numerous international awards, including Fellow of the Soil Science Society of American, International Soil Science Award, Soil Science Distinguished Service Award, the J.B. Benton Jones award for services to soil and plant analysis, and Fellow of the American Society for the Advancement of Science. He was principal author of the first two editions of ICARDA's Soil & Plant Analysis Manual and is co-author of this third edition. Currently based in Ireland, he is active professionally, particularly with the International Union of Soil Scientists and the American Society of Agronomy.