Soil and Plant Analysis Laboratory Manual

Second Edition

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The first edition of this Manual was published in 1996 under the title “A Soil and Plant Analysis Manual Adapted for the West Asia and North Africa Region,” and was authored by John Ryan, Sonia Garabet, Karl Harmsen, and Abdul Rashid.

Foreword

Soils vary greatly throughout the world; they have inherent weakness, primarily deficiency in nutrients essential to growing crops. Even when adequately supplied in the early stages of land cultivation, the nutrient-supplying capacity invariably diminishes with time. Most soils are deficient in nitrogen; it is transient in nature and plants need a lot of it. In many cases, phosphorus (P) is just as critical; soil chemical reactions reduce the effectiveness of P fertilizers. The soils of the Central and West Asia and North Africa (CWANA) region are generally well supplied with potassium, and usually don't need fertilization, especially for low-yielding rainfed crops. In recent years, a realization has grown that other elements, e.g., micronutrients, are deficient in some areas of the CWANA region.

As no essential element will substitute for another, it is critically important to identify where and when such deficiencies occur. That's where the role of soil and plant analysis comes in. Techniques have been developed to evaluate soil fertility constraints based on soil chemical extraction and analysis of the plants that grow on such soils. Both are complimentary and, when calibrated with field crop response to fertilizer, provide a rational basis to identify what elements are missing, and how much fertilizer to apply. Therefore, soil and plant analysis laboratories have a vital role in agricultural development of the CWANA region. However, the process does not end there. To be meaningful and valid, tests have to be appropriate for the purpose intended and reliable and repeatable.

As ICARDA had spearheaded the Soil Test Calibration Program within the region's National Agricultural Research Systems (NARS), and laid the basis for sound fertilizer recommendations, it was only appropriate that ICARDA should, in collaboration with NARS, address this fundamental issue. This manual is a cornerstone in ICARDA's training program and a vital link with scientists of the CWANA region.

Prof. Dr. Adel El-Beltagy
Director General
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Preface

The idea of having a common laboratory manual for the Central and West Asia and North Africa (CWANA) region was based on the fact that the soils in this arid to semi-arid area have a common suite of properties, leading to similar nutritional problems in crops. Collaborative research in the Soil Test Calibration Network in the Mediterranean zone had also revealed that a number of soil tests for assessing nutrient availability have regional applicability. In addition, there was hardly any laboratory manual on plant analysis relevant to the crop nutritional problems in the region.

A common soil-plant analysis manual is also fundamental for success of the CWANA soil fertility network. To fill the gap that existed, a comprehensive manual on all sorts of needed soil and plant analyses was developed. This manual was designed intentionally in a "cook-book" format, for the ease of laboratory technicians. On completion, the manual draft was reviewed and endorsed by a sub-committee at the Soil Fertility Meeting in ICARDA (Nov.19 - 23, 1995).

Subsequently, first edition of the manual was published in 1996, and distributed quite widely among soil-plant analysis laboratories throughout the CWANA region. The response was very encouraging. Most laboratory managers appreciated the initiative, particularly for compiling of all the needed methodologies in a single volume in an easy-to-use format for laboratory technicians.

This second edition has incorporated all the feedback from laboratory managers of the CWANA region, making the manual more comprehensive, up-to-date, and hopefully error-free. We appreciate the constructive criticism and suggestions advanced by the fellow soil scientists for improvement of the manual.

The second edition of the manual reflects the changes that have occurred within the past few years in the region. While it is still focused on production agriculture, it reflects an increasing concern about environmental pollution, mainly from nitrate, heavy metals, and toxic organic compounds. With greater sophistication in methodologies and equipment, most soil laboratories can now deal with both agricultural and environmental concerns.

We again encourage all the users of this manual to continue to provide feedback regarding its utility for their particular situations, and indicate errors, if any.

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Acknowledgements

We would like to thank all past and present ICARDA staff associated with the Soil-Plant Analysis Laboratory who have contributed to its operation and the development of various procedures.

Appreciation is extended to Dr. Abdallah Matar, who put these methods of analysis on a sound footing throughout the CWANA region through the medium of the region's Soil Test Calibration Network.

The contribution of Dr. Karl Harmsen and Miss Sonia Garabet to the authorship of the first edition of this manual is recognized.

The assistance of Mr. Samir Masri is much appreciated, particularly for his contribution regarding the soil sampling procedures presented in this manual.

The Soil-Plant Analysis Laboratory has, since ICARDA's inception, played a vital role in the research activities of the Natural Resource Management Program (NRMP) and indeed throughout the Center. The ICARDA management has always supported the Soil-Plant Laboratory; without this support, this manual of adapted soil and plant analysis procedures would not have been published.

Our thanks go to readers who have noted errors in the first edition of this manual and those who made suggestions for improvement.

We thank Dr. Adriana Bruggeman for a thorough review of the final document.

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1. INTRODUCTION

The idea that one could test or analyze a soil and obtain some information about its properties—especially its acidity or alkalinity and its nutrient status—is long established, and can be traced back to the beginning of scientific inquiry about the nature of soil. Analysis of plant to reflect fertility status of the soil in which it grew is more recent, although visual crop observations are as old as the ancient Greeks, if not older. In the last few decades, spurred on by commercialization of agriculture and the demands for increased output from limited — and even diminishing — land resources, both soil and plant analysis procedures have been developed, and are still evolving.

With the advent of chemical fertilizers, the need to know nutrient status of a soil in order to use these expensive and limited inputs more effectively became all the more crucial. However, if soil testing is to be an effective means of evaluating fertility status of soils, correct methodology is absolutely essential. A soil or a field may be assessed for its capability of providing a crop with essential nutrients in several ways:

1. Field Plot Fertilizer Trials;
2. Greenhouse Pot Experiments;
3. Crop Deficiency Symptoms;
4. Plant Analysis;
5. Rapid Tissue or Sap Analysis;
6. Biological Tests, such as Growing Microorganisms; and
7. Soil Testing prior to Cropping.

While all approaches can be used in research, the latter one is most amenable, and one upon which recommendations for farmers can be based. On the other hand, plant analysis is a *postmortem* approach and one that should be interpreted in the light of soil test results.

Soil testing is now an intrinsic part of modern farming in the West. Tests primarily focus on the elements in most demand by crops which are supplied by fertilizers: *nitrogen* (N), *phosphorus* (P), and *potassium* (K). Depending upon the soil types, in some regions tests are also conducted for secondary nutrients: *calcium* (Ca), *magnesium* (Mg), and *sulfur* (S). In drier areas, micronutrients such as iron (Fe), *zinc* (Zn), *manganese* (Mn), *copper* (Cu), and *boron* (B) are often measured, since deficiencies of these elements are more frequently associated with calcareous soils. Indeed such areas may also have excessive or toxic levels of some elements, like B and Sodium (Na).

As nutrient behavior in soils is governed by soil properties and environmental conditions, measurement of such properties is often required. These include *pH, salinity, organic matter* (OM), *calcium carbonate* ($CaCO_3$), and *texture.* In drier areas, the presence of *gypsum* ($CaSO_4.2H_2O$) is also of concern.

Soil testing involves four distinct phases:

1. **Sample Collection**: This should be such that it reliably reflects the *average* status of a field for the parameter considered.
2. **Extraction or Digestion and Nutrient Determination:** The reagent used and the procedures adopted should quantify all or a portion of the element in the soil which is related to the availability to the plant, i.e., it should be correlat ed with plant growth.
3. **Interpreting the Analytical Results:** The units of measurement should reliably indicate if a nutrient is deficient, adequate, or in excess.
4. **Fertilizer Recommendation:** This is based upon the soil test calibrated for field conditions, and considers other factors such as yield target, crop nutrient requirement, management of the crop, soil type, and method of fertilizer application, etc.

It should be emphasized, however, that a soil test, even if very *reliable* or *accurate,* is only one *factor* in making decisions about the need for *fertilization.* There are many other factors affecting crop growth and yield, such as soil type and environmental conditions, i.e., moisture, temperature, etc.

Because of varying and different forms of nutrients in soils, e.g., calcareous vs. acid soils, soil tests are equally varied, particularly for available P and micronutrients, and to a lesser extent for N (Walsh and Beaton, 1973). Being mobile in soils and subject to mineralization - immobilization, N poses particular problems to establish a reliable test. Tests for K, pH, OM, and $CaCO_3$ are more straightforward.

Since the development of the DTPA test of Lindsay and Norvell (1978) and adoption of azomethine-H as a color- developing reagent for B (Gaines and Mitchell, 1979), micronutrient (Fe, Mn, Zn, Cu, B) tests for alkaline soils have become more useful. Though tests for gypsum are developed (Richards 1954; FAO, 1990), there are unique problems for CEC measurement in such soils (Rhoades and Polemio, 1977).

The literature on soil testing is rich and varied. Some salient examples include:

1. Monographs from the American Society of Agronomy for Physical (Klute, 1986) and Chemical Analysis (Page, 1982) which give detailed descriptions of all available soil tests and their modifications.
2. Soil Science Society of America Publications (Walsh and Beaton, 1973; Westerman, 1990) that take a broader look at the philosophy, procedures, and laboratory operations for soil and plant analysis, with interpretation criteria for specific crops.
3. Soil Testing with a Textbook Format (Hesse, 1971).
4. University Publications which range from those that deal with all Soil, Water and Plant Tests (Chapman and Pratt, 1961) to more narrowly based ones (Reisenaur, 1983).
5. Publications that deal with theoretical considerations involved with sampling, correlation, and calibration, to interpretation (Brown, 1987).
6. Those that are commercially oriented and reflect "**State of the Art**" instrumentation and computer-assisted data analysis and handling (Jones, 1991; Jones *et al*., 1991).
7. Finally, publications that are written in "**Recipe/Cook-book**" style with little or no discussion; only listed are the equipment and chemicals used and the general steps involved in the procedure (Quick, 1984).

While most soil testing sources emanate from the West, publications such as those of the Food and Agriculture Organization of the United Nations (FAO, 1970; 1980) are more international in scope and assume a developing country perspective. In such countries, *soil testing is often less developed* and, in some cases, does not exist. Similarly, research pertaining to soil testing and plant analysis is often fragmentary.

This leads to a consideration of the Central and West Asia - North Africa region, which is served by the International Center for Agricultural Research in the Dry Areas (ICARDA). In this region, the development of the Soil Test Calibration Network at ICARDA served as a catalyst to promote soil testing and thus eventually lead to more efficient use of soil and fertilizer resources in the region. Its evolution and potential impact can be seen from scrutiny of the papers presented at the various workshops in Aleppo, 1986 (Soltanpour, 1987), Ankara, 1987 (Matar *et al.,* 1988), Amman, 1988 (Ryan and Matar, 1990), and in Agadir, 1991 (Ryan and Matar, 1992).

Central to the Network, and indeed ICARDA's operations, has been its Soil-Plant Analysis Laboratory. Though its facilities have been designed and developed without some of the constraints experienced by other governmental and educational laboratories in the region, some of the procedures adapted by the laboratory are based on *validated regional research*, e.g., N and P.

A key element in any worthwhile laboratory is a list of appropriate tests presented in such a manner that it can be readily followed routinely by those who actually do soil testing and plant analysis, i.e., *the laboratory technicians.* Therefore, the target audience for this manual is the cadre of technical staff throughout the region.

A brief introduction to each test is given, so that the technician should have an *elementary understanding of the importance of the work he/she is doing.* He/she should also know the range of *values to be expected* for soils and plants in the region, and therefore more readily identify gross errors. We have attempted to select the most appropriate method for each test and present it in a clear, stepwise manner.

While the manual primarily deals with soil testing, a number of important plant tests are presented, since they may complement the soil tests and are frequently needed for soil fertility and plant nutrition studies. Similarly, due emphasis has been given to physical properties; describing the tests routinely done along with chemical analysis. The importance of proper soil and plant sampling has been highlighted, and guidelines of sample collection, processing, and storage provided.

We have also presented material on *laboratory organization* and *safety-aspects* which are often *overlooked* by technical staff, but which impinge greatly on their work output and its reliability. Additionally, the appendices contain very useful information on related practiced aspects like abbreviations, conversion factors, atomic weights, solution concentrations, pH effect on soil conditions, summarized soil test methodologies, plant sampling guidelines, criteria for interpreting soil and plant analysis data, soil salinity, and boron toxicity interpretations.

Although the manual is written in English , the language of ICARDA's publications, only an elementary knowledge of English is required to follow the procedures listed. As ICARDA's mandate area is diverse in terms of languages, it is intended that French, Arabic, and Russian versions will be produced.

2. SOIL AND PLANT 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.

2.1 Soil

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 would be *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.

1. 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:

A. 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 zig-zag pattern to provide a uniform distribution of sampling sites. Some of these methods are represented in **Figure 1**.
- 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.

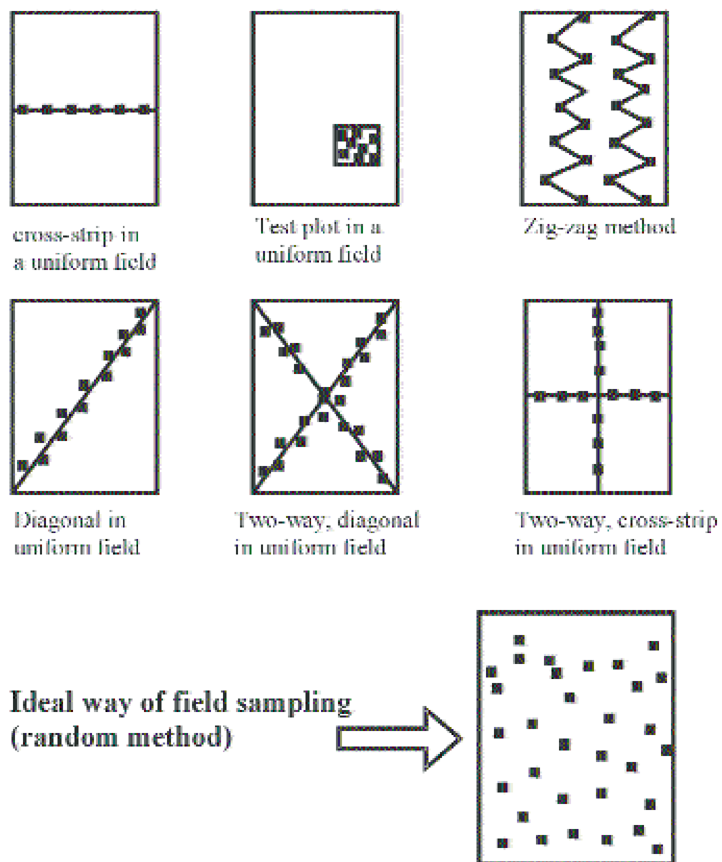


Fig. 1. Some suggested methods for soil sampling.

B. Time of Sampling

- Soil samples can be taken any time that soil conditions permit. However, 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 drawing nutrients.
- In the CWANA 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.

C. Depth of Sampling

- For most purposes, soil sampling is done to a depth of about 20-cm. Research in the CWANA region has shown that 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 and salinity.
- Depthwise soil samples should also be taken where there is a concern about B toxicity.

D. Sampling Tools

- There are two important requirements of a sampling tool: first, that a uniform slice should be taken from the surface to the depth of insertion of the tool, and second, that 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, the 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 topsoils 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.

2. Field Processing

- Soil samples must be put in plastic bags (tags and markers are required).
- Soil samples can be transported to the laboratory in cardboard boxes or sacks.

3. Laboratory Processing

- All information about samples is recorded; and each sample is given a laboratory number.
- Samples are put in a freezer for minimizing microbial activity.
- Samples are air-dried or dried in an air-forced oven at 30°C.
- When dried, samples are cleaned off any stones and plant residues.
- Samples are ground in a stainless steel soil grinder and passed through a 2-mm sieve. Samples for particle-size distribution are ground with a pestle and mortar. Some analyses call for use of sieves of a less than 1-mm size fraction.
- The sieved soils are collected, sub-sampled (~500 g), and stored in plastic bottles.
- Laboratory sub-samples are made with a Riffle-type Sample Splitter (**Fig. 2**).
- Laboratory sub-samples are given a number and sent to the laboratory for the requested analyses.

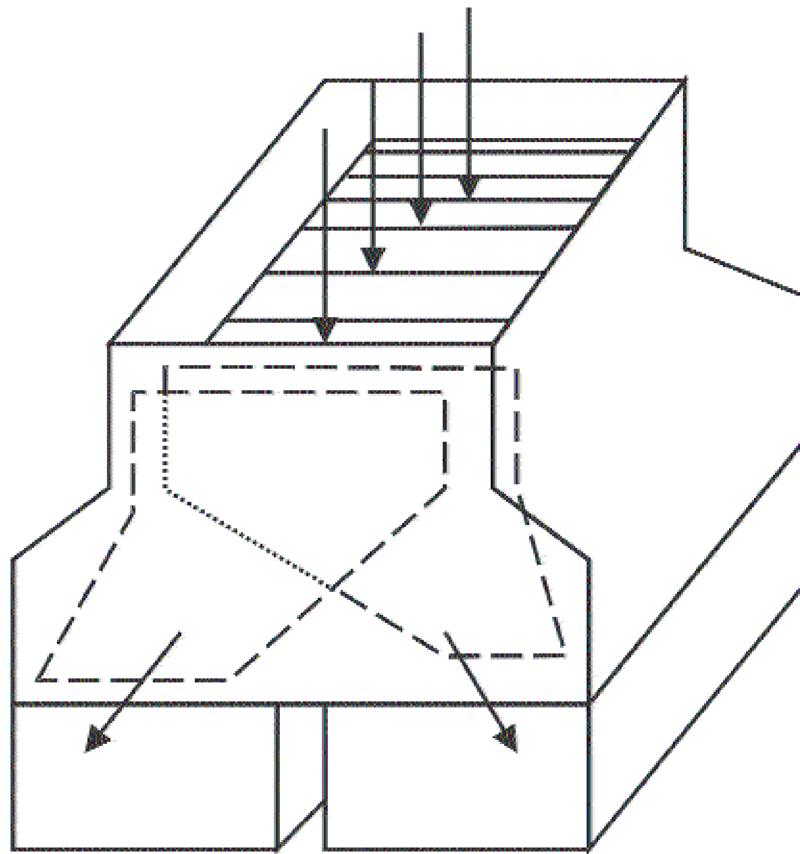


Fig. 2. Riffle-type soil sample splitter.

2.2 Plant

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. Plant analysis involves the determination of nutrient concentration in diagnostic plant part(s) sampled at recommended growth stage(s) of the crop.

The concentration of some nutrient elements may be *too low for optimum growth,* while of others may be so high as to be *detrimental to the plant's growth.* Overviews such as that of Munson and Nelson (1990) illustrate how nutrient concentrations in plants vary with the element in question, type of plant, specific plant part, growth stage, level of available soil nutrients, expected yield level, and environmental factors.

While laboratory technicians do not normally sample plants, 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*.

1. Plant Sampling

Years of research in soil fertility-plant nutrition have produced reliable sampling criteria and procedures for most of the world's commercial crops: *leaves* are most commonly chosen, but *petioles* are selected in certain cases, e.g., cotton, sugarbeet. *Seeds* are rarely used for analysis, except for assessing of B toxicity and Zn and P deficiency in certain grain crops. In some cases, e.g., cereals, the entire above-ground young plants are sampled.

When leaves are sampled, recently matured ones are taken; both new and old growth is generally avoided. However, *young emerging leaves* are sampled for diagnosing iron 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. Sampling procedures for important dry-land crops of the CWANA region are given in **Appendix 10.**

Plant samples should be transported to the laboratory immediately in properly labeled paper bags that allow for transpiration; this reduces the possibility of rotting.

2. Laboratory Processing

Five steps are followed for processing the sampled plant tissues:

1. Cleaning plant tissue to remove dust, pesticide and fertilizer residues: normally by washing the plants with de-ionized water or with 0.1 - 0.3 % P-free detergent, followed by de-ionized (DI) water. If not essentially required, samples for soluble element determination may not be washed, particularly for long periods. However, samples for total iron analysis must be washed.
2. Immediate drying in an oven to stop enzymatic activity, usually at 65°C for 24 hours.
3. Mechanical grinding to produce a material suitable for analysis, usually to pass a 60-mesh sieve; stainless steel mills are preferable, particularly when micronutrient analysis is involved.
4. Since most analytical methods require grinding of a dry sample, careful attention must be given to avoiding contamination with the element to be analyzed. Particular care is required for micronutrients.
5. Final drying at 65°C of ground tissue to obtain a constant weight upon which to base the analysis.

Moisture Factor

Weighing of perfectly oven-dried samples is, however cumbersome (involves continuous oven drying and use of desiccator, and is still prone to error) as plant material may absorb moisture during the weighing process, particularly if relative humidity is high in the laboratory.

To get around this difficulty, use of the Moisture Factor is suggested instead. The Moisture Factor for each batch of samples can be calculated, by oven-drying only a few sub-samples from the lot (e.g., 5 from a batch of 100 - 200 samples).

$$\text{Moisture Factor} = \frac{\text{Weight of air dry sample (g)}}{\text{Weight of oven dry sample (g)}} \quad \text{.................... (1)}$$

Thereafter, air dry samples are weighed, considering the Moisture Factor. For example, if Moisture Factor = 1.09, then weight of oven dry and air dry samples will be as follows:

Oven-dry weight	*Air-dry weight*
----------------------g----------------------	
0.25	*0.27*
0.50	*0.55*
1.00	*1.09*
2.00	*2.18*

The Moisture Factor approach is also used for weighing soil samples, and expressing the analytical results on oven dry soil weight basis.

3. LABORATORY FACILITIES, QUALITY CONTROL, AND DATA HANDLING

3.1 Laboratory Organization

Soil and plant analyses are carried out by various institutions in the public or government sector as well as in the private domain. Laboratories are found in Ministries of Agriculture, National Research and Teaching Institutes, International Organizations, and in Commercial Companies. Analytical services should be closely linked to the Extension/Advisory services and should maintain a functional relationship with the universities, research stations, etc.

The kind of facility for such analyses depends on the type of institution it serves, the nature of the clientele, and the volume of samples to be analyzed. Nevertheless, all *laboratories,* regardless of the size, should be designed in a manner to facilitate *operational efficiency, minimize contamination,* and produce *reliable* and *repeatable* results.

Various publications deal with management considerations in the design and operation of soil testing (e.g., Walsh and Beaton, 1973). While the advantages of standardized laboratory designs are self-evident, many laboratories in the CWANA region have apparent deficiencies in this respect (Ryan, 2000; Ryan *et al*., 1999). All too often one sees soil samples stored or, worse still, ground in wet chemistry laboratories. Similarly, many laboratories are set up in a manner that inadvertently hinders efficient use of staff resources.

Soil, Plant, and Water analysis facilities should be located in the same building and be under one unified administration. The Soil-Plant Analysis Laboratory of **ICARDA's** Natural Resource Management Program was designed with these considerations in mind **(Fig. 3)**. The various components of the facility reflect a logical activity framework.

In *Soil Preparation Room* No. 1, large bulk samples, transported by truck, are received, dried, sieved, and stored in bulk bins. An inventory or catalogue of all soil samples is maintained. All samples are retained in this area for at least two years after analysis; bulk samples of special soil types are kept indefinitely. This facility is equipped with a large oven, freezer, soil grinder, exhaust hood, and a compressed air machine.

Small lots (~1 kg) of soil samples are taken to *Soil Preparation Room* No. 2 and weighed in appropriate containers for chemical analyses. The samples are then put in the *Soil Store*. Batches of weighed samples are wheeled in a trolley to the adjacent laboratory for analysis. A separate *Kjeldahl Room* exists for plant Kjeldahl analysis. Soil extracts, where necessary, are carried to a small *Instrument Room* (atomic absorption spectrophotometer, flow injection analyzer, computer, etc.) for analysis. Where large numbers of samples are involved, and where a delay would induce bacterial changes in soil material, samples are temporarily held in the *Freezer Room*.

While no two laboratories are ever the same or have the same complement of equipment, the details presented for **ICARDA's** laboratory **(Fig. 3)** will, hopefully, serve as a general guideline for laboratory arrangement and the type of equipment needed for routine service-oriented operations.

1. Oven
2,3. Freezers
4. Soil Grinder & Exhaust Hood
5. Compressed Air
6. Sub Sampling Bench
7. Soil Grinder
8. Plant Sample Drawers
9. Vacuum Filtering System
10. Nitrogen distillation &Titration unit
11. Conductivity Meter
12. Flame Photometer
13. pH Meter
14. Expandable Ion Analyzer
15. Spectrophotometer
16. Automatic Dispensor
17,18. Nitrogen distillation & Titration unit
19. Cooled Incubator
20. Refrigerator
21. P. C. (Computer)
22. Flow Injection Analyzer
23. Atomic Absorption Spectrophotometer.
24. Muffle Furnace
25. Kjeldahl unit
26,27,28. Dispensers
29. Titration Bench
30,31. Freezers
32,33. Refrigerators

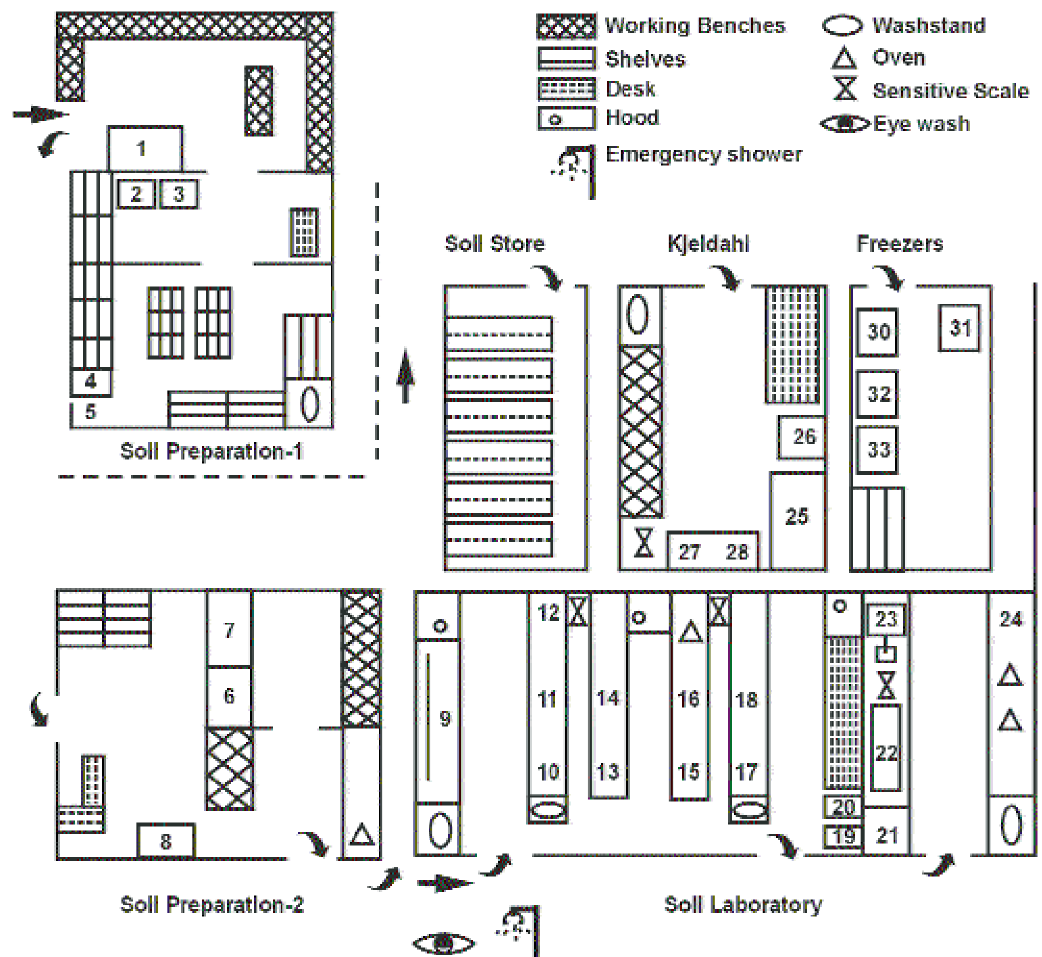


Fig. 3. Schematic layout of ICARDA's Soil-Plant Analysis Laboratory facilities.

3.2 Laboratory Safety

As with any place of work, safety is an important consideration in soil-plant analysis laboratories, and one that is frequently overlooked. Safety is in the interest of the employees who work there and the organizations that operate the laboratories.

All staff, irrespective of grade, technical skill or employment status should be briefed on all aspects of safety upon commencement of work. Periodic reminders of such regulations should be given to encourage familiarity with respect to regulations.

While rules pertaining to safety can be extensive, we have endeavored to concisely list the more important ones within different categories of concerns. These have been adapted from laboratory safety guides developed by Kalra and Maynard (1991) and Okalebo *et al*. (1993).

General Attitude

1. Develop a *positive* attitude towards laboratory safety.
2. Observe normal laboratory safety practices.
3. Maintain a safe and clean work environment.
4. Avoid working alone.

Instrument Operation

5. Follow the safety precautions provided by the manufacturer when operating instruments.
6. Monitor instruments while they are operating.
7. Atomic Absorption Spectrophotometer must be vented to the atmosphere. Ensure that the drain trap is filled with water prior to igniting the burner.
8. Never open a centrifuge cover until machine has completely stopped.

Accidents

9. Learn what to do in case of emergencies (e.g., fire, chemical spill, etc.). Fire fighting equipment must be readily accessible in the event of fire. Periodic maintenance inspections must be conducted.
10. Learn emergency **First Aid. First Aid** supplies are a necessity and laboratory staff should be well trained in their use. Replacement of expended supplies must take place in a timely fashion.

11. Seek medical attention immediately if affected by chemicals, and use **First Aid** until medical aid is available.
12. Access to eye-wash fountains and safety showers must not be locked. Fountains and showers should be checked periodically for proper operation.

Chemicals

13. Use fume hoods when handling concentrated acids, bases or other hazardous chemicals.
14. Do not pipette by mouth; *always* use a suction bulb.
15. When diluting, always add acid to water, *not* water to acid.
16. Many metals/salts are *extremely toxic* and may be *fatal* if swallowed. Wash hands thoroughly after handling such salts. Chemical spills should be cleaned promptly and all waste bins emptied regularly.
17. All reagent bottles should be clearly labeled and must include information on any particular hazard. This applies particularly to poisonous, corrosive, and inflammable substances.

Furnaces, Ovens, Hot Plates

18. Use forceps, tongs, or heat-resistant gloves to remove containers from hot plates, ovens or muffle furnaces.

Handling Gas

19. Cylinders of compressed gases should be secured at all times. A central gas facility is preferred.

Maintenance

20. All electrical, plumbing, and instrument maintenance work should be done by qualified personnel. Fume hoods should be checked routinely.
21. As most equipments operate on low wattage, an Uninterruptible Power Supply **(UPS)** provides stable power and allows the completion of any batch measurement in the event of power outage.

Eating and Drinking

22. Do *not* eat, drink, or smoke in the laboratory. This is essential both for reasons of health and to reduce contamination. Specific areas should be designated for staff breaks.
23. Do *not* use laboratory glassware for eating or drinking.
24. Do not store food in the laboratory.

Protective Equipment

25. Use personnel safety equipment as follows:
    - **Body Protection:**

      Use laboratory coat and chemical-resistant apron.
    - **Hand Protection**

      Use gloves, particularly when handling concentrated acids, bases, and other hazardous chemicals.
    - **Dust Mask**

      Essentially needed when grinding soil, plant samples, etc.
    - **Eye Protection**

      Use safety glasses with side shields. Persons wearing contact lenses should always wear safety glasses in the laboratory. Make sure that your colleagues know that you wear contact lenses. Contact lenses should never be worn around corrosives.
    - **Full-Face Shield**

      Wear face shields over safety glasses in experiments involving corrosive chemicals.
    - **Foot Protection**

      Proper footwear should be used; sandals should *not* be worn in the laboratory

Waste Disposal

26. Liquid wastes should be poured carefully down a sink with sufficient water to dilute and flush it away. Keep in mind that local ordinances often prohibit the disposal of specific substances through the public sewerage system.
27. Dispose off chipped or broken glassware in specially marked containers.

Continuing Education

- Display in a prominent place posters on **"Laboratory Safety"** which pictorially describe various phases of laboratory activities.
- Similarly, posters depicting **First Aid** after laboratories accidents should be prominently displayed. Such posters are *not* for ornamentation; they are for the *protection of laboratory personnel*, who should be thoroughly conversant with all procedures and eventualities.
- If the laboratory is a part of a large institution, the laboratory staff should know the **Safety Officer** or person responsible for safety. If it is a small operation, one laboratory staff member should be responsible for safety.

Contamination

The most insidious enemy in any laboratory is *contamination* and, therefore, its sources must be identified and eliminated. *Some common sources of contamination are:*

- External dusts blown from the surrounding environment;
- Internal dust resulting from cleaning operations;
- Cross-contamination derived from handling many samples at the same time (e.g., handling plant and soil samples together);
- Failure to store volatile reagents well away from the samples;
- Washing materials, particularly soap powder; and
- Smoking in the laboratory.

3.3 Quality Control and Standardization Procedures

What follows in this section is a synthesis from the manual of Okalebo *et al*. (1993). Quality control is an essential part of good laboratory practice. During routine analyses, errors may gradually appear due to contamination, changes in reagent quality, environmental differences, operator error, and instrument calibration or failure. *Maximum reproducibility* and *adequate accuracy of results are the important objectives*. Repeated measurement of an air-dried soil sample should provide consistent results when analyzed over time for most routine chemical procedures. The deviation of an observed value from its absolute "true" value results from either *systematic or random errors.*

Once identified, systematic errors are more easily corrected than those which occur at random. Three precautions are essential for laboratory quality control and should be routinely included among the test samples. These precautions involve the use of *blanks, repeats,* and *internal references,* as elaborated below.

Whenever a new procedure is introduced to the laboratory, its accuracy should be evaluated and compared to the test already in use. Both methods should be compared for a homogeneous test sample using ten-fold replicates, with the standard deviation calculated for each set. This provides a *measure of precision*. Then a known amount of reagent should be added to the homogeneous test sample, the procedures repeated, and the *mean* and *standard deviation* calculated. The agreement between the increase in the values obtained to the known increase in test sample concentration provides *a test of accuracy*. For procedures in which the test material is known to interact with the added reagent, as with phosphorus-sorption soils, this test can be conducted by reagent solutions.

1. Blanks

Blanks are reaction vessels that are subjected to identical procedures as the sample in a given batch which have no added test material. Blanks allow correction for any background contamination introduced from reagents, filter papers or other systemic sources of error. Provided the blank values are consistent, the mean value can be subtracted from the sample value. When blanks yield large values, this suggests excess extraneous contamination; in such cases, the entire batch analysis be repeated.

2. Repeats

At least 1 in 10 samples selected from the test materials and placed at random within the batch should be analyzed in *duplicate.* The choice of 1 in 10 is a suggested compromise between the ideal of analyzing all samples in duplicate, considering the time, effort and expense of doing so. Obviously, the analytical results for given pairs of duplicate repeats should closely resemble one another, in general, repeat values should fall within ±2.5 - 5.0% of their mean, depending on the analysis in question; any greater discrepancy must be investigated. If repeat values are not consistent, the entire batch should be re-analyzed

3. Internal References

Internal reference samples are necessary for each type of test material and analysis practiced within the laboratory. The internal sample should not be the same as the homogeneous material routinely used in the testing new methods and analytical technique. A sample obtained from a large, well-mixed and homogeneous composite bulk sample should be included in each batch analyzed. Variation from the mean as calculated over previous batches may be indicated as an error.

Analytical results for the internal reference may be plotted on a quality control chart to monitor the performance of the analyses over time. Corrective action could be taken if a single value exceeds the ± 3 standard deviation limits or if two successive values exceed the ± 2 standard deviations. Periodically, the critical limits could be re-assessed by re-calculation of the overall standard deviation of the internal reference sample as more data are accumulated.

4. Standardization of Methods

Results can only be validly compared to one another when these have been obtained using standardized methods. Collaboration between laboratories can be improved by exchanging reference materials and then comparing their results (Ryan and Garabet, 1994). Such materials are referred as "**External References**". An example of such standardization is the exchange network of ISRIC (International Soil Reference and Information Center) in Wageningen, The Netherlands, operating an international soil and plant analytical exchange programs.

Most external reference samples are costly, and their frequent use would increase operating costs of the laboratory. Internal reference samples are usually

much less expensive. Thus, if a relationship between external and internal reference samples can be firmly established, frequent use of internal reference sample with occasional use of the external reference sample can reduce costs, while still providing acceptable quality assurance.

3.4 Data Processing

A considerable amount of information is generated in any soil-plant analysis laboratory. In order to economically justify the existence of a laboratory, it is necessary to have a record of the number of samples analyzed and the types of analyses performed. With the advent of the computer, such storage is easy and retrieval is greatly facilitated. Computer processing offers the advantage of:

1. Easier manipulation of large data sets;
2. Reduced errors in calculation of recommendations;
3. Preparation of reports;
4. Automated invoicing and addressing; and
5. Ready access to historical data for preparation of soil test summaries.

The degree to which laboratories should be computerized depends on sample volume, location, and user services offered. In general, laboratories with a large volume of samples, and which offer a range of analyses have more need for computer sophistication and automation than laboratories with a small sample turnover.

In order to facilitate data processing, standard information sheets are required. These vary from laboratory to laboratory, but usually include details of analyses required for the sample and information on the crop to be grown, the soil type and previous cropping history, particularly with respect to fertilization. Such information enables one to answer questions on the extent of nutrient deficiency in any area from which the samples were obtained, and how fertility levels change over the years.

Computer programs are increasingly used to interpret soil test data and making fertilizer recommendations. Several such programs do exist. Standardized report forms for making fertilizer recommendations combine inputs of soil test data together with other soil and crop information. In order to do this, the tests used (soil NO_3^--N, available P, etc.) must be calibrated with field crop response.

With a relatively large output of analyses associated with a large number of on-station and on-farm research trials, analytical results from **ICARDA's** laboratory are stored in a computer program to generate fertilizer recommendations based on soil test values. Where soil maps and rainfall data are available, the accumulated soil test values of known locations can help establish relationships with soil type, region, climatic zone, etc.

4. SOIL PHYSICAL ANALYSIS

Soil physical measurements are numerous, depending on the objective of the study for agricultural purposes. These measurements generally include **soil *w*ater content, infiltration** and **hydraulic conductivity, evapotranspiration, heat, temperature, reflectivity, porosity, particle size, bulk density, aggregate stability, and particle size distribution** (Klute, 1986). However, only a few physical measurements are normally conducted in soil-plant analysis laboratories.

Soil moisture is routinely measured on field-moist samples, since all chemical analyses are expressed on oven-dry basis. As texture (e.g., whether sandy or clay) is quite important in relation to nutrient behavior, particle-size distribution is often carried out, especially if more precision is needed than provided by the qualitative physical "feel" approach for determining texture.

A common physical soil measurement, especially for irrigated soils, is the preparation of saturated paste. This provides an extract in which soluble anions and cations are measured; criteria for deficiency or toxicity of some elements are also based on soil extract element concentrations. For example, criteria for salinity tolerance, presented in **Appendix 14**, are based on a saturation extract. Similarly, criteria for boron (B) in relation to plant growth, shown in **Appendix 15**, are based on this extract.

4.1 Soil Moisture Content

As water is the most limiting factor in the CWANA region, soil moisture determination is of major significance. Soil moisture influences crop growth not only by affecting nutrient availability, but also **nutrient transformations** and soil **biological behavior.** Therefore at ICARDA soil moisture is routinely measured in most field trials. While it can be assessed in the field by neutron probe, the gravimetric approach is more flexible, as samples can be readily taken from any soil situation. All analyses in the laboratory are related to an air- or oven-dry basis, and therefore must consider the actual soil moisture content (Hesse, 1971).

Apparatus

Electric oven with thermostat.
Desiccator.

Procedure

1. Weigh 10 g air-dry soil (< 2-mm) into a previously dried (105°C) and weighed metal can with lid.
2. Dry in an oven, with the lid unfitted, at 105°C overnight.
3. Next day, remove from oven; fit the lid, cool in a desiccator for at least 30 minutes and re-weigh.

CALCULATIONS

$$\%\ \text{Moisture in Soil } (\theta) = \frac{\text{Wet soil (g)} - \text{Dry soil (g)}}{\text{Dry soil (g)}} \times 100 \quad \text{.......} \quad (2)$$

$$\text{Dry Soil (g)} = \frac{1}{1 + \frac{\theta}{100}} \times \text{Wet soil (g)} \quad \text{......................} \quad (3)$$

$$\textbf{Moisture Factor} = \frac{\textbf{Wet soil}}{\textbf{Dry soil}} \textbf{ or } \frac{100 + \%\theta}{100} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

4.2 Particle Size Distribution

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

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

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

Apparatus

Soil dispersing stirrer: A high-speed electric stirrer with a cup receptacle.

Hydrometer with Bouyoucos scale in g/L (ASTM 152H).

Reagents

A. Dispersing Solution

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

B. Amyl Alcohol

Procedure

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

A. Determination of Blank

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

B. Determination of Silt plus Clay

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

CALCULATIONS

Percentage **Silt** plus **Clay** in soil

$$\% \text{ [Silt + Clay] } (w/w) = (R_{sc} - R_b) \times \frac{100}{\text{Oven-dry soil (g)}} \quad \ldots\ldots\ldots\ldots\ldots(5)$$

C. Determination of Clay

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

Percentage **Clay** in soil:

$$\% \text{ Clay } (w/w) = (R_c - R_b) \times \frac{100}{\text{Oven-dry soil (g)}} \quad \text{.......................} (6)$$

Percentage **Silt** in soil:

$$\% \text{ Silt } (w/w) = [\% \text{ Silt + Clay } (w/w)] - [\% \text{ Clay } (w/w)] \quad \text{..............} (7)$$

D. Determination of Sand

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

Percentage **Sand** in soil:

$$\% \text{ Sand } (w/w) = \text{Sand weight} \times \frac{100}{\text{Oven-dry soil (g)}} \quad \text{.............} (8)$$

Where: Weight of sand follows from:

$$\text{Sand weight(g)} = [\text{Beaker + Sand (g)}] - [\text{Beaker (g)}] \quad \text{................} \quad (9)$$

Note

1. If possible, all hydrometer jars should be placed in a water bath at constant temperature (20°C); in that case, temperature corrections are not needed.
2. For temperature correction, use a value of 0.4 for each degree temperature difference from 20°C. Add or subtract this factor if the temperature is more or less than 20°C, respectively.
3. All results of mechanical analysis should be expressed on the basis of oven-dry soil (24 hours drying at 105°C).
4. In the above procedure, carbonates and organic matter are not removed from the soil.
5. The Hydrometer method, as described in this section, cannot be applied to soils that contain free gypsum (gypsiferous soils). For gypsiferous soils, see Hesse (1971).
6. Sum of % silt and clay + % sand should be 100 %. The magnitude of deviation from 100 is an indication for the degree in accuracy.

Soil Texture

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

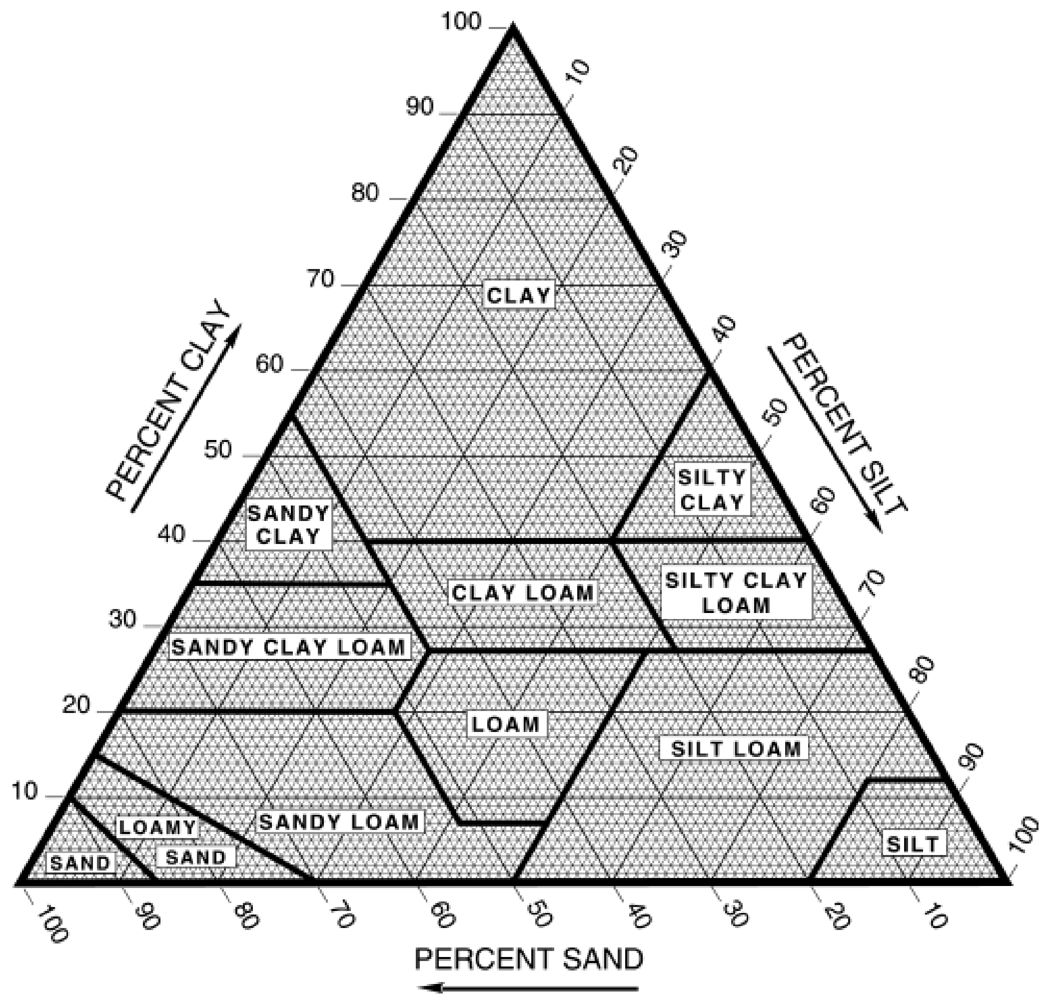


Fig. 4. The USDA Soil Textural Triangle.

4.3 Saturated Paste

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

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

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

Apparatus

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

Procedure

1. Weigh 200 - 300 g air-dry soil (< 2-mm) into a porcelain dish.
2. Slowly add DI water, and mix with a spatula until the paste glistens and flows slightly as the porcelain dish is tipped; it should slide off the spatula without collection of any free water on the surface of the paste.
3. Allow the paste to stand for 1 hour, then re-check the criteria for saturation by adding more DI water or soil, as needed.
4. Leave the paste for 6 to 16 hours, and then filter with a vacuum filtration system using a Buchner funnel fitted with Whatman No. 42 filter paper.
5. Collect filtrate in a small bottle and keep it for subsequent measurements. If the initial filtrate is turbid, re-filter.

4.4 Field Capacity Moisture and Permanent Wilting Point

Principle

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

A. Low Range: moisture at 0 - 100 kpa (0 - 1 bar) pressure

Apparatus

One-bar pressure plate extractor

One-bar ceramic plates.

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

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

Balance.

Drying oven.

Disposable aluminum dishes or soil-moisture cans.

Desiccator.

Procedure

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

9. The next morning, remove excess water from the plate with a syringe, disposable pipette, or siphon.
10. Place the triangular support in the extractor vessel on the bottom.
11. Install plate with samples in the lower-most position in the extractor. Then install the middle and top plates (the plastic spaces should be placed between plates).
12. Connect outflow tubes.
13. Close extractor and tighten, ensuring that the "O" ring is in place and all nuts are uniformly tightened. Apply desired pressure in the 0 - 100 kpa (0 - 1 bar) range. Build up the pressure in the vessel gradually.
14. Place a beaker to collect water from the outflow tubes.
15. Maintain pressure until no more water is being released (generally 18 - 20 hours, but for some soils 48 hours or even longer).
16. Release pressure from extractor (remove outflow tubes from water before turning instrument off).
17. Open extractor.
18. Without undue delay, transfer moist soil sample from ring with a wide-bladed spatula to a tarred dish. (It is not necessary to make a quantitative transfer of the entire soil.)
19. Immediately weigh wet sample (accuracy 0.01 g) and place in drying oven at 105 °C for 24 hours.
20. Place sample in desiccator, cool, and weigh.

CALCULATION

$$\% \text{ Moisture } (\theta) = \frac{\text{Wet soil (g) - Dry soil (g)}}{\text{Dry soil (g)}} \times 100 \quad ....(10)$$

B. High Range: moisture at 100 - 1500 kpa (0 - 15 bar) pressure

Apparatus

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

Procedure

1. Use 15-bar ceramic plates and follow Steps 1 - 12 of the previous method, applying 1 - 15 bar pressure (100 - 1500 kpa).
2. Place beaker to collect water from outflow tubes.
3. Leave overnight.
4. Connect outflow tube to burette partially filled with tap water.
5. Samples should stay in extractor until flow has ceased from all samples on plate and the soils have reached equilibrium (24 - 48 hours for most soil; however some fine textured and organic soils may needs up to 120 hours). No change in reading on burette would indicate that flow has stopped from all samples and equilibrium has been attained.
6. Disconnect burette to prevent backflow of tap water.
7. Release pressure from extractor.
8. Follow Steps 17 - 20 of the previous method.

CALCULATION

$$\%\ \text{Moisture}\ (\theta) = \frac{\text{Wet soil (g) - Dry soil (g)}}{\text{Dry soil (g)}} \times 100 \quad \text{...........}\ (11)$$

Note

1. If the outlets of the plates continue to bubble after a few hours of applied pressure, the plates are probably defective and should be replaced.
2. Pressure should not be allowed to fluctuate during a run. It should be checked after every 2 - 3 hours (and adjusted if necessary). If the pressure fluctuations are within the specified tolerance of the regulator, then no adjusting is needed.
3. Never remove extractor lid with pressure in the container.
4. The height of the sample in the ring should be as small as possible to reduce the time required to reach equilibrium, which is proportional to the square of the height of the sample in the ring.
5. *Available water **(AW)** or available water capacity **(AWC)** approximation:* Available water capacity is the amount of water retained in the soil reservoir that can be removed by plants. It is estimated by the difference in the soil water content between **field capacity (FC)** and **permanent wilting point (PWP).**

CALCULATION

% AWC = FC (%) - PWP (%) .... (12)

6. *Field capacity approximation*: Field capacity is commonly estimated by measuring the moisture retained at the following pressure:

Coarse-textured Soils	10 KPa	(1/10 bar)
Medium-textured Soils	33 KPa	(1/3 bar)
Fine-textured Soils	50 KPa	(1/2 bar)

7. *Permanent wilting point approximation*: Wilting point is commonly estimated by measuring the 1500-kpa (15-bar) percentage. It varies according to plant species and stage of plant growth, ranging 10 to 25 bars for mesophytic plants.

5. SOIL CHEMICAL ANALYSIS

The **18 essential nutrients** for plants are classified into four groups (Brady and Weil, 1999):

1. Major non-mineral macronutrients: these are 90 - 95% of dry plant weight, and are supplied to the plant by water absorption and photosynthesis, i.e., C, H, O;
2. Primary macronutrients, i.e., N, P, K;
3. Secondary macronutrients, i.e., Ca, Mg, S; and
4. Micronutrients, i.e., B, Cl, Co, Cu, Fe, Mn, Mo, Ni, Zn.

Three major factors contributing to plant nutrition are:

1. The amount of nutrients in the soil;
2. The soil's ability to supply the nutrients to plants; and
3. Environmental factors that affect nutrient availability and their absorption.

Measurements, which involve characterization of the *soil solution and its constituents* and *of the composition of the inorganic and organic phases in soil,* are broadly termed chemical. This encompasses all nutrient elements and soil components which directly or indirectly influence such elements or components. **This section thus constitutes the core of this manual.**

The chemical procedures presented here are extensive, though by no means exhaustive. For any one element, numerous procedures or variations of procedures can be found in the literature (Walsh and Beaton, 1973; Page, 1982; Westerman, 1990). We have endeavored to select procedures which, in our experience, are appropriate for soils of the CWANA region, i.e., where a good relationship exists between the test value and crop growth. Where alternative methods are appropriate, we have presented the salient features of these methods. A bibliography of soil testing information is provided for users who may need to expand or modify their range of soil testing procedures.

We have initially presented analyses which are routinely done to characterize a soil sample or soil type in terms of background information, i.e., pH, salinity, calcium carbonate, organic matter, cation exchange capacity, and gypsum. With regard to nitrogen, the dominant fertility factor in the CWANA region soils, we have dealt with the most convenient methods for measuring different forms or fractions of N in soils. This is subsequently followed by procedures for P, soluble and exchangeable cations, soluble anions, and micronutrients. Where appropriate, we have given guidelines in the appendix **for interpreting the data** produced with the **analytical procedures** listed.

5.1 pH

The pH is defined as the negative log of the hydrogen ion activity. Since pH is logarithmic, the H-ion concentration in solution increases ten times when its pH is lowered by one unit. The pH range normally found in soils varies from 3 to 9. Various categories of soil pH may be arbitrarily described as follows: *strongly acid* (pH < 5.0), *moderately* to *slightly acid* (5.0 - 6.5), *neutral* (6.5 - 7.5), *moderately alkaline* (7.5 - 8.5), and *strongly alkaline* (> 8.5).

Significance of pH lies in its influence on availability of soil nutrients, solubility of toxic nutrient elements in the soil, physical breakdown of root cells, cation exchange capacity in soils whose colloids (clay/humus) are pH-dependent, and on biological activity. At high pH values, availability of phosphorus (P) and most micronutrients, except boron (B) and molybdenum (Mo), tends to decrease.

Acid soils are rare in semi-arid dryland areas of the world; they tend to occur in *temperate and tropical areas* where rainfall is substantial; conversely, soils of drier areas are generally alkaline, i.e., above pH 7.0, as a result of the presence of calcium carbonate ($CaCO_3$); they visibly effervesce (fizz) when 10% hydrochloric acid is added dropwise to the soil. Most soils in the Central and West Asia and North Africa region have pH values of 8.0 - 8.5. Calcareous soils with gypsum have somewhat lower pH values, while those with excess sodium (Na) have values over 8.5 (sodic soils).

Thus, soil pH is one of the most common measurements in soil laboratories. It reflects whether a soil is *acid, neutral, basic or alkaline*. Procedure for determining soil pH in a 1:1 (soil: water) suspension (McKeague, 1978; McLean, 1982) is:

Apparatus

pH meter with Combined Electrode.
Glass rod.
Glass beaker.

Reagents

A. Deionized water.
B. pH 7.0 buffer solution.
C. pH 4.0 buffer solution.

Procedure

1. Weigh 50 g air-dry soil (< 2-mm) into a 100-mL glass beaker.
2. Add 50 mL DI water using a graduated cylinder or 50-mL volumetric flask
3. Mix well with a glass rod, and allow to stand for 30 minutes.
4. Stir suspension every 10 minutes during this period.
5. After 1 hour, stir the suspension.
6. Put the Combined Electrode in suspension (about 3-cm deep). Take the reading after 30 seconds.
7. Remove the Combined Electrode from the suspension, and rinse thoroughly with DI water in a separate beaker, and carefully dry excess water with a tissue.

Note

1. Make sure that the combined electrode contains *saturated* KCl solution and some solid KCl.
2. Calibrate the pH meter using at least two buffer solutions of different pH values, usually 4.0 and 7.0. *First,* measure the temperature of the solution and adjust the *"temperature"* knob. *Second,* dip the combined electrode in pH 7.0 buffer solution, check for actual pH at measured temperature, and adjust with the *"buffer"* knob. Then, dip the combined electrode in the pH 4.0 buffer solution and adjust with *"sensitivity"* knob. Repeat until pH meter gives correct reading of both buffer solutions.
3. At ICARDA, pH is measured in a 1:1 (soil: water) suspension. For special purposes, pH can be measured in a saturated soil paste, or in more dilute suspensions. In some laboratories, pH is measured in a suspension of soil and 1 *N* KCl or 0.01 *M* $CaCl_2$. The main advantage of the measurement of soil pH in salt solution is the tendency to eliminate interference from suspension effects and from variable salt contents, such as fertilizer residues. However, this is hardly needed in alkaline-calcareous soils of CWANA.
4. Air-dry soils may be stored several months in closed containers without affecting the pH measurement.
5. If the pH meter and combined electrodes are not to be used for extended periods of time, the instructions for storage published by the instrument manufacturer should be followed.
6. For soil samples very high in organic matter, use a 1:2 or 1:5 (soil: water) ratio.

5.2 Electrical Conductivity

Soil salinity refers to the concentration of soluble inorganic salts in the soil. It is normally measured by extracting the soil sample with water (1:1 or 1:5 soil: water ratio, w/v) or in an saturated paste extract. However, soil: solution ratios of a 1:1 or wider ratio are more convenient where the soil sample is limited. Such extracts are rapid and salinity is measured by electrical conductivity (EC) using a conductivity bridge. *The total salt content of a soil can be estimated from this measurement.* A more precise method involves evaporation of the aqueous extract and weighing the residue.

Salinity is an important laboratory measurement since it reflects the extent to which the soil is suitable for growing crops. On the basis of a saturation extract, values of 0 to 2 dS/m (or mmhos/cm) are safe for all crops; yields of very sensitive crops are affected between 2 to 4 dS/m; many crops are affected between 4 and 8 dS/m; while only tolerant crops grow well above that level (Richards, 1954).

While salinity is largely a concern in irrigated areas of the CWANA region and in areas with saline soils, it is not so important in rainfed agriculture. However, with increasing use of irrigation, there will be greater emphasis on EC measurement in the future. The methodology of EC measurement is given in USDA Handbook 60 (Richards, 1954).

Apparatus

Vacuum filtration system.
Conductivity bridge.

Procedure

1. Prepare a 1:1 (soil: water) suspension, as for pH determination.
2. Filter the suspension using suction. *First,* put a round Whatman No. 42 filter paper in the Buchner funnel. *Second,* moisten the filter paper with DI water and make sure that it is tightly attached to the bottom of the funnel and that all holes are covered.
3. Start the vacuum pump.
4. Open the suction, and add suspension to Buchner funnel.
5. Continue filtration until the soil on the Buchner funnel starts cracking.
6. If the filtrate is not clear, the procedure must be repeated.
7. Transfer the clear filtrate into a 50-mL bottle, immerse the Conductivity Cell

in the solution, and take the reading.

8. Remove the conductivity cell from the filtration, rinse thoroughly with DI water, and carefully dry excess water with a tissue.

Note

1. Readings are recorded in milli-mhos per centimeter (mmhos/cm) or deci-Siemens per meter (dS/m). The use of the unit deci-Siemens is preferred over the unit milli-mhos. Both units are equal, that is, **1 dS/m = 1 mmho/cm.**
2. Reading are usually taken and reported at a standard temperature of 25°C.
3. Check accuracy of the EC meter using a **0.01 *N* KCl** solution, which should give a reading of **1.413 dS/m at 25°C.**

5.3 Calcium Carbonate

Inorganic carbonate, either as calcium (calcite) or magnesium (dolomite) carbonate or mixtures of both, occurs in soils as a result of weathering, or is inherited from the parent material. *Most soils of arid and semi-arid regions are calcareous.* In fact, soils of the CWANA region may contain up to 50% $CaCO_3$-equivalent or even more.

As with alkaline pH, soils with free $CaCO_3$ tend to have lower availability of P and of some micronutrient cations. Consequently, $CaCO_3$ equivalent is normally determined in most laboratories of the CWANA region.

While some laboratories also determine "active" $CaCO_3$, it is less common than "total" $CaCO_3$, being mainly in areas of French influence since it was developed by Drouineau (1942) in France. It basically reflects surface area or reactivity of $CaCO_3$ particles, mainly the clay-size fraction. Measurement is based on reaction with excess ammonium oxalate followed by titration with permanganate in an acid medium.

Active $CaCO_3$ is usually related to total $CaCO_3$ equivalent, being about 50% or so of the total value. Proponents of its use claim that this fraction is more closely related to nutrient behavior, such as involved with iron chlorosis.

Principle

A given weight of soil is reacted with an excess of acid. In this reaction, CO_2 gas is released and the acid not used in the dissolution of carbonates is back-titrated with sodium hydroxide solution (FAO, 1974). Some methods of carbonate determination in soils are based on the collection of CO_2 gas, and the measurement of CO_2 pressure which develops if acid is added to a calcareous soil in a closed flask. In the titrimetric method, two equivalents of acid are assumed to react with one mole of $CaCO_3$. Hence, one equivalent of acid is assumed to be equivalent to one-half mole of $CaCO_3$.

Apparatus

Hot plate.
Burette.
Erlenmeyer flask.
Volumetric pipette.

Reagents

A. Hydrochloric Acid Solution (HCl), 1 *N*

Dilute 82.8 mL *concentrated hydrochloric acid* (37%, sp. gr. 1.19) in DI water, mix well, let it cool, and bring to 1-L volume with DI water.

B. Sodium Hydroxide Solution (NaOH), 1 *N*

Dissolve 40 g *sodium hydroxide* in DI water, and transfer to a 1-L volume, let it cool, and bring to volume with DI water.

C. Phenolphthalein Indicator [$C_6H_4COOC (C_6H_4\text{-}4\text{-}OH)_2$]

Dissolve 0.5 g *phenolphthalein* indicator in 100-mL ethanol (ethyl alcohol).

D. Methyl-Orange Indicator [$4\text{-}NaOSO_2C_6H_4N: NC_6H_4 /\text{-}4\text{-}N (CH_3)_2$]

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

E. Ethanol (C_2H_5OH), 95%

F. Sodium Carbonate Solution (Na_2CO_3), 1 *N*

Dissolve 53 g *anhydrous sodium carbonate* in DI water, and bring to 1-L volume with DI water.

Procedure

1. Weigh 1 g air-dry soil (0.15-mm) into a 250-mL Erlenmeyer flask.
2. Add 10 mL 1 *N* **hydrochloric acid** solution to the flask with a volumetric pipette.
3. Swirl and leave the flask overnight, or heat to 50 - 60°C, and let the flask cool.
4. Add 50 - 100 mL DI water using a graduated cylinder, and add 2 - 3 drops **phenolphthalein** indicator.
5. Titrate with 1 *N* **sodium hydroxide** solution while swirling the flask. Continue the titration until a faint pink color develops, and take the reading, **R.**

CALCULATION

Percentage **Calcium Carbonate** in soil:

$$\% \, CaCO_3 = [(10 \times N_{HCl}) - (R \times N_{NaOH})] \times 0.05 \times \frac{100}{Wt} \quad \text{..... (13)}$$

Where:

N_{HCl} = Normality of HCl solution.
R = Volume of NaOH solution used (mL)
N_{NaOH} = Normality of NaOH solution.
Wt = Weight of air-dry soil (g)

Standardization of Solutions

1. Hydrochloric Acid (HCl), 1 *N*

Pipette 10 mL 1 *N Sodium Carbonate* solution into a 100-mL Erlenmeyer flask, add 2 drops *methyl-orange* indicator, and titrate this solution against 1 *N hydrochloric acid* (in the burette). The solution color changes from light to dark orange.

HCl normality is:

$$N_{HCl} = \frac{10 \times N_{Na_2CO_3}}{V_{HCl}} \quad \text{.................................... (14)}$$

Where:

N_{HCl} = Normality of HCl solution.
V_{HCl} = Volume of HCl solution used (mL)
$N_{Na_2CO_3}$ = Normality of Na_2CO_3 solution.

2. Sodium Hydroxide (NaOH), 1 *N*

Pipette 10 mL standardized 1 *N hydrochloric acid* solution into a 100-mL Erlenmeyer flask, add 2 drops *phenolphthalein* indicator, and titrate against 1 *N sodium hydroxide* solution. The solution color changes from colorless to pink.

NaOH normality is:

$$N_{NaOH} = \frac{10 \times N_{HCl}}{V_{NaOH}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (15)$$

Where:
N_{NaOH} = Normality of NaOH solution.
V_{NaOH} = Volume of NaOH solution used (mL)
N_{HCl} = Normality of HCl solution.

Note

1. It requires some experience to accurately determine **color change of the suspension from colorless to pink.**
2. 10 mL 1 *N* **HCl** would dissolve up to 0.5 g $CaCO_3$. That is, if a soil contains 50% $CaCO_3$ or more, 10 mL 1 *N* **HCl** would not be sufficient. In that case, 15 or 20 mL would have to be added.
3. When a soil is reacted with acid to dissolve carbonates, other soil components may also consume acid. Most of the latter reactions are assumed to be reversible, i.e., if the suspension is back-titrated, the acid is released again. For this reason it is *not recommended* to filter the suspension and titrate the clear filtrate. The color change is easier to determine in a clear solution, but the titration value may overestimate the actual $CaCO_3$ content of the soil.
4. Not all reactions involving acid and soil components are completely reversible, and therefore the acid titration of the soil suspension may also slightly over-estimate the actual soil carbonate content. The acid titration method may be calibrated against the **Calcimeter,** if available; however, it is rarely used nowadays.

5.4 Organic Matter

Soil organic matter represents the remains of roots, plant material, and soil organisms in various stages of decomposition and synthesis, and is variable in composition. Though occurring in relatively small amounts in soils, *organic matter (OM) has a major* influence on *soil aggregation*, *nutrient reserve* and its *availability, moisture retention,* and *biological activity*.

Organic carbon (OC) ranges from being the dominant constituent of peat or muck soils in colder regions of the world to being virtually absent in some desert soils. Cultivated, temperate-region soils normally have more than 3 - 4 % OM, while soils of semi-arid rainfed areas, such as in the CWANA region, have normally less than 1% OM.

Most laboratories in the region perform analysis for soil organic matter. The most common procedure involves reduction of potassium dichromate ($K_2Cr_2O_7$) by OC compounds and subsequent determination of the unreduced dichromate by oxidation-reduction titration with ferrous ammonium sulfate (Walkley, 1947; FAO, 1974). While the actual measurement is of oxidizable organic carbon, the data are normally converted to percentage organic matter using a constant factor, assuming that **OM contains 58% organic carbon**. However, as this proportion is not in fact constant, we prefer to report results as **oxidizable organic carbon**, or **multiplied by 1.334 as organic carbon.**

Apparatus

Magnetic stirrer and teflon-coated magnetic stirring bar.
Glassware and pipettes for dispensing and preparing reagents.
Titration apparatus (burette).

Reagents

A. Potassium Dichromate Solution ($K_2Cr_2O_7$), 1*N*

- Dry *potassium dichromate* in an oven at 105°C for 2 hours, cool in a desiccator (silica gel), and store in a tightly stoppered bottle.
- Dissolve 49.04 g *potassium dichromate* in DI water, and bring to 1-L volume with DI water.

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

C. Orthophosphoric Acid (H_3PO_4), concentrated

D. Ferrous Ammonium Sulfate Solution [$(NH_4)_2SO_4.FeSO_4.6H_2O$], 0.5 *M*

Dissolve 196 g *ferrous ammonium sulfate* in DI water, and transfer to a 1-L volume, add 5 mL *concentrated sulfuric acid,* mix well, and bring to volume with DI water.

E. Diphenylamine Indicator $(C_6H_5)_2NH$

Dissolve 1 g diphenylamine indicator in 100 mL concentrated sulfuric acid.

Procedure

1. Weigh 1 g air-dry soil (0.15 mm) into a 500-mL beaker.
2. Add 10 mL 1 *N* **potassium dichromate** solution using a pipette, add 20 mL **concentrated sulfuric acid** using a dispenser, and swirl the beaker to mix the suspension.
3. Allow to stand for 30 minutes.
4. Add about 200 mL **DI water,** then add 10 mL **concentrated orthophosphoric acid** using a dispenser, and allow the mixture to cool.
5. Add 10 - 15 drops **diphenylamine** indicator, add a teflon-coated magnetic stirring bar, and place the beaker on a magnetic stirrer.
6. Titrate with 0.5*M* **ferrous ammonium sulfate** solution, until the color changes from violet-blue to green.
7. Prepare two blanks, containing all reagents but no soil, and treat them in exactly the same way as the soil suspensions.

CALCULATIONS

Percentage **Organic Matter** in soil:

$$M = \frac{10}{V_{blank}} \quad \text{......} \quad (16)$$

$$\% \text{ Oxidizable Organic Carbon (w/w)} = \frac{[V_{blank} - V_{sample}] \times 0.3 \times M}{Wt} \quad .. (17)$$

$$\% \text{ Total Organic Carbon(w/w)} = 1.334 \times \% \text{ Oxidizable Organic Carbon} \quad .... (18)$$

$$\% \text{ Organic Matter (w/w)} = 1.724 \times \% \text{ Total Organic Carbon} \quad .... (19)$$

Where: M = Molarity of ferrous ammonium sulfate solution (approx. 0.5 M)

V_{blank} = Volume of ferrous ammonium sulfate solution required to titrate the blank (mL)

V_{sample} = Volume of ferrous ammonium sulfate ferrous ammonium sulfate solution required to titrate the sample (mL)

Wt = Weight of air-dry soil (g)

0.3 = $3 \times 10^{-3} \times 100$, where 3 is the equivalent weight of C.

Note

1. For soils high in organic matter (1% Oxidizable Organic Carbon or more), more than 10 mL **potassium dichromate** is needed.
2. The factors 1.334 and 1.724 used to calculate ***TOC*** and ***OM*** are approximate; they may vary with soil depth and between soils.
3. Soils containing large quantities of chloride (Cl^-), manganese (Mn^{--}) and ferrous (Fe^{++}) ions will give higher results. The chloride interference can be eliminated by adding **silver sulfate** (Ag_2SO_4) to the oxidizing reagent. No known procedure is available to compensate for the other interferences.
4. The presence of $CaCO_3$ up to 50% causes no interferences.

5.5 Cation Exchange Capacity

Many minerals in soils are negatively charged and, as a consequence, can attract and retain cations such as potassium (K^+), sodium (Na^+), calcium (Ca^{++}), magnesium (Mg^{++}), ammonium (NH_4^+), etc. Cation exchange is a reversible process. Thus, elements or nutrients can be held in the soil and not lost through leaching, and can subsequently be released for crop uptake.

Certain organic compounds also contribute to cation exchange capacity (CEC). Additionally, CEC is influenced by soil pH. A certain portion of the total negative charge is permanent, while a variable portion is pH-*dependent*.

Several methods are available for CEC determination (Rhoades, 1982). Most involve saturation of the soil with an index cation (NH_4^+), removal by washing of excess cation, and subsequent replacement of the adsorbed index cation by another cation (Na^+) and measurement of the index cation in the final extract (Richards, 1954). Modified procedures have been introduced because of high calcium solubility in calcareous and gypsiferous soils (FAO, 1990; Rhoades and Polemio, 1977).

Cation exchange capacity is reported as milliequivalents per 100 g soil or more recently as cmol (+)/*kg soil* **(S.I. unit)**; the actual numbers being the same **(1 meq/100 g = 1 cmol (+)/kg)**. Values of CEC are in the range of 1.0 to 100 meq/100g, least for sandy soils and most for clay soils. Similarly, higher CEC values reflect the dominance of 2:1 clay minerals, and lower values reflect the presence of 1:1 clay minerals.

Apparatus

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

Reagents

A. Sodium Acetate Solution (NaOAc), 1 *N*

- Dissolve 136 g *sodium acetate trihydrate* ($CH_3COONa.3H_2O$) in about 950 mL DI water, mix well, and let the mixture cool.
- Adjust pH to 8.2 by adding more *acetic acid* or *sodium hydroxide*, and bring to 1-L volume with DI water.

B. Ethanol (C_2H_5OH), 95%

C. Ammonium Acetate Solution (NH_4OAc), 1*N*

- Add 57 mL *concentrated acetic acid* (CH_3COOH) to 800 mL DI water, then add 68 mL *concentrated ammonium hydroxide* (NH_4OH), mix well, and let the mixture cool.
- Adjust to pH 7.0 by adding more *acetic acid* or *ammonium hydroxide,* and bring to 1-L volume with DI water.

D. Standard Stock Solution

- Dry about 5 g *sodium chloride* (NaCl) in an oven at 105°C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 2.5418 g dried *sodium chloride* in DI water, and bring to 1-L volume with DI water. This solution contains 1000 ppm Na (*Stock Solution*).
- Prepare a series of Standard Solutions from the *Stock Solution* as follows: Dilute 2, 4, 6, 8, 10, 15, and 20 mL *Stock Solutions* to 100 mL final volume by adding 1 *N* ammonium acetate solution, and 25 mL LiCl *(Diluted Stock Solution)*. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm Na, with each containing the same concentration of LiCl (25 ppm).

Procedure

1. Weigh 4 g (for medium to fine textured) or 6 g (for coarse textured) air-dry soil into a 40-mL centrifuge tube, and add 33 mL 1 *N* **sodium acetate trihydrate** solution, stopper tube, and shake for 5 minutes.
2. Remove stopper from tube and centrifuge at 3000 rpm until supernatant liquid is clear. Decant the supernatant as completely as possible and discard.
3. Repeat with 33-mL portions 1 *N* **sodium acetate trihydrate** solution, a total of four times, discarding the supernatant liquid each time. Then add 33-mL 95% **ethanol,** stopper tube, and shake for 5 minutes, unstopper tube, and centrifuge until the supernatant is clear and decant.
4. Wash the sample with 33 mL portions 95% **ethanol,** a total of three times, discarding the supernatant liquid each time. The electrical conductivity (EC) of the supernatant liquid from the third washing should be less than 400 µS/cm.

5. Replace the adsorbed sodium (Na^+) from the sample by extraction with three 33-mL portions 1 *N* **ammonium acetate** solution. Each time shake for 5 minutes, and centrifuge until supernatant liquid is clear.
6. Decant the three supernatant liquids as completely as possible into a 100-mL volumetric flask, bring to volume with 1 *N ammonium acetate* solution, and mix well.
7. Run a series of suitable Na standards, and draw a calibration curve.
8. Measure the samples (soil extract) and take the emission readings by a **Flame Photometer** at 767 nm wavelength.
9. Calculate sodium (Na) concentration according to the calibration curve.

CALCULATION

For **Cation Exchange Capacity** in soil:

$$\text{CEC (meq/100 g)} = \text{meq/L Na (from calibration curve)} \times \frac{A}{Wt} \times \frac{100}{1000} \quad (20)$$

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

Note

Though quite laborious, the method of Rhoades and Polemio (1977) is more appropriate for soils containing carbonates, gypsum, and zeolite.

5.6 Gypsum

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

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

Apparatus

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

Reagent

Acetone

Procedure (Quantitative)

1. Weigh 10 to 20 g air-dry soil (medium to fine textured) into a 250-mL bottle, and add a measured volume of **DI water** sufficient to dissolve the gypsum present.
2. Stopper the bottle and shake by hand six times at 15-minute intervals or agitate for 15 minutes in a mechanical shaker.
3. Filter the extract through filter paper of medium porosity, and transfer a 20-mL aliquot of filtered extract into a 50-mL conical centrifuge tube.
4. Add 20 mL **acetone,** mix well, and let stand until precipitate is flocculated. This usually requires 5 to 10 minutes.
5. Centrifuge at 4000 rpm for 3 minutes, decant supernatant liquid, invert tube, and drain on filter paper for 5 minutes.
6. Disperse precipitate and rinse tube wall with a stream of 10 mL blown from a pipette.

7. Again, centrifuge for 3 minutes, decant supernatant liquid, invert tube, and drain on filter paper for 5 minutes.
8. Add exactly 40 mL **DI water** to tube, stopper, and shake until the precipitate is completely dissolved. Measure electrical conductivity of solution, and correct conductivity reading to 25°C.
9. Determine gypsum concentration in the solution by reference to a graph showing the relationship between the concentration and EC constructed by means of the following data from the International Critical Tables (Richards, 1954, **Fig. 2**).

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

CALCULATIONS

For **Gypsum** in soil:

$CaSO_4.2H_2O$ in aliquot (meq) = $CaSO_4.2H_2O$ from conductivity reading (meq/L) ×
water used to dissolve precipitate (mL)/1000 .... (21)

Gypsum (meq/100 g) = 100 × $CaSO_4.2H_2O$ in aliquot (meq)/(soil water) ratio ×
(soil - water) extract used (mL) .... (22)

Note

1. Sodium and potassium sulfates, when present in sufficiently high concentrations, are also precipitated by acetone. The maximum concentrations of sodium sulfate and of potassium that may be tolerated are 50 and 10 meq/L, respectively.
2. At a 1:5 (soil: water) ratio, water will dissolve approximately 15 meq gypsum per 100 g soil. If it is found that the gypsum content of the soil approaches 15 meq/100 g using a 1:5 (soil: water) extract, the determination should be repeated, using a diluted extract.
3. In some soils from the Euphrates Basin, gypsum may be well over 25%, in which case dilution's of 1:500 or 1:1000 (soil: water) ratio have to be used.
4. **Qualitative test** for gypsum should be made on all soils as a routine in order to save time later when analyzing for gypsum. Pipette 5 mL of the soil extract into a small centrifuge tube and add 5 mL acetone. Mix well, and allow to stand for 10 minutes if a flocculate white precipitate forms, the soil contains gypsum; if no precipitate forms, the soil is considered to have no gypsum.

6. SOIL NUTRIENTS, SODIUM AND ANION ANALYSIS

6.1 Nitrogen

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

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

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

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

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

6.1.1 Kjeldahl Nitrogen

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

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

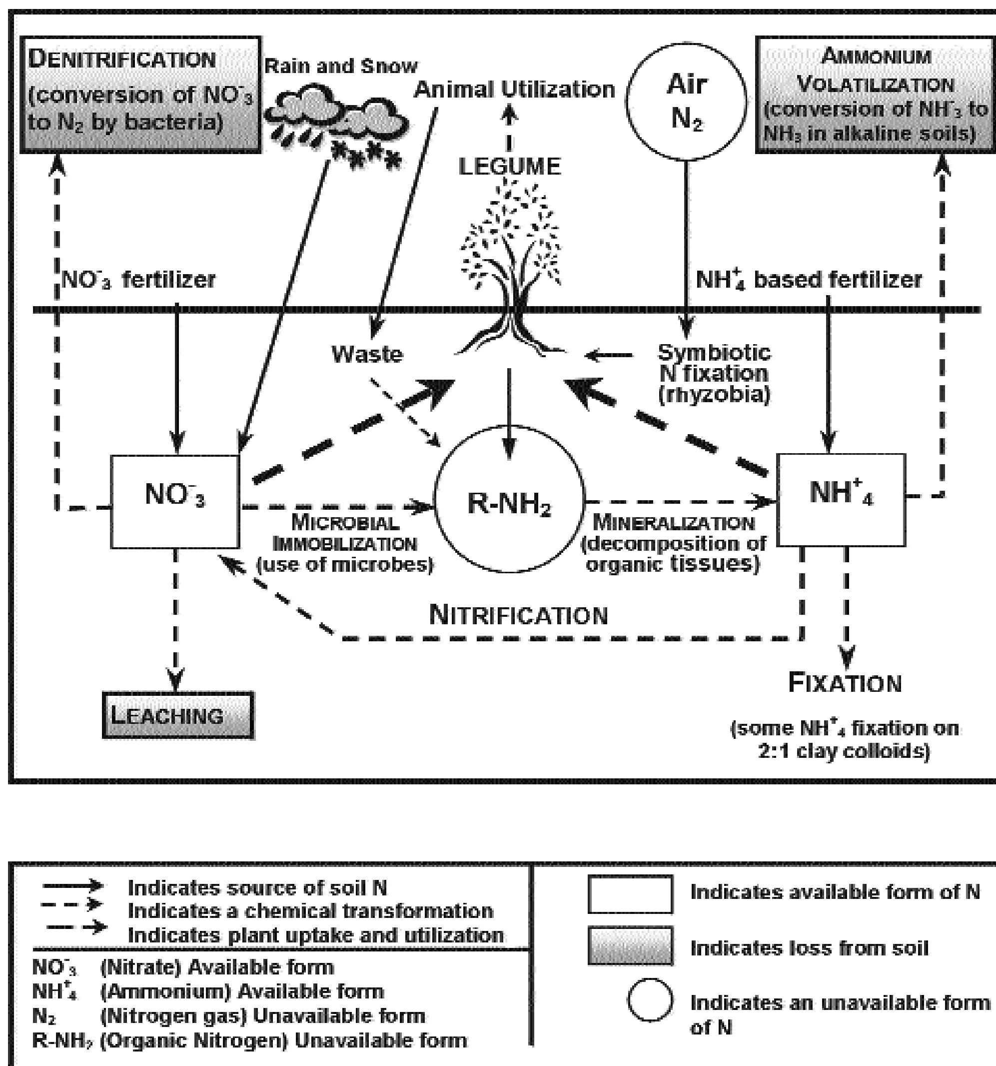


Fig. 5. The Nitrogen Cycle (Hach Company, 1992).

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

Apparatus

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

Reagents

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

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

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

C. Sodium Hydroxide Solution (NaOH), 10 N

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

D. Boric Acid Solution (H_3BO_3), saturated

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

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

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

F. Sulfuric Acid Solution (H_2SO_4), 0.01 N

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

G. Standard Stock Solution

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

Procedure

A. Digestion

1. Weigh 1 g air-dry soil (0.15 mm) into a 100-mL calibrated digestion tube.
2. Add about 5.0 - 5.5 g catalyst mixture, a few pumice boiling granules, 15 mL concentrated sulfuric acid (in the fume hood), and swirl carefully. Place a glass funnel in the neck of the tube, then place tubes in the rack, and leave overnight.
3. Place the tubes rack in the block-digester, and slowly increase temperature setting to about 370°C. The H_2SO_4 should condense about half-way up the tube neck; and when solution clears, continue heating for about 3 hours.
4. Lift the tubes rack out of the block-digester, carefully place on a rack holder, and let tubes cool to room temperature.
5. Slowly add about 15 mL DI water to the tubes, cool, and bring to volume with DI water. If tube contents are solidified and do not dissolve, heat the tubes again until the precipitate (gypsum) dissolves. Then cool with tap water.
6. Each batch of samples for digestion should contain at least one reagent blank (no soil), and one chemical standard (no soil, 1 mL of the Stock Solution).

B. Distillation

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

   H_2SO_4 normality is:

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

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

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

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

CALCULATIONS

Percentage recovery of Ammonium-N standard:

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

Percentage Nitrogen in soil:

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

Where:

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

Note

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

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

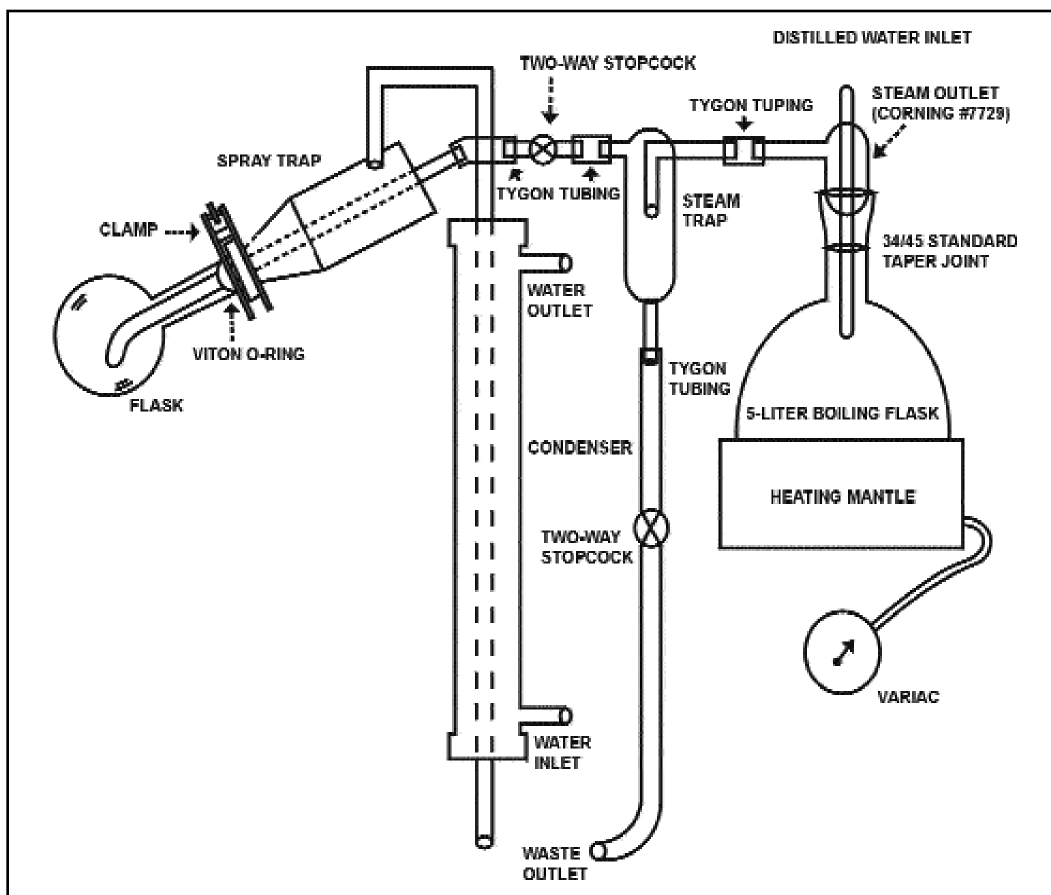


Fig. 6. Diagram of a Distillation Unit.

6.1.2 Total Nitrogen

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

Reagents

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

B. Potassium Permanganate Solution ($KMnO_4$)

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

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

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

D. Reduced Iron

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

E. N-Octyl Alcohol Solution

F. Catalyst Mixture

Prepare as in Kjeldahl-N.

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

Store in a desiccator.

H. Sodium Hydroxide Solution (NaOH), 10 N

Prepare as in Kjeldahl-N.

I. Boric Acid Solution (H_3BO_3), saturated

Prepare as in Kjeldahl-N.

J. Tris

Prepare as in Kjeldahl-N.

K. Sulfuric Acid Solution (H_2SO_4), 0.01 N

Prepare as in Kjeldahl-N.

Procedure

A. Digestion

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

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

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

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

B. Distillation

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

3. Carry out distillations as follows:

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

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

CALCULATIONS

Percentage recovery of EDTA standard:

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

Percentage Nitrogen in soil:

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

Where:

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

6.1.3 Mineral Nitrogen

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

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

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

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

Reagents

A. Potassium Chloride Solution (KCl), 2 M

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

B. Magnesium Oxide (MgO), powder

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

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

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

D. Boric Acid Solution (H_3BO_3), saturated

Prepare as in Kjeldahl-N.

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

Prepare as in Kjeldahl-N.

G. Sulfuric Acid Solution (H_2SO_4), 0.01 N

Prepare as in Kjeldahl-N

H. Standard Stock Solution

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

Procedure

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

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

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

CALCULATIONS

For Ammonium-N in air-dry soil:

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

For Ammonium-N in oven-dry soil:

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

Where:

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

Note

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

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

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

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

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

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

6.1.4 Nitrate-Nitrogen

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

Apparatus

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

Reagents

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

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

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

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

C. Sulfuric Acid (H_2SO_4), concentrated

D. Standard Stock Solution

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

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

Procedure

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

CALCULATION

For Nitrate-N in soil:

$$NO_3 - N\ (ppm) = ppm\ NO_3 - N\ (\text{from calibration curve}) \times \frac{A}{\quad} \times \frac{10}{\quad}$$

...(30)

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

Note

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

6.1.5 Microbial Biomass Nitrogen and Carbon

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

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

Apparatus

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

Reagents

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

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

B. Potassium Sulfate Solution (K_2SO_4), 0.5 M

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

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

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

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

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

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

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

F. 1.10-Phenanthroline Indicator

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

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

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

Procedure

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

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

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

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

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

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

1. Determination of Nitrogen

A. Digestion

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

B. Distillation

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

CALCULATIONS

For Biomass Nitrogen in soil:

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

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

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

2. Determination of Carbon

A. Digestion

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

B. Titration

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

CALCULATION

For Biomass Carbon in soil

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

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

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

B = Digested blank titration volume (mL)

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

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

Wt = Weight of fresh soil (g)

V = Aliquot used for soil digest measured (mL)

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

Note

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

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

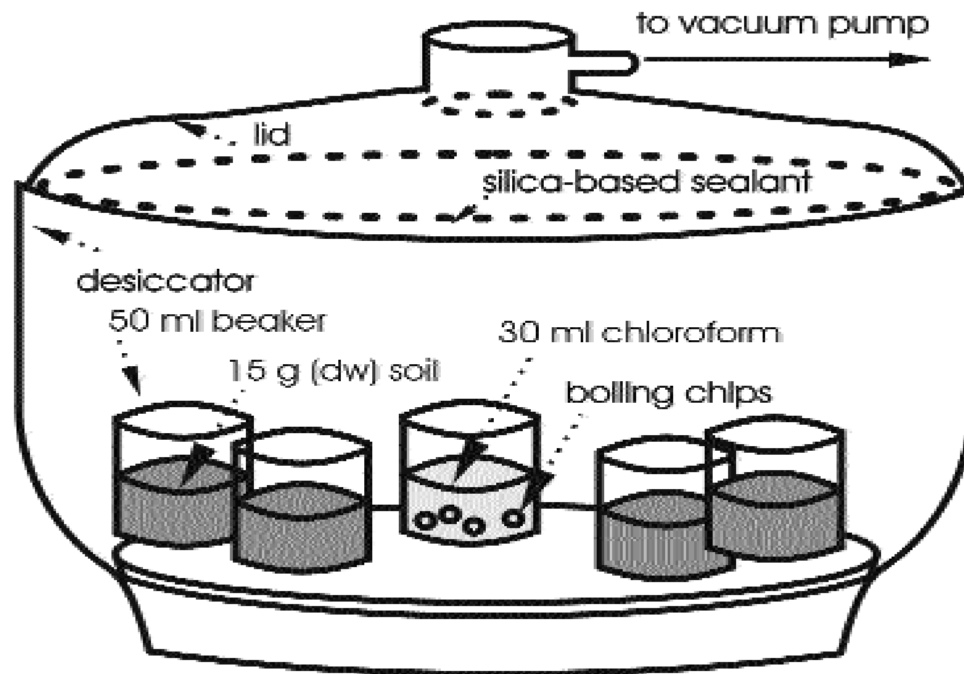


Fig. 7. Experimental Apparatus and Sample Arrangement in the Fumigation Procedure (Okalebo et al., 1993)

6.2 Phosphorus

6.2.1 Extractable Phosphorus

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

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

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

Apparatus

Spectrophotometer or colorimeter, 882-nm wavelength.

Mechanical shaker, reciprocating.

Extraction bottle, 250 mL with stopper.

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

Reagents

A. Sodium Hydroxide Solution (NaOH), 5 N

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

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

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

C. Sulfuric Acid Solution (H_2SO_4), 5 N

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

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

E. Standard Stock Solution

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

F. Reagent A

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

mL DI water.

- Dissolve 0.2908 g antimony potassium tartrate ($KSbO.C_4H_4O_6$) in 100 mL DI water.
- Add both dissolved reagents to a 2-L volumetric flask, and add 1-L 5 N H_2SO_4 (148 mL concentrated H_2SO_4 per liter) to the mixture.
  Mix thoroughly, and dilute to 2-L volume with DI water. Store in a Pyrex bottle in a dark, cool place.

G. Reagent B

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

Procedure

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

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

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

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

CALCULATION

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

For Extractable Phosphorus in soil:

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

Note

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

3. If the sample solutions are too dark-colored for measurement against the highest standard, a smaller soil extract aliquot should be taken, and the calculation modified accordingly. Once the blue color has developed, the solution cannot be diluted.

4. Glassware used in P analysis should not be washed with detergents containing P (and remember that most detergents do contain P).

5. As glass tube density may vary, it is best to use the same tube (cuvette) for each absorbance reading on a spectrophotometer.

6. If AB-DTPA test (described at 6.10.2) is used for evaluating micronutrient status of the soil, then P can also be determined in the same extract. The beauty of this 'universal' test for alkaline soils is that macro - (NO_3-N, P, K)

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

6.2.2 Total Phosphorus

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

Apparatus

Spectrophotometer or colorimeter, 410-nm wavelength.

Block-digester.

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

Vortex tube stirrer.

Reagents

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

B. Ammonium Heptamolybdate-Ammonium Vanadate in Nitric Acid

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

C. Standard Stock Solution

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

bottle.

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

Procedure

A. Digestion

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

Note

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

B. Measurement

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

with DI water.

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

CALCULATION

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

For Total Phosphorus in soil:

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

6.2.3 Organic Phosphorus

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

Apparatus

Spectrophotometer or colorimeter, 882-nm wavelength.

Muffle furnace, for igniting soils at 550°C.

Mechanical shaker.

Centrifuge, capable of 1500 rmp.

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

Reagents

A. Sulfuric Acid (H_2SO_4), 1 N

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

B. Sodium Hydroxide Solution(NaOH), 5 N

Prepare as for Extractable Phosphorus in soils.

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

D. Reagent A

Prepare as for Extractable Phosphorus in soils.

E. Reagent B

Prepare as for Extractable Phosphorus in soils.

F. Standard Stock Solution

Prepare as for Extractable Phosphorus in soils.

Procedure

A. Digestion:

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

B. Measurement

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

CALCULATION

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

For organic Phosphorus in soil:

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

Wt= Weight of air-dry soil (g)

V = Volume of digest used for measurement (mL)

6.3 Potassium

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

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

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

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

6.3.1 Extractable Potassium

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

Apparatus

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

Reagents

A. Ammonium Acetate Solution (NH_4OAc), 1 N

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

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

B. Standard Stock Solution

- Dry about 3 g potassium chloride (KCl) in an oven at 120°C for 1 - 2 hours and cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 1.907 g dried potassium chloride in DI water, and bring to 1-L volume with DI water. This solution contains 1000 ppm K (Stock Solution).
- Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 2, 4, 6, 8, 10, 15 and 20 mL Stock Solution to 100-mL final volume of each by adding DI water or 1 N ammonium acetate solution. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm K, respectively.

Note

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

Procedure

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

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

CALCULATION

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

For Extractable Potassium in soil:

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

6.3.2 Soluble Potassium

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

Procedure

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

CALCULATION

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

For Soluble Potassium in soil:

6.3.3 Exchangeable Potassium

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

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

For Exchangeable Potassium in soil:

Note

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

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

6.4 Sodium

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

Reagents

A. Lithium Chloride (LiCl), 1000 ppm

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

B. Standard Stock Solution

- Dry about 5 g sodium chloride (NaCl) in an oven at 110°C for 3 hours cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 2.5418 g dried sodium chloride in DI water, and bring to 1-L final volume with DI water. This solution contains 1000 ppm Na (Stock Solution)
- Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 2, 4, 6, 8, 10, 15, and 20 mL Stock Solution to 100 mL final volume by adding DI water or 1 N ammonium acetate solution, and 25 mL LiCl Diluted Stock Solution. These solutions contain 20, 40, 60, 80,100, 150, and 200 ppm Na, with each containing the same concentration of LiCl (25 ppm).

Note

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

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

Procedure

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

CALCULATIONS

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

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

For Extractable or Soluble Sodium in soil:

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

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

6.5 Soluble Calcium and Magnesium

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

Reagents

A. Buffer Solution (NH_4Cl-NH_4OH)

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

B. Eriochrome Black Indicator

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

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

Dissolve 2 g ethylene diaminetetraacetic acid, and 0.05 g magnesium chloride ($MgCl_2$) in DI water, and bring to 1-L volume with DI water.

D. Sodium Hydroxide Solution (NaOH), 2 N

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

E. Ammonium Purpurate Indicator ($C_8H_8N_6O_6$)

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

F. Standard Stock Calcium Chloride Solution ($CaCl_2.2H_2O$), 0.01 N

Dissolve 0.5 g pure calcium carbonate ($CaCO_3$ dried for 3 hours at 100°C), in 10 mL 3 N hydrochloric acid and bring to1-L volume with DI water. This can also be prepared by dissolving 0.735 g calcium chloride dihydrate

($CaCl_2.2H_2O$) in 1-L volume with DI water.

Procedure

A. Calcium

1. Pipette 10 - 20 mL soil saturation extract, having not more than 1.0 meq Ca, into a 250-mL Erlenmeyer flask.
2. Dilute to 20 - 30 mL with DI water, add 2 - 3 mL 2 N sodium hydroxide solution, and about 50 mg ammonium purpurate indicator.
3. Titrate with 0.01 N EDTA. The color change is from red to lavender or purple. Near the end point, EDTA should be added one drop every 10 seconds since the color change is not instantaneous.
4. Always run a blank containing all reagents but no soil, and treat it in exactly the same way as the samples; and subtract the blank titration reading from the readings for all samples.

B. Calcium plus Magnesium

1. Pipette 10 - 20 mL soil saturation extract into a 250-mL flask, dilute to 20 - 30 mL with DI water. Then add 3 - 5 mL buffer solution. And a few drops eriochrome black indicator.
2. Titrate with 0.01 N EDTA until the color changes from red to blue.

CALCULATIONS

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

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

For Soluble Calcium or Magnesium in soil:

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

B = Blank titration volume (mL)

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

N = Normality of EDTA solution.

Wt= Weight of air-dry soil (g)

Standardization of EDTA

- Pipette 10 mL 0.01 N calcium chloride solution, and treat it as in determining Ca and Ca+Mg procedure, respectively.

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

- Take the reading, and calculate EDTA normality:

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

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

N_{CaCl_2} = Normality of $CaCl_2$ solution

Note

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

saturation extract is sufficient to determine Ca and Mg.

6.6 Carbonate and Bicarbonate

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

Reagents

A. Methyl Orange Indicator [4-$NaOSO_2C_6H_4N$:NC_6H_4/-4-$N(CH_3)_2$], (F.W. 327.34), 0.1%

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

B. Sulfuric Acid Solution (H_2SO_4), 0.01 N

- Dilute 28 mL concentrated sulfuric acid (98 %, sp.gr.1.84) in DI water, mix well, let it cool, and bring to 1-L volume with DI water. This solution contains 1 N H_2SO_4 solution.
- Then dilute 100 times (10 mL to 1-L volume) to obtain 0.01 N H_2SO_4 solution.

C. Phenolphthalein Indicator, 1%

Dissolve 1 g phenolphthalein indicator in 100 mL ethanol.

Procedure

1. Pipette 10 - 15 mL soil saturation extract into a wide-mouthed porcelain crucible or a 150-mL Erlenmeyer flask.
2. Add 1 drop phenolphthalein indicator. If pink color develops, add 0.01 N sulfuric acid by a burette, drop by drop, until the color disappears.
3. Take the reading, y.
4. Continue the titration with 0.01 N sulfuric acid after adding 2 drops 0.1% methyl orange indicator until the color turns to orange.
5. Take the reading, t.
6. Always run two blanks containing all reagents but no soil, and treat them in exactly the same way as the samples. Subtract the blank titration reading from the readings for all samples.

CALCULATIONS

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

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

For Carbonate and Bicarbonate in soil:

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

N = Normality of H_2SO_4 solution.

Wt = Weight of air-dry soil (g)

6.7 Chloride

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

Reagents

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

- Dissolve 5 g potassium chromate in 50 mL DI water.
- Add dropwise 1 N silver nitrate ($AgNO_3$) until a slight permanent red precipitate is formed.
- Filter, and bring to 100-mL volume with DI water.

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

- Dry about 3 g silver nitrate in an oven at 105°C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 1.696 g dried silver nitrate in DI water, and bring to 1-L volume with DI water.

C. Sodium Chloride Solution (NaCl), 0.01 N

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

Procedure

1. Pipette 5 - 10 mL soil saturation extract into a wide-mouth porcelain crucible or a 150-mL Erlenmeyer flask.
2. Add 4 drops potassium chromate solution.
3. Titrate against silver nitrate solution until a permanent reddish-brown color appears.
4. Always run two blanks containing all reagents but no soil, and treat them in exactly the same way as for the samples. Subtract the blank titration

reading from the readings for all samples.

CALCULATION

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

For Chloride in soil:

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

B = Blank titration volume (mL)

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

N = Normality of $AgNO_3$ solution.

Wt = Weight of air-dry soil (g)

Standardization of $AgNO_3$

- Titrate 10 mL 0.01N of sodium chloride solution against 0.01 N silver nitrate solution after adding 4 drops potassium chromate solution until a permanent reddish-brown color appears.

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

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

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

N_{AgNO_3} = Amount of $AgNO_3$ solution used (mL)

N_{NaCl} = Normality of NaCl solution.

6.8 Sulfate

6.8.1 Turbidimetric Method

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

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

Apparatus

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

Reagents

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

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

B. Hydrochloric Acid Solution (HCl), 6 M

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

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

D. Sorbitol, 70% aqueous solution

E. Standard Stock Solution

- Dissolve 0.5434 g potassium sulfate (K_2SO_4) in DI water, and bring to 1-L volume with DI water. This solution contains 100 ppm SO_4-S (Stock Solution).

- Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 5, 10, 20, 30, 40, and 50 mL Stock Solution to 100-mL final

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

Procedure

A. Extraction

1. Weigh 5 g air-dry soil (2-mm) into a 150-mL Erlenmeyer flask.
2. Add 25 mL 0.15% calcium chloride dihydrate solution (don't use a rubber stopper, or wrap the rubber stopper in thin polyethylene. Errors result from gradual oxidation of sulfur compounds present in the stopper).
3. Shake for 30 minutes on a reciprocal shaker (180+ oscillations per minute).
4. Filter the suspension through Whatman No. 42 filter paper. This procedure yields almost colorless extracts.

B. Measurement of SO_4-S

1. Pipette 10-mL aliquot of the extract into a 50-mL test tube, or a smaller aliquot diluted to 10 mL with DI water.
2. Add 1 mL 6 M hydrochloric acid solution followed by 5 mL 70% sorbitol solution from a pipette with an enlarged jet. Finally, add about 1 g barium chloride crystals (using a measuring spoon).
3. Shake vigorously (on a test tube shaker for 30 seconds) to dissolve the barium chloride and obtain a homogeneous suspension.
4. Prepare a standard curve as follows:
    - Pipette 10 mL of each standard (0 - 50 ppm), and proceed as for the samples.
    - Also make a blank with 10 mL 0.15% calcium chloride dihydrate solution, and proceed as for the samples.
    - Read the absorbance (turbidity) of the blank, standards, and samples at 470-nm wavelength.
5. Prepare a calibration curve for standards, plotting absorbance against the respective SO_4-S concentrations.

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

CALCULATION

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

For Turbidimetric of Sulfate in soil:

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

Note

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

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

6.8.2 Precipitation Method

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

Apparatus

Mechanical shaker, reciprocating.
Muffle furnace.

Reagents

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

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

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

Mix equal portions of concentrated hydrochloric acid with DI water.

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

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

Procedure

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

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

7. Transfer crucible to a muffle furnace heated to 550°C, and leave to dry ash for 2 - 3 hours.

8. Take crucible out of the muffle furnace, and place in a desiccator to cool, weigh crucible on an analytical balance, and take the reading, t.

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

CALCULATION

For precipitate of Sulfate in soil:

Where: t = Weight of crucible + $BaSO_4$ precipitate (g)

b = Weight of empty crucible (g)

V = Volume of extract used for measurement (mL)

6.9 Boron

6.9.1 Hot-Water Method

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

Apparatus

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

Spectrophotometer or colorimeter, 420-nm wavelength.

Polypropylene test tubes, 10-mL capacity.

Reagents

A. Buffer Solution

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

B. Activated Charcoal (Boron-free)

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

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

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

D. Standard Stock Solution

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

Procedure

A. Extraction

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

B. Measurement

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

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

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

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

CALCULATION

For Extractable Boron in soil:

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

Note

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

6.9.2 Dilute Hydrochloric Acid Method

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

Reagents

A. Buffer Solution

Prepare as for hot-water extractable B.

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

Prepare as for hot-water extractable B.

C. Activated Charcoal (Boron-free)

Prepare as for hot-water extractable B.

D. Standard Stock Solution

Prepare as for hot-water extractable B.

E. Hydrochloric Acid (HCl), 0.05 N

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

Procedure

A. Extraction

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

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

B. Measurement (by Azomethine-H Method)

Same as in hot-water extractable B in soils.

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

CALCULATION

For Boron in soil:

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

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

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

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

6.10.1 DTPA Method

Apparatus

Mechanical shaker, reciprocal
Atomic absorption spectrophotometer

Reagents

A. DTPA Extraction Solution

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

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

B. Standard Stock Solutions

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

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

Procedure

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

Note

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

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

CALCULATION

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

Note

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

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

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

6.10.2 Ammonium Bicarbonate-DTPA Method

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

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

Apparatus

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

Reagents

A. Extracting Solution

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

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

B. Mixed Reagent for Phosphorus

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

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

C. Color Developing Solution for Phosphorus

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

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

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

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

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

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

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

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

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

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

G. Color Developing Solution for Nitrate-Nitrogen

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

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

H. Standard Stock Solutions

Nitrate-N:

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

Phosphorus:

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

Potassium:

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

Micronutrients:

Prepare working standards for:

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

Procedure

1. Extraction Method

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

2. Nitrate-N

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

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

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

3. Phosphorus

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

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

4. Potassium

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

The standard solutions are made in the extracting solution.

5. Micronutrients

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

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

CALCULATIONS

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

For Nitrate-N in soil:

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

For Phosphorus in soil:

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

For Potassium in soil:

For Micronutrient in soil:

Note

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 H_2SO_4 and H_2O_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 (K_2SO_4-Se), 100: 1 w/ w ratio

B. Sulfuric Acid (H_2SO_4), concentrated

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

D. Sodium Hydroxide Solution (NaOH), 10 N

E. Boric Acid Solution (H_3BO_3), saturated

F. Sulfuric Acid Solution (H_2SO_4), 0.01 N

G. Standard Stock Solution: 1.2 g NH_4^+-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_2SO_4 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:

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

Where: V = Volume of 0.01 N H_2SO_4 titrated for the sample (mL).
B = Distillate blank titration volume (mL)
N = Normality of H_2SO_4 solution.
C = Volume of NH_4-N standard solution (mL)
D = Concentration of NH_4-N standard solution (µg/mL)
14.01= Atomic weight of N.

Percentage recovery of EDTA standard:

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

Percentage Nitrogen in plant:

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

Where: R = Ratio between total digest volume and distillation volume.

B_1 = Digested blank titration volume (mL)

Wt_1 = Weight of EDTA (g)

Wt_2 = 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 H_2O_2-H_2SO_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 (H_2SO_4), concentrated

B. 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 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 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 sulfuric acid.

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

C. Sodium Thiosulfate ($Na_2S_2O_3.5H_2O$), 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°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_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 0.1g 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_2SO_4 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

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

Percentage Nitrogen in plant:

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

Where: V = Volume of 0.01 N H_2SO_4 titrated for the sample (mL).
B = Digested blank titration volume (mL)
N = Normality of H_2SO_4 solution.
14.01= Atomic weight of N.
R = Ratio between total digest volume and distillation volume.
Wt_1 = Weight of EDTA (g)
Wt_2 = 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 [$(NH_4)_6Mo_7O_{24}$. $4H_2O$] in 400 mL DI water (a).
- Dissolve 1.25 g ammonium metavanadate (NH_4VO_3) in 300 mL hot DI water (b).
- Add (b) to (a) in a 1-L volumetric flask, and let the mixture cool to room temperature.
- Slowly add 250 mL concentrated nitric acid (HNO_3) to the mixture, cool the solution to room temperature, and bring to 1-L volume with DI water.

B. Standard Stock Solution

- Dry about 2.5 g potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C for 1 hour cool in desiccator, and store in a tightly stoppered bottle.
- Dissolve 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 to100-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

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

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

Wt = 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 HNO_3-$HClO_4$ 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 HNO_3-$HClO_4$ 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

Dilute165.6 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 (H_2SO_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:

$$B\ (ppm) = ppm\ B\ (from\ calibration\ curve) \times \frac{A}{Wt} \quad ....\ (65)$$

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 HNO_3-$HClO_4$. 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 photophotometer.

Reagent

Nitric Acid-Perchloric Acid (HNO_3-$HClO_4$), 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°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°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 Photophotometry.

CALCULATIONS

For Micronutrient Cations in plant:

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

For Alkaline Earth Cations in plant:

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

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 (Fe^{++}) in fresh tissue by o-phenathroline 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 ($C_{12}H_8N_2$), 1.5% in HCl-buffer with pH 3.0

Add 15 g 1 - 10 o-phenathroline to about 850 mL DI water. Dropwise, add 1 N hydrochloric acid by continuously stirring solution until last traces of 1 - 10 o-phenathroline 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 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

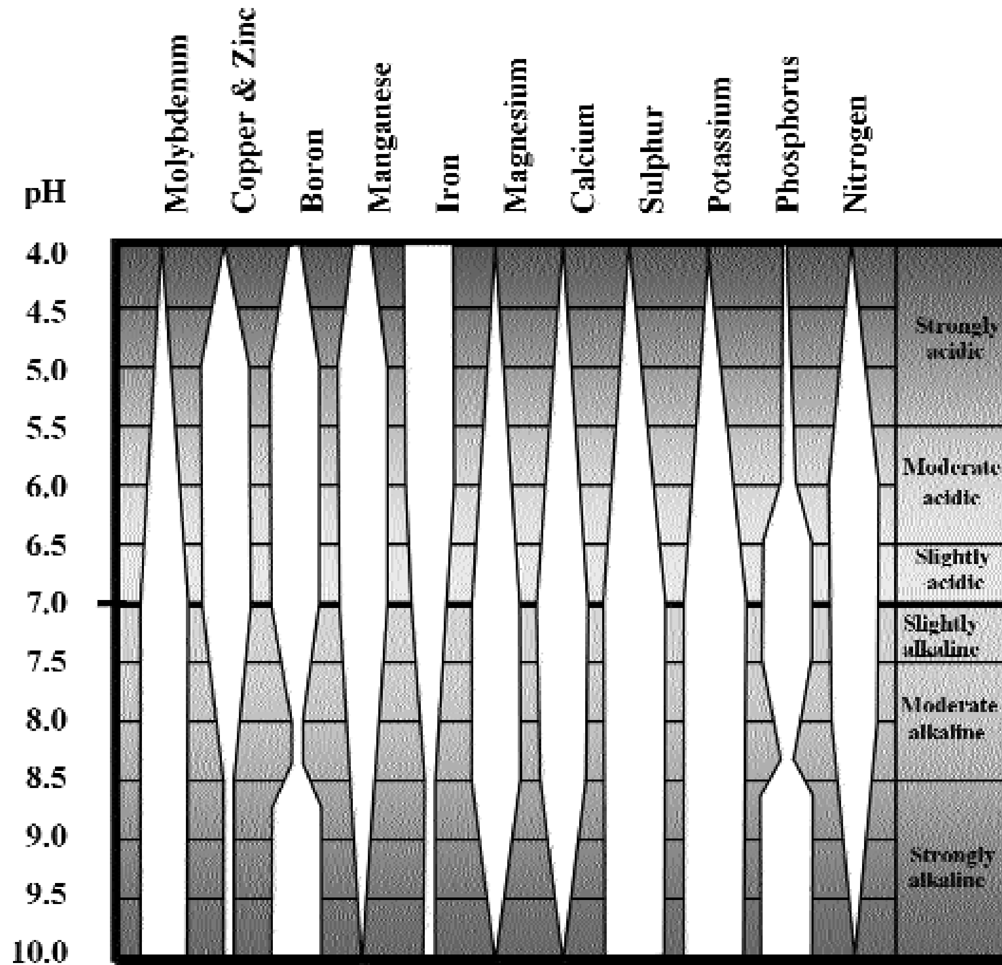
For Ferrous Iron in fresh plant tissue:

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

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

Box 1

INFLUENCE OF SOIL pH ON PLANT NUTRIENT AVAILABILITY



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 NO_3-N, NH_4-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 (NO_3-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 ($dS\ m^{-1}$) at $25^{o}C$.

CALCULATIONS

$$\text{Sodium Adsorption Ratio (SAR)} = \frac{Na^{+}}{\sqrt{(Ca^{++} + Mg^{++})/2}} \quad \text{.............................. (69)}$$

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

$$\text{Residual Sodium Carbonate (RSC)} = (CO_3^{--} + HCO_3^{-}) - (Ca^{++} + Mg^{++}) \quad \text{......... (70)}$$

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:

Quality	EC (dS/m)	Sodium Adsorption Ratio	ResidualSodium Carbonate (meq/L)
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).

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Appendix 1. Abbreviations.

Weight

g	gram
mg	milligram
µg	microgram

Length

m	meter
cm	centimeter
mm	millimeter

Concentration

mg/L	milligram per liter
meq/L	milli equivalent per liter

Salt Concentration

dS	decisiemens
mS	millisiemens
µS	microsiemens

$mS\ cm^{-1} = dS\ m^{-1} = S\ m^{-1} \times 10$

$mS\ cm^{-1} \times 0.1 = S\ m^{-1}$

Volume

L	Liter
mL	milliliter

Area

ha	hectare
da	decare
du	Dunums
A	Acre

Temperature

°C	degree Celsius

Miscellaneous

AAS	Atomic absorption spectrophotometer
CEC	Cation exchange capacity
SAR	Sodium adsorption ratio
EC_e	Electrical conductivity of the saturation extract
nm	Wavelength
OM	Organic matter
sp gr	Specific gravity
pH_s	pH of saturated soil paste
ESP	Exchangeable sodium percentage

Appendix 2. Conversion Factors for SI and Non-SI Units.

To convert Column 1 into Column 2, multiply by:	Column 1 SI Unit	Column 2 non-SI Unit	To convert Colum 2 into Column 1, multiply by:
	Length		
0.621	kilometer, km (10^3 m)	mile, mi	1.609
1.094	meter, m	yard, yd	0.914
3.28	meter, m	foot, ft	0.304
1.0	micrometer, µm (10^{-6} m)	micron	1.0
3.94×10^{-2}	millimeter, mm (10^{-3} m)	inch, in	25.4
10	nanometer, nm (10^{-9} m)	Angstrom, Å	0.1
	Area		
2.47	hectare, ha	acre	0.405
247	square kilometer, km^2 $(10^3\ m)^2$	acre	4.05×10^{-3}
0.386	square kilometer, km^2 $(10^3\ m)^2$	square mile, mi^2	2.590
2.47×10^{-4}	square meter, m^2	acre	4.05×10^3
10.76	square meter, m^2	square foot, ft^2	9.29×10^{-2}
1.55×10^{-3}	square millimeter, mm^2 (10^{-3} m^2)	square inch, in^2	645
	Volume		
9.73×10^{-3}	cubic meter, m^3	acre-inch	102.8
35.3	cubic meter, m^3	cubic foot, ft^3	2.83×10^{-2}
6.10×10^4	cubic meter, m^3	cubic inch, in^3	1.64×10^{-5}
3.53×10^{-2}	liter, L (10^{-3} m^3)	cubic foot, ft^3	28.3
0.265	liter, L (10^{-3} m^3)	gallon	3.78
33.78	liter, L (10^{-3} m^3)	ounce (fluid), oz	2.96×10^{-2}
2.11	liter, L (10^{-3} m^3)	pint (fluid), pt	0.473
	Mass		
2.20×10^{-3}	gram, g (10^{-3} kg)	pound, lb	454
3.52×10^{-2}	gram, g (10^{-3} kg)	ounce (avdp), oz	28.4
2.205	kilogram, kg	pound, lb	0.454
0.01	kilogram, kg	quintal (metric), q	100
1.10×10^{-3}	kilogram, kg	ton (2000 lb), ton	907
1.102	megagram, Mg (tonne)	ton (U.S.), ton	0.907
1.102	tonne, t	ton (U.S.), ton	0.907
	Yield and Rate		
0.893	kilogram per hectare, kg ha^{-1}	pound per acre, lb $acre^{-1}$	1.12
0.107	liter per hectare, L ha^{-1}	gallon per acre	9.35
893	tonnes per hectare, t ha^{-1}	pound per acre, lb $acre^{-1}$	1.12×10^{-3}
893	megagram per hectare, Mg ha-1	pound per acre, lb $acre^{-1}$	1.12×10^{-3}
0.446	megagram per hectare, Mg ha^{-1}	ton (2000 lb) per acre, ton $acre^{-1}$	2.24

(Contd...)

Appendix 2 (Contd...)

To convert Column 1 into Column 2, multiply by:	Column 1 SI Unit	Column 2 non-SI Unit	To convert Colum 2 into Column 1, multiply by:
	Pressure		
9.90	megapascal, MPa (10^6 Pa)	atmosphere	0.101
10	megapascal, MPa (10^6 Pa)	bar	0.1
1.00	megagram per cubic meter, Mg m^{-3}	gram per cubic centimeter, g cm^{-3}	1.00
2.09×10^{-2}	Pascal, Pa	pound per square foot, lb ft^{-2}	47.9
1.45×10^{-4}	Pascal. Pa	pound per square inch, lb in^{-2}	6.90×10^3
	Temperature		
1.00 (K-273)	Kelvin, K	Celsius, °C	1.00 (°C+273)
(9/5 °C) + 32	Celsius, °C	Fahrenheit, °F	5/9 (°F - 32)
	Electrical Conductivity, Electricity, and Magnetism		
10	siemen per meter, S m^{-1}	millimho per centimeter, mmho cm^{-1}	0.1
10^4	tesla, T	Gauss, G	10^{-4}
	Water Measurement		
9.73×10^{-3}	cubic meter, m^3	acre-inches, acre-in	102.8
9.81×10^{-3}	cubic meter per hour, m^3 h^{-1}	cubic feet per second, ft^3 s^{-1}	101.9
4.40	cubic meter per hour, m^3 h^{-1}	U.S. gallons per minute, gal min^{-1}	0.227
8.11	hectare-meter, ha-m	acre-feet, acre-ft	0.123
97.28	hectare-meters, ha-m	acre-inches, acre-in	1.03×10^{-2}
8.1×10^{-2}	hectare-centimeters, ha-cm	acre-feet, acre-ft	12.33
	Concentrations		
1	centimole per kilogram, cmol kg^{-1} (ion exchange capacity)	milliequivalents per 100 grams, meq 100 g^{-1}	1
0.1	gram per kilogram, g kg^{-1}	percent, %	10
1	milligram per kilogram, mg kg^{-1}	parts per million, ppm	1
	Plant Nutrient Conversion		
2.29	P	P_2O_5	0.437
1.20	K	K_2O	0.830
1.39	Ca	CaO	0.715
1.66	Mg	MgO	0.602

Source: Tekalign *et a*l. (1991).

Appendix 3. Symbols, Atomic Number, and Atomic Weights of Elements.

Element	Symbol	Atomic No.	Atomic Weight	Element	Symbol	Atomic No.	Atomic Weight
Actinium	Ac	89	227	**Iodine**	I	53	126.904
Aluminum	Al	13	26.9815	**Iridium**	Ir	77	192.2
Americium	Am	95	243	**Iron**	Fe	26	55.847
Antimony	Sb	51	121.75	**Krypton**	Kr	36	83.8
Argon	Ar	18	39.948	**Lanthanum**	La	57	138.91
Arsenic	As	33	74.9216	**LawrenciumLr**		103	257
Astatine	At	85	210	**Lead**	Pb	82	207.19
Barium	Ba	56	137.34	**Lithium**	Li	3	6.94
Berkeliom	Bk	97	249	**Lutetium**	Lu	71	174.97
Beryllium	Be	4	9.0122	**Magnesium**	Mg	12	24.312
Bismuth	BI	83	208.98	**Manganese**	Mn	25	54.938
Boron	B	5	10.81	**Mendelevium**	Md	101	258
Bromine	Br	35	79.909	**Mercury**	Hg	80	200.59
Cadmium	Cd	48	112.4	**Molybdenum**	Mo	42	95.94
Calcium	Ca	20	40.08	**Neodymium**	Nd	60	144.24
Californium	Cf	98	251	**Neon**	Ne	10	20.183
Carbon	C	6	12.011	**Neptunium**	Np	93	237
Cerium	Ce	58	140.12	**Nickel**	Ni	28	58.71
Cesium	Cs	55	132.905	**Niobium**	Nb	41	92.906
Chlorine	Cl	17	35.453	**Nitrogen**	N	7	14.0067
Chromium	Cr	24	51.996	**Nobelium**	No	102	254
Cobalt	Co	27	58.9332	**Osmium**	Os	76	190.2
Copper	Cu	29	63.54	**Oxygen**	O	8	15.9994
Curium	Cm	96	247	**Palladium**	Pd	46	106.4
Dysprosium	Dy	66	162.5	**Phosphorus**	P	15	30.9738
Einsteinium	Es	99	254	**Platinum**	Pt	78	195.09
Erbium	Er	68	167.26	**Plutonium**	Pu	94	239
Europium	Eu	63	151.96	**Polonium**	Po	84	209
Fermium	Fm	100	25	**Potassium**	K	19	39.102
Fluorine F	9	18.9984		**Praseodymium**	Pr	59	140.907
Francium	Fr	87	233	**Promethium**	Pm	61	145
Gadolinium	Gd	64	157.25	**Protactinium**	Pa	91	231
Gallium	Ga	31	69.72	**Radium**	Ra	88	226
Germanium	Ge	32	72.59	**Radon**	Rn	86	222
Gold	Au	79	196.967	**Rhenium**	Re	75	186.2
Hafnium	Hf	72	178.49	**Rhodium**	Rh	45	102.905
Helium	He	2	4.0026	**Rubidium**	Rb	37	85.47
Holmium	Ho	67	164.93	**Ruthenium**	Ru	44	101.07
Hydrogen	H	1	1.0079	**Samarium**	Sm	62	150.35
Indium	In	49	114.82	**Scandium**	Sc	21	44.956

(Contd...)

Appendix 3 (Contd...)

Element	Symbol	Atomic No.	Atomic Weight	Element	Symbol	Atomic No.	Atomic Weight
Selenium	Se	34	78.96	**Thulium**	Tm	69	168.934
Silicon	Si	14	28.086	**Tin**	Sn	50	118.69
Silver	Ag	47	107.87	**Titanium**	Ti	22	47.9
Sodium	Na	11	22.9898	**Tungsten**	W	74	183.85
Strontium	Sr	38	87.62	**Uranium**	U	92	238.03
Sulfur	S	16	32.064	**Vanadium**	V	23	50.9412
Tantalum	Ta	73	180.948	**Xenon**	Xe	54	131.3
Technetium	Tc	43	99	**Ytterbium**	Yb	70	173.04
Tellurium	Te	52	127.6	**Yttrium**	Y	39	88.906
Terbium	Tb	65	158.925	**Zinc**	Zn	30	65.37
Thallium	TI	81	204.37	**Zirconium**	Zr	40	91.22
Thorium	Th	90	232.038				

Source: Tekalign *et al*. (1991).

Appendix 4. Solution Concentrations.

System Name	Abbreviation	Definition
Molar	M	gram-molecular weight (mole of solute) per liter of solution.
Molal	M	gram-molecular weight (mole of solute) per kilogram of solvent.
Formal	F	gram-formula weight of solute per liter of solution.
Normal	N	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	Vol % or v/v %	Volume of solute × 100 per volume of solution.
Weight percent	wt % or w/w %	Weight of solute X 100 per 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.

Source: Tekalign *et al*. (1991).

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, and Amount of Concentrated Acids and Bases to Make 1-L of 1 *N* Solution.

Acid or Base	Chemical Properties				Solution
	Specific Gravity	Percent by Weight	Grams per Liter	Approximate Normality *(N)*	Needed[1] (mL)
Acetic acid	1.05	99.0	1042.0	17.45	58
Ammonium hydroxide	0.90	28.3	255.0 (NH_3)	15.0	67
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*.

Source: Tekalign *et al*. (1991).

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.	Soil cation exchange capacity is production near 100% base saturation.
7.5 - 8.4	Free lime ($CaCO_3$) exists in soil	Usually excellent filtration and percolation of water due to high Ca content of 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 of Alkaline Soils.

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

AAS=Atomic Absorption Spectrophotometry.
Source: Soil and Plant Analysis Council (1992).

Appendix 9. Generalized Guidelines for Interpretation of Soil Analysis Data.

Measurement	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	>0.2
	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 pentoacetic 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*. (1992).

Appendix 10. Suggested Plant Tissue Sampling Procedures for Selected Dryland Crops[1].

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)	All the 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 initial flowering	Two or three fully developed Leaves at the top of the plant	20 - 30
	Peanut	
Maximum tillering	Recently matured leaflets	25
	Alfalfa, Clover and other Legumes	
Prior to or at 1/10th bloom stage	Mature leaf blades taken about One-third of the way down the plant	40 - 50
	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 initiation.

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

Appendix 11. Generalized Interpretation of Cereal Tissue Analysis Data.

Nutrient	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
Phosphorus	<0.15	0.15 - 0.19	0.20 - 0.50	>0.50
Potassium	<1.25	1.25 - 1.49	1.50 - 3.00	>3.00
Calcium				
(wheat, oats)		<0.20	0.20 - 0.50	>0.50
(barley)		<0.30	0.30 - 1.20	>1.20
Magnesium		<0.15	0.15 - 0.50	>0.50
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] -dS/m-	Crop	ECe[1] -dS/m-	Crop
		FORGE CROPS	
22.0	Kallar grass (*Leptochloa fusca*)	14.4	Sudangrass (*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 offic-narum)*
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 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*)	
Pecan (*Carya illinoensis*)		Squash (*Cucurbita pepo*)	
Cowpea (*Vigna sinensis*)		Muskmelon (*Cucumis melo*)	
Onion (*Allium cepa*)			
Garlic (*Allium sativum*)		**TOLERANT CROPS**	
Sweet potato (*Ipomoea batatas*)		Sorghum (*Sorghum bicolor*)	
Wheat (*Triticum aestvium*)		Alfalfa (*Medicago sativa*)	
Mung bean (*Phaseolus aureux*)	0.046	Purple vetch (*Vicia benghlensis)*	0.19
Strawberry (*Fragaria sp*.)	0.074	Oat *(Avena vulgare)*	0.37
Kindney bean (*Phaseolus vulgaris*)		Parsley (*Petroselium crispum*)	
		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	-

Source: Tekalign *et al*. (1991).

Appendix 17. Equivalent Weights.

Symbol/Formula	Equivalent Weight---g----	Common Name
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	Aluminium sulfate
$FeSO_4 \cdot 7H_2O$	139.01	Ion sulfate (ferrous)

Conversion of milliequivalents per liter to parts per million:
ppm = milliequivalents/liter × equivalent weight.