3. Soil Sampling and Processing

While the main focus of this manual is to present an easy-to-use methodology for soil testing and, to a lesser extent, for plant analysis, their related aspects are worthy of due emphasis. Therefore, a brief description of such aspects follows.

While much attention is given to laboratory procedures, the process of obtaining soil for analysis, i.e., soil sampling, is often ignored or poorly considered. A good sampling plan should provide a measure of the average fertility level of a field and a measure of how variable it is. If a sample is not representative of the field or is incorrectly taken, the resulting analytical data are meaningless, or at best, difficult to interpret. The error in field sampling is generally much greater than that due to chemical analysis. Therefore, obtaining a representative soil sample from a field is the most important step for making a meaningful soil analysis.

3.1. Soil Sampling

A soil sample should be composed of several sub-samples representing a seemingly uniform area or field with similar cropping and management history. There is no universally accepted numbers of sub-samples for different field situations. However, the following points can serve as guidelines:

Composite Sampling

- At ICARDA, eight sub-samples are taken per hectare (ha) in a diagonal pattern for obtaining one composite sample.
- Other plans range from 5 to 25 borings or sub-samples per composite sample, with sample units varying from 2 to 8 ha.
- Fewer sub-samples are needed where little or no fertilizer has been used. Sampling areas are often traversed in a zigzag pattern to provide a uniform distribution of sampling sites. Some of these methods are represented in Figure 2 and 3.
- Correspondingly, more sub-samples are needed where fertility is variable due to hand broadcasting of fertilizers and/or with cropping-livestock systems. Indeed, banding of fertilizer poses serious problems for reliable sampling.
- Thus, the number of sub-samples taken by farmers should be realistic, considering the particular field situation.

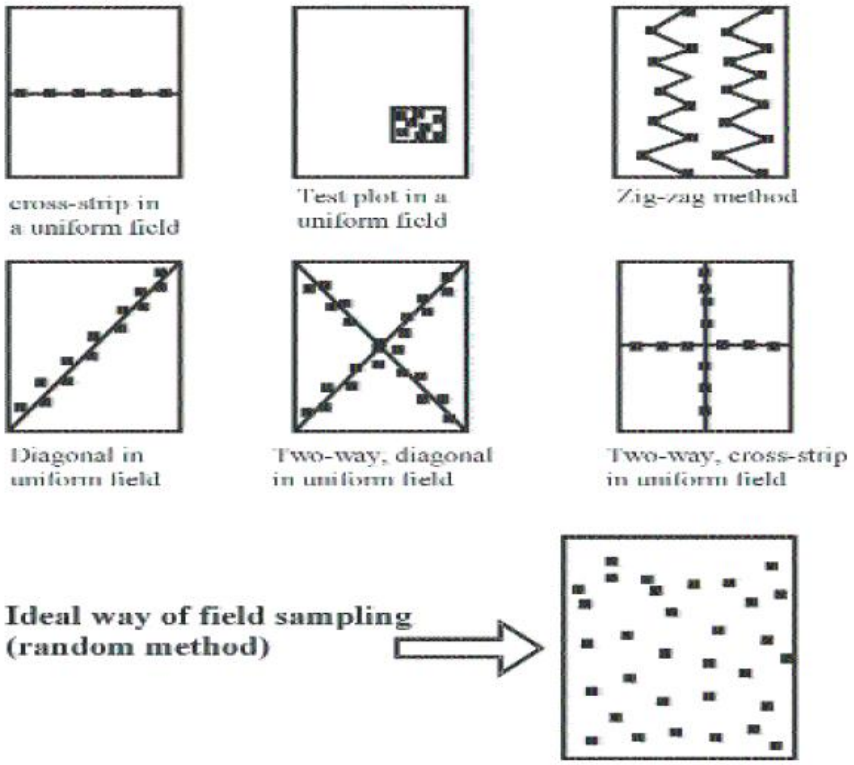

Figure 2. Some suggested methods for soil sampling; each dot represents a sample point, with formation of a sample pattern within the fielded

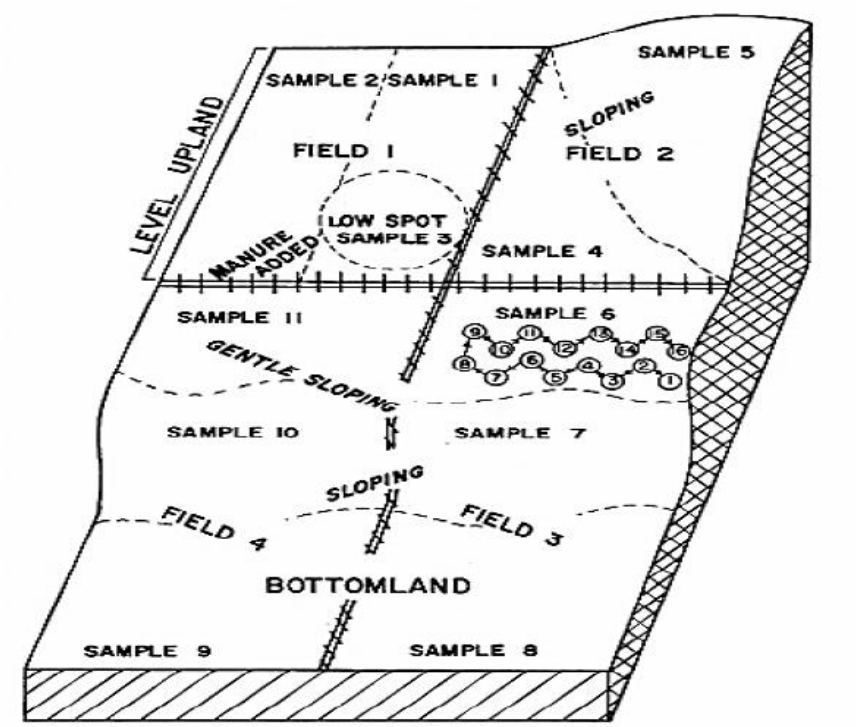

Figure 3. Sampling pattern for fertility test in a non-uniform land (sample numbers refer to composite sample; Tarzi, 1984)

Sampling Time

- Soil samples can be taken any time that soil conditions permit, but sampling directly after fertilization or amendment application should be avoided.
- Samples taken during the crop growth period will help in knowing the nutrient status of the soil in which plants are actively taking up nutrients.
- In the WANA region, it is recommended that sampling be carried out in autumn (before planting) if fertilization is intended at planting.
- It is important to sample at similar times year after year for comparing analysis at regular time intervals.

Sampling Depth

- For most purposes, soil sampling is done to a depth of about 20-cm. Available P, NO_3-N, and micronutrients in such samples are related to crop growth, and nutrient uptake.
- In some cases, especially in irrigated areas, sampling to a depth of 60-100 cm is desirable, especially for monitoring nitrate (NO_3-N) leaching.
- Depth-wise soil samples should also be taken where there is a concern about B toxicity.

Soil profile

Sampling Tools

- A uniform slice should be taken from the surface to the depth of insertion of the tool; the same volume of soil should be obtained in each sub-sample.
- Augers generally meet these requirements. In areas where the topsoil is dry, e.g., during summer, topsoil sampling can be done by a metal ring, by digging out the soil inside the ring, because it is almost impossible to sample dry topsoil with an auger.
- Soil samples for micronutrient analysis should be taken using a stainless steel auger, or at least ungalvanized auger (because galvanized coating is zinc oxide).
- Researchers generally use augers for field sampling. Farmers or Extension Agents can use shovels or trowels, with almost the same result.

Soil sampling tool: Auger

- If you do not have sampling tools, use a spade as follows:

- Dig a V-shaped hole 15 to 20 cm deep. Then take a fine thick slice from the smooth side (see Figure 4).

- Trim the sides leaving a fine strip then dump this strip into a clean bucket. Break the clods, and mix thoroughly. Remove large rocks, pieces of sod, earthworms, etc. Put the soil into the sample container and label the box clearly.

- For a moist soil, the tube auger or spade is considered satisfactory. For harder soil, a screw auger may be more convenient.

Figure 4. Soil sampling tool: Spade

Instructions for Field Processing

- Disturbed soil samples should be put in plastic bags (tags and markers are required), or aluminum or stainless steel boxes
- Depending on the subsequent analysis samples may be kept cool until laboratory analysis.
- Bags should be examined for cleanliness as well as for strength.
- Soil samples can be transported to the laboratory in cardboard boxes or sacks.
- All information about samples is recorded, and each sample is given a laboratory number.
- Sketch your field. Diagram it the way you sampled it. Be sure 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).
- Information sheet should be clearly written with copying pencil. Fill out the information sheets. The more information you can provide with each sample, the better your recommendation will be.
- Do not sample unusual area, like unevenly fertilized, old channel, old bunds, area near the tree, and site of previous compost piles and other unrepresentative sites.
- Avoid any type of contamination at all stages. Soil samples should never be kept in the store along with fertilizer materials and detergents. Contamination is likely when the soil samples are spread out to dry in the vicinity of stored fertilizers or on floor where fertilizers were stored previously.
- Collect samples from the middle of the rows, when crops have been planted in rows, so as to avoid the area where fertilizer has been band-placed.

3.2. Preparation Laboratory Processing

Handling in the laboratory

- As soon as the samples are received at the soil preparation facility, they should be checked with the accompanying information list (including sample number, depth, and date of sampling should be written on the bag from outside, and on a sample card placed inside the bag). Information regarding samples should be entered in a register and *each sample be given a laboratory number*.
- The soil-fresh sample received in the laboratory should be analyzed directly after sampling for determination of nitrate, nitrite and ammonium. ***These samples should not be dried, and the results are expressed on oven-dry basis by separately estimating moisture content in the samples.*** If short-term storage is unavoidable, this must be done in a fridge at temperature close to 0 °C (but not below zero!). Lag time between field sampling and analysis must be minimized. Otherwise, storage time will inevitably introduce an additional factor influencing analysis results.

Drying of the soil samples

- The soil-fresh samples received in the laboratory should be dried in wooden or enameled trays. The trays can be numbered or a plastic tag could be attached, and care should be taken to maintain the identity of each sample at all stages of preparation.
- During drying, the soils are allowed to dry in the air. Alternatively, the trays may be placed in racks in a hot air cabinet (the temperature should not exceed 35 °C and humidity should be between 30 and 60 %). In general, excessive oven-drying of the soil affects the availability of most of the nutrients present in the sample, and should be avoided.

Ovens

- Only air-drying is recommended for some of the nutrients present in the sample. Such as, drying has negligible effect on total N content but the NH_4 and NO_3 content in the soil changes with time and temperature. The microbial biomass is also significantly affected by drying at high temperature.

Preparation of soil samples

- After drying, the samples are ground with a wooden pestle and mortar in preparation room (which is separate from the main laboratory), and clods and large aggregates are crushed and mixed. Remember that:
- Pebbles, concretions, and stones should not be broken during grinding.
- Care should be taken not to break the individual soil particles during the grinding process.
- The entire sample should be passed through the sieve, except for concretions and pebbles of more than 2-mm.
- The purpose of grinding is to reduce heterogeneity and to provide maximum surface area for physical and chemical reactions. Various devices are used for crushing and grinding soils. However, choice of equipment depends on:
  - Amount of sample to be crushed or ground
  - Degree of fineness to be attained
  - Contamination that can be tolerated
  - The analysis in question

- After grinding, the soil is screened through a 2-mm sieve. The coarse portion on the sieve should be returned to the mortar for further grinding (except for concretions, pebbles, and organic residues). Repeat sieving and grinding till all aggregate particles are fine enough to pass the 2-mm sieve.
- It is necessary to reduce the size of the large sample for ease of storage and handling. To achieve this goal, a random method of sub-sampling is essential. Sample splitting can be performed with a mechanical sample splitter, such as a **Riffle-type Sample Splitter**, by which the sample is divided in half by a series of chutes. This process can be repeated as many times as necessary.
- Another way for reduction of sample size is by **quartering**. The sample is spread uniformly over a plastic or paper sheet and divided into four equal portions. For example, portions 2 and 3 are collected and thoroughly mixed, whereas the remainder is discarded.
- Following the drying and preparing processes, half of the amounts of the dried soil sub-sample are placed in a clean container and then transferee into the soil testing laboratory for the requested analysis, the rest should be stored in cardboard boxes in a store room.
- Remember, if the soil is to be analyzed for trace elements, containers made of copper (Cu), zinc (Zn) and brass must be avoided during grinding and handling. Sieves of different sizes can be obtained in stainless steel. Aluminum or plastic sieves are useful alternative for general purposes.

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 °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 °C, because higher temperatures may cause volatilization loss.
- Drying at temperatures less than 70 °C may not remove all combined water and may result in poor homogenization and incorrect analytical results.
- Drying temperatures above 70 °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 °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 °C before analysis.

Technical Remarks

1. If samples are placed in a cool (4 °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.