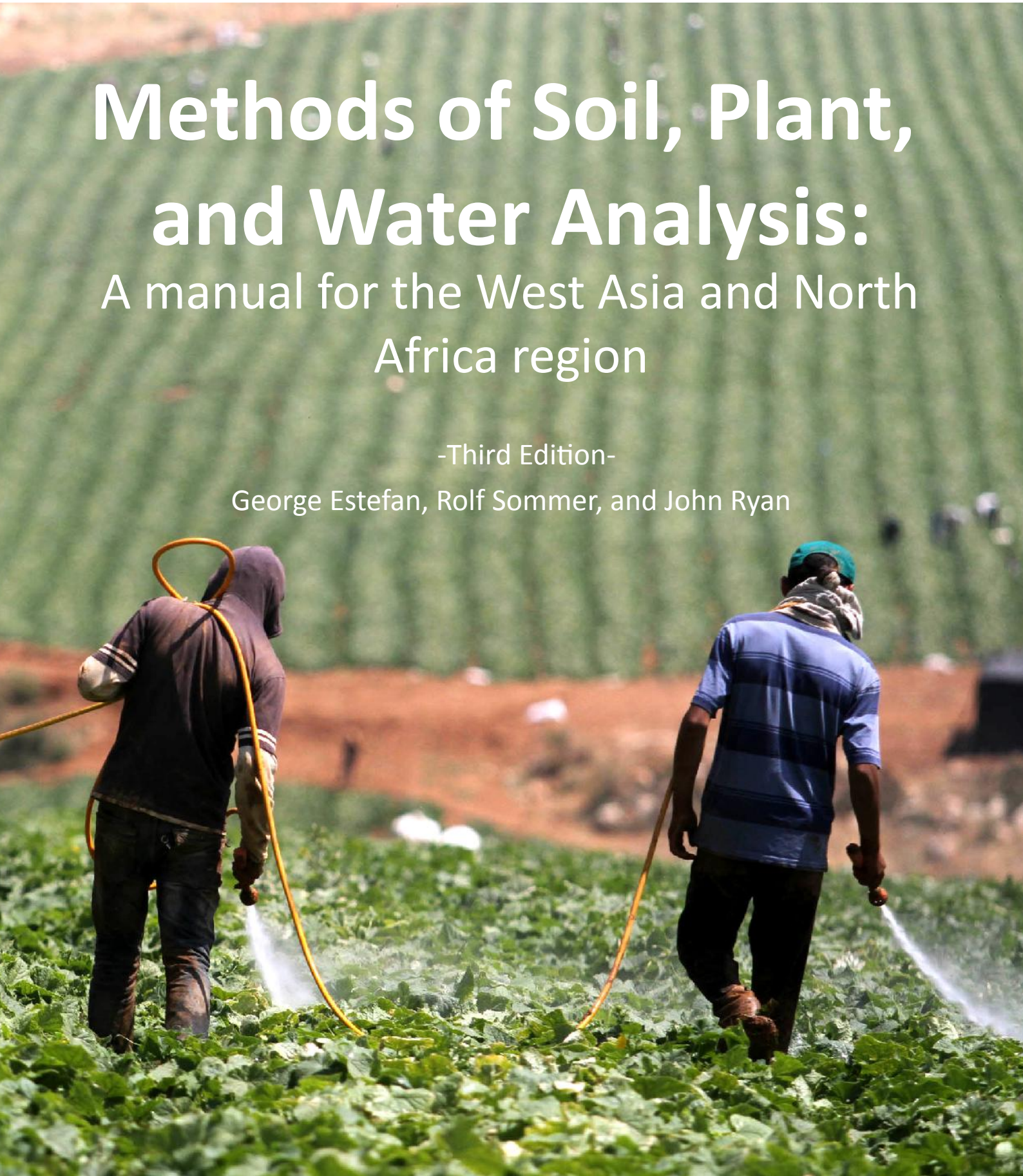
ICARDA International Center for Agricultural Research in the Dry Areas

Methods of Soil, Plant, and Water Analysis:

A manual for the West Asia and North Africa region

-Third Edition-

George Estefan, Rolf Sommer, and John Ryan



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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 two editions of this manual and those who made suggestions for improvement.

Key words:;

Feedback
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Foreword

Soil is a non-renewable resource upon which mankind depends for survival. Historically, the rise of great civilizations has been linked to the quality of soil and the availability of water. Equally, the demise of such civilizations is often attributed to mismanagement of soil and land in its broadest sense. Crop productivity and soil fertility are thus synonymous.

In today's overcrowded world, the challenge to feed and clothe the burgeoning populations of developing countries is a daunting task. Yields have to be increased from existing land areas; adding fertility to the soil to satisfy the demands of higher-yielding crops is essential. Soils vary greatly in their capacity to grow crops without fertilizer; even the richest soils experience declining yields without man's intervention. In essence, soil is not always a perfect medium for growing plants; it is, however, the only one that is available.

Soils vary greatly throughout the world; they have inherent weakness, primarily deficiencies in nutrients that are 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 (N); 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 West Asia and North Africa (WANA) region are generally well supplied with potassium (K), and usually don't need fertilization, especially for low-yielding rainfed crops. In recent years, there is a growing realization has grown that other elements, e.g., micronutrients, are deficient in some areas of the region.

As no essential element can 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 complementary and, when calibrated with field crop responses to fertilizer, provide a rational basis to identify what elements are missing, and how much fertilizer, whether organic or inorganic, to apply. Therefore, soil and plant analysis laboratories have a vital role in agricultural development of the WANA 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.

The idea for this soil, plant and water analysis manual grew out of the Soil Test Calibration Program within the region's national agricultural programs that laid the basis for sound fertilizer recommendations. Thus, it was appropriate that ICARDA should address this fundamental issue. If soil and plant tests are not reliable, the process of sampling and analysis is meaningless and undermines the validity of any agronomic trials. This manual is a cornerstone in ICARDA's soil and water-related research program as well as its training program and is a vital link with agricultural scientists of the ICARDA's mandate region.

Dr. Mahmoud Solh

Director General of ICARDA

Preface

This laboratory manual of analytical methods has been compiled to be used primarily by research assistants, technicians and student trainees working in the laboratory. The methods have been selected from different sources and adapted for routine analyses in the WANA region. A detailed description of the methods can be found in the original publications listed in the references. As analytical techniques are developed and improving, this manual will be subject to continuous revision. Any helpful suggestion and feedback from users is most appreciated.

The idea of having a common laboratory manual for the WANA 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 (Ryan and Matar, 1990, 1992). In addition, there was hardly any laboratory manual on plant analysis relevant to the crop nutritional problems of the region.

A common soil, plant, and water analysis manual is also fundamental for success of the WANA soil fertility network. To fill the gap that existed, a comprehensive manual on all essential soil, plant, and water analyses was developed. This manual was designed intentionally in a “cookbook” format, for the ease of laboratory technicians. On completion, the manual draft was reviewed and endorsed at the Soil Fertility Meeting in at Tel Hadya in 1995 (Ryan, 1997).

Subsequently, the First Edition of the manual (Ryan. J, S. Garabet, K. Harmsen, and A. Rashid, “A soil and plant analysis manual adapted for the WANA region”) was published in 1996 by ICARDA, and distributed widely among soil and plant analysis laboratories throughout the 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.

The Second Edition of the manual (Ryan. J, G. Estefan, and A. Rashid, 2001, “Soil and plant analysis laboratory manual”) had incorporated all the feedback from laboratory managers of the WANA region, making the manual more comprehensive, up-to-date, and with minimum technical or typographical errors. We appreciate the constructive criticism and suggestions advanced by the fellow soil scientists for improvement of the manual. The Second Edition was translated and published in Arabic, Russian, and Farsi.

The Third Edition of the manual reflects the changes that have occurred within the past few years in the region, particularly will respect to water and irrigation. While it is still focused on production agriculture, it reflects an increasing concern about environmental pollution, mainly from nitrates, 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. In addition, improvements have occurred in electronic handling of data and establishing databases. As with the Second Edition, we anticipate translating the Third Edition into Arabic for the WANA region in general as well as into French, particularly for North Africa.

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

George Estefan, Rolf Sommer, and John Ryan

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1. Introduction

The idea that one could test or analyze a soil and obtain some information about 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. Analyses of plants to reflect the fertility status of the soil in which they 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 such 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
7. Soil testing prior to cropping

While all these 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 *post-mortem* 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, as well as in many developing countries. 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, such as B, and high levels of elements such as Na and Mg, which can adversely affect soil physical properties. 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 and aggregate stability. 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 reagents used and the procedures adopted should extract and reflect all or a portion of the element in the soil which is related to the availability to the plant, i.e., it should be correlated with plant growth.
3. ***Interpreting the Analytical Results***: The units of measurement should reliably indicate if a nutrient is *deficient*, *adequate,* or in *excess* (in some cases toxic to plants).
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 every 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 and temperature. 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; Page et al., 1982; Sparks et al., 1966). 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 tests for alkaline soils (e.g., Fe, Mn, Zn, Cu, and B) have become more valid and therefore more common. Though tests for gypsum are developed (Richards 1954; FAO, 1990), there are unique problems for cation exchange capacity (CEC) measurement in such soils (Rhoades and Polemio, 1977). Special mention has to be made of the original USDA Handbook 60, jointly authored by the Staff of the Salinity Laboratory in Riverside, California, with Richards (1954) as lead author. Despite its age, this iconic manual is the basis for most analyses of salinity parameters in relation to soils, water and plants, and the ensuing interpretative criteria. Though many methods have been modified, the influence of this manual ("Handbook 60" as it is called) still persists.

The literature on soil testing is rich and varied. Some salient examples include: 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. Soil Science Society of America publications (Walsh and Beaton, 1973; Page et al., 1982; Westerman, 1990; Sparks et al., 1996) take a broader look at the philosophy, procedures, and laboratory operations for soil and plant analysis, with interpretation criteria for specific crops. These monographs are regularly updated. The ranges of soil and plant analysis publications include:

1. Soil Testing with a textbook format (Hesse, 1971).
2. University publications that range from those that deal with all soil, water and plant tests (Chapman and Pratt, 1961) to more narrowly based ones (Reisenaur, 1983).
3. Publications that deal with theoretical considerations involved with sampling, correlation, calibration, and interpretation (Brown, 1987).
4. 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).
5. 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 and of questionable relevance.

This leads to a consideration of the West Asia – North Africa (WANA) 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, e.g., Aleppo, 1986 (Soltanpour, 1987), Ankara, 1987 (Matar et al., 1988), Amman, 1988 (Ryan and Matar, 1990), and in Agadir, 1991 (Ryan and Matar, 1992), and finally in Aleppo, 1995 (Ryan, 1997).

Central to the Network, and indeed ICARDA's operations, has been its Soil, Plant and Water 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, most of the procedures adapted by the laboratory are based on validated regional research.

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 methods 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, plant and water sampling has been highlighted, and guidelines of sample collection, processing, and storage have been 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 useful information on related practiced aspects such as *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 *B toxicity interpretations*.

2. Laboratory Facilities, Quality Control and Data Handling

2.1. Laboratory Organization

Soil, plant, and water analyses are carried out by various institutions in the public or government sector, as well as in the private domain. Laboratories are operated by several entities, including Ministries of Agriculture, National Research and Teaching Institutes, International Organizations, and Commercial Companies.

To be much effective, analytical services should be closely linked to the extension/advisory services and should maintain a functional relationship with the universities and research stations. 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, minimizes contamination, and produces 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 WANA 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, and Water Analysis Laboratory of ICARDA was designed in the 1980 with these considerations in mind. The various components of the laboratory reflect a logical activity framework. While no two laboratories are ever the same or have the same complement of equipment, the details presented for ICARDA's laboratory will, hopefully, serve as a general guideline for laboratory arrangement and the type of equipment needed for routine service-oriented operations.

The Soil, Plant and Water Analysis Laboratory of ICARDA is represented in the following parts:

1. *Soil Preparation Room*

Where large bulk samples, transported by truck, are received, dried and sieved. This facility is equipped with a large-capacity oven, freezers, soil grinder, containers and trays, stainless steel soil sieve sets, vacuum pumps, sample dividers, different types of soil sampling (augers, spade, and metal rings), exhaust hood, and a compressed-air machine. Soil samples (~0.5 kg) are dried and placed in clean containers and then transferee to the soil testing laboratory for requested analysis.

2. *Soil Store Room*

Where all samples are retained for at least 2 years after analysis; bulk samples of special soil types are kept indefinitely. An inventory or catalogue of all soil samples is maintained.

3. *Freezer Room*

In some cases it is necessary to temporarily store large numbers of samples in cold conditions, such as a freezer room, pending analysis; without such conditions, any delay in analysis would result in bacterial changes in soil samples that would invalidate the analytical results.

4. *Chemical Analysis Room*

The chemical analysis room is where the sub-sample of dried soil is received in the soil testing laboratory for requested analyses. This facility is normally has various equipment, such as N digestion/distillation, CN-elemental analyzer, flame photometer, spectrophotometer, pH meter, and conductivity meter.

The more traditional analytical processes of weighing, stirring, shaking, filtering under suction, heating, drying, incubating and centrifuging are done almost exclusively with the aid of electrical machines and devices. In addition, for the digestion and preparation of reagents, chemicals carry out in the Fume Hood.

5. *Instrument Room*

This is where soil extraction, where necessary, is carried out for analysis. This facility is normally equipped with various equipment, e.g., atomic absorption spectrophotometer, computer, refrigerator, etc.

6. *Physical Analysis Room*

This is where the dried soil sub-samples are received in the soil testing laboratory for requested analyses. This facility is equipped with various equipment, such as pF instrument for field capacity and permanent wilting point, soil dispersing stirrer (a high-speed electric stirrer with a cup receptacle) for particle size distribution, aggregate stability (for wet and dry methods) instruments, balances, permeability apparatus, and water bath.

7. Water Analysis Room

In this room the more traditional analytical processes are carried out, e.g., pH, electrical conductivity, and anions and cations.

General Equipment

Various items of equipment and associated furnishings are generally found in soil, plant and water analysis laboratories, as indicated as follows:

- Laboratory working tables
- Appropriate racks
- Weighing benches
- Cupboards
- Laboratory desks and chairs
- Fixed suction unit
- Fume hood or exhaust systems mounted above the flame photometer, atomic absorption spectrometer, muffle furnace, and Kjeldahl digestion
- Washing-sinks for cleaning glassware
- Drawing desk facilities for storing standard forms and documentation

2.2. Laboratory Safety

As with any place of work, safety is an important consideration in soil, plant and water analysis laboratories, and one that is frequently overlooked. A safe working in a chemical laboratory needs special care, both in terms of design and construction of the laboratory building, and handling and use of chemicals. For chemical operations, the release of gases and fumes in some specific analytical operation are controlled through a fume hood or trapped in acidic/alkaline solutions and washed through flowing water. Also, ***some chemical reactions during the process of analysis, if not handled well, may cause an explosion*.**

Analytical processes normally carried out at room temperature can be affected by differences in temperature so that an analysis performed in a "cold" room can give a different result to one performed in a "hot" room. Many chemicals are affected by the temperature and humidity conditions under which they are stored, particularly if these conditions fluctuate. The air temperature of the laboratory and working rooms should ideally be maintained at a constant level (usually between 20 and 25 °C). Humidity should be kept at about 50 %.

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. Ideally, posters relatively to laboratory safely should be prominently displayed in the laboratory.

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

1. Follow the safety precautions provided by the manufacturer when operating instruments.
2. Monitor instruments while they are operating.
3. Atomic Absorption Spectrophotometer must be vented to the atmosphere. Ensure that the drain trap is filled with water prior to igniting the burner.
4. Never open a centrifuge cover until machine has completely stopped.
5. Use of balances:
    - The warming-up time of the balances is 30 minutes
    - Spilled chemicals should be removed immediately
    - Never blow away the spilled product
    - Brushes are supplied with the balances

Accidents

1. 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.
2. Learn emergency **First Aid**, such 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.
3. Seek medical attention immediately if affected by chemicals, and use **First Aid** until medical aid is available.
4. Access to eye-wash fountains and safety showers must not be locked. Fountains and showers should be checked periodically for proper operation.

Chemicals

1. Use fume hoods when handling concentrated acids, bases or other hazardous chemicals.
2. Do not pipette by mouth; **always use a suction bulb**.
3. When diluting, **always add acid to water, not water to acid**.
4. Some metal salts are extremely toxic and may be fatal if swallowed. **Wash hands thoroughly after handling such salts or indeed any chemical regardless of toxicity**. Chemical spills should be cleaned promptly and all waste bins emptied regularly.
5. All reagent bottles should be clearly labeled and must include information on any particular hazard. This applies particularly to poisonous, corrosive, and inflammable substances.
6. For the preparation of reagents, only **distilled water** (**DI**) is used. Note that volatile acids, ammonia, nitrite, chlorine and carbon dioxide have to be removed by means of a column containing resin (deionizer) which will exchange the charged ions, is needed.

Furnaces, Ovens, Hot Plates

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

Handling Gas

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

Maintenance

1. All electrical, plumbing, and instrument maintenance work should be done by qualified personnel.
2. **Fume hoods** should be checked routinely.
3. As most equipment operates on low wattage, an Un-interruptible Power Supply (UPS) provides stable power and allows the completion of any batch measurement in the event of power outage.

Maintenance of Pipettes

1. At the end of the working day, wash the pipette with tap water and then several times with distilled water.
2. Dry the pipette in an oven.
3. Keep the pipette upside down in a special clamp.

Eating and Drinking

1. **Do not eat or drink in the laboratory**. This is essential both for reasons of health, to eliminate any possibly of poisoning, and to reduce contamination. Specific areas should be designated for staff breaks.
2. Do not use laboratory glassware for eating or drinking.
3. Do not store food in the laboratory.

Protective Equipment

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
A mask is needed when grinding soil, plant samples, etc.

Eye Protection
Use safety glasses with side shields. Contact lenses should never be worn around corrosives. Make sure that your colleagues know if you wear contact lenses.

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

1. 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.
2. Dispose-off chipped or broken glassware in specially marked containers.

Continuing Education

1. Display in a prominent place posters on "**Laboratory Safety**" which pictorially describe various phases of laboratory activities.
2. 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.
3. 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

Contamination is a most serious problem in any laboratory; 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 while 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
- Smoking in the laboratory

Technical Remarks

1. The air-dry moisture in a soil sample taken straight from a hot and humid storeroom (or a very cold one) may be different from that in a similar sample kept in an air-conditioned laboratory; both may be weighed for analysis at the same time as “air-dry’ samples.
2. The tap water supplied to a laboratory should be entirely free of pollution, as free as possible from insoluble matter, and under good and steady hydrostatic pressure. It may be necessary to filter the supply to certain pieces of equipment.
3. Drainage should be to a main drain if possible or to good-sized “soak-away”. Effluents from soil laboratories contain considerable quantities of waste soil in addition to acid and alkaline liquids. The facilities should be provided in the design of the drainage system for periodic cleaning and removal of solid matter.

2.3. 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**. Known amounts of reagent should be added to the homogeneous test sample, the procedures repeated, and the mean and standard then deviation calculated. The agreement between the increases 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-sorptive soils, this test can be conducted by reagent solutions.

Blanks

Blanks are reaction vessels that are subjected to identical procedures as the sample in a given batch which has 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.

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.

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.

Standardization of Methods

Results can only be validly compared to one another when they 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, and operating international soil and plant analytical exchange programs.

Most external reference samples are costly, and their frequent use increases 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.

Errors in Quantitative Analysis

In dealing with analysis, the concepts of accuracy and precision (See Figure.1) are important.

Accuracy: A measure of systematic error or the degree of agreement of an experimental value with the true or expected value of the quantity of concern. Accuracy of the value is important, but it is important to know when to use a given analytical method and to know its limitations.

$$\boldsymbol{Accuracy} = \frac{\boldsymbol{mean - true\ value}}{\boldsymbol{true\ value}}$$

$$\boldsymbol{Recovery}\ (\%) = \frac{\boldsymbol{measured\ value}}{\boldsymbol{Know\ value}} \times \mathbf{100}$$

Precision: A measure of reproducibility affected by random error. It is usually described by the standard deviation, standard error, or confidence interval.

$$\boldsymbol{RSD\ or\ CV}\ (\%) = \frac{\boldsymbol{S}}{\boldsymbol{mean}} \times \mathbf{100}$$

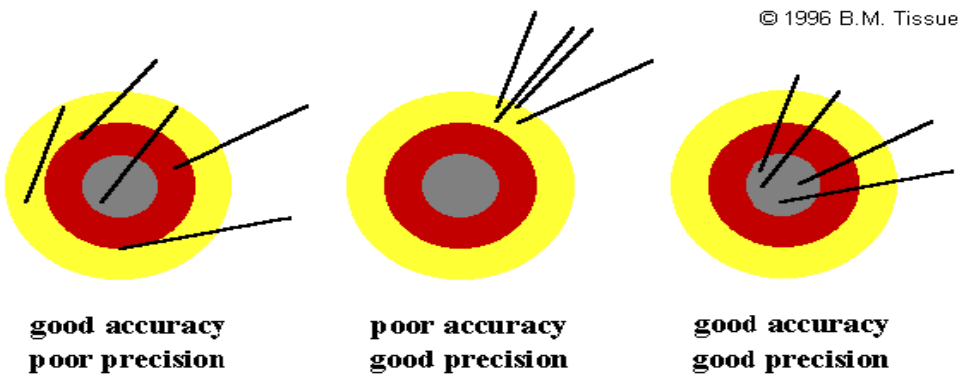


Figure. 1 Illustration of Accuracy and Precision

2.4. Data Processing

A considerable amount of information is generated in any soil, plant, and water 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:

- Easier manipulation of large data sets,
- Reduced errors in calculation of recommendations,
- Preparation of reports,
- Automated invoicing and addressing, and
- 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 turn-over.

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, and climatic zone.

3. Soil Sampling and Processing

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

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

3.1. Soil Sampling

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

Composite Sampling

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

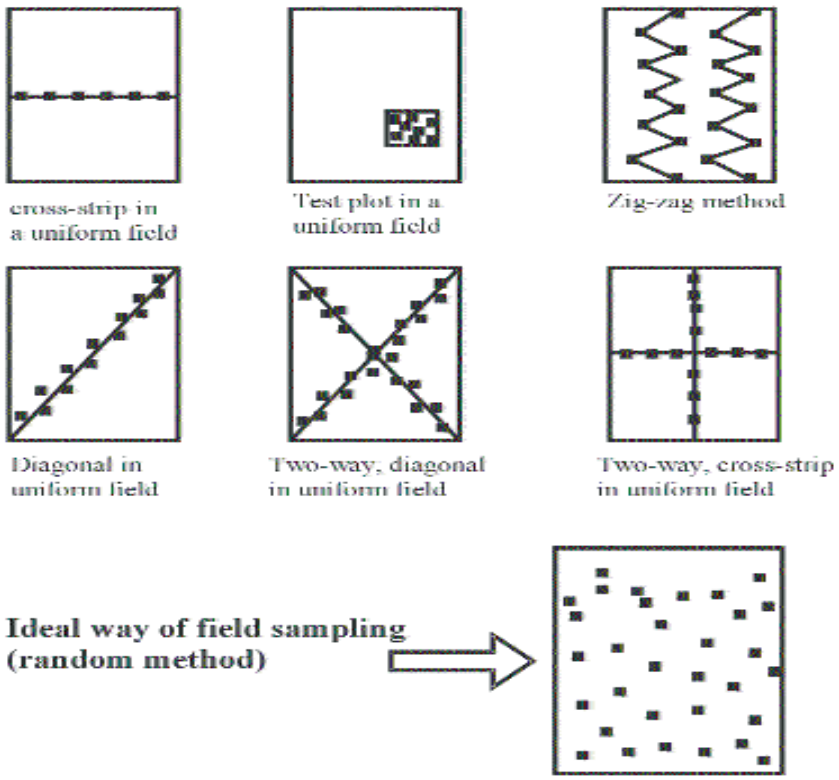


Figure 2. Some suggested methods for soil sampling; each dot represents a sample point, with formation of a sample pattern within the fielded

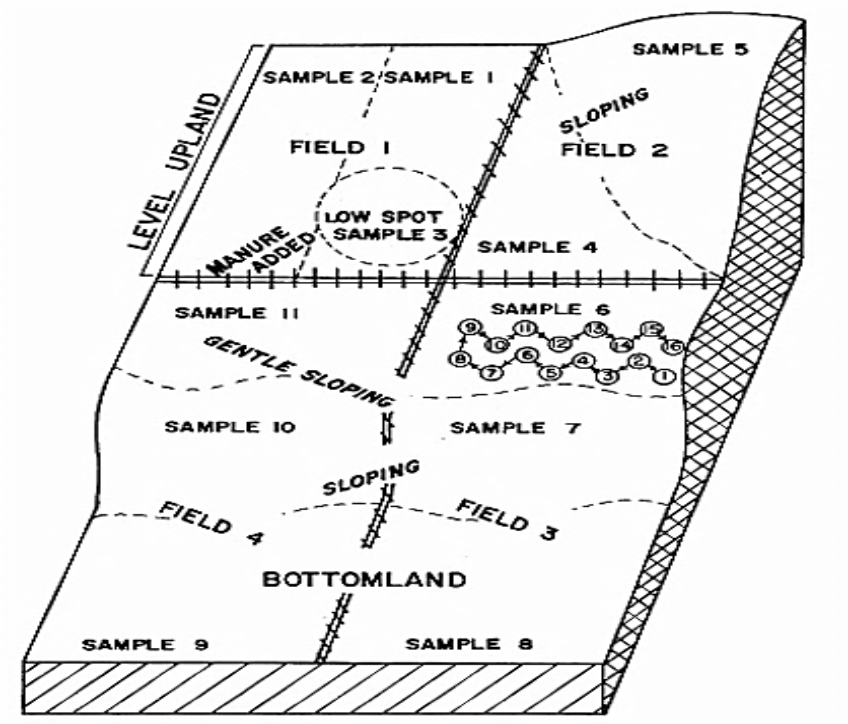


Figure 3. Sampling pattern for fertility test in a non-uniform land (sample numbers refer to composite sample; Tarzi, 1984)

Sampling Time

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

Sampling Depth

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

Soil profile

Sampling Tools

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

Soil sampling tool: Auger

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

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

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

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

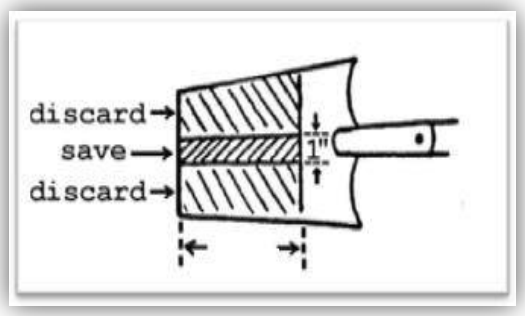


Figure 4. Soil sampling tool: Spade

Instructions for Field Processing

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

3.2. Preparation Laboratory Processing

Handling in the laboratory

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

Drying of the soil samples

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

Ovens

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

Preparation of soil samples

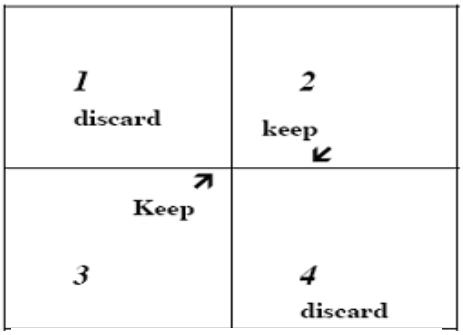
- After drying, the samples are ground with a wooden pestle and mortar in preparation room (which is separate from the main laboratory), and clods and large aggregates are crushed and mixed. Remember that:
- Pebbles, concretions, and stones should not be broken during grinding.
- Care should be taken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

- Amount of sample to be crushed or ground
- Degree of fineness to be attained
- Contamination that can be tolerated
- The analysis in question

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

Riffle-type soil samples splitter (sample dividers)

Soil grinder



Reduction of soil sample size

4. Soil Physical Analysis

Soil physical measurements are numerous, depending on the objective of the study for agricultural purposes. These measurements generally includes soil water purpose on the content, infiltration and hydraulic conductivity, evapotranspiration, heat, temperature, reflectivity, porosity, particle size, bulk density, aggregate stability, and particle size distribution.

Soil moisture is routinely measured on field-moist samples, since all physical analyses are expressed on oven-dry basis (16-18 hours drying at 105 ^{o}C). 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.

4.1. Soil Moisture Content

As water is the most limiting factor in the arid to semi-arid areas, 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 the 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* (Sparks et al., 1966).

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 (normally for 24 hours).
3. Next day, when the soil has dried, remove the container from the oven, using tongs; fit the lid, cool in a desiccator for at least 30 minutes and re-weigh.

Calculation

$$\boldsymbol{Soil\ Moisture}\ (\theta) = \frac{\boldsymbol{wet\ soil}\ (\boldsymbol{g}) - \boldsymbol{dry\ soil}(\boldsymbol{g})}{\boldsymbol{dry\ soil}\ (\boldsymbol{g})}$$

$$\boldsymbol{Dry\ Soil}\ (\boldsymbol{g}) = \frac{\mathbf{1}}{\mathbf{1} + \frac{\boldsymbol{\theta}}{\mathbf{100}}} \times \boldsymbol{Wet\ soil}$$

$$\boldsymbol{Moisture\ Factor} = \frac{\boldsymbol{Wet\ soil}(\boldsymbol{g})}{\boldsymbol{Dry\ soil}\ (\boldsymbol{g})}\ \boldsymbol{or}\ \frac{\mathbf{100} + \%\,\boldsymbol{\theta}}{\mathbf{100}}$$

Technical Remarks

1. The wet soil sample should be kept loosely in the container.
2. Care should be taken to avoid over-heating of the soil sample by maintaining the oven temperature at 105-110 °C.
3. Dry soil sample should not be left uncovered before weighing.
4. To determine the moisture content of litter and humus samples, dry samples at 70 °C for 48 h.
5. Moisture content in air-dry is called hygroscopic moisture. It varies from less than 0.2% for sand to more than 8% for similar with leaf litter/OM, depending upon the relative humidity in the storage area, and fineness of soil particles. Samples should be air-dried prior to moisture content determination.
6. Moisture content values reproducible to within ± 0.5 % can be achieved.
7. The oven is monitored periodically to ensure that temperature fluctuation does not exceed 5 ^{o}C.
8. The water content at field capacity, wilting point, and the hygroscopic coefficient are all based on the oven-dry reference mass. The percentage of water held under each of these conditions can therefore be used to define the following and other forms of soil water. Each of these forms of water can be calculated from the appropriate soil mass.

$$\boldsymbol{Hygroscopic\ water}\ (\%) = \boldsymbol{Hygroscopic\ coefficient}$$

$$\textit{Capillary water (\%)} = \textit{Field capacity - Hygroscopic coefficient}$$

$$\textit{Available water (\%)} = \textit{Field capacity} - \textit{Wilting point}$$

$$\textit{Unavailable water (\%)} = \textit{Wilting point}$$

$$\textit{Gravitational water (\%)} = \textit{Water content} - \textit{Field capacity}$$

4.2. Water Holding Capacity

The water-holding capacity *(WHC) is defined as the amount of water held in the soil after the excess gravitational water has drained away and after the rate of downward movement of water has materially ceased. Stage of field capacity is attained in the field after 24 to 72 hours of saturation; this is the upper limit of plant-available soil moisture. We must distinguish between* ***soil water content****, (the percent water on an oven-dry weight basis), and* ***the soil water potential*** *(the energy status of water in the soil), which is usually expressed in pressure units (Pascal or bar). However, as indeed we are dealing with a tension – a negative pressure - units are usually considered to be negative.*

Apparatus

Polythene sheets	Funnel (glass or plastic)
Spade	Tubing (to attach to bottom of funnel)
Soil auger	Clamp (to secure tubing)
Moisture boxes/cans	Filter paper (to line funnel)
Balance	Beakers (250-mL)
Oven	Graduated cylinder
Ring stand	Stirring rod (long)

Procedure

A. Field Processing

1. Select a uniform plot measuring (5 m x 5 m) and make a flat and horizontal area.
2. Remove any loose material from the surface (weeds, pebbles, etc.).
3. Make bunds around the plot.
4. Fill sufficient water in the plot to completely saturate the soil.
5. Cover the plot area with a polythene sheet to check evaporation.
6. Take soil sample from the centre of the plot from the desired layer, starting after 24 h of saturation and determine moisture content daily till the values of successive days are nearly equal.
7. Record the weight of the oven-dry soil.
8. Repeat above on next day and so on till a constant oven-dry soil value is reached.

B. Laboratory Processing

1. Thoroughly air-dry compost and soil samples.
2. Attach and clamp tubing to bottom of funnel and attach funnel to ring stand.
3. Place filter paper in funnel.
4. Fill funnel with the 100 mL sample – do not compact.
5. Measure out 100 mL of water using the graduated cylinder.
6. Gradually add water to the sample until covered. Record the amount of water added.
7. Stir gently and let sit until sample is fully saturated.
8. Release the clamp and collect excess water in the graduated cylinder (water drained, mL).
9. Record the amount of water in the cylinder.
10. Calculate how much water was retained in the 100-mL sample of compost, soil or compost/ soil mixture and then calculate the water-holding capacity.

Calculations

$$\frac{\boldsymbol{Water\ retained\ (mL)}}{\boldsymbol{100\ mL\ sample}} = \boldsymbol{Water\ added\ (mL) - Water\ drained\ (mL)}$$

$$\boldsymbol{Water\ Holding\ Capacity\ (mL\ /L)} = \frac{\boldsymbol{Water\ retained\ (mL)}}{\boldsymbol{100\ mL\ sample}} \times \mathbf{10}$$

Note

Water-holding capacity is expressed as the amount of water retained per liter of soil, so the next step is to multiply by 10 to convert from the 100 mL sample to the full liter.

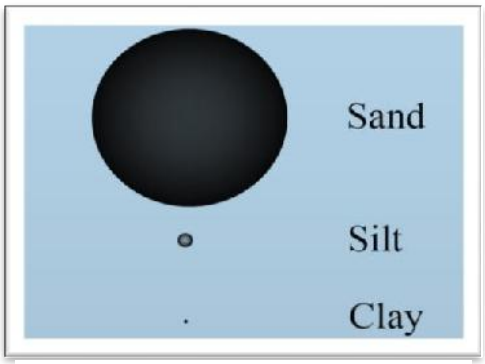
Technical Remarks

1. Estimates of soil WHC, wilting point and texture can be made from the saturated moisture content. The method is generally reproducible within ± 12%, dependent on the soil textural class.
2. Plot the daily readings on a graph paper. The lowest reading is taken as the value of field capacity of the soil.

4.3. 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 for soil water, aeration, and nutrient availability to plants. Also, soil texture is an important property for agricultural soil, road beds, septic disposal systems, influences fertility, drainage, water-holding capacity, aeration, tillage, and bearing strength of soils. Because texture is so important, it is a standard physical soil test.



Relative size of soil separates

As primary soil particles are usually cemented together by organic matter, this has to be removed by hydroxide peroxide (H_2O_2) treatment. However, if substantial amounts of calcium carbonate ($CaCO_3$) 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 or the pipette-gravimetric method.

4.4. Hydrometer Method

*The hydrometer method of silt and clay measurement relies in the effect of particle size on the differential settling velocities within a water column. By this method (using Hydrometer with Bouyoucos scale in g/L) after 40 second all sand-sized particles (0.02 mm and larger) settle out of the suspension and after 4 h, particles larger than clay (0.002 mm) settle out of the suspension. **Theoretically**, the particles are assumed to be spherical having a specific density 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**).*

$$V = \frac{2\,r^2\left(P_{dp} - P_{dl}\right)g}{9\,\eta}$$

Where:

V = Velocity of fall (cm/sec)

r = "equivalent" radius of the particle (cm)

ρ_{dp} = Density of particle (g/cm^3), 2.65 is a (usual) good approximation

ρ_{dl} = Density of liquid (g/cm^3), for water this is ~1.0

g= Acceleration of gravity (cm/sec), value at the sea level is 981

η= Viscosity of liquid (g/cm/sec)

***In practice**, the density of water and its viscosity are both affected by temperature. 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)

Interval timer

Hot plate

Drying oven

Balance, accurate to 0.5 g

Desiccator

Beakers

Measuring cylinders, 1000-mL

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. 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. Next day, 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 (hydrometer jar), and bring to volume with water.

A. Determination of Blank

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

B. Determination of Silt plus Clay

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

C. Determination of Clay

1. Mix suspension in the hydrometer jar with paddle; withdraw the paddle, leave the suspension undisturbed.
2. After 4 h, insert the hydrometer, and take hydrometer reading, R_C.

E. Determination of Sand

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

Calculations

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

$$Clay\ (\%\ w/w) = (R_c - R_b) \times \frac{100}{Oven - Dry\ soil\ (g)}$$

$$Silt\ (\%\ w/w) = [\ Silt + Clay\ (\%w/w)] - [Clay\ (\%w/w)]$$

$$Sand\ (\%\ w/w) = Sand\ weight\ (g) \times \frac{100}{Oven - Dry\ soil\ (g)}$$

$$Sand\ weight\ (g) = [Beaker + Sand\ (g)] - [Beaker\ (g)]$$

Technical Remarks

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 (16-24 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.
7. The material not passing the sieve is weighed and reported as percentage of the air-dry weight of the whole sample.
8. Dispersion is achieved by mechanical means (stirring for larger aggregates), by chemical dispersion using sodium hexametaphosphate, and by eliminating cementing agents such as organic matter (oxidized with hydrogen peroxide) and calcium carbonate (dissolved with hydrochloric acid).
9. **Sodium hexametaphosphate is an effective dispersing agent for two reasons**:

- The sodium monovalent cation replaces polyvalent cations (predominantly calcium) usually adsorbed on soil colloids, thereby breaking one type of inter-particle linkage. The polyvalent cations are then reduced in activity by reacting with the phosphate and precipitating.
- The adsorbed sodium cations are highly hydrated and raise the electro-negativity of the colloids until these particles repel each other and remain dispersed. The mixture of dispersed soil particles in water is called a soil suspension.

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. Within the textural triangle are various soil textures which depend on the relative proportions of the soil fractions. **Twelve soil textural classes are recognized and their compositions are designed on a textural triangle**. The class name can be determined by plotting these values on a textural triangle using the following technique:

1. Clay; extend line horizontal from the percent clay (parallel with base of triangle, sand).
2. Silt; extend line downward from percent silt at 60° (parallel with right side of triangle, clay).
3. Sand; extend line upward from percent sand at 120° (parallel with left site of triangle, silt).

For example, if a soil is 50 % sand, 35 % silt, and 25 % clay, the texture is sandy clay loam (see Figure. 5).

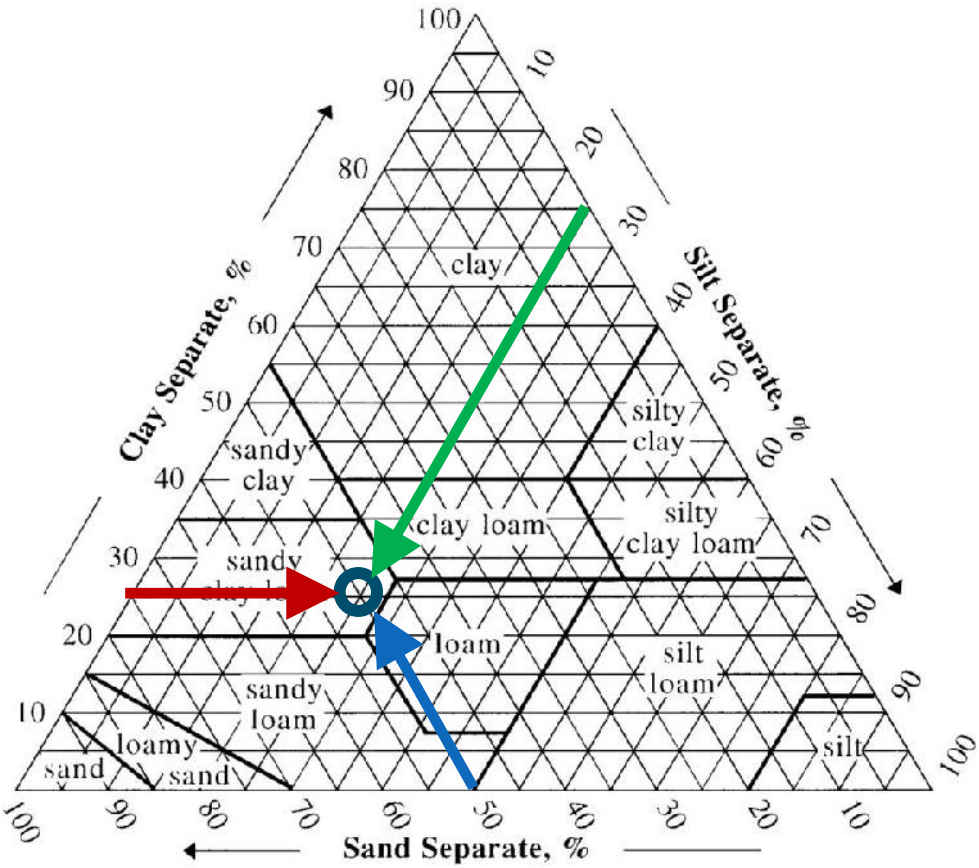


Figure 5. USDA soil textural triangle; indicated example, sandy clay loam: 50 % sand, 35 % silt, and 25 % clay

4.4.1. Pipette Method

The pipette *is a laboratory instrument used to transport a measured volume of suspension. Therefore, the pipette method is based on the difference in sedimentation velocity between large and small soil particles. The sedimentation of the particles is the result of two opposite forces, i.e., gravity and friction as a result of movement in a liquid medium. In the* ***pipetting method****, a sample is pipette from a suspension in a graduated measuring cylinder at various times and depths. Times and depths are determined with Stokes' law. The pipetted suspension is evaporated down and dehydrated, and the mass percentage of the pipetted fraction is determined by weighing.*

Apparatus

Cylinders (1000-mL) provided with rubber stoppers
Soil dispersing stirrer: a high-speed electric stirrer with a cup receptacle
Water bath
Pipette apparatus consists of:

- Frame with a runner with pipette holder fixed on top of it.
- Pipette balloon.
- Glass tank (113 L capacity).
- Heating element with thermostat and stirring device.

Reagents

A. Amyl Alcohol
B. Deionized or distilled water
C. 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.

Procedure

A. Pre-treatment

1. Each sample is given a laboratory number.
2. The sample is spread uniformly over a plastic or paper sheet, let them air dry.
3. Remove any straw, roots, dead leaves and large stones.
4. Prepare the subsamples by '**quartering**'. The mixed soil material is coned in the center of the mixing sheet with care to make it symmetrical with respect to fine and coarse soil material.
5. Calculate the oven dry weight of the soil sub-sample (soil moisture content).

B. Sand fraction determination

1. The pretreated soil suspension is passed through the appropriate sized sieve (usually 200 µm sieve). Therefore, the soil should be crushed by pestle and porcelain mortar and pass through 200 µm sieve.
2. Repeat until 20% of the soil is retained on the top of the 200 µm sieve.
3. The sand fraction is washed, and quantitatively transfer the sediment suspension through a 270 mesh (53 um) sieve and wash with DI water using a wash bottle. Transfer the sand to a tare beaker, dry at 105 ^{o}C and weigh. The dried sand may be placed in nested sieves to determine individual sand fraction size analysis.

C. Silt and clay determination

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. Next day, quantitatively transfer contents of the beaker to a soil stirring cup, and fill the cup to about three quarters with water using a soil stirring device, stir suspension at high speed for 2 minutes.
5. Transfer suspension quantitatively into a 1-L calibrated cylinder (**hydrometer jar**), and bring to volume with water.
6. Place the volumetric cylinders in the large temperature-controlled water bath (should be placed on a vibration-free table). The temperature of the water of the controlled water bath must be kept as constant as possible with the aid of the heating element with thermostat and stirring element.
7. Place the stirring paddle in the volumetric cylinder. Shake up-down and mix the suspension intensively for 20 times. Remove stirring paddle and wait until the swirling motion of the practices has just given way to steady settling under gravity.
8. Start the timer or stop-watch. Leave this to settle for 4 hours.

Note

The suspension must be complete, since/ otherwise particles could inter-aggregate. One indication of incomplete suspension is the forming of stratification of the suspension in the measuring cylinder.

9. About 30 seconds before the appropriate sampling time, lower the pipette (previously fixed above the centre of the cylinder) slowly until the tip just touches the surface of the suspension. Note the vertical scale reading and then lower the pipette to the required depth. Calibrated and fill the pipette by gentle and steady suction (capacity of the pipette around 20 mL (see below the using of the calibrated pipette).
10. Remove the pipette from the suspension. Transfer the aliquot to an evaporating dish and wash the pipette twice with water, adding the washing to the dish.
11. Dry the evaporating dishes with the suspension (evaporated down).
12. Determine the mass percentage of the pipette fraction by weighing.
13. Decant the cylinder sediments and suspension into analytical sieves to calculate the sand fractions.
14. Repeat the sampling a second time after remixing the suspension with the stirring paddle.
15. Evaporate to dryness on a water bath, dry the residue in an oven at 105 ^{o}C for 16-18 h, cool in desiccators and weigh to the nearest 0.2 mg.

Technical Remarks

Using of the calibrated pipette

1. Make sure that the two-way cock (K_1) and the cock (K_3) of the pipette are closed, and the two-way cock (K_2) of the pipette (P) is opened toward the pipette before you place the pipette (see sketch of pipette) over the measuring cylinder with the suspension, 30 second before pipetting (see Figure.6).
2. Insert the pipette into the suspension at the required depth (lower the pipette slowly to avoid disturbance).
3. Connect exhaust point (A) to the pipette balloon.
4. Open the two-way cock (K_1) and steadily draw the pipette full until the liquid level is above two-way cock (K_2).
5. Close the two-way cocks (K_1) and (K_2).
6. Move the runner to the right (outside the glass tank).
7. Open two-way cock (K_2) above drain (D) to drain off any excess suspension.

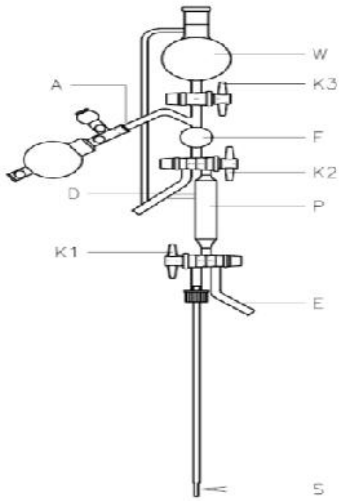


Figure 6. Calibrated pipette

8. Rinse overflow ball (F) with water from storage ball (W) by opening cock (K_3).
9. Open the two-way cock (K_2) above pipette (P).
10. Open two-way cock (K_1) above drain (E) and empty the pipette in an evaporating dish.
11. Rinse the pipette (P) with water from the storage ball (W). Collect also this scouring water in the same evaporating dish.
12. Open two-way cock (K_1) above pipette point (S) and remove the pipette from the suspension.
13. Collect the suspension of the pipette in evaporating dish.

Determination of the in-feed depth of the pipette

1. Determine the temperature of the blank and read the in-feed depth of the pipette and the sedimentation time of the desired fraction from the table below.
2. Mix the suspension intensively for at least 1 minute and start the chronometer. Pipette (after the prescribed sedimentation time) the particles ≤ 2 µm to determine the lutum content (lower the pipette slowly to avoid disturbance).
3. To determine the particle-size distribution, first pipette the fraction smaller than or equal to 35 µm, and subsequently the other desired fractions in descending order according to particle size.

Temperature of the suspension in °C	In-feed depth of the pipette in cm at		Temperature of the suspension in °C	In-feed depth of the pipette in cm at	
	Upper limit particle fraction in µm			Upper limit particle fraction in µm	
	35	2		35	2
	After a sedimentation time of			After a sedimentation time of	
	90 second	4 hours		90 second	4 hours
15.0	8.4	4.4	**20.5**	9.7	5.1
15.5	8.6	4.5	**21.0**	9.8	5.1
16.0	8.7	4.5	**21.5**	9.9	5.2
16.5	8.8	4.6	**22.0**	10.1	5.3
17.0	8.9	4.6	**22.5**	10.2	5.3
17.5	9.0	4.7	**23.0**	10.3	5.4
18.0	9.1	4.8	**23.5**	10.4	5.4
18.5	9.2	4.8	**24.0**	10.6	5.5
19.0	9.3	4.9	**24.5**	10.7	5.6
19.5	9.5	5.0	**25.0**	10.8	5.6
20.0	9.6	5.0			

Calculation the depth of pipette sampling

The depth of pipette sampling is based on the velocity (**Stokes' Law**). The re-arranged equation is:

$$T = \frac{18\,\eta h}{\left[(V - g)\left(P_{dp} - P_{dl}\right)\right]X^2}$$

Where:

T = Time of pipetting

h = Depth of pipette

V = Velocity of fall (cm/sec)

P_{dp}= Density of particle (g/cm^3)

X^2= Particles diameter

ρ_{dl} = Density of liquid (g/cm^3), for water this is ~1.0

g= Acceleration of gravity (cm/sec), value at the sea level is 981

η= Viscosity of liquid (g/cm/sec)

Assumptions used in applying Stokes' Law to soil sedimentation measurements are as follows:

1. Terminal velocity is attained as soon as settling begins.
2. Settling and resistance are entirely due to the viscosity of the fluid.
3. Particles are smooth and spherical.
4. There is no interaction between individual particles in the solution.
5. It is recommended to use horizontal reciprocating to disperse the samples. The use of electric stirrer at high revolutions per minute (rpm) may result in significant grinding of sample primary minerals.
6. For soils having clay particle densities <2.65 g/cm^3, settling time increases, and for soils >2.65 g/cm^3, settling time decreases.
7. An error of 0.001 ± g in the dry weight of the pipette sample results in an error of ± 0.32 % for clay size fraction.
8. Since soil particles are not smooth and spherical, the radius of the particle is considered an equivalent rather than an actual radius. In this method, particle density is assumed to be 2.65 (g/cm^3) and density of liquid is assumed to be 1 (g/cm^3).
9. Water density and viscosity at different temperatures.

Temperature (^{o}C)	**10.0**	**12.5**	**15.0**	**17.5**	**20.0**	**22.5**	**25.0**	**27.5**	**30.0**
Density (g/cm^3)	0.99973	0.99947	0.99913	0.99871	0.99823	0.99768	0.99707	0.99640	0.99567
Viscosity (g/cm/sec)	0.01301	0.01219	0.01138	0.01072	0.01006	0.00950	0.00895	0.00847	0.00800

4.5. Soil Structure

Soil structure is defined as the arrangement of the soil particles. With regard to structure, soil particles refer not only to sand, silt and clay but also to the aggregate or structural elements, which have been formed by the aggregation of smaller mechanical fractions. The size, shape and character of the soil structure varies, which could be cube, prism and platter likes. ***On the basis of size, the soil structure is classified as follows****:* ***very coarse*** *(>10 mm),* ***coarse*** *(5-10 mm);* ***medium*** *(2-5 mm);* ***fine*** *(1-2 mm); and* ***very fine*** *(<1 mm). Depending upon the stability of the aggregate and the ease of separation,* ***the structure is characterized as follows: Poorly developed, weekly developed, moderately developed, well developed, and highly developed.***

4.5.1. Dry Aggregate Analysis

An aggregate analysis *aims to measure the percentage of water-stable secondary particles in the soil and the extent to which the finer mechanical separates are aggregated into coarser or larger fractions.* ***The resulting aggregate-size distribution depends on the manner and condition in which the disintegration is brought about****. For the measurements to have practical significance, the disruptive forces causing disintegration should closely compare with the forces expected in the field. The field condition, particularly with respect to soil moisture, should be compared with the moisture condition adopted for soil disintegration in the laboratory. The sampling of soil and subsequent disintegration of clods in relevance to seed bed preparation for upland crops should be carried out under air dry conditions for dry sieve analysis. A rotary sieve shaker would be ideal for dry sieving.*

Apparatus

- Nest of sieves having different diameter round openings with a pan and a lid
- Metal ring (ring is 20-cm diameter and 10- cm height)
- Rotary sieve shaker
- Aluminum cans
- Balance
- Spade
- Brush
- Polyethylene bags
- Labels

Procedure

A. Field processing

1. Collect the soil sample with metal ring by pressing on the bulk soil sample (better when moist) until level with the surface. Avoid excessive compaction or fragmentation of soil Remove the loose soil within the ring and collect it in a polyethylene bag.
2. Record all the information about samples (depth and profile), put one label inside the bag and tie the other label to the bag. Then bring the soil samples to the soil preparation laboratory.

B. Laboratory processing

1. Each sample is given a laboratory number.
2. Spread samples uniformly over a plastic or paper sheet, and let them air dry. Prepare the subsamples by '***quartering***'. The mixed soil material is coned in the center of the mixing sheet, with care to make it symmetrical with respect to fine and coarse soil material.
3. Calculate the oven-dry weight of the soil sub-sample (soil moisture content).
4. Weigh the soil subsamples to the nearest 0.1 g.
5. Prepare the sieving tower and stack sieves starting with the smallest sieve opening, and add a pan at the bottom of the set.

Note

To avoid leakage, make sure that the O-rings are placed between individual sieves, the bottom sieve and the sieve pan, and the top sieve and the clamping lid.

6. Gently pour the soil aggregates collected on the top of the nest of sieves (having different diameter round openings usually 5.0, 2.0, 1.0, 0.5 and 0.25 mm)
7. Cover the top sieve with the lid and place the nest of sieves on a rotary shaker.
8. Switch on the shaker for 10 minutes, remove the sieves, collect the soil retained on each sieve in the pre-weighed aluminum cans, with the help of a small brush, and weigh the cans with the soil.
9. Analyze the duplicate sample following the same procedure and calculate the percent distribution of dry aggregates retained on each sieve.

Calculations

$$\boldsymbol{Wt\,(g)} = (\boldsymbol{Wt_1} - \boldsymbol{Wt_2})$$

$$\boldsymbol{Distribution\ of\ aggregates}\ (\%) = \frac{\boldsymbol{Wt}}{\boldsymbol{Wt_3}} \times \mathbf{100}$$

$$\boldsymbol{Oven - dry\ weight\ of\ aggregates}\ (\%) = \frac{\boldsymbol{Weight\ of\ air - dry\ soil}}{\mathbf{100} + \boldsymbol{Moisture}\%} \times \mathbf{100}$$

Where:

Wt = Weight of aggregates in each sieve group (g)

Wt_1 = Weight of aggregates in each sieve group plus can (g)

Wt_2 = Weight of empty can (g)

Wt_3 = Total weight of soil (g)

Technical Remarks

1. If the percentage of dry aggregates on 5-mm sieve exceeds 25 %, transfer these aggregates to a nest of sieves with 25, 10 and 5-mm sieves along with a pan. Then, cover the top sieve containing the aggregates with a lid and place the nest of sieves on the rotary sieve shaker. Switch on the motor for 10 minutes and proceed (as above Steps 5, 6, 7) for the estimation of aggregate size distribution.
2. Use work gloves to protect hands, and a mask and other personal protective equipment to protect from dust.
3. The fume hood is the best place to use the rotary sieve shaker.
4. The number and sizes of aggregate-size classes collected depends on the objective of the experiment.

4.5.2. Wet Aggregate Analysis

The soil sample is taken when it is moist and friable. It is broken by applying mild stress into smaller aggregates which can pass through 8-mm screen. The sieved soil sample is taken on a watch glass for wetting by either vacuum soaking or immersion method. ***Aggregates of different sizes can be obtained through several methods such as, samples under vacuum and sieving under water****. Among the different procedures adopted, wetting the samples under vacuum is suggested because the rate of wetting influences slaking of crumbs. The time of sieving ranges from 10 to 30 minutes depending upon the type of wetting.* ***However, sieving under water compares more closely with the disruptive actions of water and other mechanical forces****.*

Apparatus

Yoder -type wet sieve shaker (mechanical oscillator powered by a gear reduction motor)

Two sets of sieves, i.e., sieves-nest (20-cm diameter. and 5-cm height) with screen openings of 0.5, 0.2-mm diameter.

Filter paper

Aluminum metal pot (Al-pot)

Plastic dishes (small)

Standard metal ring

Soil dispersion stirrer: high-speed electric with a cup receptacle

Reagents

A. Sodium Hexametaphosphate [($NaPO_3$)13], 5%

Dissolve 50 g *Na-hexametaphosphate* in DI water, and bring to 1- L volume. This solution deteriorates with time and should not be kept for more than 1 to 2 weeks.

B. Sodium Hydroxide (NaOH), 4%

Dissolve 40 g *NaOH* in DI water, and bring to 1-L volume.

Procedure

A. Field processing

1. Collect the soil sample with metal ring by pressing on the bulk soil sample (better when moist) until level with the surface. Avoid excessive compaction or fragmentation of soil.
2. Remove the loose soil within the ring and collect it in a polyethylene bag.
3. Record all the information about samples (depth and profile), put one label inside the bag and tie the other label to the bag. Then bring the soil samples to the soil preparation laboratory.

B. Laboratory processing

1. Each sample is given a laboratory number.
2. Spread the samples uniformly over a plastic or paper sheet to air-dry. Field-moist instead of air-dry samples may be used, if air-drying is assumed to affect aggregates ("self-mulching soils"). Prepare the subsamples by 'quartering'. The mixed soil material is coned in the center of the mixing sheet with care to make it symmetrical with respect to fine and coarse soil material.
3. Calculate the oven-dry weight of the soil sub-sample (soil moisture content).
4. Weigh 50 g air-dry soil (<2-mm).
5. Gently pour the soil aggregates collected into standard metal ring (place under the metal ring a filter paper and a small plastic dish).
6. Add 50 mL tap water (allowing the water to soak up underneath) to saturate the soil sample. Leave the wetted soil samples for 30 minutes.
7. Transfer the set saturated soil sample into the 2 sieves 0.5 mm (above) and 0.2 mm (bottom). Add any larger size sieve above the first sieve to reach the limit of the surface water in the tank.
8. Remove the bottom pan, and attach the nest of sieves to the Yoder-type wet sieve shaker.
9. Fill the drum (which holds the set of sieves) with salt-free water at 20-25 ^{0}C to a level somewhat below that of the screen in the top sieve of the nest of sieves, when the sieves are in the highest position. Then lower the sieves-nest to wet the soil for 10 minutes.
10. Bring the nest of sieves to the initial position and adjust the level of water so that the screen in the top sieve is covered with water in its highest position. Then switch on the mechanical oscillator to move the sieves-nest up and down with a frequency of 30-35 cycles/minute and a stroke of 3.8 cm.
11. Sieve for 10 minutes.
12. Remove the sieves-nest from the water and allow it to drain for some time.
13. Transfer the soil resting on each screen with a stream of distilled water and brush into a big Al-metal pot (known weight). Leave the washed water-stable aggregates in the pot for 4-6 hours to precipitate all the suspended fractions and decant some of the water pot to reduce the drying period.
14. Dry the soil aggregate sample in an oven at 70 ^{0}C. Weigh the soil and Al-pot to nearest to 0.01 g.

Calculations

$$\textbf{Soil particle in each size group } (\%) = \frac{Wt_{(agg+s)} \times I}{W} \times 100$$

$$\textbf{Sand particle in each size group } (\%) = \frac{Wt_s \times I}{Wt} \times 100$$

Where:

Wt = Weight of oven-dry soil particle in each sieve group (g)

$Wt_{(agg+s)}$ = Weight of oven-dry aggregates and sand (g)

Wt_s = Weight of oven-dry sand (g)

I = Size group

4.6. Soil Bulk Density

Soil bulk density *(BD) is ratio of the mass (oven-dry weight) of the soil to the bulk volume expressed in grams per cubic cm (g/cm^3) or tons per cubic meter (t/m^3), which includes the volume of both solids and pore space at a specified soil water content (usually the moisture content at sampling).* ***The BD may be dependent on soil conditions at sampling time****. Changes in soil swelling due to changes in water content can alter BD.* ***Soil mass remains fixed, but the volume of soil may change as water content changes****.*

Bulk density is a parameter that indicates soil structure and void space. The BD is required to calculate porosity when particle density is known, to convert weights to volume, and to estimate weights of soil volumes too large to weigh. It is also required to convert data from a weight to a volume basis, to estimate ***saturated hydraulic conductivity*** *(Ks), and to identify compacted horizons. For example, the volumetric content of water in a soil layer is obtained by multiplying the gravimetric water content by the product of the BD and the volume of the soil layer.*

Soil BD is an indirect measure of the total pore space which is also affected by texture and structure*. The BD of fine textured mineral soils usually ranges from about 1.0 to 1.5 g/cm^3, and that of sandy soils from 1.3 to 1.7 g/cm^3. The bulk density of organic soils is usually much less than that of mineral soils and may be as low as 0.4 g/cm^3. Bulk density and total pore space are readily altered by tillage operations.*

Two methods are commonly used to determine soil BD*: one with samples of disturbed soil, and the other with undisturbed soil. The second method uses consolidated soil masses, e.g., clods and cores.*

Apparatus

Trowel or shovel

Sampling cylinders

Plastic bags

Hammer

Knife

4.6.1. Soil bulk density - disturbed samples

This method is used when it is not possible to take a consolidated sample of soil, as in sandy soils and soils of greenhouses and nurseries, because they are loose and very friable.

Procedure

1. Remove any loose material from the surface, i.e., the O horizon or any other material at the surface. When sampling subsurface horizons, dig a large pit to the required depth.
2. Make a flat and horizontal area a little larger than the cylinder (metal ring).
3. Place the cylinder on the soil surface (or subsurface horizon); put one end of the cylinder (usually 75-mm and 50-mm height ring with sharp cutting edge at the bottom and removable one at the top) while holding the other end with one hand. Hammer the cylinder into the soil with blows until the top of the cylinder is flush with the surface. Stop regularly and check how much deeper the cylinder has to go.

Note

If you strike too often, the soil in the cylinder will be compressed and the results will be wrong.

4. Dig around and under the cylinder and carefully remove it. With a knife, trim any protruding soil flush with the ends of the cylinder.
5. If the ends have small holes, these may be filled with soil, but if holes are too big, the sample must be discarded.
6. Put the sample in a plastic bag and label it appropriately.

Note

Do not put the cylinder itself in the bag (the Sharpe edges my perforate the bag). Take a replicate sample and put it in a bag.

7. The volume of your samples is equivalent to the volume of the cylinder.
8. In the laboratory, calculate the oven-dry weight of the soil in the cylinder, after drying it in an oven at 104 °C until the weight is constant (usually after 24 h).

Calculation

$$BD\ (g/cm^3) = \frac{W}{V}$$

Where:

BD = Bulk density (g/cm^3)

W = Weight of oven-dry soil (g)

V = Volume of soil sample (cm^3)

Technical Remarks

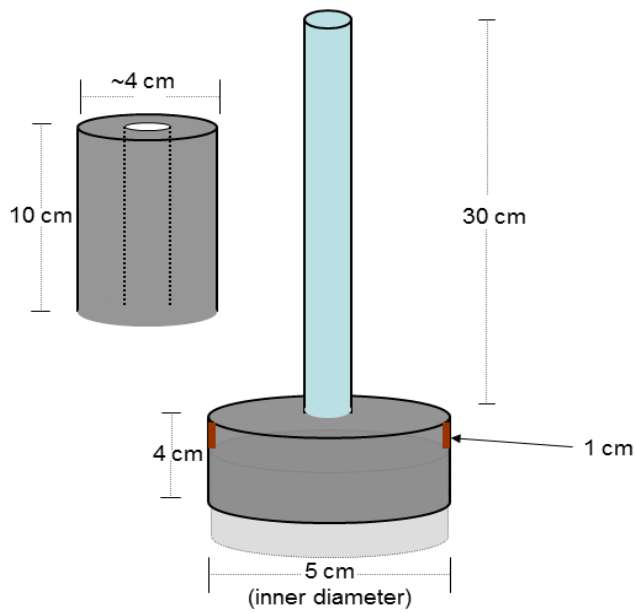
1 The BD is commonly calculated on an oven-dry basis, but for certain uses it is calculated on a wet-soil basis.

2 If the sample contains rock fragments, put the wet sample through a 2-mm sieve. Dry, weigh, and record weight of the rock fragments that are retained on the sieve. Determine the volume of the rock fragments based on their weight and an assumed a particle density of 2.65g/cm^3 -unless better knowledge is available. Subtract the weight of the rock fragments from the total weight of the sample and subtract their volume from the total volume of the sample.

3 The BD measurement should be performed at the soil surface and /or in a compacted zone (plow pan, etc.) if one is present.

4 To get a more representative BD measurement of the area, additional samples may be taken.

5 If BD is sampled frequently a special sampling device like the one sketched below may be acquired. The advantage of such a sampler is that the weight used for hammering (dark gray shaded piece to the left) is guided by the handle allowing for convenient and pain-free hammering. The diameter of base part must be adapted so as to hold a usual standard core ring (light gray-shaded). It is also advisable to leave a ~1 cm edge (indicated in red) at the lower part of the cylinder holding the core rings. This facilitates taking samples without compacting them.



4.6.2. Soil bulk density - undisturbed samples

4.5.2.1. Clod method

The basis for this method is the measurement of the volume of a clod by utilizing the buoyancy principle, as follows: When a solid material (density greater than water) is immersed in water (an upward directed force equal to the weight of the water displaced) is exerted upon it. Thus, if a solid object is weighed first in air (the buoyant force of air can be considered negligible in this case) and then in water, the difference between the two weights represents the weight of displaced water. The weight of displaced water in grams equals its volume in cm^3 *(assuming that the density of the water is 1 g/cm^3).When measuring the volume of a soil clod, the clod is coated with paraffin to keep water out of the pores. The volume of water displaced by the clod is equal to the volume of the paraffin coated minus the volume of the paraffin.*

Reagent

Melted wax (paraffin wax)

Procedure

1. Carefully collect soil clods from a soil profile. If roots are present, cut them carefully with scissors.
2. Tie and hold the clod with a fine copper wire and carefully weigh it.
3. Dip the clod in the melted wax, hang it to dry for 30 minutes. Additional melted wax coatings may be applied to make the clod more water-proof.
4. Weigh the coated clod and wire in air (***Wt_a***).
5. To determine the volume of the clod, weigh it while suspended in water with a balance that can accept the clod hanging on the balance beam by the thin copper wire (***Wt_w***). The drop in weight (***Wt_a – Wt_w***) is equal to the weight or volume of the water displaced, which is the volume of the clod.
6. If such a balance is not available, immerse the clod completely in a graduated cylinder half full with water, and detect the change in water volume in the cylinder (***V***).
7. On a separate sample, determine the moisture content of the soil sample and calculate the oven-dry weight of the soil (***Wt***) or break the clod and take a sample for the determination of soil moisture content.

Calculations

$$BD\ (g/cm^3) = \frac{Wt}{Wt_a - Wt_w}$$

$$BD\ (g/cm^3) = \frac{Wt}{V}$$

Where:

Wt = Weight of oven-dry solid particles in the clod (g)

Wt_a – Wt_w = Weight of the water displaced by the clod (g) or volume of the clod (cm^3)

V = Change in volume of water in the graduated cylinder (cm^3)

Technical Remarks

1. The clod method may yield higher BD values than other methods.
2. If such a wax is not available, a resin, such as Dow Saran F 310 can be use (general purpose non-crystalline copolymer). Dow Saran (F 310) is dissolved in acetone at a saran: solvent ratio of 1:7. **Acetone is flammable**; therefore, this mixture should be prepared in a fume hood and a tightly closed container to prevent volatilization.
3. The difference between the weight of the paraffin clod in air and its weight in water is the weight of water displaced by the clod, assuming the density of water is 1 g/cm^3. (The actual value is 0.9982 g/cm^3 at 20 °C). Hence, the weight of water displaced in grams is equal to the volume of the clod and paraffin in cm^3.
4. To obtain the volume of the clod alone, subtract the volume of the paraffin. The paraffin's volume is calculated from its weight and density which is 0.80 g/cm^3.
5. The BD is determined by dividing the oven-dry weight of the soil by its bulk volume.
6. Melted wax (paraffin wax) could be used as a replacement to the dissolved saran resin.

4.5.2.2. Core method

Apparatus

Metal ring
Cylinder
Wood and a hammer
Oven

Procedure

1. Remove any loose material from the surface, i.e., the O horizon, or any other material at the surface. When sampling subsurface horizons a large pit has to be dug to the required depth.
2. Make a flat and horizontal area a little larger than the cylinder (metal ring).
3. Carefully drive cylinder (known volume) into the soil with a block of wood and a hammer. Be careful to avoid compaction of the soil during collection of the cores. After careful removal of the soil core, examine it and trim the ends carefully.
4. Weigh the soil and cylinder ($\boldsymbol{Wt_{sc}}$).
5. Calculate the weight of the soil sample alone by subtracting the weight of the cylinder.
6. Take a portion of this soil for the determination of soil moisture and calculate the oven-dry weight of the soil in the sample.
7. Calculate BD in g/cm^3.

Calculation

$$\boldsymbol{BD\ (g/cm^3) = \frac{Wt_{sc}}{V}}$$

Where:

Wt_{sc} = Weight of oven-dry soil core (g)

V = Volume of soil core (cm^3)

Technical Remark

The BD value of undisturbed cores is practical significance as it indicates soil aggregation and structure under field conditions.

4.7. Particle Density

Soil particle density *(PD) is defined as the ratio of the mass (oven dry weight) of the soil particles to the particle volume expressed in grams per cubic centimeter (only solid no pore space). The volume of soil is determined by measuring the volume of water displaced by the particles. The magnitude of PD depends on the type of minerals in the particles, and the content of organic matter (OM) in the soil. The particle density of most soils varies from 2.60 to 2.75 g/cm^3. When the average PD is not known, a value of 2.65 g/cm^3 is generally assumed. Organic soils have a lower PD since OM has a density of 1.2 – 1.5 g/cm^3. However,* ***PD is used with BD to calculate soil porosity****. Two methods for PD determination are common. The first method uses a graduated cylinder and the second method a Pycnometer (volumetric flask) which is a specific gravity flask fitted with a ground glass stopper that is pierced lengthwise by a capillary opening. Both analyses are simple and rapid.*

4.7.1. Graduated Cylinder Method

Apparatus

Graduated cylinder	Oven
Stirring rod	Desiccators

Procedure

1. Weigh 40 g oven-dry solids (***Wt$_s$***) in a 100-mL graduated cylinder.
2. Add 50 mL water to the soil in the cylinder. Be sure that no soil material is on the inner walls of the cylinder.
3. Stir thoroughly with a stirring rod to displace the air, and rinse the stirring rod and the inner walls of the cylinder with 10 mL water.
4. Allow the mixture to stand for 5 minutes and record the volume of the soil plus 60 mL water.
5. On a separate sample, determine the moisture content of the soil sample and calculate the oven-dry weight of the soil.
6. Add the amount of moisture to the amount of added water to obtain the total amount of water used.
7. Calculate PD in g/cm^3.

Calculation

$$PD\ (g/cm^3) = \frac{Wt_s}{V_s}$$

Where:

Wt_s = Weight of oven-dry soil (g)

V_s = Volume of the solids (cm^3)

4.7.2. Pycnometer Method

Apparatus

Pycnometer
Hot plate
Oven
Desiccators

Procedure

1. Weigh a clean and dry Pycnometer flask including the stopper in air (Wt_P).
2. Weigh about 10 g air-dry soil (2-mm) into a weighted Pycnometer.

Note: The Pycnometer should be cleaned of any soil (the outside and neck) that might have spilled during transfer.

3. Weigh the Pycnometer, including stopper and soil sample (Wt_{P+S}).
4. On a separate sample, determine the gravimeter water content of the soil sample and calculate the weight of oven-dry soil from the duplicate sample (Θ_m).
5. Fill the Pycnometer about one-half full with DI water (DI water is boiled and cooled to room temperature), washing into the flask any soil adhering to the inside of the neck.
6. Remove entrapped air by gently boiling the water over a hot plate; with frequent gentle agitation of the contents to prevent soil loss by foaming (boil for several minutes till no air bubbles are visible).
7. Cool the Pycnometer and its contents to room temperature in a vacuum desiccator.
8. Add enough DI water at room temperature to fill the Pycnometer.
9. Insert the stopper and seat it carefully. Thoroughly dry and clean the outside of the flask with a dry cloth, using care to avoid drawing water out of the capillary.
10. Weigh the Pycnometer and its contents (Wt_{P+S+W}).
11. Remove the soil from the Pycnometer, and thoroughly wash it.
12. Fill the Pycnometer with DI water at room temperature, insert the stopper, and remove all excess water and weigh the Pycnometer including stopper and water (Wt_{P+W}).
13. Correct the weight of the soil sample to oven-dry.
14. Calculate PD in g/cm^3.

Calculations

$$Wt_s = \frac{Wt_{p+s} - Wt_p}{(\theta_m + 1)}$$
$$V_p = \frac{Wt_{p+w} - Wt_p}{\rho_w}$$
$$V_w = \frac{Wt_{p+s+w} - Wt_p - Wt_s}{\rho_w}$$
$$PD\ (g/cm^3) = \frac{Wt_s}{V_p - V_w}$$

Where:

PD = Particle density (g/cm^3)
Wt_s = Correct the weight of the soil sample to oven-dry (g)
Wt_{p+s} = Weigh the Pycnometer including stopper and soil sample (g)
Wt_{p+s+w} = Weight of the Pycnometer and its contents (g)
Wt_{p+w} = Weight of the Pycnometer including stopper and water (g)
Wt_p = Weight of the clean and dry Pycnometer flask including the stopper in air (g)
θ_m = Gravimetric water content
V_p = Volume of the Pycnometer (m^3)
V_w = Volume of the water occupying the Pycnometer together with the sample (m^3)
ρ_w = Density of water (g/cm^3)

4.7.3. Volumetric Flask Method

Apparatus

Volumetric flask

Oven

Desiccators

Procedure

1. Fill a pre-weighed volumetric flask to the mark with boiled DI water (cooled to room temperature).
2. Weigh the flask plus water (***Wt_1***), and then discard the water and dry the flask thoroughly.
3. Weigh accurately 50 g air-dry soil (2-mm) (***Wt***).
4. Transfer quantitatively to the volumetric flask, which should be cleaned of any soil that might have spilled during transfer.
5. Add 50 mL with boiled and cooled DI water into the flask, washing down any soil particles adhering to the neck.
6. Heat the flask gently on a hot plate until the water starts to boil in order to drive out air from the soil.
7. Cool the content and bring up to the 100-mL mark with DI water.
8. Weigh the volumetric flask with its content (***Wt_2***).
9. On a separate sample, determine the moisture content of the soil by the gravimetric method to find the oven-dry weight of the soil in the flask.
10. Calculate first the volume of soil particles and then PD.

Calculations

$$V\ (cm^3) = Wt + (Wt_1 - Wt_2)$$

$$PD\ (g/cm^3) = \frac{Wt}{V}$$

Where:

V = Volume of air-oven soil particles (cm^3)

Wt = Weight of air-dry soil (g)

Wt_1 = Weight of the flask plus water (g)

Wt_2 = Weight the volumetric flask with its content (g)

4.8. Total Pore Space and Porosity

The total pore space *(TPS) consists of the PS between adjacent sand, silt and clay particles and those between aggregates.* ***Percent pore space*** *(PS) is the ratio of the volume of voids in a soil to the total volume (bulk volume) of the soil times 100.* ***Texture and structure are the main factors governing the amount of PS in soil. The OM affects pore space indirectly by improving structure****. If the aggregation of a particular soil is increased, the TPS increases, and the weight per unit volume or BD of the soil decreases. A medium textured mineral soil that is in good structural condition for plant growth has a PS of about 50 %. This PS is important for gas exchange (O_2 and CO_2) between the soil and the atmosphere, and water storage and movement. The PS is filled with air and water. Depending on pore size, pore spaces are given the name macro-pores (large) or micro-pores (small).* ***There is no sharp line of demarcation between the two pore sizes****.*

Calculation

$$Pore\ Space\ (\%) = \frac{PD - BD}{PD} \times 100$$

Where:

PD = Particle density (g/cm^3)

BD= Bulk density (g/cm^3)

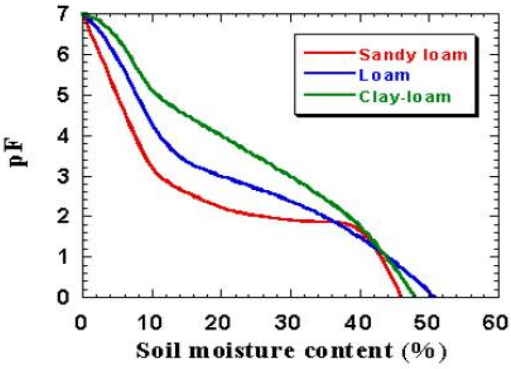
4.9. Soil Water Retention Curve (pF-curve)

Soils are equilibrated with water at various tensions and later the moisture content is determined. *The ability of soil to retain water depends on several factors, e.g., texture, OM content (due to its hydrophilic nature), nature of mineral colloids, arrangement of particles (soil structure) and its bulk density.* ***The relation between soil moisture tension and soil moisture content is called water retention curve***. *Usually the tension is expressed as the logarithmic value of cm water and the graph is referred to as a pF-curve. The pF-value is obtained from soil water pressure head according to the formula:*

$$\boldsymbol{PF} = \mathbf{log}_{\mathbf{10}}(-\mathbf{h})$$

Where, h is the soil water pressure head (cm). The soil water pressure head is zero when the soil is fully saturated (no air in the pores), ***the permanent wilting point*** *(PWP) is at approximately -15000 cm, i.e. pF = 4.2, and* ***the field capacity*** *(FC) ranges between -100 cm (pF = 2) to -500 cm (pF = 2.69) depending on the soil texture. Water can exist in soil under a tension varying from pF = 0 (no tension) to pF = 7.0 (high tension). A positive value of a tension in-situ, i.e., a pressure, indicates that water is ponded on top of the soil.*

The below figure displays the pF-curve of three soil with different soil texture



The FC is the point at which water is held in soil after excess water has drained out driven by gravity. The PWP is the point at which water is held in soil at a force that cannot be removed by common crops. Water in air-dry soil is held with a tension of pF 6.0 to 7.0.

The available water capacity *(AWC) or the plant-avaialbe water (PAW) is the amount of water retain in the soil that can be removed by plants. It is estimated as the difference in the soil water content between FC and PWP.*

$$\boldsymbol{AWC}\ (\%) = \boldsymbol{FC}\ (\%) + \boldsymbol{PWP}\ (\%)$$

***Soil moisture characteristic are used to determine**: 1) the water that can be readily absorbed by plant roots (for irrigation purposes), 2) the effective pore space and porosity, 3) specific yield (for drainage design), and 4) indicate changes in the structure of a soil (caused by tillage, mixing of soil layers). Sometimes the relationship between soil moisture tension and other soil physical properties, e.g., capillary conductivity, thermal conductivity, clay and OM content is subject of research.*

4.9.1. Ceramic Plate Method (0.1 to 15 bar)

***The ceramic plate equipment** is suitable for determination of pF-curves in the pF range of 2.0-4.2 (0.1-15 bar of suction). Soil moisture is removed from the soil samples by raising air pressure in an extractor. A porous ceramic plate serves as a hydraulic link for water to move from the soil to the exterior of the extractor. The high-pressure air will not flow through the pores in the plate since the pores are filled with water. The smaller the pore size, the higher the pressure that can be exerted before air will pass through.*

Low range: moisture at 0-100KPa (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. Soak the pressure plates thoroughly in DI water overnight prior to use.
2. Place pressure plates on a workbench.
3. Place labeled rubber rings in order the plate (each plate accommodates 12 samples).
4. Fill ring with air-dry soil (2-mm) using a spatula (about 20 g sample). In order to avoid particle-size segregation, place entire soil sample into the ring, and then level, but don't pack.
5. Add water between the rings until there is an excess of water (at least 3-mm deep) on the plate.
6. Cover plate and samples with plastic sheet.
7. Allow samples to stand overnight.
8. The next morning, remove excess water from the plate with a certain tool (e.g., syringe, disposable pipette, or siphon).
9. Place the triangular support in the extractor vessel on the bottom.
10. Install plate with samples in the lower-most position in the extractor. Then install the middle and top plates (the plastic disks should be placed between plates).
11. Connect outflow tubes.
12. 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.
13. Place a beaker to collect water from the outflow tubes.
14. Leave overnight.

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 0C for 24 hours.
20. Place samples in a desiccator, cool, and weigh.

Calculation

$$\boldsymbol{Moisture\ (\theta)\%} = \frac{\boldsymbol{Wet\ soil\ (g) - Dry\ soil\ (g)}}{\boldsymbol{Dry\ soil\ (g)}} \times \mathbf{100}$$

High range: moisture at 100-1500KPa (0-15 bar) pressure

Apparatus

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

Procedure

1. Use 15-bar ceramic plates and follow Steps 1-14 of the previous method, applying 1-15 bar pressure (100-1500 KPa).
2. 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). Stop the water outflow tubes from extractor which indicate the equilibrium has been attained.
3. Release pressure from extractor (remove outflow tubes from water before turning instrument off).
4. Follow Steps 17–20 of the previous method.

Calculation

$$\boldsymbol{Moisture\ (\theta)\%} = \frac{\boldsymbol{Wet\ soil\ (g) - Dry\ soil\ (g)}}{\boldsymbol{Dry\ soil\ (g)}} \times \mathbf{100}$$

Technical Remarks

1 ***Checking the pressure plate***: Install the pressure plate to be used in a pressure cooker. Fill with water, fasten the lid on the cooker, and measure the rate of outflow of water from the ceramic plates at a pressure of 15 bar, the rate should be about 1 mL per cm^2/h/atm. Pressure difference or greater for satisfactory operation of the porous plates.

2 ***Checking the pressure of the ceramic plate***: push a free air pressure inside the ceramic pressure plate and then close the outflow valve by the finger, then submerge the ceramic pressure plate in a tank filled with water. If there is air leaks at the ceramic pressure plate gasket or attendant connections, the ceramic pressure plate should be replaced.

3 If the outlets of the plates (cooker) continue to bubble after a few hours of applied pressure, the plates are probably defective and should be replaced (air leaks cause troubles with air-pressure control and serious errors in retentively determination through direct loss of water vapor from the soil sample).

4 Pressure should not be allowed to fluctuate during a run. It should be checked after every 2-3 h (adjusted if necessary). If the pressure fluctuations are within the specified tolerance of the regulator, no adjusting is needed.

5 During an experimental run, equilibrium is reached when water flow from the outflow tube ceases. At equilibrium, there is an exact relationship between the air pressure in the extractor and the soil suction (and hence the moisture content) in the samples. Accuracy of equilibrium values will be no more accurate than the regulation of air supply; therefore the pressure control panel has independent double regulators.

6 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.

7 Never remove extractor lid with pressure in the container.

8 The FC is commonly estimated by measuring the moisture retained at the following pressure:

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

9 Pascal is the standard unit for the expression of soil suction. By definition, 100 kPa is equal to 1 bar or 0.987 atmospheres or 14.5 psi. The latter units should not be used anymore.

10 The various pressure plate cells are not suitable for extracting solution from soils for chemical analysis. The immense surface are within the porous ceramic plate can cause disturbance and contamination of the soil solution.

11 Pressure plates are washed after use and dried with a coating of sand over the ceramic. This prevents fine air-borne particles from lodging in the pores of the ceramic. Then dry the sand can be brushed off and the plate stored until required.

4.9.2. Sandbox Method

Sandbox *can be used to apply suction from pF 0 (saturation) to pF 2.0 (-100 hPa). Sand is used to convey the suction from the drainage system to the soil samples. As the surface of the sand is flexible, the contact between the sample and the sand can be established more easily, which makes sand a better suction material than a stiff porous plate.*

Results of measurements taken with this sandbox correspond with points on the drying curves of the relevant samples; associated with decreasing pressure. These pressure values are usually standard water potential increments. The wetting curve, on the other hand, is determined by graphing the water content against increasing pressure values. This curve is not identical to the drying curve, because the water content does not respond instantaneously to changes in pressure. This phenomenon is called Hysteresis.

Apparatus

Assembling the sandbox

Soil sample ring

Procedure

A. Field processing

1. Collect the soil sample by pressing a metal ring on the bulk soil sample (better when moist) until it levels with the surface. If the sampled soil volume is larger than the volume of the core ring, carefully remove excess soil by 'chipping' it off with a sharp edged tool. Prevent smearing the sample surface so as not to affect the physical properties of the soil. Avoid excessive compaction or fragmentation of soil.
2. Cap the core sample ring from both sides.
3. Record all the information about samples (depth and profile), put one label inside the bag and tie the other label to the bag. Then bring the soil samples to the soil preparation laboratory.

B. Laboratory processing

1. Each core ring is given a laboratory number.
2. Uncap the core soil sample ring, and then fix a piece of nylon cloth to the bottom side (sharp edged) of the sample with an elastic-band, or an O-ring.
3. Ensure that a 0.5 cm layer of water is covering the surface of the sand in the sandbox.
4. Place the core soil sample with the bottom side down in a homogeneous water saturated fine sand surface. Let the sample adapt for 1 hour. The sand surface usually is covered with the same type of cloth that is used for the samples.
5. To saturate the sample, slowly raise the water level to 1-cm below the top of the sample ring.
6. Place a lid on the basin (to prevent evaporation) and allow the sample to saturate for 2 days (sandy soils) or up to 1 week (clayey soils).

7. Mark the rings, and draw a sketch of the box, so that the rings can be replaced in exactly the sample place after removal.
8. Take the ring carefully out of the water basin and wipe off any water drops hanging underneath the sample before weighing it. Weigh the core soil sample with an accuracy of 0.01 g. This weight (including ring, cloth and elastic) is used to calculate water content at saturation, pF 0 (***weight A***).

Notes

- It is difficult to transfer the saturated sample to a balance without changing water content, especially with sandy samples.
- The middle of the soil sample is used as the reference level for zero pressure, but the free water level (h = 0) is in fact 1 cm below the top of the sample ring. The moisture tension thus ranges from +1 cm at the bottom of the sample, to - 4 cm at the top of the sample. Note that at lower pressures this difference due to sample size becomes less important.

9. Put the sample back to its previous positions in the sandbox. Press the ring slightly, to improve soil-sand contact.
10. Apply a suction of -2.5 cm (=-2.5 hPa, = 0.25 kPa) to the centre of the samples.
11. Leave the sample to equilibrate, (with the lid on the box to stop evaporation). This will take a few days for sandy soil, and up to a week for clayey soils.
12. Gently remove the samples and weigh them.
13. To check equilibrium, place the sample back into the sandbox (take care that the contact between sand and sample is restored) and weigh the sample again the next day. In case of equilibrium with the created tension, the difference in water content will not exceed 0.002 in volume fraction.
14. If equilibrium between soil moisture content and pressure has been established, record the weight of the sample.
15. Moisten the sand surface with a wet sponge. Do not remove the filter cloth; just clean and smoothen it at the same time to remove air bubbles and impressions.
16. Place the soil samples on the sand at exactly the same position as they were previously - use the sketch made earlier.
17. Apply the next suction level and repeat the above steps.

Calculation

$$\boldsymbol{Moisture}\ (\%) = \frac{\boldsymbol{Wt - Wt_1}}{\boldsymbol{Wt_1 - Wt_2}} \times \mathbf{100}$$

Where:

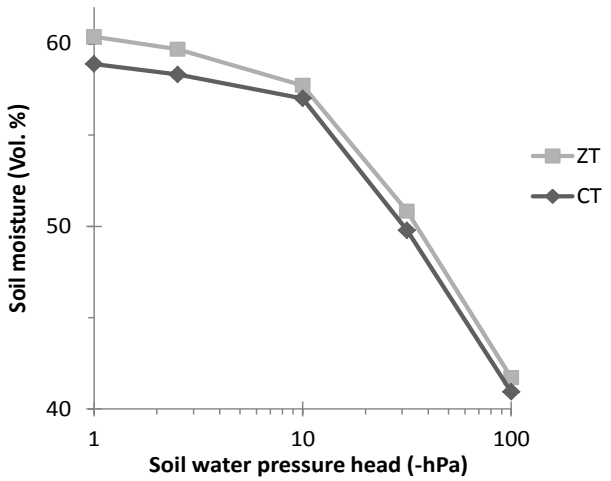
Wt = Weight of the water content at the various pF values (g)

Wt_1 = Weight of the ring (with sample, cloth and elastic) (g)

Wt_2 = Weight of the ring, cloth and elastic (g)

As usually the volume of the soil is known – it is equal to the volume of the metal ring used – the above calculation can be adapted to calculate volumetric moisture content (g water/cm^3 soil).

The below figure shows provides an example of a soil water retention curve obtain with the sandbox method (ZT = zero-tillage, CT = conventional tillage; soil is a heavy clay).



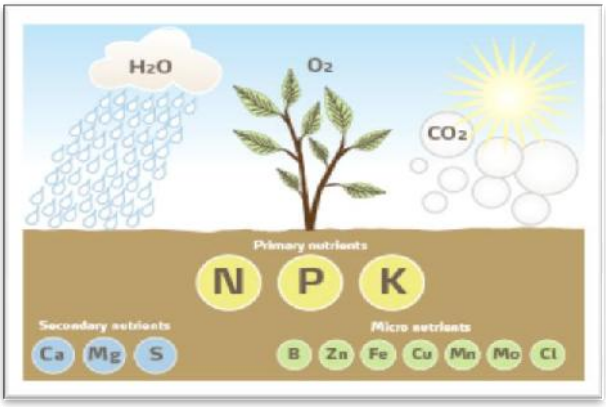
Technical Remarks

1. Because of the major influences of both pore size distribution and soil structure on moisture retention, especially at the high matrix potentials, care should be taken not to disturb the original setting of the soil and to completely fill the ring, when pressing the core rings into the soil.
2. Sampling conditions are best when the soil is approximately at field capacity.
3. Do not freeze the samples because soil structure will be influenced.
4. The Laboratory should have a constant temperature between measurements, since temperature changes affect water viscosity and therefore water retention values.
5. If the soil volume is less than the volume of the ring, or if the sample has been damaged during transport, the sample should not be used for analysis. Also samples with large projecting stones may have to be discarded.
6. Fast raising of the water levels in the sandbox (step 5 above) will entrap air and may damage soil structure.
7. Vibration may cause a leak between the sidewalls of the box and the sand.
8. Add (0.01 mg/l) copper sulfate to the water used in the sandbox to eliminate microbiological activity.

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., carbon (C), hydrogen (H), and oxygen (O);*
2. ***Primary macronutrients**, i.e., nitrogen (N), phosphorus (P), potassium (K);*
3. ***Secondary macronutrients**, i.e., calcium (Ca), magnesium (Mg), sulfur (S); and*
4. ***Micronutrients**, i.e., boron (B), chloride (Cl), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), zinc (Zn).*



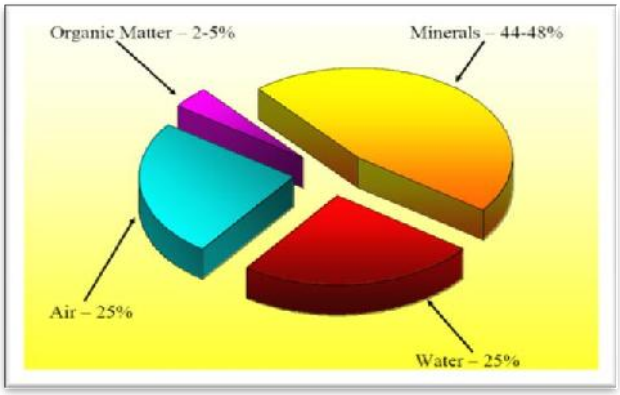
Primary, secondary and micronutrients

***Three major factors contributing to plant nutrition are**:*

- The amount of available nutrients in the soil;
- The soil's ability to supply the nutrients to plants; and
- Environmental factors that affect nutrient availability and plant 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; Sparks et al., 1996). **We have endeavored to select procedures, which in our experience are appropriate for soils of the Mediterranean 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.*



General composition of soil

*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 ($CaCO_3$), organic matter (OM), cation exchange capacity (CEC), and gypsum ($CaSO_4.2H_2O$). With regard to N, the dominant fertility factor in 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 **for interpreting the data produced** with the **analytical procedures** listed (see Appendix 9).*

5.1. 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 B toxicity tolerance by various plant species have been also developed for such an extract (see Appendix 15).Thus; a saturation extract is routinely used where salinity is a concern. The cations analyzed in saturation extracts are Ca, Mg, K, and Na, while the anions are* SO_4 *(sulfate),* CO_3 *(carbonate),* HCO_3 *(bicarbonate) and Cl (chloride). Boron (B) 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 soil paste:

- Does not have free-standing water on the surface of the paste.
- Slides freely and cleanly off a spatula (**does not apply to clay soils, > 40% clay**).
- Flows slightly when the container is tipped to a 45 ^{o} from horizontal.
- Soil surface glistens as it reflects light.
- Consolidates easily by tapping after a trench is formed in the paste with the flat side of a spatula (**does not apply to sandy soils, >70% sand**).

3. Allow the paste to stand for 1 hour, and 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.

Technical Remarks

1. Do not stir soil until the entire soil mass is wet.
2. After first wetting, the paste usually stiffens and loses its shine on standing. Adding water and re-mixing gives a mixture that retains the characteristic of a saturated paste.
3. **Gypsum soil samples** should not be oven-dried above 70 ^{o}C, prior to extracting for soluble salts, because heating to 105 °C partially converts gypsum ($CaSO_4.2H_2O$) to partially hydrated gypsum ($CaSO_4.½H_2O$), which is more soluble in water than the fully hydrated gypsum.

4. **For organic and peat soils** (>16 % OM) it is advisable to start with a 150 mL water and slowly add soil material and water. Soak overnight wetting to obtain a definite end point for the saturated paste. Some high organic matter soils swell considerably upon addition of water. In such cases, the method must be repeated until the saturated paste characteristics are stable.
5. **Fine textured soils** (> 40% clay) may puddle easily. To minimize puddle and obtain more definite endpoint for the saturated paste, water should be added with a minimum amount of stirring, especially in the early stages of wetting. The method must be repeated until the saturated paste characteristics are stable.
6. **For some soil types** (coarse textured soils, sandy loam and loamy sand with less than 15% clay), may not exhibit saturated paste characteristics of finer textured soils. Therefore, the relative accuracy of the method declines and should be noted when making soil comparisons.
7. **For salinity appraisal**, the saturated paste can be extracted after 4 hours.
8. **For sodic soil samples**, it should stand 16 or more hours.
9. For the soluble B, it should stand 24 hours or more (saturated paste equilibration is required).
10. For $CaCO_3$ if precipitates are noted in the extract, dilute paste extract 1:1 with DI water and note dilution in subsequent analysis. The paste extract may be refrigerated (4 ^{o}C) for storage (do not allow to freeze) more than 30 days by adding small quantities (2-mL) of toluene to minimize microbial activity.
11. Approximately one quarter to one third of the water added in making the saturated paste can be recovered as extract.
12. Because KCl from the pH electrode can contaminate the paste, it is recommended that a separate paste should be made for EC determination. If, however, there are not enough samples for two separate pastes, then the same paste can be used for both analyses; ensure that the pH electrode is not left in the paste unnecessarily.

5.1.1. Saturated Paste Percentage

Soil moisture, as determined on a separate sample, is taken into consideration by adding it to the amount of water used in preparing the saturation paste. Report the ***saturation percentage*** *(SP) to the nearest 0.1 %. The SP is calculated by dividing the total amount of water added (mL) by the oven-dry weight of the soil (g) and multiplying by 100.*

Procedure

1. Weigh a 30-50 g sub-sample of the paste into a dried and weighed metal can with lid.
2. Dry in an oven, with the lid unfitted, at 105 °C for 4 hours.
3. Remove from oven, fit the lid, and cool in a desiccator for at least 30 minutes.
4. Re-weigh and calculate SP.

Note: Oven-dry moisture values will be slightly higher than the direct method as air-dry soil will retain 3-5 % moisture, dependent on clay and salt content.

Calculations

$$\boldsymbol{Weight\ of\ oven-dry\ soil} = \frac{\boldsymbol{weight\ of\ air-dry\ soil}}{(\boldsymbol{100+air-dry\ soil\ mositure\ percentage})} \times \boldsymbol{100}$$

$$\boldsymbol{Total\ water} = (\boldsymbol{water\ added}) + (\boldsymbol{water\ in\ air-dry\ soil})$$

Or

$$\boldsymbol{Total\ water} = (\boldsymbol{weight\ of\ water\ added}) + (\boldsymbol{weight\ of\ air-dry\ soil}) - (\boldsymbol{weight\ of\ oven-dry\ soil})$$

$$\boldsymbol{SP}\ (\%) = \frac{\boldsymbol{total\ weight\ of\ water}}{\boldsymbol{weight\ of\ oven-dry\ soil}} \times \boldsymbol{100}$$

Technical Remarks

1. The SP is directly related to field moisture range (e.g., PWP is about one fourth of SP, and FC is about one half of SP).
2. Oven-dry moisture values will be slightly higher than the direct method as air-dry soil will retain 3-5 % moisture, dependent on clay and salt content.
3. **For mineral soils**, about twice of the FC is generally the amount of water required to obtain SP.
4. The SP is related to soil texture (based on OM contents less than 3%), as follows: sand or loamy sand (<20); sandy loam (20-35); loam or silt loam (35-50); clay loam (50-65); clay (65-135); and organic soils (> 81).
5. **For fine-textured soils and those high in Na** (SAR > 10), the SP cannot be used to estimate FC and PWP values.

5.2. pH

Soil pH *is a crucial soil indicator, and 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)**

The 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, and CEC in soils whose colloids (clay/humus) are pH-dependent and biological activity. At high pH values, availability of P, and most micronutrients, except B and Mo, tends to decrease (see Box No. 2 for more details).

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* $CaCO_3$*, and will visibly effervesce (fizz) when 10% hydrochloric acid (HCl) is added dropwise to the soil. Most soils in the WANA region have pH values of 8.0 – 8.5. Calcareous soils with gypsum have somewhat lower pH values, while those with excess Na have values over 8.5 (sodic soils).*

Apparatus

pH meter with combined electrode

Reference electrode, saturated KCl

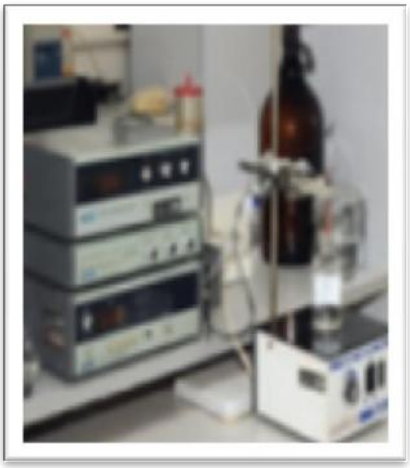
Measuring cylinder

Glass rod

Glass beaker

Interval timer

Wash bottle, plastic



Reagents

De-ionized water

pH 7.0 buffer solution

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 Calibrate the pH meter (see Box No. 3 for more details).

7 Put the combined electrode in suspension (about 3-cm deep). Take the reading after 30 seconds with one decimal.

8 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.

Technical Remakes

1. Make sure that the combined electrode contains saturated KCl solution and some solid KCl.
2. 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* $CaCl_2$.
3. The pH measured in 0.01 *M* $CaCl_2$ is about 0.5 unit lower than that measured in water (soil: liquid ratio of 1:2).
4. For soil samples very high in organic matter, use a 1:2 or 1:5 (soil: water) ratio.
5. 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.
6. Air-dry soils may be stored several months in closed containers without affecting the pH measurement.
7. Air-drying can cause changes in pH values (e.g., through oxidation of sulfides). The determination of pH of field moist samples presents two limitations: taking a representative samples is difficult; and biological activity can affect pH during storage of soils in their natural state of moisture. Air-drying prevents development of acidity during moist storage.
8. Presence of clay may slow the electrode response. To avoid this, thoroughly clean electrode between samples. If electrode response is slow, clean by immersing in weak HCl solution overnight.
9. Suspended colloids influence pH through the junction potential effect. In the presence of negatively charged colloids (e.g., clay particles or organic matter), pH measured in the suspension will usually be lower than measurement in the supernatant liquid. This is the suspension effect. This effect is extremely pronounced in peat soils because there is often little supernatant and soil sediment. In every sample, therefore, place the electrode junction at the same distance above the surface of the soil to maintain uniformity in pH reading. The CO_3 status of some soils changes with time; therefore, undue delay in taking a reading after introducing the electrode should be avoided. The initial pH of a non-alkaline soil may be as much as 0.5 pH unit greater than the reading taken after the sample has stood for 30 minutes or longer.

5.3. 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 extract saturated paste. However, soil: solution ratios of a 1:1 or wider are more convenient where the quantity of soil 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 (total dissolved solids, TDS).*

Salinity *is an important laboratory measurement since it reflects the extent to which the soil is suitable for growing crops. However, salinity affects plants at all stages of development and for some crops sensitivity varies from one growth stage to another. While salinity is largely a concern in irrigated areas and in areas with saline soils, it is often less important in rainfed agriculture. However, with increasing use of irrigation, there will be greater emphasis on EC measurement in the future. The basic methodology and principles of EC measurement is given in USDA Handbook 60 (Richards, 1954).*

On the basis of a saturation extract, values of 0 to 2 dS/m 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 reasonably well above that level (see Appendix 14).

Apparatus

Conductivity bridge
Vacuum filtration system

Reagent

Potassium Chloride Solution (KCl), 0.01 *N*

- Dry a small quantity of reagent-grade *KCl* in an oven at 60 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 0.7456 g *KCl* in DI water and transfer to 1-L flask, mix well, and bring to volume. This solution gives an electrical conductivity of 1.413 dS/m at 25 °C.

Procedure

1. Weigh 50 g air-dry soil (< 2-mm) into a 100-mL glass beaker, as for pH determination.
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. 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.
7. Start the vacuum pump.
8. Open the suction, and add suspension to Buchner funnel.
9. Continue filtration until the soil on the Buchner funnel starts cracking. If the filtrate is not clear, repeat the procedure.
10. Calibrate the conductivity meter according to maker's instructions (see Box No. 4 for more details).
11. Transfer the clear filtrate into a 50-mL bottle, immerse the conductivity cell in the solution, and take the reading.
12. Remove the conductivity cell from the solution, rinse thoroughly with DI water, and dry excess water with a tissue.

Technical Remarks

1. Readings are recorded in **deci-Siemens per meter** (dS/m).
2. Exposure of the saturated soil sample or extracting solution to the atmosphere may cause changes in conductivity due to loss or gain of dissolved gasses: CO_2 and NH_3-N. Freshly distilled water has a conductivity of 0.005 - 0.002 dS/m increasing after a few weeks to 0.002 -0.004 dS/m. This is a special concern on samples with very low ECe.
3. The EC should be measured as soon as the extracts are prepared because of possible changes in ionic content due to microbial activity during storage at room temperature. If needed, however, extracts can be stored for a week under refrigeration (4 °C) before measuring EC.

5.4. 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 arid/ semi-arid regions may be mostly of $CaCO_3$. As with alkaline pH, the soil retention of P, Mn, Zn and Cu is directly related to carbonate content and to the distribution of total and active $CaCO_3$ between the clay and silt fractions. Consequently, $CaCO_3$ equivalent is normally determined in most laboratories. 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.*

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 Fe chlorosis. Some methods of $CaCO_3$ 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.*

5.4.1. Total Calcium Carbonate

Carbonate in the sample is dissolved in the excess of hydrochloric acid (HCl). The remainder of the acid is titrated against sodium hydroxide (NaOH). However, 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 NaOH solution. In the titration 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	Erlenmeyer flask
Burette	Volumetric pipette

Reagents

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

Dilute 82.8 mL *concentrated HCl* (37%, sp. gr. 1.19) in DI water, mix well, let it cool, and bring to 1-L volume. Standardize with 1 *N* Na_2CO_3 solution, and determine the exact normality of the HCl solution.

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

Dissolve 40 g *NaOH* in DI water, and transfer to a 1-L flask, let it cool, and bring to volume. Standardize with 1 *N HCl* solution, and determine the exact normality of the *NaOH* solution.

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 [$4\text{-}NaOSO_2C_6H_4N\text{: }NC_6H_4\ /\text{-}4\text{-}N\ (CH_3)_2$]

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

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

Dissolve 53 g *anhydrous* Na_2CO_3 in DI water, and bring to 1-L volume. Do not keep longer than one week.

F. Ethanol (C_2H_5OH), 95%

Procedure

1. Weigh 1 g air-dry soil (0.15-mm) into a 250-mL Erlenmeyer flask.
2. Add 10 mL **1 *N* HCl** 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* NaOH** solution while swirling the flask. Continue the titration until a faint pink color develops, and take the reading, ***R***.
6. In order to standardize the hydrochloric acid (1*N*) and sodium hydroxide (1*N*) solutions used in the determination of $CaCO_3$:

- Pipette 10 mL **1 *N* Na_2CO_3** solution into a 100-mL Erlenmeyer flask, add 2 drops *methyl-orange* indicator, and titrate this solution against **1 *N* HCl** (in the burette). The solution color changes from light to dark orange. The **HCl** normality is:

$$N_{HCl} = \frac{10 \times N_{Na_2CO_3}}{V_{HCl}}$$

- Pipette 10 mL standardized **1 *N* HCl** solution into a 100-mL Erlenmeyer flask, add 2 drops *phenolphthalein* indicator, and titrate against **1 *N* *NaOH*** solution. The solution color changes from colorless to pink. The **NaOH** normality is:

$$N_{NaOH} = \frac{10 \times N_{HCl}}{V_{NaOH}}$$

Where:

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

Calculation

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

Where:

Wt = Weight of air-dry soil (g)

V = Volume of NaOH solution used (mL)

0.05 is equivalent weight of $CaCO_3$ (50/1000)

Technical Remarks

1. Some experience is needed 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 $CaCO_3$**. If a soil contains 50% $CaCO_3$ or more, 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 over-estimate the actual soil $CaCO_3$ content.
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 can be calibrated against the **Calcimeter method**.
5. The retention or precipitation of P, Mn, Zn and Cu is often related to the total amount of carbonates.

Active Calcium Carbonate

Measurement is based on reaction with excess ammonium oxalate followed by titration with permanganate in an acid medium. Most of the reversibly adsorbed Ca (on clay minerals) is released and precipitated as calcium oxalate. Part of the $CaCO_3$ in soil is dissolved and the Ca precipitated as the oxalate. The soluble "active" $CaCO_3$ is assumed to include the smaller particles (less than 2-mm) and some amorphous forms of $CaCO_3$. The reaction of ammonium oxalate with $CaCO_3$ can be written as follows:

$$CaCO_3 + 2\ NH_4 + C_2O_4 \rightarrow CaC_20_4\ (s) + 2NH_3 + H_20 + CO_2$$

*The oxalate also reacts with dissolved and exchangeable Ca to form oxalate; therefore, the procedure should more appropriately be called an **"active" Ca procedure** rather than an **"active" CO_3 procedure**. The reaction of un-reacted ammonium oxalate (following acidification with H_2SO_4) is summarized by the following equation:*

$$2MnO_4 + 5\ H_2C_20_4 + 6\ H \rightarrow 2\ Mn + 10\ CO_2 + 8\ H_20$$

After equilibration the suspension is filtered and the remaining oxalate is titrated with permanganate in an acid medium. Once all the oxalate is oxidized, the solution turns pink, because of the permanganate.

Apparatus

Hot plate	Erlenmeyer flask
Burette	Volumetric pipette

Reagents

A. Ammonium Oxalate Solution [$(NH_4)_2C_2O_4$], 0.2 *N*

Dissolve 12.61 g *ammonium oxalate* in DI water, and bring to 1- L volume.

B. Potassium Permanganate Solution ($KMnO_4$), 0.02 *N*

- Dissolve 3.16 g of $KMnO_4$ in about 600-800 mL DI water (DI water is boiled and cooled).
- Keep the solution at a gentle boil for about 1 hour, cover, and let stand overnight.
- Filter and bring to 1- L volume, store the solution in amber glass bottle.

C. Oxalic Acid ($H_2C_2O_4$), 0.2 *N*

Dissolve 6.3 g $H_2C_2O_4$ in 500 mL DI water.

D. Sodium Oxalate (NaC_20_4), 0.2 *N*

- Dry about 8 g of NaC_20_4 at 110 °C for at least 1 hour. Cool in desiccators.
- Dissolve 6.7 g dried NaC_20_4 in DI water, and bring to 500-mL volume.

E. Sulfuric Acid (H_2S0_4), concentrated (98%, sp.gr. 1.84)

Procedure

1. Weigh 1 g air-dried soil (0.15 mm) in a 500-mL beaker.
2. Add 100 mL **0.2 *N* ammonium oxalate** solution, using a volumetric flask.
3. Shake on a mechanical (orbital) shaker for 2 hours at 250-300 revolution per minute (rpm).
4. Filter the suspension using suction (discard the first few mL).
5. Pipette 10 mL clear filtrate into a 500-mL Erlenmeyer flask, and add about 75 mL **DI water** and 5 mL **concentrated H_2SO_4** with a dispenser.
6. Heat the mixture to 60-70 ^oC on a hot plate.
7. Titrate the hot solution with **0.04 *N* potassium permanganate** solutions, until the pink color becomes stable for at least 1 minute (V_{sample}).
8. Prepare a blank in the same manner using 10 mL **0.2 *N* ammonium oxalate** solution, and record the volume of the titration used (V_{blank}).
9. In order to standardize the potassium permanganate solution used in the determination of active $CaCO_3$:
   - Pipette 20 mL **0.2 *N* oxalic acid** in 500-mL Erlenmeyer flask, add about 75 mL **DI water** and 5 mL **concentrated H_2SO_4** Heat to 70 ^oC on hot plate, and titrate with **potassium permanganate** solution.
   - Pipette 20 mL **0.2 *N* ammonium oxalate** solution in 250-mL Erlenmeyer flask, add about 75 mL DI water and 5 mL **concentrated H_2SO_4**. Heat to 70 ^oC on hot plate, and titrate with standardized **potassium permanganate** solution.

Calculation

$$\boldsymbol{Active\ CaCO_3}\ (\%) = (V_{blank} - V_{sample}) \times N \times \frac{V_1}{V_2} \times \frac{100}{Wt} \times \frac{5}{1000}$$

Where:

V_{blank}= Volume of $(NH_4)_2C_2O_4$ solution required to titrate the blank (mL)

V_{sample} = Volume of $(NH_4)_2C_2O_4$ solution required to titrate the sample (mL)

N = Normality of $KMnO_4$ solution

V_1 = Total volume of sample (mL)

V_2 = Volume of soil extract used for measurement (mL)

Wt = Weight of air-dry soil (g)

0.1 meq MnO_4 reacts with 5 mg $CaCO_3$

Technical Remarks

1. This method should not be applied to soils that contain gypsum ($CaSO_4.2H_2O$), or that are high in organic matter (OM).
2. The potassium permanganate can be standardized by titrating with the primary standard sodium oxalate ($Na_2C_2O_4$), which is dissolved in H_2SO_4 to form oxalic acid:

$$\mathbf{5H_2C_2O_4 + 2MnO_4 + 6H \rightarrow 10CO_2 + 2Mn + 8H_2O}$$

3. The solution must be heated to 80 – 90 ^{o}C for rapid reaction. The reaction is catalyzed by Mn produced and it goes very slowly at first until some Mn is formed. The first persistent pink color (30 seconds) should be taken as the end point. Determine a blank by titrating an equal volume of 1 *M* H_2SO_4
4. Precautions should be taken to ensure uniformity of sample handling and shaking procedures. Major sources of error include solution and exchangeable Ca and the inability of oxalate to react with Mg in Mg-substituted calcite.
5. The maximum quantity of "active" $CaCO_3$ that can be dissolved by the above procedure is 200 g/ kg (20%) soil. If "active" $CaCO_3$ contents greater than about 150 g/kg (17%) are obtained, then the analysis should be repeated with a smaller amount of soil and/or a larger volume of ammonium oxalate.

5.5. Organic Matter

Soil organic matter *(SOM) 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.* ***Soil Organic Carbon (SOC) 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 often than 3 – 4 % SOM, while soils of semi-arid rainfed areas, such as in the WANA region, have normally less than 1.5 % SOM.*

Most laboratories in the region perform analysis for SOM, which can be measured by. either the loss after ignition method, i.e., weight change destruction of organic compounds by H_2O_2 treatment or by ***ignition*** *at high temperature, or by* ***wet combustion analysis*** *of soils by chromic acid digestion , which is the standard method for determining total C. Also, organic matter/organic carbon can be estimated by volumetric and colorimetric methods. However, 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. This method is referred to as the* ***Walkley-Black method*** *(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 1.33 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 $K_2Cr_2O_7$ 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 $K_2Cr_2O_7$ in DI water, and bring to 1-L volume.

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 flask, add 5 mL *concentrated H_2SO_4*, mix well, and bring to volume.

E. Diphenylamine Indicator $(C_6H_5)_2NH$

Dissolve 1 g *diphenylamine* indicator in 100 mL *concentrated H_2SO_4*.

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 H_2SO_4** 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 H_3PO_4** 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

$$M = \frac{10}{V_{blank}}$$

$$Oxidizable\ Organic\ Carbon\ (\%) = \frac{[V_{blank} - V_{sample}] \times 0.3 \times M}{Wt}$$

$$Total\ Organic\ Carbon\ (\%) = 1.334 \times Oxidizable\ Organic\ Carbon\ (\%)$$

$$Organic\ Matter\ (\%) = 1.724 \times Total\ Organic\ Carbon (\%)$$

Where:

M = Molarity of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution (about 0.5 *M*)

V_{blank} = Volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution required to titrate the blank (mL)

V_{sample} = Volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ 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.

Technical Remarks

1. **The conversion factor for organic carbon to total organic matter** for surface soils varies from 1.7 to 2.0. In the soils of arid and semi-arid regions; and a value of 1.724 (=1/0.58) is commonly used. The factors 1.334 and 1.724 used to calculate *TOC* and *OM* are approximate, and may vary with soil depth and between soils.
2. **For soils high in OM** (1 % oxidizable OM or more), more than 10 mL potassium dichromate is needed.
3. Soils containing large quantities of chloride (Cl), manganese (Mn) and ferrous (Fe) ions give higher results. The Cl 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 % of sample weight causes no interferences.
5. **The Walkley-Black method for the determination of SOC** in soils gives about 89% recovery of carbon as compared to the dry combustion method. The conversion factor 0.336 was obtained by dividing 0.003, the milli-equivalent weight of carbon, by 89% and multiplying by 100 to convert to percentage. Chloride interference is eliminated by the addition of the silver sulfate to the digesting acid as indicated. The presence of nitrates and carbonates up to 5 % and 50 %, respectively, do not interfere.
6. **The concentration of H_2SO_4 should be about 6*M***. For this reason only 30 mL water are added. (10 mL $K_2Cr_2O_7$ solution plus 20 mL concentrated H_2SO_4 plus 30 mL H_20 give about 6 *M* H_2SO_4).
7. Air-dried soils seldom contain sufficient amounts of Fe (II) to cause interference. Water-logged soils often contain large quantities of Fe (II), but in most cases this can be oxidized by drying the soil samples prior to analysis.
8. Chloride is oxidized to chromyl chloride, which is volatilized, resulting in high OM values. If high amounts of Cl are present in the sample, add 15 g Ag_2SO_4 to 1-L H_2SO_4.
9. **Sulfuric acid readily absorbs water**. Therefore, use a fresh reagent.
10. **Elemental C** (e.g., charcoal) is not attacked by dichromate solution in this method.
11. Grinding of the samples is required only to reduce sub-sampling error. It is generally not necessary to pass the ground sample through a sieve (if required use a non-ferrous sieve).

5.6. Particulates Organic Matter

The Particulate Organic Matter *(POM) fraction is a relatively labile, sand-sized fraction. It acts as a micro-aggregate binding agent, and is a sensitive indicator for management practice.*

Apparatus

Mechanical shaker, reciprocating

Sieve (53 µm)

Oven

Balance, accurate to 0.001 g

Standard laboratory glassware

Reagent

Sodium hexametaphosphate $(NaPO_3)_{13}$, 5 g/L

Dissolve 5 g $(NaPO_3)_{13}$ in DI water, mix well, and bring to 1-L volume.

Procedure

1. Weigh 10 g air-dry soil (2-mm) into a pre-weighed and dried beaker.
2. Add 30 mL $(\mathbf{NaPO_3})_{13}$, and shake for 15 hours on a reciprocal shaker.
3. Wash quantitatively all the suspension in the beaker, through a 53-µm sieve with plenty of DI water, into a pre-weighed and dried beaker (600-mL), until water flow out is clear (now the suspension in this beaker is called **mineral-associated organic matter** (MOM), with a particle size is < 53 µm).
4. Wash all the retained material in the sieve to anther pre-weighed and dried beaker (600-mL), (now this suspension is called **particulate organic matter**, POM, with a particle size is > 53 µm).
5. Dry the beakers in an oven at 45 ^{o}C, overnight.
6. Remove the beakers from oven; cool in desiccator for at least 30 minutes and re-weigh for MOM and POM fractions.
7. Determine OM-content of the MOM and POM by using the OM method

$$\boldsymbol{POM\ or\ MOM}\ (\%) = (\boldsymbol{Wt_1} - \boldsymbol{Wt_2}) \times \mathbf{100}$$

Where:

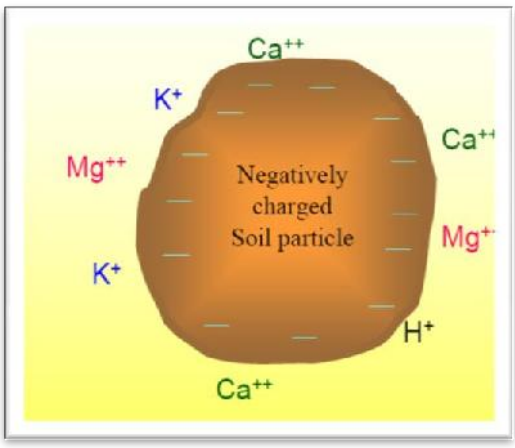
Wt_1 = Weight of air-dry soil plus dried beaker (g)

Wt_2 = Weight of dried beaker (g)

5.7. 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** (M), **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 contribute to **cation exchange capacity** (CEC). Also, the presence of high concentrations of Ca, especially in the form of gypsum, interferes with the determination of CEC, which is an important parameter for soil fertility and mineralogical characterization. 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 methods 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 Ca solubility in calcareous and gypsiferous soils (FAO, 1990; Rhoades and Polemio, 1977).*



Soil particles

*Cation exchange capacity is reported as centimoles of positive charge per kg of soil (cmol (+)/kg). The old unit milli-equivalents per 100 g (meq/100g), whereas **1 meq/100 g = 1 cmol (+)/kg**), should no longer be used. Values of CEC are in the range of 1.0 to 100 cmol (+)/kg, 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 (*$CH_3COONa.3H_2O$*), 1 *N*

- Dissolve 136 g *sodium acetate trihydrate ($CH_3COONa.3H_2O$)* in about 950 mL DI water, mix well, and let the mixture to cool.
- Adjust pH to 8.2 by adding more *acetic acid (CH_3COOH)* or *NaOH* and bring to 1-L volume.

B. Ethanol (C_2H_5OH), 95%

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

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

D. Standard Stock Solution

- Dry 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 *NaCl* in DI water, and bring to 1-L volume. 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 numbered flask by adding 1 *N ammonium acetate* solution, and 25 mL LiCl (*Diluted Stock Solution*), and then bring to volume. 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.
2. Add 33 mL **1 *N* sodium acetate trihydrate** solution, stopper tube, and shake for 5 minutes.
3. Remove stopper from tube and centrifuge at 3000 rpm until supernatant liquid is clear. Decant the supernatant as completely as possible and discard.
4. Repeat with 33-mL portions **1 *N* sodium acetate trihydrate** solution, a total of four times, discarding the supernatant liquid each time.
5. Add 33-mL **95 % ethanol**, stopper tube, and shake for 5 minutes, unstopped tube, and centrifuge until the supernatant is clear and decant.
6. Wash the sample with 33 mL portions **95 % ethanol** three times, discarding the supernatant liquid each time. The electrical conductivity (EC) of the supernatant liquid from the third washing (EC should be <400 µS/cm).
7. Replace the adsorbed 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.
8. Decant the three supernatant liquids as completely as possible into a 100-mL flask, and bring to volume with **1 *N ammonium acetate*** solution, mix well.
9. Run a series of suitable Na standards, and draw a calibration curve.
10. Measure the samples (soil extract) and take the emission readings by a **Flame Photometer** at **767-nm wavelength**.
11. Calculate Na concentration according to the calibration curve.

Calculation

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

Where:

V = Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

Technical Remarks

1. Though quite laborious, the method of Rhoades and Polemio (1977) is more appropriate for soils containing carbonates, gypsum, and zeolite.
2. Clay mineral CEC is pH-dependent.
3. Kaolinite clays minerals have a low CEC.
4. Montmorillinitic clay minerals have a high CEC due to the negative charges developed through loss of cations during formation of these clays.
5. Organic matter has a large CEC, but it too is pH-dependent.
6. Practices such as fertilization, liming, irrigation and addition of organic manures can increase CEC.

5.8. Gypsum

Soils with variable contents of gypsum ($CaSO_4.2H_2O$) are common in many countries of the WANA region. ***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 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 (CH_3COH_3)

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 minutes, 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 (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 **acetone** 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.
9. Measure EC of solution, and correct conductivity reading to 25 °C.
10. 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 Richards (1954).

Gypsum Concentration (meq/L)	1.0	2.0	5.0	10.0	20.0	30.5
Electrical Conductivity (dS/m)	0.121	0.226	0.500	0.900	1.584	2.205

Calculations

$$\boldsymbol{Gypsum\ in\ aliquot\ (meq) = \frac{meq/L\ gypsum(\ from\ calibration\ curve) \times A}{1000}}$$

$$\boldsymbol{Gypsum\ (meq/100g) = \frac{100 \times\ \ gypsum\ in\ aliquot(meq)}{A_1 \times A_2}}$$

$$\boldsymbol{Gypsum\ (\%) =\ Gypsum\ (meq/100g) \times 0.086}$$

Where:

A = Amount of water used to dissolve precipitate (mL)

A_1 = Soil: water ratio

A_2 = Soil - water extracted used (mL)

Example

The **Electrical Conductivity** reading from 10 : 50 (soil : water ratio) is **1.211 mS/cm**.

1	Gypsum from calibration curve (meq/L)	$= 11.629 \times (1.211)^{1.1765} = 14.57$
2	Water used to dissolve precipitate (mL)	= 40
3	Gypsum in aliquot (meq)	= (14.57×40)/1000 = 0 .58
4	Soil : water ratio	= 10 gram : 50 mL = 5
5	Soil-water extract used (mL)	= 20 mL
6	Weight of soil used from 20 mL extract	= 20/5 = 4 g
7	Gypsum (meq/100g)	= (100 × 0.58)/4 = 14.57
8	Gypsum (%)	= 14.57 × 0.086 = 1.25

Technical Remarks

1. Sodium and potassium sulfate (K_2SO_4), when present in sufficiently high concentrations, is also precipitated by acetone. The maximum concentrations of sodium sulfate ($NaSO_4$) and K that may be tolerated are 50 and 10 meq/L, respectively.
2. At a 1:5 (soil: water) ratio, water will dissolve about 15 meq gypsum / 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, gypsum may be well over 25 %, in which case a dilution 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 flocculated white precipitate forms, the soil contains gypsum; if no precipitate forms, the soil is considered to be free of gypsum.

5.9. Nitrogen

In view of high N requirements of crops and the low levels of available-N in many soils, N is the most important nutrient in agriculture*. Monitoring N fertilizer dynamics in soils is also important from the environmental perspective, as Nitrate is one of major pollutants of Groundwater, river water, lakes etc. Nitrogen in soils occurs in many forms, it is either organic or inorganically bound. With increasing aridity, organic and total soils N tend to decrease.*

The organic fraction constitutes the majority of total N in soils (usually >95 %). It is composed mostly of plant and microbial remains, in variable composition. The inorganic phase of soil N is composed of ***ammonium*** *(NH_4),* ***nitrate*** *(NO_3), and-very little though-****nitrite*** *(NO_2) forms. Environmental (temperature and moisture) and agronomic management (fertilization, cropping, etc.) factors influence its dynamic relationship with the organic fractions and also within the inorganic forms. The NH_4-N and NO_3-N forms are routinely measured in soil laboratories, as they reflect the extent of mineralization, and the forms of N are taken up by plants. In the WANA region, NO_3-N content in soils 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.*

5.9.1. 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 NH_4-N. The NH_4-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.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****. If desired, nitrate-N can be included through the reduced iron or salicylic acid modifications of the Kjeldahl procedure (see following section).*

Apparatus

- Distillation unit
- Block-digester
- 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 in a porcelain pestle and mortar to pass a 60-mesh screen (0.250 mm), **taking care not to breath *Se* dust or allow *Se* to come in contact with skin**.

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

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

Dissolve 400 g *NaOH* in DI water, transfer the solution to a 1-L heavy walled Pyrex flask, let it cool, and bring to volume.

D. Boric Acid Solution (H_3BO_3), saturated

- Add 500 g H_3BO_3 to a 5-L flask.
- Add 3 L *DI water*, and swirl vigorously.
- Leave overnight.

Note

There should always be solid H_3BO_3 on the bottom of the flask.

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

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

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

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

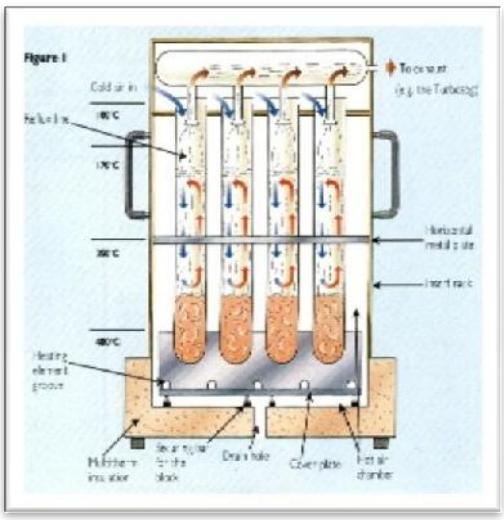
G. Standard Stock Solution

- Dry reagent-grade *ammonium sulfate* $(NH_4)_2SO_4$ in an oven at 100 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 5.6605 g dried $(NH_4)_2SO_4$ in DI water, and bring to 1-L volume. This solution contains 1.2 g NH_4-N / L (*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 H_2SO_4** (in the fume hood), and swirl carefully. Place a glass funnel in the neck of the tube, and 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. If tube contents are solidified and do not dissolve, heat the tubes again until the precipitate (gypsum) dissolves, then cool with tap water.



Block digester

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

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the **0.01 *N* H_2SO_4** in the **Auto-Titrator** by titrating three separate 10-mL aliquots of the primary standard, **0.01 *N* Tris** solution, to pH 5.0. The titrations should agree within 0.03 mL, if not; titrate further aliquots until agreement is found. The **H_2SO_4** normality is:

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

3. Carry out distillations as follows:
   - Dispense 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
   - Pipette 10-mL aliquot into a 100-mL distillation flask, and add 10 mL **10 *N* NaOH** 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 an **Auto-Titrator**.
   - Each distillation should contain at least two standards and two blanks (reagent blanks). Recovery of NH_4-N should be at least 96 %.

Notes

- After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish.
- Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100-mL empty distillation flask to distillation unit. Place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 second.

Calculations

$$\boldsymbol{Recovery}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \mathbf{14.01}}{\boldsymbol{V_3} \times \boldsymbol{C}} \times \mathbf{100}$$

$$\boldsymbol{N}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \boldsymbol{V_1} \times \mathbf{14.01}}{\boldsymbol{Wt} \times \boldsymbol{V_2} \times \mathbf{1000}} \times \mathbf{100}$$

Where:

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

V_1= Total volume of the digest (mL)

V_2= Volume of soil digest used for distillation (mL)

B = Digested blank titration volume (mL)

N = Normality of H_2SO_4 solution

14.01 = Atomic weight of N

Wt = Weight of air-dry soil (g)

V_3 = Volume of NH_4-N standard solution (mL)

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

Technical Remarks

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 sound bottom flask in the heating mantle to trap any NH_3 present. Also, add *Teflon Boiling Chips* to ensure smooth boiling.
3. The precision of the method depends upon complete conversion of organic N into NH_4- N, the digestion temperature and time, solid: acid ratio and the type of catalyst used.
4. The ideal temperature for digestion is 320-370 ^oC. At lower temperature, the digestion may not be complete, while above 410 ^{o} C, loss of NH_3 may occur.
5. **The catalysts** (the mixture of $CuSO_4$ or Se) are used to hasten the digestion process. Potassium sulfate is added to raise the boiling point of the acid so that loss of acid by volatilization is prevented.
6. Check all the joints of the Kjeldahl apparatus to prevent any leakage and loss of NH_3.
7. Hot Kjeldahl flasks should neither be washed immediately with cold water nor allowed to cool for long to avoid deposits that settle at the bottom and are difficult to remove.
8. Where frothing occurs and passes through to the boric acid, such samples should be discarded and a fresh distillation done.
9. **Opening NH_3 bottles in the laboratory should be strictly prohibited while distillation is on**. The titration should be carried out in NH_4-free atmosphere.
10. When the titration is not carried out immediately, the distillate should be stored in NH_4-free cupboards after tightly stoppering the flasks.

5.9.2. Total Nitrogen

The difference between Kjeldahl-N and total-N in soil is normally very small*, due mainly to the presence of NO_3-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 *$KMnO_4$* in DI water, and bring to 1-L volume. Store the solution in an amber bottle.

C. Sulfuric Acid Solution, 50% v/v ratio

Slowly add 1-L *concentrated H_2SO_4* 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 (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 in a porcelain pestle and mortar to pass a 60-mesh screen (0.250 mm), taking care not to breath *Se* dust or allow *Se* to come in contact with skin.

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

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

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

I. Boric Acid Solution (H_3BO_3), saturated

- Add 500 g H_3BO_3 to a 5-L flask.
- Add 3 L DI water, and swirl vigorously.
- Leave overnight.

Note: **There should always be solid H_3BO_3 on the bottom of the flask.**

J. 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 flask, and bring to volume.

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

- Add 28 mL concentrated H2SO4 to about 600 – 800 mL DI water in a 1-L flask, mix well, let it cool, and bring to 1-L volume. This solution contains 1 N H2SO4 solution (Stock Solution).
- Pipette 10 mL Stock Solution to 1-L flask, and bring to volume with DI water. This solution contains 0.01 N H2SO4.

L. Standard Stock Solution

- Dry reagent-grade ammonium sulfate (NH4)2SO4 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 (NH4)2SO4 in DI water, and bring to 1-L volume. This solution contains 1.2 g NH4-N / L (Stock Solution).

Procedure

A. Pre-treatment

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.

B. Digestion

1. Weigh 0.5 g air-dry soil (0.15 mm) and place into a 250-mL calibrated digestion tube.
2. Determine moisture on soil sub-samples to express results on a dry-weight basis.
3. Add 10 mL **DI water** to each tube and swirl thoroughly to wet the soil. Allow wet soil to stand for 30 minutes.
4. Prepare a blank digest, weigh 0.1 g **EDTA standard digest** (accurately weighed to 0.1 mg) with each batch.
5. Add 10 mL **potassium permanganate** solution, swirl well, allow to stand for 30 second, then hold the digestion tube at 45 ° angle and slowly add 20 mL **50 % H_2SO_4** in a manner which washes down material adhering to the tube neck.
6. Allow to stand for 15 minutes, and then swirl well. Do not swirl digestion tube immediately after adding acid because this may result in excessive frothing.
7. Add 2 drops **N-octyl alcohol** solution.
8. Add a few **pumice boiling granules** to the blank, EDTA, and sample digest tubes.

9. Add 2.5 g **reduced iron** through a long-stem funnel and immediately place a 5-cm (ID) glass funnel (with stem removed) in the tube neck, and swirl.
10. Excessive frothing at this stage may be halted by pouring 5 mL **DI water** through the 5-cm glass funnel; do not swirl.
11. Allow the tubes to stand overnight.
12. 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.
13. Samples should be swirled at 45 minutes.
14. Remove tubes from the block-digester, and cool. Rapid cooling may be affected in tap water.
15. Leave overnight.
16. Add about 5 g **catalyst mixture** through a long stem funnel. Then add 25 mL **concentrated H_2SO_4** to each tube, and swirl (more acid may be required if larger amount of soil is used).
17. 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.
18. 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.
19. Continue boiling off the water for 1 hour after reaching 240 °C.
20. After the water has been removed, replace the funnels and raise the temperature to 380 °C.
21. Set the timer on the block-digester, and digest for 4 hours at this temperature.
22. 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.
23. After cooling, add DI water to the 250-mL mark.

C. Distillation

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

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

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

3. Carry out distillations as follows:

- Dispense 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
- Prior to distillation, shake the digestion tube to mix thoroughly its contents.
- Pipette 50 mL soil digestion into a 250-mL distillation flask and carefully add 25 mL **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 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.

- 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 an **Auto-Titrator**.
- Each distillation should contain at least two standards and two blanks (reagent blanks). Recovery of NH_4-N should be at least 96 %.

Notes:

- After finishing titration, the Teflon-coated magnetic stirring bar, the burette tip and the combined electrode wash 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 second.

Calculations

$$\boldsymbol{Recovery\ of\ EDTA}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \boldsymbol{V_1} \times \mathbf{186.1}}{\boldsymbol{Wt_1} \times \boldsymbol{V_2} \times \mathbf{1000}} \times \mathbf{100}$$

$$\boldsymbol{N}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \boldsymbol{V_1} \times \mathbf{14.01}}{\boldsymbol{Wt_2} \times \boldsymbol{V_2} \times \mathbf{1000}} \times \mathbf{100}$$

Where:

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

V_1 = Total volume of the digest (mL)

B= Digested blank titration volume (mL)

N= Normality of H_2SO_4 solution

14.01= Atomic weight of N

V_2 = Volume of the digest sample used for distillation (mL)

Wt_1= Weight of EDTA (g)

Wt_2= Weight of air-dry soil (g)

186.1= Equivalent weight of the EDTA

5.9.3. Mineral Nitrogen

Nitrogen *is absorbed by plant roots in two forms, NH_4-N and NO_3-N.* ***Ammonium ions*** *are produced in soils through breakdown of OM, manures, or urea or ammonium-containing fertilizer.* ***Nitrate ions*** *are the final form of N breakdown/reactions (excluding gaseous forms of N). It can also be supplied to soil by fertilizers.*

Nitrogen 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 – unless positive charges are present (Anion exchange capacity). 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 NO_3 leaching is often the consequence of inadequate N-fertilizer management.*

Nitrate *is highly soluble in water, and a number of solutions including water have been used as extractants. Exchangeable NH_4 can be extracted at room temperature with a neutral K salt solution. Various extractants at different molarities have been used, such as 0.05M K_2SO_4, 0.1M KCl, 1M KCl, and 2M KCl.*

At ICARDA, mineral-N is determined using 2M KCl as the extracting solution in a 1:5 (soil: water) ratio. Ammonium and 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).

Field-fresh soil samples are to be preferred over air-dried or oven-dried soil samples, as the process of drying may alter the concentrations of NH_4 and NO_3 (see for instance Frye and Hutcheson 1981, Soil Sci. Soc. Am. J.). Soil samples may be stored in the fridge for a few days until analysis.

Apparatus

Distillation unit

Automatic titrator connected to a pH-meter

Suction

Reagents

A. Potassium Chloride Solution (KCl), 2 *M*

Dissolve 150 g reagent-grade *KCl* in DI water, and bring to 1-L volume.

B. Magnesium Oxide (MgO), powder

Heat the heavy *MgO* in a muffle furnace at 600-700 °C for 2 hours, 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

- Add 500 g H_3BO_3 to a 5-L flask.
- Add 3 L DI water, and swirl vigorously.
- Leave overnight.

Note

There should always be solid H_3BO_3 on the bottom of the flask.

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

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

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

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

G. Standard Stock Solution

- Dry reagent-grade *ammonium sulfate* $(NH_4)_2SO_4$, 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 *$(NH_4)_2SO_4$* and 8.6624 g *KNO_3* in DI water, mix well, and bring to 1-L volume This solution contains (1.2 g NH_4-N, and 1.2 g NO_3-N)/L (*Stock Solution)*.
- Prepare a *Standard Solution* from the *Stock Solution* as follows:

  Dilute 50 mL *Stock Solution* to 1-L flask by adding 2 *M KCl* 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

A. Extraction

1. Weigh duplicate 20 g fresh soil samples into a 250-mL Erlenmeyer flask.
2. Determine moisture on soil sub-samples to express results on a dry-weight basis.
3. Add 100 mL **2 *M* KCl** solution (1:5 soil: solution ratio) to determine NH_4-N. Add to the second flask 100 mL **DI water** to determine NO_3-N. Stopper flasks.
4. Shake for 1 hour on an orbital shaker at 200 – 300 rpm.
5. Filter the NO_3-N suspensions using Whatman No. 42 filter paper, and leave the NH_4-N suspensions flask for at least 1 hour until the soil is precipitated.

B. Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the **0.01 *N* H_2SO_4** in the **Auto-Titrator** by titrating three separate 10-mL aliquots of the primary standard, **0.01 *N* Tris** solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found. The **H_2SO_4** normality is:

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

3. Carry out distillations as follows:

To determine NH_4-N:

- Pipette 20-mL an aliquot of 2 *M* KCl soil extract into a 100-mL distillation flask.
- Pipette 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 50-mL Pyrex evaporating dish or beaker, which is placed underneath the condenser tip, with the tip touching the solution surface.
- Add about 0.2 g **heavy MgO**, with a calibrated spoon, to the distillation flask.
- Immediately, attach the distillation flask to the distillation unit with a clamp.
- Start distillation, and continue for 3 minutes, then lower the dish to allow distillate to drain freely into the Pyrex evaporating dish or beaker.
- After 4 minutes, when 35-mL distillate or more is collected, turn off the steam supply and remove the distillation flask.
- Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NH_4-N from *Diluted Stock Solution*) and two blanks (pipette 20 mL 2 *M* KCl). Recovery of NH_4-N should be at least 96 %.

To determine NO_3-N and NO_2-N:

- Pipette 20-mL an aliquot of DI water soil extract into a 100-mL distillation flask.
- Pipette another 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 50-mL Pyrex evaporating dish or beaker, which is placed underneath the condenser tip, with the tip touching the solution surface.
- Add about 0.2 g **heavy MgO** and 0.2 g **Devarda's alloy**, with a calibrated spoon, to the distillation flask.
- Immediately, attach the distillation flask to the distillation unit with a clamp.
- Start distillation, and continue for 3 minutes, then lower the dish to allow distillate to drain freely into the Pyrex evaporating dish or beaker.
- After 4 minutes, when 35-mL distillate or more is collected, turn off the steam supply and remove the distillation flask.
- Wash tip of the condenser into Pyrex evaporating dish or the beaker with a small amount of DI water.
- Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NO_3-N from *Diluted Stock Solution*) and two blanks (pipette 20 mL DI water). Recovery of NO_3-N should be at least 96 %.

C. Titration

Titrate the both distillates, separately, to pH 5.0 with standardized **0.01 *N* H_2SO_4** using an **Auto-Titrator**.

Notes

- 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. Proceed as for NH_4-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 NH_4-N (determined by MgO), and NO_3-N (by Devarda's alloy) in the same sample.

Calculations

$$NO_3 - N \text{ or } NH_4 - N\ (ppm) = \frac{(V - B) \times N \times V_1 \times 14.01 \times 1000}{Wt \times V_2}$$

$$NO_3 - N \text{ or } NH_4 - N\ (ppm) = \frac{(V - B) \times N \times V_1 \times 14.01 \times 1000}{(Wt - \theta) \times V_2}$$

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

V_1 = Total volume of the extract (mL)

V_2 = Volume of the soil extract used for distillation (mL)

Wt = Weight of air-dry soil (g)

θ = Weight of water (g) per air-dry soil

Technical Remarks

1. 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.
2. **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.
3. Often there is confusion about the relationship between NO_3 and NO_3-N. The nitrate ion is a combination of one N atom and three O_2 atoms. The total mass of NO_3 is 14 + 48 = 62. So, in 62 g NO_3 contains. 14 g N and 48 g O_2. 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.
4. Errors caused by NH_4 and NO_3 contamination from filter paper can be significant.
5. In samples low in NH_4 and NO_3 (such as those from deeper mineral soil horizons) the soil-to-solution ratio could be decreased to obtain detectable amounts of NH_4 and NO_3.

5.9.4. Nitrate-Nitrogen (by chromotropic acid)

Nitrate-N *is measured by a spectrophotometric method (using chromotropic acid), which is quite rapid, used originally for water and later for soils. 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
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 *$CuSO_4.5H_2O$* in DI water, and bring to 2-L volume.

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 H_2SO_4*. Keep solution in a dark bottle for 2 weeks.

C. Sulfuric Acid (H_2SO_4), concentrated

D. Standard Stock Solution

- Dry 4-5 g *potassium nitrate* (*KNO_3*) in an oven at 100 °C for 2 hours, cool in a desiccator, and store in tightly stoppered bottle.
- Dissolve 3.6092 g *KNO_3* in 500 mL 0.02 *N copper sulfate ($CuSO_4.5H_2O$) solution* (*Stock Solution*).

- Dilute 10 mL *Stock Solution* to 200-mL flask by adding 0.02 *N* $CuSO_4.5H_2O$ 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 numbered flasks by adding 0.02 *N* $CuSO_4.5H_2O$ solution, and then bring to volume. 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* $CuSO_4.5H_2O$** 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 H_2SO_4**.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.
8. Read the absorbance of blank, standards, and samples after 45 minutes on the **Spectrophotometer** at **430-nm wavelength**.
9. Prepare a calibration curve for standards, plotting absorbance against the respective NO_3-N concentrations.
10. Read NO_3-N concentration in the unknown samples from the calibration curve.

Calculation

$$NO_3\text{-}N(ppm) = ppm\ NO_3\text{-}N\ (from\ calibration\ curve) \times \frac{V}{Wt} \times \frac{V_2}{V_1}$$

Where:

V = Total volume of the extract (mL)

Wt = Weight of air-dry soil (g)

V_1 = Volume of soil extract used for measurement (mL)

V_2 = Volume of flask used for measurement (mL)

Technical Remarks

1. Where soils contain less than 1 ppm NO_3-N, add 0.1 mL *sulphamic acid* to 3-mL sample solution.
2. If filter paper gives purple solutions, wash with distilled water and dry before use.

5.9.5. Microbial Biomass Nitrogen and Carbon

Microbial biomass *as determined by the fumigation/incubation technique subjects a fresh soil to chloroform fumigation which causes cell walls to lyse and denature and the cellular contents become 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
- 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* H_2SO_4. In a separation funnel, separate the acid and then rinse repeatedly (8 – 12 times) in DI water. Store the washed chloroform in a dark bottle.

B. Potassium Sulfate Pentahydrate Solution (K_2SO_4), 0.5 *M*

Dissolve 87.13 g K_2SO_4 in DI water, and bring to 1-L volume.

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

Dissolve 49.94 g $CuSO_4.5H_2O$ in DI water, and bring to 1-L volume.

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

Dissolve 19.616 g $K_2Cr_2O_7$ in DI water, and bring to 1-L volume.

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* H_2SO_4 mix well, and bring to 1-L volume.

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.

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

Add 1-L concentrated H_2SO_4 to 0.5-L concentrated H_3PO_4

Procedure

1. Weigh duplicate 30 g fresh soil samples into a 100-mL beaker.
2. Determine moisture on soil sub-samples to express results on a dry-weight basis.
3. Place the beakers into two desiccators. The first desiccator contains fumigated control samples. Place a 100-mL beaker containing 50 mL **$CHCl_3$ (**add **pumice boiling granules** to the $CHCl_3$ to assist rapid volatilization of the chloroform) into the center of the desiccator.
4. The second desiccator contains non-fumigated control samples, which apart from fumigation-evacuation to be handled in the same fashion. Close the lids of the desiccators, paying particular attention that the sealant is uniformly distributed (**Figure. 7**).
5. Apply vacuum to the fumigated treatment until the chloroform is rapidly boiling. Evacuate the fumigated treatments using a vacuum pump repeatedly (8 – 12 times).
6. Close the desiccator and store under darkened conditions for 72 hours at room temperature.
7. Open the desiccators; release the **$CHCl_3$** vapor under the fume hood, and transfer the fumigated/non-fumigated soil samples to 250-mL Erlenmeyer flasks.
8. Add 100 mL **0.5 *M* K_2SO_4** solution. Shake on an orbital shaker for 1 hour.
9. To obtain a clear extract, filter the soil suspensions using Whatman No. 42 filter paper or a centrifuge.

Remember: 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.

1. Determination of Nitrogen

A. Digestion

1. Pipette 50 mL soils extract into a 250-mL calibrated digestion tube, and add 1 mL **0.2 *M* $CuSO_4.5H_2O$** solution.
2. Add 10 mL **concentrated H_2SO_4**, and a few **pumice boiling granules**, and then place the tubes in the rack.
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 lifts 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

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

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

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

3. Carry out distillations as follows:

- Dispense 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
- Pipette 50 mL aliquot into a 100-mL distillation flask, and add 15 mL **10 *N* NaOH** 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 an **Auto-Titrator**.
- Each distillation should contain at least two standards and two blanks (reagent blanks). Recovery of digested EDTA should be at least 96 %.

Notes: After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish. Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100-mL empty distillation flask to distillation unit. Place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds.

Calculations

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

$$Microbial\ Biomass\ N\ (ppm) = (N_{fumigated} - N_{control})$$

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 oven-dry soil (g)

V_1 = Volume of soil extract used for digestion (mL)

V_2 = Volume of soil digest used for distillation (mL)

14.01 = Atomic weight of N

θ = Weight of water (g) per oven-dry soil

2. Determination of Carbon

A. Digestion

1. Pipette 8 mL soils extract into a 100-mL calibrated digestion tube, and add 2 mL **0.4 *N* $K_2Cr_2O_7$** solution.
7. Add 0.07 g **mercury (II) oxide (HgO)**, 15 mL **(2:1) H_2SO_4: H_3PO_4** mixture, and a few **pumice boiling granules**, and then place the tubes in the rack.
2. Place the tubes rack in the block-digester, increase temperature setting to 150 °C and digest for 30 minutes.
3. 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

1. Add 2-3 drops **1.10-phenalthroline** indicator.
2. Titrate the digested sample with **0.2 *N* ferrous ammonium sulfate** until the color changes from bluish-green to reddish- brown.

Calculations

$$\boldsymbol{Biomass\text{–}C\ (ppm) = (B - V) \times N \times 0.003 \times \frac{100 + \theta}{Wt} \times \frac{1000}{V_1} \times 1000}$$

$$\boldsymbol{Microbial\ Biomass\ C\ (ppm) = (C_{\ fumigated} - C_{\ control})}$$

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 oven-dry soil (g)

V_1 = Volume of soil digest used for measurement (mL)

θ = Weight of water per oven-dry soil (g)

Technical Remarks

1. 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 Brookes 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.

2. The fumigation is done in a desiccators lined with paper or tissue. Place the beakers with soil (after pre-incubating for 1- week) in the desiccators, together with a beaker containing 20 mL ethanol-free $CHCl_3$ and a few anti-bumping granules.

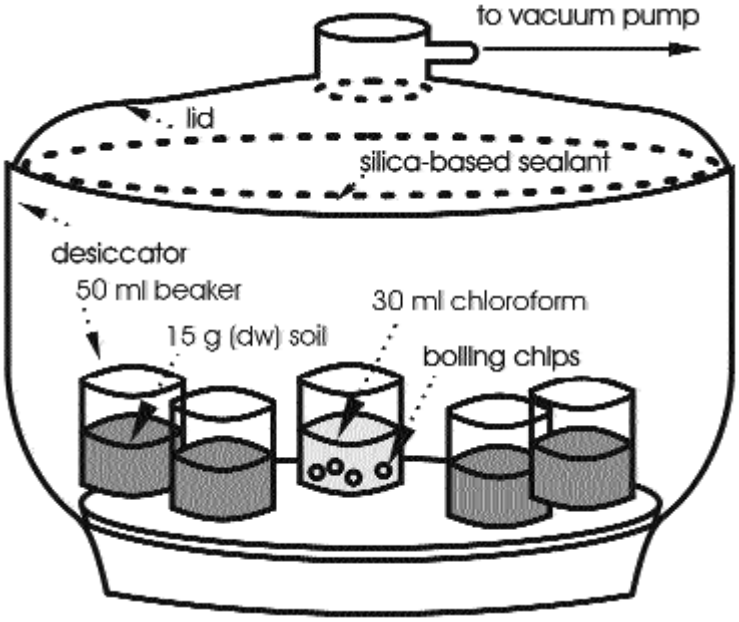


Figure 7. Experimental apparatus and sample arrangement in the fumigation procedure

5.10. Phosphorus

5.10.1. 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 WANA 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.*

The sodium bicarbonate ($NaHCO_3$) procedure *of Olsen et al. (1954) 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 WANA 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 $CuSO_4.5H_2O$ 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 OM) 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 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 *NaOH* in DI water, and transfer the solution to a 1-L heavy-walled Pyrex flask, let it cool, and bring to volume.

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

- Dissolve 42 g $NaHCO_3$ in about 900 mL DI water; adjust to pH 8.5 with 5 *N NaOH*, and bring to 1-L volume.
- 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 140 mL *concentrated* H_2SO_4 solution (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume.

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

E. Reagent-A

- Dissolve 12 g *ammonium heptamolybdate* $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250 mL DI water **(a)**.
- Dissolve 0.2908 g *antimony potassium tartrate* $(KSbO.C_4H_4O_6)$ in 100 mL DI water **(b)**.
- Add both dissolved Reagents **(a)** and **(b)** to a 2-L flask.
- Slowly add 1-L 5 *N* H_2SO_4 to the *mixture*. Mix thoroughly, and bring to 2-L volume.
- Store in a dark Pyrex bottle, cool place.

F. Reagent-B

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

G. 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 KH_2PO_4 in DI water, and bring to 1-L volume. This solution contains 500 ppm P (*Stock Solution*).
- Dilute 50 mL *Stock Solution* to 250-mL 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 numbered flasks by adding DI water, and then bring to volume. These solutions contain 1, 2, 3, 4, and 5 ppm P, respectively.

Procedure

A. Extraction

1. Weigh 5 g air-dry soil (2-mm) into a 250-mL Erlenmeyer flask; add 100 mL **0.5 *M* $NaHCO_3$** 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 suspension using a Whatman No. 40 filter paper.

B. Measurement

1. Pipette 10 mL clear filtrate into a 50-mL flask.
2. Add the required acid to all the unknown solutions (adding 1 mL 5 *N* H_2SO_4 is adequate to acidify each 10 mL $NaHCO_3$ extract to pH 5).

Notes

- To acidify the unknown solution to pH 5. Take 10 mL **0.5 *M* $NaHCO_3$** solution and determining the amount of acid required to bring the solution pH to 5, using **P-nitrophenol** indicator, the color changes from yellow to colorless.
- Do not swirl flasks immediately after adding 1 mL **5 *N* H_2SO_4**, because this may results is excessive frothing.

3. Dilute to 40-mL volume by adding DI water, add 8 mL **Reagent-B**, mix well, and then bring to volume.
4. Prepare a standard curve as follows:

- Pipette 2 mL of each standard (1 – 5 ppm), and proceed as for the samples.
- Make a blank with 10 mL 0.5 *M* $NaHCO_3$ solution, and proceed as for the samples.

5. Read the absorbance of blank, standards, and samples after 10 minutes on the **Spectrophotometer** at **882-nm wavelength**.
6. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
7. Read P concentration in the unknown samples from the calibration curve.

Calculation

$$\boldsymbol{Extractable\ P\ (ppm) = ppm\ P\ (from\ calibration\ curve)\ \times \frac{V}{Wt} \times \frac{V_2}{V_1}}$$

Where:

V = Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

V_1 = Volume of soil extract used for measurement (mL)

V_2 = Volume of flask used for measurement (mL)

Technical Remarks

1. 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.
2. 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.
3. **Intensity of blue color changes slightly with every batch of molybdate reagent**. Check the standard curve every day by using 2 or 3 dilutions of the standard P solution. If the standard curve is not the same as before, draw a new standard curve with fresh molybdate reagent. The intensity of blue color changes slightly with every batch of molybdate reagent.
4. Glassware used in P analysis should not be washed with detergents containing P (most detergents 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 Section 5.18.2) 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 macronutrients (NO_3-N, P, and K) and micronutrients (Zn, Fe, Mn, and Cu) can be determined in a single extract.
7. Color is stable for 24 hours.
8. Dissolved organic matter does not interfere with the method.
9. If glass container is used to store extracting solution, pH tends to increase with time, resulting in higher values for extractable P.
10. In general, the Bray P-1 method extracts about the same amount P as the Olsen method in the low range, and more in the medium and high ranges, except on highly calcareous soils, where it extracts less P.
11. The most commonly used methods for determination of available P in soils are **Bray's method** for acidic soils and **Olsen's method** for neutral and calcareous/alkaline soils.

5.10.2. 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 and organic P forms of minerals. This measurement is usually employed only for soil genesis or mineralogical studies (Olsen and Sommers, 1982).*

Apparatus

Spectrophotometer or colorimeter

Block-digester

Standard laboratory glassware: beakers, volumetric flasks, pipettes, and funnels

Vortex tube stirrer

Reagents

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

B. Ammonium Heptamolybdate- Ammonium Vanadate in Nitric Acid (HNO_3)

- 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 both dissolved Reagents **(b)** and **(a)** to a 1-L flask, and let the mixture cool to room temperature.
- Slowly add 250 mL *concentrated* HNO_3 to the mixture, cool the solution to room temperature, and dilute to 1-L volume.

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 KH_2PO_4 in DI water, and bring to 1-L volume. 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 numbered flasks by adding DI water, and then bring to volume. 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% $HClO_3$** and a few **pumice-boiling granules**. Mix well, and then place the tubes in the rack.
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 $HClO_3$ 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 **60% $HClO_3$** usually requires about 40 minutes.
6. Cool the mixture, and bring to volume, mix the contents, and filter through Whatman No. 1 filter paper.

Note

If the soil samples are high in organic matter (OM), add 20 mL concentrated HNO_3 before Step 2 and cautiously heat to oxidize OM.

B. Measurement

1. Pipette 5 mL clear filtrate into a 50-mL 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.
   - Make a blank with 10 mL ammonium-vanadomolybdate reagent, and proceed as for the samples.
4. Read the absorbance of blank, standards, and samples after 10 minutes on the **Spectrophotometer** at **410-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

$$\boldsymbol{Total\ P\ (ppm)\ = ppm\ P\ (from\ calibration\ curve)\ \times \frac{V}{Wt} \times \frac{V_2}{V_1}}$$

Where:

V = Total volume of the digest tube (mL)

Wt = Weight of air-dry soil (g)

V_1 = Volume of soil digest used for measurement (mL)

V_2 = Volume of flask used for measurement (mL)

Caution!

$HClO_4$ is explosive in presence of easily oxidizable organic matter. Avoid this hazard by taking the following precautions:

(**a**) Do not add $HClO_4$ to a hot solution containing high OM content.

(**b**) Always pre-treat samples containing OM with HNO_3 before adding $HClO_4$.

5.10.3. Organic Phosphorus

Organic Phosphorus *(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

Muffle furnace

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* H_2SO_4 solution (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume.

B. Sodium Hydroxide (NaOH), 5 *N*

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

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

D. Reagent-A

- Dissolve 12 g *ammonium heptamolybdate* $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250 mL DI water (**a**).
- Dissolve 0.2908 g *antimony potassium tartrate* $(KSbO.C_4H_4O_6)$ in 100 mL DI water (**b**).
- Add both dissolved Reagents (**a**) to (**b**) to a 2-L flask.
- Slowly add 1-L 5 *N* H_2SO_4 to the *mixture*. Mix thoroughly, and dilute to 2-L volume.
- Store in a Pyrex bottle in a dark, cool place.

E. Reagent-B

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

F. 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 KH_2PO_4 in DI water, and bring to 1-L volume. This solution contains 500 ppm P (*Stock Solution*).
- Dilute 50 mL *Stock Solution* to 250 mL 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 numbered flasks by adding DI water, and then bring to volume. These solutions contain 1, 2, 3, 4, and 5 ppm P, respectively.

Procedure

A. Digestion

1. Weigh 1 g air- dry soil (0.15 mm) for ignited soil 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 air- dry soil (0.15 mm) for un-ignited soil.
5. Add 50 mL **1 *N* H_2SO_4** to both samples (ignited and un-ignited soils), and place the tubes on a shaker for 16 hours.
6. Centrifuge the samples at 1500 rmp for 15 minutes (if the extract is not clear, use acid-resistant filter paper).

B. Measurement

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

Calculation

$$\boldsymbol{Ignited\ P\ (ppm) = \ ppm\ Ignited\ P\ (from\ calibration\ curve)\ \times \frac{V}{Wt} \times \frac{V_2}{V_1}}$$

$$\boldsymbol{Unignited\ P\ (ppm) = \ ppm\ Unignited\ P\ (from\ calibration\ curve)\ \times \frac{V}{Wt} \times \frac{V_2}{V_1}}$$

$$\boldsymbol{Organic\ P\ (ppm)\ = \ Ignited\ P(ppm) - \ Unignited\ P\ (ppm)}$$

Where:

V = Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

V_1 = Volume of extract used for measurement (mL)

V_2 = Volume of flask used for measurement (mL)

5.11. 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 forms available to plants, i.e., water-soluble and exchangeable-K. The highly weathered acid soils (of tropical regions) are more frequently deficient in plant available K, whereas soils arid and semi-arid areas tend to be well supplied with K. Thus, soils of the WANA region are generally adequate in K. A possible exception is sandy soils and irrigated soils grown to high K-requiring crops, e.g., sugar beet and potatoes.*

The determination of plant available-K is complicated as, in addition to K soluble in water, it includes part of the exchangeable-K. Another factor which complicates the determination of plant available-K in arid and semi-arid regions is that extracting solutions used for exchangeable-K may also extract part of the extractable-K from K-aluminum silicate clay minerals, i.e., K-feldspars and micas, which is not readily available. The moisture content of the soil sample and the method of drying the sample also affect K extractability. 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 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.

5.11.1. Extractable Potassium

This fraction of soil K is the sum of ***water-soluble*** *and* ***exchangeable K****. The method uses a neutral ammonium acetate solution (1 N) to replace the cations present on the soil exchange complex. This is considered as plant available K in the soils, and is commonly measured by the* ***flame photometer****.* ***However, the cation concentrations 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"****.*

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

B. Standard Stock Solution

- Dry 3 g *potassium chloride* (KCl) in an oven at 120 °C for 1-2 hours. Cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 1.907 g dried *KCl* in DI water, and bring to 1-L volume. 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 numbered flasks by adding 1 *N* ammonium acetate solution, and then bring to volume. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm K, respectively.

Procedure

A. Extraction

1. Weigh 10 g air-dry soil (< 2-mm) into a 250-mL flask.
2. Add 50 mL **1 *N* NH_4OAc** solution (ratio 1:5).
3. Shake for 30 minutes on a reciprocate shaker at 200-300 rpm.
4. Filter suspension using a Whatman No.1 filter paper to exclude any soil particles, and bring the extract to a 50-mL volume with **1 *N* NH_4OAc** solution.

Or as an alternative procedure

1. Weigh 5 g air-dry soil (< 2-mm) into a 50-mL centrifuge tube.
2. Add 33 mL **1 *N* NH_4OAc** solution, and shake for 5 minutes on a reciprocating shaker at 200-300 rpm.
3. Centrifuge at 2000 rpm until the supernatant liquid is clear and collect the extract in a 100-mL flask through a Whatman No. 1 filter paper to exclude any soil particles. Repeat this process two more times and collect the extract each time.
4. Dilute the combined **1 *N* NH_4OAc** extracts to 100-mL volume with **1 *N* NH_4OAc** solution.

Note

For both two methods above, the tubes or flasks should be stoppered with a clean rubber or polyethylene stopper, but not corks, which may introduce errors.

B. Measurement

1. Operate **Flame Photometer** according to the instructions provided.
2. Run a series of suitable K standards, and draw a calibration curve.
3. Measure K in the samples (soil extracts) by taking the emission readings on the **Flame Photometer** at **767-nm wavelength**.
4. Calculate K concentrations according to the calibration curve.

Calculations

$$\boldsymbol{Extractable\ K\ (ppm)\ = ppm\ K\ (from\ calibration\ curve)\ \times \frac{V}{Wt}}$$

$$\boldsymbol{Extractable\ K\ (ppm) = meq/L\ K\ (from\ calibration\ curve) \times \frac{V}{Wt} \times 39.1}$$

Where:

V= Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

5.11.2. Soluble Potassium

This fraction is a measure of the amount of K extracted from the soil by water (can be obtained in a water extract from a saturated paste as for Electrical Conductivity (EC).

Procedure

A. Extraction

1. Soluble K can be obtained in a water extract from a saturated paste as for pH and EC determinations.
2. Filter suspension using a Whatman No.1 filter paper to exclude any soil particles.

B. Measurement

1. Run a series of suitable K standards, and draw a calibration curve.
2. Measure K in the samples (soil extracts) by taking the emission readings on the **Flame Photometer** at **767-nm wavelength**.
3. Calculate K concentrations according to the calibration curve.

Calculation

$$\boldsymbol{Soluble\ K\ (ppm) = ppm\ K\ (from\ calibration\ curve)}$$

5.11.3. 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:

$$\boldsymbol{Exchangeable\ K\ (ppm)\ =\ Extractable\ K\ (ppm) - Soluble\ K\ (ppm)}$$

Technical Remarks

1. Exchangeable Na, Ca and Mg can be measured in the same way as derived for exchangeable K. Extractable-Na, Ca, and Mg are measured in the NH_4OAc extract and soluble Na, Ca, and Mg in the water extract (saturated paste). The difference represents exchangeable Na, Ca, and Mg.
2. **After extraction**, the filtrate containing K, Mg, Ca, and Na should not be stored for longer than 24 hours unless refrigerated or treated to prevent bacterial growth.
3. Soils can be stored in an air-dry condition for several months without any effect on the exchangeable K, Na, Ca, and Mg content.
4. Standard Solutions for measuring soluble-K should be prepared in DI water, but for measuring extractable-K the standards should be made in NH_4OAc solution.
5. The routine laboratory tests for determining plant available-K do not reflect the true situation under field conditions, because of the variation in the clay mineralogy of the soils.
6. **Available K** in soils is mainly in the water-soluble and exchangeable-fractions, and constitutes only a small part (not more than1%) of total K in soils.

5.12. 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****, where Na emits a sparkling yellowish-red color.*

5.12.1. Extractable Sodium

Reagents

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

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

B. Lithium Chloride (LiCl), 1000 ppm

- Dissolve 6.109 g dry *LiCl* 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 volume. This solution contains 100 ppm LiCl (*Diluted Stock Solution*).

C. Standard Stock Solution

- Dry 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 *NaCl* in DI water, and bring to 1-L volume. 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 numbered flasks by adding 1 *N ammonium acetate* solution, and 25 mL LiCl (*Diluted Stock Solution*), and then bring to volume. These solutions contain 20, 40, 60, 80,100, 150, and 200 ppm Na, with each containing the same concentration of LiCl (25 ppm).

Procedure

A. Extraction

1. Weigh 10 g air-dry soil (< 2-mm) into a 250-mL flask.
2. Add 50 mL **1 *N* NH_4OAc** solution (ratio 1:5).
3. Shake for 30 minutes on a reciprocate shaker at 200-300 rpm.
4. Filter suspension using a Whatman No.1 filter paper to exclude any soil particles, and bring the extract to a 50-mL volume with **1 *N* NH_4OAc** solution (each 50-mL flask should contain 25 ppm LiCl).

Or as an alternative procedure

1. Weigh 5 g air-dry soil (< 2-mm) into a 50-mL centrifuge tube.
2. Add 33 mL **1 *N* NH_4OAc** solution, and shake for 5 minutes on a reciprocating shaker at 200-300 rpm.
3. Centrifuge at 2000 rpm until the supernatant liquid is clear and collect the extract in a 100-mL flask through a Whatman No. 1 filter paper to exclude any soil particles. Repeat this process two more times and collect the extract each time.
4. Dilute the combined **1 *N* NH_4OAc** extracts to 100-mL flask with **1 *N* NH_4OAc** solution (each 50-mL volume flask should contain 25 ppm LiCl).

Note

Both two methods above, the tubes or flasks should be stoppered with a clean rubber or polyethylene stopper, but not corks, which may introduce errors.

B. Measurement

1. Operate **Flame Photometer** according to the instructions provided for the equipment.
2. Run a series of suitable Na 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 Na concentrations by inferring to the calibration curve.

Calculations

$$\boldsymbol{Extractable\ Na}\ (\mathbf{meq/L}) = \mathbf{meq/L}\ \boldsymbol{Na}\ (\boldsymbol{from\ calibration\ curve}) \times \frac{\boldsymbol{V}}{\boldsymbol{Wt}}$$

$$\boldsymbol{Extractable\ Na}\ (\mathbf{ppm}) = \mathbf{meq/L}\ \boldsymbol{Na}\ (\boldsymbol{from\ calibration\ curve}) \times \frac{\boldsymbol{V}}{\boldsymbol{Wt}} \times \mathbf{23}$$

Where:

V = Total volume of the soil extract (mL)
Wt = Weight of air-dry soil (g)
23 = Atomic weight of Na

5.12.2. Soluble sodium

This fraction is a measure of the amount of Na extracted from the soil by water (can be obtained in a water extract from a saturated paste as for pH and EC).

Procedure

A. Extraction

1. Soluble Na can be obtained in a water extract from a saturated paste as for pH and EC determinations.
2. Filter suspension using a Whatman No.1 filter paper to exclude any soil particles.

B. Measurement

1. Operate **Flame Photometer** according to the instructions provided for the equipment.
2. Run a series of suitable Na 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 Na concentrations by inferring to the calibration curve.

Calculation

$$\boldsymbol{Soluble\ Na}\ (\boldsymbol{ppm}) = \boldsymbol{ppm\ Na}\ (\boldsymbol{from\ calibration\ curve})$$

5.12.3. Exchangeable Sodium

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

$$\boldsymbol{Exchangeable\ Na\ (ppm) = Extractable\ Na\ (ppm) - Soluble\ Na\ (ppm)}$$

5.13. Calcium and Magnesium

Exchangeable Ca and Mg are usually determined in a ***neutral normal ammonium acetate*** *extract of soil. Soluble Ca and Mg are obtained by extracting the soil by water or from a saturated paste as for pH and EC. Extraction is carried out by shaking the soil: extractant mixture followed by filtration or centrifugation, and measurement of their concentrations in the extract by titration with EDTA.*

The ***EDTA titration method*** *is preferred on account of its accuracy, simplicity and speed. However, Ca and Mg in the extracts can also be measured by atomic absorption spectrophotometer after the removal of ammonium acetate and organic matter.*

5.13.1. Extractable Calcium and Magnesium

The method is based on the principle that calcium, magnesium and a number of other cations form stable complexes with versenate (ethylendiaminetetraacetic acid disodium salt, EDTA) at different pH. A known volume of standard calcium solution is titrated with standard 0.01N EDTA solution using Murexid (ammonium purpurate) indicator in the presence of NaOH solution.

The ***end point*** *is a change of color from orange red to purple at pH 12 when the whole of Ca forms a complex with EDTA. Calcium plus Mg are also present in the solution and can be titrated with 0.01N EDTA using* ***buffer solution*** *and a few drops* ***Eriochrome Black*** *T indicator. The end point is a change of color from red to blue at pH 10. Beyond pH 10, magnesium is not bound strongly to* ***Erichrome Black-T*** *indicator to give a distinct end point.*

Reagents

A. Buffer Solution (NH_4Cl-NH_4OH)

Dissolve 67.5 g *NH_4Cl* in 570 mL *concentrated NH_4OH*, and transfer the solution to a 1-L flask, let it cool, and bring to volume.

B. Eriochrome Black Indicator

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

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

Dissolve 2 g *EDTA*, and 0.05 g *magnesium chloride* (*$MgCl_2$*) in DI water, and bring to 1-L volume.

D. Sodium Hydroxide Solution (NaOH), 2 *N*

Dissolve 80 g *NaOH* in about 800 mL DI water, transfer the solution to a 1-L flask, cool, and bring to volume.

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

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

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

- Dry 2-3 g *calcium carbonate* (*$CaCO_3$*) in an oven at 100 ˚C for 3 hours. Cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 0.5 g dried *$CaCO_3$* in 10 mL 3 *N HCl* and bring to1-L volume with DI water. This can also be prepared by dissolving 0.735 g *calcium chloride dehydrate* (*$CaCl_2.2H_2O$*) in 1-L flask, then dilute to volume with DI water.

Procedure

A. Extraction

1. Weigh 10 g air-dry soil (< 2-mm) into a 250-mL flask.
2. Add 50 mL **1 *N* NH_4OAc** solution (ratio 1:5).
3. Shake for 30 minutes on a reciprocate shaker at 200-300 rpm.
4. Filter suspension using a Whatman No.1 filter paper to exclude any soil particles, and bring the extract to a 50-mL volume with **1 *N* NH_4OAc** solution.

Or as an alternative procedure

1. Weigh 5 g air-dry soil (< 2-mm) into a 50-mL centrifuge tube,
2. Add 33 mL **1 *N* NH_4OAc** solution, and shake for 5 minutes on a reciprocate shaker at 200-300 rpm.
3. Centrifuge at 2000 rpm until the supernatant liquid is clear and collect the extract in a 100-mL vol. flask through a Whatman No. 1 filter paper to exclude any soil particles. Repeat this process two more times and collect the extract each time.
4. Dilute the combined **1 *N* NH_4OAc** extracts to 100 mL volume with **1 *N* NH_4OAc** solution.

Note

For both two methods above, the tubes or flasks should be stoppered with a clean rubber or polyethylene stopper, but not corks, which may introduce errors.

B. Measurement

Calcium

1. Pipette 5 – 10 mL soil extract, having not more than 1 meq Ca, into a 250-mL Erlenmeyer flask.
2. Dilute to 20 – 30 mL with **DI water**, add 2-3 mL **2 *N* NaOH** solution.
3. Add about 50 mg **ammonium purpurate** indicator.
4. Titrate with **0.01 *N* EDTA** until the color changes 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.
5. Always run a blank containing all reagents but no soil, and treat it in exactly the same way as the samples; subtract the blank titration reading from the readings for all samples.

Calcium plus Magnesium

1. Pipette 5 – 10 mL soil extract into a 250-mL Erlenmeyer flask.
2. Dilute to 20 – 30 mL with **DI water**, add 3-5 mL **buffer solution.**
3. Add a few drops **eriochrome black** indicator.
4. Titrate with **0.01 *N* EDTA** until the color changes from red to blue.
5. In order to standardize the EDTA solution used in the determination of Ca and Mg:
   - Pipette 10 mL **0.01 *N calcium chloride*** solution, and treat it as in determining Ca and Ca + Mg procedure, respectively.
   - Take the reading, and calculate **EDTA** normality:

$$N_{EDTA} = \frac{10 \times N_{CaCl_2}}{V_{EDTA}}$$

Where:

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

Calculations

$$Extractable\ Ca\ or\ Ca + Mg\ (meq/L) = \frac{(V - B) \times N \times V_1 \times 1000}{Wt \times V_2}$$

$$Extractable\ Mg\ (meq/L) = Extrractable\ (Ca + Mg) - Extractable\ Ca$$

Where:

V = Volume of EDTA titrated for the sample (mL)
B = Blank titration volume (mL)
V_1 = Total volume of the soil extract (mL)
V_2 = Volume of soil extract used for titration (mL)
N = Normality of EDTA solution
Wt = Weight of air-dry soil (g)

5.13.2. Soluble Calcium and Magnesium

This fraction is a measure of the amount of Ca and Mg extracted from the soil by water (can be obtained in a water extract from a saturated paste as for pH and Electrical Conductivity (EC).

Procedure

A. Extraction

1. Soluble Ca and Mg can be obtained in a water extract from a saturated paste as for pH and EC determinations.
2. Filter suspension using a Whatman No.1 filter paper to exclude any soil particles.

B. Measurement

Calcium

1. Pipette 10-20 mL soil extract, into a 250-mL Erlenmeyer flask.
2. Dilute to 20-30 mL with **DI water**, add 2-3 mL **2 *N* NaOH** solution.
3. Add about 50 mg **ammonium purpurate** indicator.
4. Titrate with **0.01 *N* EDTA** until the color changes from red to lavender or purple. Near the end point, EDTA should be added one drop every 10 second since the color change is not instantaneous.
5. 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.

Calcium plus Magnesium

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

Calculations

$$\boldsymbol{Soluble\ Ca\ or\ Ca\ +\ Mg\ (meq/L) = \frac{(V - B)\ \times\ N\ \times\ 1000}{V_1}}$$

$$\boldsymbol{Soluble\ Mg\ (meq/L) =\ Soluble(Ca + Mg) - Soluble\ Ca}$$

Where:

V = Volume of EDTA titrated for the sample (mL)
B = Blank titration volume (mL)
V_1 = Volume of soil extract used for titration (mL)
N = Normality of EDTA solution

5.13.3. Exchangeable Calcium and Magnesium

Exchangeable Ca and Mg, or that held on the exchange sites or surfaces of clay minerals, is normally the dominant portion of total extractable Ca and Mg. It can be detected by difference:

$$\boldsymbol{Exchangeable\ Ca\ (meq/L) = Extractable\ Ca - Soluble\ Ca}$$

$$\boldsymbol{Exchangeable\ \ Mg\ (meq/L) = Extractable\ Mg - Soluble\ Mg}$$

Technical Remarks

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 (ratio 1:5) can be prepared. Shake for 30 minutes, filter, and use 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.
4. **The interference of Cu, Zn, Fe, and Mn** is prevented by the use of 2 % NaCN solution or carbonate. In irrigation waters and water extracts of soil, the quantities of interfering ions are negligible and can be neglected.

5.14. Carbonate and Bicarbonate

Carbonate *(CO_3) and* ***bicarbonate*** *(HCO_3) ions are species of the same acid, carbonic acid. Their proportionate content is a function of pH. The CO_3 starts to form as pH rises above 8.4. 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.*

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 DI water, and bring to 100-mL volume.

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

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

C. Phenolphthalein Indicator, 1%

Dissolve 1 g *phenolphthalein* indicator in 100 mL *ethanol*.

Procedure

A. Extraction

1. Soluble carbonate and bicarbonate can be obtained in a water extract from a saturated paste as for pH and EC determinations.
2. Filter suspension using Whatman No.1 filter paper to exclude any soil particles.

B. Measurement

1. Pipette 10 – 15 mL soil saturation extract in 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* H_2SO_4** by a burette, drop by drop, until the color disappears.
3. Take the reading, ***y***.
4. Continue the titration with **0.01 *N* H_2SO_4** 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

$$\textbf{Soluble } CO_3\ (meq/L) = \frac{2y \times N \times 1000}{V}$$

$$\textbf{Soluble } HCO_3\ (meq/L) = \frac{(t - 2y) \times N \times 1000}{V}$$

Where:

2 = Valance of carbonate
Y = Volume of titrant against phenolphthalein indicator (mL)
t = Volume of titrant against methyl orange indicator (mL)
V = Volume of soil extract used for titration (mL)
N = Normality of H_2SO_4 solution

Technical Remark

Carbonate and bicarbonate should be determined soon after preparing extracts because $CaCO_3$ precipitates on standing. If other analyses (Ca, Mg, K, Na, Cl, and SO_4) cannot be performed immediately, refrigerate the extracts.

5.15. Chloride

Soluble chloride *(Cl) 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****. This method quantifies the concentration of Cl (meq/L) in the saturation paste extract. Chloride may be determined using anion selective electrode (potentiometric). Plant tolerance to Cl can be related to its concentration in the soil saturation paste extract. The method detection limit is approximately 0.1 meq/L, dependent on the method of analysis, and is generally reproducible within ± 10 %.*

Reagents

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

- Dissolve 5 g K_2CrO_4 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 3 - 4 g $AgNO_3$ 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 $AgNO_3$ in DI water, and bring to 1-L volume.

C. Sodium Chloride Solution (NaCl), 0.01 *N*

- Dry 2-3 g *NaCl* in an oven at 110 °C for 3 hours. Cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 0.585 g dried *NaCl* in DI water, and bring to 1-L volume.

Procedure

A. Extraction

1. Soluble Cl can be obtained in a water extract from a saturated paste as for pH and EC determinations.
2. Filter suspension using Whatman No.1 filter paper to exclude any soil particles.

B. Measurement

1. Pipette 5-10 mL soil saturation extract in a wide-mouth Erlenmeyer flask (150-mL).
2. Add 4 drops **5 % K_2CrO_4** solution.
3. Titrate against **0.01 *N* $AgNO_3$** 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.
6. In order to standardize the $AgNO_3$ solution used in the determination of Cl:
   - Pipette 10 mL **0.01*N* NaCl** solution in a wide-mouth Erlenmeyer flask.
   - Add 4 drops **5 % K_2CrO_4** solution.
   - Titrate against **0.01 *N* $AgNO_3$** solution, until a permanent reddish-brown color appears.
   - Take the reading, and calculate **$AgNO_3$** normality:

$$N_{AgNO_3} = \frac{10 \times N_{NaCl}}{V_{AgNO_3}}$$

Where:

N_{AgNO} = Normality of $AgNO_3$ solution
V_{AgNO} = Volume of $AgNO_3$ solution used (mL)
N_{NaCl} = Normality of NaCl solution

Calculation

$$Soluble\ Cl\ (meq/L) = \frac{(V - B) \times N \times 1000}{V_1}$$

Where:

V = Volume of 0.01 *N* $AgNO_3$ titrated for the sample (mL)
B = Blank titration volume (mL)
V_1 = Volume of extract used for titration (mL)
N = Normality of $AgNO_3$ solution

Technical Remarks

1. Clean all glassware prior to analysis. Wash all glassware with 0.2 *N* HNO_3 and wash by DI water.
2. Samples containing Cl concentrations greater than the highest standard will require dilution.

5.16. Sulfate

Sulfur *(S) is present in soils in organic and inorganic forms.* ***Organic S*** *is an important constituent of proteins and amino acids. The major inorganic sources of S include gypsum ($CaSO_4$), and pyrite (Fe_2S). Sulfur is added to soil as fertilizers containing S, such as K_2SO_4 and some pesticides. It exists in soil and soil solution mainly as the sulfate (SO_4-S) anion in combination with the cations Ca, Mg, K, Na or NH_4. Phosphate ions (as monacalcium phosphate) are generally preferred for replacement of the adsorbed SO_4 ions. The extraction is also carried out using $CaCl_2$ solution. However, the former is considered to be better for more efficient replacement of SO_4 ions.*

Under aerobic condition*, the form of elemental S is oxidized to form SO_4 and under anaerobic condition, elemental SO_4 is reduced by microorganisms into SO_3 and S; waterlogged or paddy soils, especially in swamps, provide a suitable environment for the formation of hydrogen sulphide (H_2S).*

The total S content in soils varies widely from soil to soil*. Sandy soils in the humid regions are generally low in S (0.002%). In contrast, soils in arid regions may contain 5% SO_4-S. Plants absorb S mostly in the SO_4 form, which is the available form of S. Sulfate is extractable by water, NaCl, $CaCl_2$, NH_4OAc, $NaHCO_3$ and Ca $(H_2PO_4)_2$ solutions.*

5.16.1. Precipitation Method

Sulfate in soil extractants is determined normally by barium sulfate precipitation.

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 HCl* with DI water.
C. Barium Chloride Solution ($BaCl_2.2H_2O$), 1 *N*
Dissolve 122 g *$BaCl_2.2H_2O$* in DI water, and bring to 1-L volume.

Procedure

A. Extraction

1. Soluble SO_4 can be obtained in a water extract from a saturated paste as for pH and EC determinations.
2. Filter suspension using Whatman No.1 filter paper to exclude any soil particles.

B. Measurement

1. Pipette 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 volume.
2. Add 1 mL **1:1 HCl** solution and 2-3 drops **methyl orange**; if the color does not turn pink; add more 1:1 HCl.
3. Put beakers on a hotplate, heat to boiling, then add 10 mL **1 *N* $BaCl_2.2H_2O$** solution in excess to precipitate SO_4 as barium sulfate.

4. Boil for 5 to 10 minutes, cover with a watch-glass, 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 Cl remains. The presence of Cl 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 (***Wt_1***) 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, ***(Wt_2)***.

Calculation

$$\boldsymbol{Soluble\ SO_4 - S\ (meq/L)} = \frac{\boldsymbol{Wt_2 - Wt_1}}{\boldsymbol{V}} \times \frac{\boldsymbol{1000}}{\boldsymbol{0.1165}}$$

Where:

Wt_2 = Weight of crucible + $BaSO_4$ precipitate (g)
Wt_1 = Weight of empty crucible (g)
V = Volume of soil extract used for measurement (mL)
0.1165 g $BaSO_4$ equal to 1 meq of SO_4^{2-}

5.16.2. Turbidimetric Method

The commonly used method for S determination in alkaline soils is the extraction of SO_4-S with 0.15% $CaCl_2.2H_2O$ and measurement of SO_4-S concentration in the extracts by a turbidimetric procedure using barium chloride. A critical range of 10-13 mg/kg $CaCl_2$-extractable SO_4-S has commonly been reported for cereal (e.g., wheat, maize), oilseed (e.g., mustard), and crops (Tandon, 1991).

Apparatus

Mechanical shaker, reciprocal
Spectrophotometer or colorimeter

Reagents

A. Calcium Chloride Dihydrate Solution ($CaCl_2.2H_2O$), 0.15%
Dissolve 1.5 g *$CaCl_2.2H_2O$* in about 700 mL DI water, and bring to 1-L volume.

B. Hydrochloric Acid Solution (HCl), 6 *M*
Dilute 496.8 mL *concentrated HCl* (37%, sp. gr. 1.19) in DI water, mix well, let it cool, and bring to 1-L volume.

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. 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 numbered flasks by adding 0.15 % *calcium chloride dihydrate* solution, and then bring to volume. 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% $CaCl_2.2H_2O$** solution (do not use a rubber stopper, or wrap the rubber stopper in thin polyethylene. Errors result from gradual oxidation of S 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

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* HCl** solution followed by 5 mL **70 % sorbitol** solution from a pipette with an enlarged jet.
3. Add about 1 g **$BaCl_2.2H_2O$** crystals (using a measuring spoon).
4. Shake vigorously (on a test tube shaker for 30 seconds) to dissolve the **$BaCl_2.2H_2O$** and obtain a uniform suspension.
5. 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 % $CaCl_2.2H_2O$ solution, and proceed as for the samples.

6. Read the absorbance (turbidity) of the blank, standards, and samples on the **Spectrophotometer** at **470-nm wavelength**.
7. Prepare a calibration curve for standards, plotting absorbance against the respective SO_4-S concentrations.
8. Read SO_4-S concentration in the unknown samples from the calibration curve.

Calculation

$$\boldsymbol{Soluble\ SO_4 - S\ \ (ppm) = ppm\ SO_4 - S\ (from\ calibration\ curve)\ \times \frac{V}{Wt}}$$

Where:

V = Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

Technical Remarks

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 about the same time to standards and unknowns between shaking and turbidimetric reading.
3. Use of Ca salts has a distinct advantage over those of Na or K as Ca prevents deflocculating in heavy textured soils and leads to easy filtration. The SO_4 in the extract can be estimated turbidimetrically using a spectrophotometer. When the amount of extracted S is too low to be measured, add a solution of known S concentration to the extract to raise the concentration to easily detectable level.
4. Soils rich in iron, hydrogen sulfide (H_2S) usually precipitate as Pyrite (Fe_2S), which imparts a black color to the soil.
5. Accumulate of the H_2S in soils, it is not only toxic to soil organisms, but also it creates environmental problems. Some bacteria present in soils are capable of oxidizing the H_2S into elemental S and SO_4.

5.17. Boron

5.17.1. Hot-Water Method

Boron *(B) is a non-metal, in contrast to the other micronutrient elements. Boron is present in small amounts in igneous, sedimentary and metamorphic rocks. It is an essential micronutrient element for plant growth (needed for cell division), hence for the growth of young shoots.* ***Boron in soils is primarily of importance in soil fertility and plant nutrition****.The major inorganic sources of B are borate and boron-silicate minerals. Borate minerals occur mostly in arid regions and are formed by the evaporation of water in enclosed salt-water lakes and basins in arid regions.*

The hot-water extraction procedure*, introduced by Berger and Truog (1939), modified later is still the most popular method for measuring "available" soil B or the fraction of B related to plant growth in alkaline soils. Water soluble B, the available form of B, is extracted from the soil by water suspension. Boron in soil extracts is measured calorimetrically using reagent* ***Azomethine-H*** *(Bingham, 1982). Also, B can be analyzed by colorimetric methods using reagents such as* ***Carmine****, and most recently by* ***Inductively Coupled Plasma (ICP****) and* ***Atomic Emission Spectrometry****. The colorimetric method, using reagent Azomethine-H, is preferable because the use of AAS poses some limitations as B is not a metal.*

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. The normal B concentration in plant tissue is reported to be between 20 and 100 µg/mg dry matter of mature leaves. It is essential in sugar translocation and in the synthesis of hormones and protein in plants.

Apparatus

Erlenmeyer flasks (Pyrex), 50-mL volume
Spectrophotometer or colorimeter
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* and 1 g *L-ascorbic acid* in 100 mL DI water. 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. 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 numbered flasks by adding DI water, and then bring to volume. 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 250-mL Erlenmeyer flask (Pyrex).
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.

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, pipette 1 mL DI water, and proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 30 minutes on the **Spectrophotometer** at **420-nm wavelength**.
6. Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations.
7. Read B concentration in the unknown samples from the calibration curve.

Calculation

$$\boldsymbol{B\ (ppm) = ppm\ B\ (from\ calibration\ curve)\ \times \frac{V}{Wt}}$$

Where:

V = Total volume of the soil extract (mL)

Wt= Weight of air-dry soil (g)

Technical Remarks

1. Use of glassware should be minimal; always use **concentrated HCl-treated glassware** (for a week) where absolutely essential.
2. The use of Azomethine-H is an improvement over that of ***carmine*** and ***curcumin***, since the procedure involving this chemical does not require the use of concentrated acid.
3. The amount of charcoal added may vary with the OM content of the soil and should be just sufficient to produce a colorless extract after 5 minutes of boiling on a hot plate. Excess amounts of charcoal can reduce extractable B values
4. **In humid regions soils**, borate ions may tend to leach from soils. Therefore, soluble B concentrations are low in highly leached soils. Since highly leached soils usually exhibit low soil pH, acid soils are deficient in B.
5. **In arid region soils**, borate ions are usually not affected by leaching. Therefore, strongly basic in reaction, they may contain excessive amounts of B for plant growth.
6. Boron deficiency is more likely to occur in Ultisols and Oxisols, whereas B toxicity could be noted in Aridisols. This is in contrast with the other micronutrients.

5.17.2. Dilute Hydrochloric Acid Method

*Though **the hot water extraction method** (HWE) is quite popular for predicting B fertility 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 the dilute HCl method of Ponnamperuma et al. (1981), **originally designed for acid soils, to be equally effective in diagnosing B deficiency in alkaline and calcareous soils**. The HCl method is simple, economical, and more efficient.*

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

Dissolve 0.45 g *Azomethine-H* and 1 g *L-ascorbic acid* in 100 mL DI water. Fresh reagent should be prepared weekly and stored in a refrigerator.

C. 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. Check B in the filtrate by *Azomethine-H* color development; continue washing until it is B-free.

B. Hydrochloric Acid (HCl), 0.05 *N*

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

C. Standard Stock Solution

- Dissolve 0.114 g *boric acid* (H_3BO_3) in DI water, and bring to 1-L volume. 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 numbered flask by adding DI water, and then bring to volume. 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 polypropylene tube or into a 50-mL Erlenmeyer flask (Pyrex).
2. Add about 0.2 g **activated charcoal** (B-free).
3. Add 20 mL **0.05 *N* HCl** solution.
4. Shake for 5 minutes, and then filter the suspension using a Whatman No. 40 filter paper.

B. Measurement (Azomethine-H method)

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, pipette 1 mL DI water, and proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 30 minutes on the **Spectrophotometer** at **420-nm wavelength**.
6. Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations.
7. Read B concentration in the unknown samples from the calibration curve.

Calculation

$$B\ (ppm) = ppm\ B\ (from\ calibration\ curve)\ \times \frac{V}{Wt}$$

Where:

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

5.18. Micronutrient Cations

Though required by plants in much smaller amounts than the major plant nutrients (e.g., 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 WANA region are alkaline****, micronutrient deficiencies are common and are becoming more frequent and widespread with intensification of cropping.*

The extracted elements *can be estimated by various methods, which include volumetric analysis, spectrometry and atomic absorption spectroscopy. Volumetric methods such as* ***ethylene diaminetetraacetic acid solution*** *(EDTA) and $KMnO_4$ titrations are used for extraction and estimation of Zn, Mn, and Fe, respectively. Copper can be estimated by titration with $Na_2S_2O_3$.*

The Atomic Absorption Spectroscopy *method is commonly used to measure of Zn, Cu, Mn, and Fe. Running parameters that are specific to a particular model are given in the software provided with the equipment manual. Accordingly, the current supply, wavelength of hollow cathode lamp, integration time and anticipated estimation ranges are fixed.*

5.18.1. DTPA Method

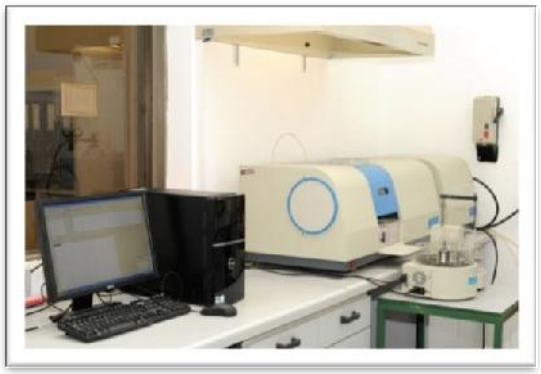
The diethylene triamine pentaacetic Acid *(DTPA) test of Lindsay and Norvell (1978) is commonly used for evaluating fertility status with respect to micronutrient cations, i.e., Fe, Zn, Mn, and Cu. The DTPA method is an important and widely used chelating agent, which combines with free metal ions in the solution to form soluble complexes of elements. The DTPA method has a capacity to complex each of the micronutrient cations as 10 times of its atomic weight. The capacity ranges from 550 to 650 mg/kg depending upon the micronutrient cations. However, the universal soil test for alkaline soils (i.e., AB-DTPA described in Section* ***5.18.2****) 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.*

Apparatus

Atomic absorption spectrophotometer
Mechanical shaker, reciprocal

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 volume.
- Into another beaker, weigh 14.92 g (or add 13.38 mL) *Triethanolamine* (*TEA*), transfer with DI water into the 1-L flask, and then bring to about 900-mL volume.
- Adjust the pH to exactly 7.3 with 6*N hydrochloric acid* (*HCl*), and bring to 1-L volume. This solution contains 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:

1. Iron (Fe) standard solution

- Pipette 10 mL Fe *Stock Solution* (1000 ppm) in 100-mL flask and then dilute to volume with DTPA solution. This solution contains 100 ppm Fe (*Diluted Stock solution*).
- Pipette 1, 2, 3, 4 and 5 mL *Diluted Stock Solution* to 100-mL numbered flask and then dilute to volume DTPA solution. These solutions contain 1, 2, 3, 4, and 5 ppm Fe, respectively.

2. Zinc (Zn) standard solution

- Pipette 10 mL Zn *Stock Solution* (1000 ppm) in 100 mL flask, and then dilute to volume with DTPA solution. This solution contains 100 ppm Zn (*Diluted Stock Solution*).
- Pipette 10 mL *Diluted Stock Solution* to 100-mL flask, and then dilute to volume DTPA solution. This solution contains 10 ppm Zn (*Second Diluted Stock Solution*).
- Pipette 1, 2, 4, 6, 8 and 10 mL *Second Diluted Stock Solution* in 50-mL numbered flasks, and then dilute to volume DTPA solution. These solutions contain 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ppm Zn, respectively.

3. Copper (Cu) standard solution

- Pipette 10 mL Cu *Stock Solution* (1000 ppm) in 100-mL flask, and then dilute to volume with DTPA solution. This solution contains 100 ppm Cu (*Diluted Stock Solution*).
- Pipette 10 mL *Diluted Stock Solution* to 100-mL flask, and then dilute to volume DTPA solution. This solution contains 10 ppm Cu (*Second Diluted Stock Solution*).
- Pipette 2, 3, 4, 5, 6 and 7 mL *Second Diluted Stock Solution* in 50-mL numbered flasks, and then dilute to volume DTPA solution. These solutions contain 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ppm Cu, respectively.

4. Manganese (Mn) standard solution

- Pipette 10 mL Mn *Stock Solution* (1000 ppm) in 100-mL flask and then dilute to volume with DTPA solution. This solution contains 100 ppm Mn (*Diluted Stock Solution*).
- Pipette 10 mL *Diluted Stock Solution* to 100-mL flask, and then dilute to volume DTPA solution. This solution contains 10 ppm Mn (*Second Diluted Stock Solution*).
- Pipette 2, 3, 4, 5, 6 and 7 mL *Second Diluted Stock Solution* in 50-mL numbered flasks, and then dilute to volume DTPA solution. These solutions contain 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ppm Mn, respectively.

Procedure

A. Extraction

1. Weigh 10 g air-dry soil (2-mm) into a 125-mL Erlenmeyer flask.
2. Add 20 mL **extraction solution.**
3. Shake for 2 hours on a reciprocal shaker.
4. Filter the suspension through a Whatman No. 42 filter paper.

B. Measurement

1. Operate **Atomic Absorption Spectrophotometer** according to the instructions provided for the equipment.
2. Run a series of suitable **Micronutrient Cations** (Zn, Cu, Fe, and Mn) standards, and draw a calibration curve.
3. Measure **Micronutrient Cations** in the samples (soil extracts) by an **Atomic Absorption Spectrophotometer** using appropriate lamp for each element.
4. Calculate **Micronutrient Cation** concentrations according to the calibration curve.

Calculation

$$\boldsymbol{Micronutrient\ Cation\ (ppm) = ppm\ MC\ (from\ calibration\ curve) \times \frac{V}{Wt}}$$

Where:

MC= Micronutrient cation

V= Total volume of the extract (mL)

Wt = Weight of air-dry soil (g)

Technical Remarks

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 di-sodium salt).
3. To avoid excessive dissolution of $CaCO_3$, which may release occluded micronutrients that are not available to crops in calcareous soils and may give erroneous results, the extractant is buffered in slightly alkaline pH.
4. **Triethanolamine** (TEA) is used as buffer because it burns clearly during atomization of extractant solution while being measured on the AAS.
5. **Extracting solution** can be stored for 2 weeks under mineral oil, and then the pH adjusted to 7.6 if necessary.
6. **The time of shaking** is important because trace elements continue to dissolve (non equilibrium extraction). Therefore, factors as shaking time, speed and shape of vessel are critical and should be standardized in every laboratory.

5.18.2. 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
Accurate automatic dilutor
Flame photometer
Mechanical shaker, reciprocal

Reagents

A. Extracting Solution

- Add 1.97 g diethylene triamine pentaacetic acid (DTPA) to 800 mL DI water; and then add 2 mL 1:1 ammonium hydroxide (NH_4OH) to facilitate dissolution and to prevent effervescence when bicarbonate is added. This solution contains 0.005 M DTPA.
- When most of the DTPA is dissolved, add 79.06 g ammonium bicarbonate (NH_4HCO_3) and stir gently until dissolved.
- Adjust pH to 7.6 with NH_4OH, and then bring to 1-L volume with DI water.

B. Mixed Reagent for Phosphorus

- Dissolve 12 g *ammonium heptamolybdate* $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250 mL DI water (**a**).
- Dissolve 0.2908 g *antimony potassium tartrate* ($KSbO.C_4H_4O_6$) in 100 mL DI water (**b**).
- Add both dissolved Reagents (**a**) and (**b**) to a 2-L flask.
- Slowly add 1-L 5 *N* H_2SO_4 (148 mL concentrated H_2SO_4 /L) to the *mixture*. Mix thoroughly, and dilute to 2-L volume.
- Store in a dark Pyrex bottle, 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 $H_2N_2H_2.H_2SO_4$in 750 mL DI water, mix well, and then bring to 1-L volume (*stock solution)*.

Prepare* $H_2N_2H_2.H_2SO_4$ *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 $CuSO_4.5H_2O$ in 800 mL DI water, mix well, and then bring to 1-L volume *(stock solution)*.

Prepare* $CuSO_4.5H_2O$ *working solution by diluting 6.25 mL *stock solution* to 1-L volume with DI water.

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

Dissolve 60 g NaOH in 500 mL DI water, cool, and bring to 1-L volume with DI water (*stock solution)*.

Prepare* NaOH *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* ($C_6H_8N_2O_{25}$), and then add 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 then bring to 500-mL volume with DI water.
- This reagent should be prepared as required, as it cannot be used after appearance of pink color.

H. Standard Stock Solutions

1. **Nitrate-N**: Prepare working standards containing 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ppm NO_3-N.
2. **Phosphorus**: Prepare working standards containing 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ppm P.
3. **Potassium:** Prepare working standards containing 0, 5, and 10 ppm K.
4. **Micronutrients**: Prepare working standards for:
   Fe: 0, 1, 2, 3, 4, 5 ppm
   Cu: 0, 1, 2, 3, 4 ppm
   Mn: 0, 1, 1.5, 2, 2.5 ppm
   Zn: 0, 0.2, 0.4, 0.6, 0.8, 1 ppm

Procedure

A. Extraction

1. Weigh 10 g air-dry soil (2-mm) into a 125-mL conical flask.
2. Add 20 mL **extracting solution**.
3. Shake on a reciprocal shaker for 15 minutes at 180 cycles/minute with flasks kept *open*.
4. Filter the suspensions using Whatman No. 42 filter paper.

B. Measurement

1. Nitrate Nitrogen

1. Transfer 1 mL soil extract to 25-mL test tube.
2. Add 3.0 mL **copper sulfate** working solution.
3. Add 2 mL **hydrazine sulfate** working solution.
4. Add 3 mL **NaOH** working solution. Mix well and heat in water bath (38 $^{\circ}$C) for 20 minutes. Remove from water bath.
5. For NO_3-N measurement, add 3 mL **color-developing reagent**, mix well.
6. The blank and standards are developed the same way as described above.
7. Read the absorbance of blank, standards, and samples after 20 minutes on the **Spectrophotometer** at **540-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.

2. Phosphorus

1. Dilute 1 mL aliquot of the soil extract to 10 mL with DI water.
2. Add 2.5 mL **color developing reagent** carefully to prevent loss of sample due to excessive foaming. Stir well.
3. The blank and standards are developed the same way as described above.
4. Read the absorbance of blank, standards, and samples after 30 minutes on the **Spectrophotometer** at **880-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.

3. Potassium

1. Determine K in soil extracts directly either by a **Flame Photometer**, or by an **Atomic Absorption Spectrophotometer** using a K hollow cathode lamp.
2. Operate the instrument according to the instructions provided for the equipment.
3. Run a series of suitable K standards, and draw a calibration curve.
4. Measure K in the samples (soil extracts) by taking the emission readings on the **Flame Photometer** at **767-nm wavelength**.
5. Calculate K concentrations according to the calibration curve.

4. Micronutrients

1. Determine micronutrients (Zn, Fe, Cu, and Mn) in soil extracts directly by **Atomic Absorption Spectrophotometer**.
2. Operate the instrument according to the instructions provided for the equipment.
3. Run a series of suitable micronutrients standards, and draw a calibration curve.
4. Measure micronutrients in the samples (soil extracts) by taking the emission readings on the **Atomic Absorption Spectrophotometer** at suitable wavelength for each element.
5. Calculate micronutrients concentrations according to the calibration curve.

Calculations

$$\boldsymbol{NO_3 - N\ (ppm)\ = NO_3 - N(ppm\ in\ extract) \times Dilution\ Factor}$$

$$\boldsymbol{P(ppm) = ppm\ P\ (from\ calibration\ curve) \times Dilution\ Factor}$$

$$\boldsymbol{K\ (ppm) = ppm\ K\ (from\ calibration\ curve) \times Dilution\ Factor}$$

$$\boldsymbol{Micronutrient\ Cation(ppm) = ppm\ MC\ (from\ calibration\ curve) \times Dilution\ Factor}$$

Technical Remarks

1. The DTPA reagent should be of the acid form.
2. The extracting solution can be stored for 2 weeks under mineral oil, and then the pH adjusted to 7.6 if necessary.
3. The AB-DTPA soil test data can be interpreted using the generalized guidelines in Appendix 9.

5.19. Heavy Metals

*The heavy metals of concern in agriculture, especially as it relates to the environment are **Cadmium** (Cd), **Lead** (Pb), **Mercury** (Hg), **Chromium** (Cr), **Selenium** (Se), Nickel (Ni), as well as **Cobalt** (Co), **Molybdenum** (Mo), and **Arsenic** (As). For the release of mineral elements from soil and sediments, **wet oxidation** of sample is carried out. Wet oxidation employs oxidizing acids (e.g., HNO_3-$HClO_4$-HF tri acid mixture or HNO_3-$HClO_4$ di-acid mixture). The di-acid oxidation method is easier, less time-consuming and convenient but it is not a total digestion as soil does not dissolve completely, particularly silicate minerals, therefore, di-acid digestion is known as pseudo digestion or partial digestion.*

5.19.1. Di-Acid (HNO3-HClO4), Block Digester

Apparatus

Digestion tube

Block-digester

Atomic Absorption Spectrophotometer

Reagent

Nitric Acid (HNO_3), concentrated

Perchloric Acid ($HClO_4$), concentrated

Procedure

1. Weigh 0.5-1.0 g air-dry soil (0.15-mm) into a 300-mL calibrated digestion tube.
2. Add 3 mL **concentrated HNO_3** (in the fume hood), and swirl carefully, and then place tubes in the rack.
3. Place the tubes rack in the block-digester, and then place a glass funnel in the neck of the tubes. Slowly increase temperature setting to about 145 $^{\circ}$C for 1 hour.
4. Add 4 mL **concentrated $HClO_4$** and heat it to 240 ^{o}C for further 1 hour.
5. Lift the tubes rack out of the block-digester, carefully place on a rack holder, and let tubes cool to room temperature.
6. Filter through Whatman No. 42 filter paper and bring to 50-mL volume.
7. Each batch should contain at least one reagent blank (no soil).
8. Determine Fe, Mn, Cu, Zn, Cd, Ni, Pb, Co, and Cr by **Atomic Absorption Spectrophotometer**.

5.19.2. Di-Acid (HNO3-HCl), Microwave

Apparatus

Vessels, MF/HF

Microwave

Atomic absorption spectrophotometer

Reagent

Nitric Acid (HNO_3), concentrated
Hydrochloric acid (HCl), concentrated

Procedure

1. Weigh 0.5 g air-dry soil (0.15 mm) into MF/HF vessels of microwave.
2. Add 5 mL **concentrated HNO_3** (in the fume hood), swirl carefully, and then add 1 mL **concentrated HCl**. Insert the vessels into the rotor of microwave.
3. Set the microwave system as; Temperature 100 ^{o}C/ vessel, ramp 10 minutes, hold 10 minutes and cool 30 minutes.
4. After cooling, filter through Whatman No. 42 filter paper and bring to 50-mL volume.
5. Determine Fe, Mn, Cu, Zn, Cd, Ni, Pb, Co, and Cr by **Atomic Absorption Spectrophotometer.**

Notes

- Microwave digestion procedures require the use of specially designed ovens to handle acid fumes.
- The microwave exhaust should be removed to a scrubber before being released to a fume exhaust system.
- Special safety precautions are required for microwave digestion. Therefore, read the manufacturer's specifications for details.

5.19.3. Tri-Acid (HCl04-HF- HCl), Block Digester

Apparatus

Teflon beaker
Hot plate
Polyethylene bottle
Atomic Absorption Spectrophotometer

Reagent

Perchloric Acid ($HClO_4$), concentrated
Hydrofluoric Acid (HF), concentrated
Hydrochloric acid (HCl), concentrated

Procedure

1. Weigh 0.5-1.0 g air-dry soil (0.15 mm) into a clean 100-mL Teflon beaker, wet with 5 mL DI water.
2. Add 2 mL **concentrated $HCl0_4$** and 12 mL **concentrated % HF**, heat to incipient (near dryness).
3. Add 8 mL **concentrated HF** and heat to dryness.
4. Add 2 mL **concentrated $HCl0_4$** and about 5 mL **DI water** and heat to incipient.
5. Dissolve the remaining residue in 8 mL **concentrated HCl** and then add 20 mL DI water.
6. Bring to 100-mL volume, and store in polyethylene bottle.
7. Determine **Micronutrient Cation** (MC) and **Heavy Metal** (HM) (e.g., Fe, Mn, Cu, Zn, Cd, Ni, Pb, Co, and Cr) by **Atomic Absorption Spectrophotometer**.

Calculation

$$\boldsymbol{MC\ or\ HM\ (ppm) = ppm\ MC\ or\ HH\ (from\ calibration\ cutve) \times \frac{V}{Wt}}$$

Where:

MC= Micronutrient cation
HM= Heavy metals
V = Total volume of the soil extract (mL)
Wt = Weight of air-dry soil (g)

Technical Remarks

1. Use of $HClO_4$ avoids volatilization loss of K and provides a clear solution.
2. Using HF solution helps removing silica.
3. If tube contents are solidified and do not dissolve, heat the tubes again until the precipitate (gypsum) dissolves. Then cool with tap water.

5.20. Soluble Silicon

Soluble Silicon *(Si) corresponds to the amount of Si available in soil solution. It is the most labile form of Si and consists primarily of monomeric silica acid (H_3SiO_4). Extraction with 0.01 M $CaCl_2$ solution provides for low interference of the method with high levels of $CaCO_3$ in the soil. The H_3SiO_4 can be determined colorimetrically by reaction with molybdate; blue (this procedure) and yellow analyses methods are usually distinguished.*

Apparatus

Ventilated drying oven
Mortar and pestle
Sieve (2-mm)
Plastic bottles/cups (150-mL)
Shaker, horizontal
Magnetic stirrer
Plastic cups (50-mL)
Volumetric flasks (100, 200, and 1000-mL)
Spectrophotometer

Reagents

A. Calcium Chloride Solution ($CaCl_2 \cdot 2H_2O$), 0.01 *M*
Dissolve 1.47 g *$CaCl_2 \cdot 2H_2O$* in DI water, mix well, and bring to 1-L volume.

B. Ammonium Sulfuric-Heptamolybdate Solution ($(NH_4)_6Mo_7O_{24}$. $4H_2O$) + H_2SO_4)), 75 g/ L
Dissolve 7.5 g *ammonium heptamolybdate tetrahydrate* in 75 mL DI water, add 10 mL 18 *N H_2SO_4,* mix well, and then bring to a 100 mL volume with DI water.

C. Tartaric Acid Solution ($C_4H_6O_6$), 200 g/ L
Dissolve 20 g *tartaric acid* (2, 3-dihydroxybutanedioic acid, $C_4H_6O_6$) in 50 mL DI water. Transfer this solution into a 100-mL flask and bring to volume.

D. Ascorbic Acid Solution ($C_6H_8O_6$), 3 g/ L
Dissolve 0.3 g *ascorbic acid* in 50 mL DI water. Transfer this solution into a 100-mL flask and bring to volume with DI water.

E. Standard Stock Solution ($SiCl_4$ in 14 % NaOH)

- With Merck Titrisol® Silicon standard 1000 mg solution ($SiCl_4$ in 14 % NaOH; Merck-I.N.: 109947): Open the capsule and let the Si-solution flow into a 1-L flask (to make sure that the 1000 mg of SI in the capsule is transferred completely into the flask, rinse the capsule thoroughly with DI water), and then bring up with DI water (*stock solution*).

Note: The Silicon standard 1000 mg solution is not a 1000 ppm solution! Pipette 4 mL *stock solution* to 200-mL, and bring volume with DI water. This solution contains 20 ppm Si (*Diluted stock solution*).

Note: This reagent should be prepared as required because it does not keep for more than 24 hours.

Procedure

A. Pre-treatment

Manual grinding (mortar) might be preferable to avoid potential contamination of samples with previous residues in the mechanical soil grinder.

B. Extraction

1. Weigh 10 g air-dry soil (2-mm) into a 150-mL plastic bottle.
2. Add 100 mL **0.01 *M* $CaCl_2 \cdot 2H_2O$** solution. Stopper plastic bottle.
3. Shake for 1 hour on horizontal shaker at 250 rpm.
4. Let solution settle for ±15 minutes and filter suspensions using Whatman No. 42 filter paper.

Note

Instead of filtering, the clear supernatant solution can be decanted after letting the soil particle settle over night.

C. Measurement

1. Pipette 10-mL an aliquot of the soil extract into a 50-mL plastic cup.
2. Prepare a series of standard solution from the *Diluted stock solution* 20 ppm as follows:
   - Dilute 0, 2, 5 and 10 mL into 100-mL flasks and bring to volume with DI water.
   - Pipette 10-mL an aliquot of each standard into a 50-mL plastic cup.
3. Add 1 mL **ammonium sulfomolybdate** solution to samples and standards. Depending on the concentration of Si, a more or less intensive yellow color develops.
4. After 10 minutes, add 2 mL **tartaric acid solution** (This will complex phosphate in the solution)
5. After 5 minutes, add 10 mL **L-ascorbic acid** solution. This will provide for a reduction of Si, and the color of the solution will turn from yellow to blue.
6. Read the absorbance of blank, standards, and samples on the **Spectrophotometer** at **660-nm wavelength**.
7. Prepare a calibration curve for standards, plotting absorbance against the respective Si concentration.
8. Read Si concentration in the unknown samples from the calibration curve.

Calculation

$$\boldsymbol{Soluble\ Si\ (ppm) = ppm\ Soluble\ Si\ (from\ calibration\ curve) \times \frac{V}{Wt}}$$

Where:

V = Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

Technical Remark

Glassware might be a source of Si. Thus a blank sample should be analyzed for Si, and if significant, the concentration subtracted from the soil sample results. Also, if available, plastic flasks instead of glass should be used throughout.

6. Plant Sampling and Processing

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

6.1. Field Processing

Preparation for Sampling

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

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

Where to Take Sample?

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

Uniform Fields

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

Non-uniform Fields

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

Sampling Time

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

Amount of Plant Material

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

What to Sample

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

What Not to Sample

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

Shipment of the Plant Material Sample

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

6.2. Laboratory Processing

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

Handling in the laboratory

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

Decontamination

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

A. Reagents and Apparatus

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

B. Cleaning processing

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

Drying

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

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

Grinding and Mixing

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

A. Apparatus

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

B. Procedure

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

Notes

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

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

Storage

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

Apparatus

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

Procedure

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

Technical Remarks

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

7. Plant Analysis

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

The concentration of nutrients in plant tissues can be measured in a plant extract obtained from fresh plant material, (i.e., tissue analysis), as well as in whole dried plant material. The former test is qualitative and is appropriate only for quick measurements on a growing crop. Total plant analysis is quantitative in nature and is more reliable and useful. Generalized ranges of deficiency, adequacy, and excess of nutrient- concentrations in cereal crops are given in the Appendix 11. Of prime concern are forms of N, as well as P, B, and micronutrient cations. More detailed interpretative guidelines for plant analysis data are available in Reuter and Robinson (1986, 1997), Munson and Nelson (1990) and Jones et al. (1991).

Effective Uses for Plant Analyses

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

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

7.1. Moisture Factor

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

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

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

7.2. Nitrogen

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

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

7.2.1. Kjeldahl Nitrogen (with Catalyst)

Apparatus

Block-digester

Distillation unit

Automatic titrator connected to a pH-meter

Vortex tube stirrer

Reagents

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

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

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

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

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

D. Boric Acid Solution (H_3BO_3), saturated

- Add 500 g H_3BO_3 to a 5-L flask.
- Add 3 L DI water, and swirl vigorously.
- Leave overnight.

Note

There should always be solid H_3BO_3 on the bottom of the flask.

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

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

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

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

G. Standard Stock Solution

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

Procedure

A. Pre-treatment

1. Mix and spread finely ground (Cyclone mill) plant sample in a thin layer on a sheet of paper until it looks uniform.
2. Select representative sub-samples of about 1 g by taking at least 10 small portions from all parts of the sample with a spatula, and put them into a plastic vial.
3. Dry the sub-sample at 60 °C in an oven (overnight), and then cool in a desiccator.

B. Digestion

1. Weigh 0.25 g (grain) or 0.50 g (straw) of dry plant material, and transfer quantitatively into a 100-mL digestion tube.
2. Add a few **pumice boiling granules**, and add about 3 g **catalyst mixtures** using a calibrated spoon.
3. Add 10 mL **concentrated H_2SO_4** using a dispenser, and stir with Vortex tube stirrer until mixed well.
4. Place tubes in a block-digester set at 100 °C for 20 minutes, and remove the tubes to wash down any material adhering to the neck of the tube with the same **concentrated H_2SO_4**.
5. Thoroughly agitate the tube contents, and then place the tubes back on the block-digester set at 380 °C for 2 hours after clearing.
6. After digestion is complete, remove tubes, cool, and bring to 100-mL volume with DI water.
7. Each batch of samples for digestion should contain at least one reagent blank (no plant), and one chemical standard (weigh 0.1 g EDTA standard digest), and one standard plant sample (internal reference).

C. Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the **0.01 *N* H_2SO_4** in the **Auto-Titrator** by titrating three separate 10-mL aliquots of the primary standard, **0.01 *N* Tris** solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found. The **H_2SO_4** normality is:

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

3 Carry out distillations as follows:

- Dispense 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
- Pipette 10-mL aliquot into a 100-mL distillation flask.
- Carefully add 10 mL **10 *N* NaOH** solution, and immediately connect the flask to the distillation unit and begin distillation.
- Collect about 35 mL distillate in the collecting dish.
- Remove distillation flask and connect an empty 100-mL distillation flask to the distillation unit. Drain water from the condenser jacket and steam out apparatus for 90 seconds before connecting the next sample.
- Titrate the distillate to pH 5.0 with standardized **0.01 *N* H_2SO_4** using an **Auto-Titrator**; record titration volume of acid.
- Each distillation should contain at least two standards (pipette 10 mL digested solution), two blanks (pipette 10 mL digested solution). Recovery of N should be at least 96 %.

Notes: After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish. Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100-mL empty distillation flask to distillation unit. Place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds. Each distillation should contain at least two standards and two blanks (reagent blanks).

Calculations

$$\boldsymbol{Recovery}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \mathbf{14.01} \times \mathbf{100}}{\boldsymbol{V}_{\mathbf{1}} \times \boldsymbol{C}}$$

$$\boldsymbol{Recovery\ of\ EDTA}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}_{\mathbf{1}}) \times \boldsymbol{N} \times \boldsymbol{V}_{\mathbf{2}} \times \mathbf{186.1} \times \mathbf{100}}{\boldsymbol{Wt}_{\mathbf{1}} \times \boldsymbol{V}_{\mathbf{3}} \times \mathbf{1000}}$$

$$\boldsymbol{N}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}_{\mathbf{1}}) \times \boldsymbol{N} \times \boldsymbol{V}_{\mathbf{2}} \times \mathbf{14.01} \times \mathbf{100}}{\boldsymbol{Wt}_{\mathbf{2}} \times \boldsymbol{V}_{\mathbf{3}} \times \mathbf{1000}}$$

Where:

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

V_2 = Tolat volume of the plant digest (mL)

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

B = Distillate blank titration volume (mL)

B_1 = Digested blank titration volume (mL)

Wt_1 = Weight of EDTA (g)

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

Wt_2 = Weight of dry plant (g)

N = Normality of H_2SO_4 solution

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

14.01 = Atomic weight of N

186.1 = Atomic weight of EDTA

Technical Remarks

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

7.2.2. Kjeldahl Nitrogen (Without Catalyst)

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

Reagents

Sulfuric Acid (H_2SO_4), concentrated

Hydrogen Peroxide (H_2O_2), 30%

Procedure

1. Weigh 0.5 g dry-plant material into a 100-mL digestion tube.
2. Add 3-4 **pumice boiling granules**, and then add 5 mL **concentrated H_2SO_4**, mix well.
3. Keep overnight.
4. Heat on a block-digester at a moderate temperature 100 – 150 °C.
5. Swirl to restrict foaming. If foaming enters the neck of the digestion tube, add 2 mL **30 % H_2O_2**.
6. Heat the tubes for 30 – 60 minutes on the block-digester.
7. Cool the tubes, and then add 2 mL **30 % H_2O_2**.
8. Raise the temperature of the block-digester to 280 °C.
9. Heat the tubes for 10 minutes at 280 °C.
10. Cool, then add 2 mL **30 % H_2O_2**, and heat for 10 minutes.
11. Repeat Steps 9 and 10 until solution remains clear after 10 minutes of heating.
12. Cool, and make up to 100-mL volume with DI water.

Measurement

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

Note

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

7.2.3. Total Nitrogen (Sulfuric-Salicylic Acid Mixture)

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

Reagents

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

B. Catalyst Mixture (K_2SO_4 - Se), 100:1 w/w ratio
Grind reagent-grade chemicals separately and mix. If caked, grind the mixture in a porcelain pestle and mortar to pass a 60-mesh screen (0.250 mm), taking care not to breath *Se* dust or allow *Se* to come in contact with skin.

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

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

Procedure

A. Pre-treatment

1. Mix and spread finely ground plant sample in a thin layer, on a sheet of paper or plastic until the sample looks uniform.
2. Take a representative sub-sample of about 1 g by systematically withdrawing at least 10 small portions from all parts of the sample with a spatula, and put them into a plastic vial.
3. Dry the sub-sample at 60 °C in an oven (overnight), and then cool in a desiccator.

B. Digestion

4. Weigh 0.25 g (grain) or 0.50 g (straw) dry-plant material, and then transfer quantitatively into a dry 250-mL digestion tube.
5. Add 20 mL **sulfuric-salicylic acid mixture** while rotating the tube to wash down any sample adhering to the neck of the tube, and allow to stand 2 hours or longer with occasional swirling.
6. Add 2.5 g **sodium thiosulfate** through a long-stemmed funnel to the contents of the tube and swirl gently a few times, and allow to stand overnight.
7. Add 4 g **catalyst mixture**, and 3 – 4 **pumice boiling granules**, and place tubes on the block-digester pre-heated to 400 °C.
8. Place a small glass funnel in the mouth of the tubes to ensure efficient refluxing of the digestion mixture and prevent loss of H_2SO_4, and proceed with the digestion until the mixture clears.
9. Remove the tubes from the block-digester and allow them to cool for about 20 minutes. Then wash down any material adhering to the neck of the tube with a minimum quantity of DI water.
10. Thoroughly agitate the tube contents, place tubes back on the block-digester, and digest for 2 hours after clearing. No particulate material should remain in the tube after digestion.
11. After the digestion is finished, allow the digest to cool, and add water slowly shaking until the liquid level is about 2-cm below the graduation mark.
12. Allow tube to cool to room temperature, and add DI water to bring the volume to the 250-mL mark.
13. Each batch of samples for digestion should contain at least one reagent blank (no plant), and one chemical standard (weigh 0.1 g EDTA standard digest), and one standard plant sample (***internal reference***).

C. Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the **0.01 *N* H_2SO_4** in the **Auto-Titrator** by titrating three separate 10-mL aliquots of the primary standard, **0.01 *N* Tris** solution, to pH 5.0. The titrations should agree within 0.03 mL; if not, titrate further aliquots until agreement is found. The **H_2SO_4** normality is:

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

3. Carry out distillations as follows:

- Dispense 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
- Prior to distillation, shake the digestion tube to thoroughly mix its contents, and pipette an aliquot in a 300-mL distillation flask.
- Carefully add 7 mL **10 *N* NaOH** solution for 25-mL aliquot (or 15 mL **10 *N* NaOH** solution for 50-mL aliquot), and immediately connect flask to distillation unit and begin distillation.
- Collect about 35-mL distillate in the collecting dish.
- Remove distillation flask and connect an empty 100-mL distillation flask to the distillation unit. Drain water from the condenser jacket and steam out apparatus for 90 seconds before connecting the next sample.
- The distillate is then titrated to pH 5.0 with standardized **0.01 *N* H_2SO_4** using an **Auto-Titrator**; record titration volume of acid.
- Each distillation should contain at least two standards and two blanks (reagent blanks). Recovery of EDTA, corrected for reagent blank, should be at least 97%.

Notes: After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish. Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100-mL empty distillation flask to distillation unit. Place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds.

Calculations

$$\textbf{Recovery } \boldsymbol{of\ EDTA}(\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \boldsymbol{V_1} \times 186.1 \times 100}{\boldsymbol{Wt_1} \times \boldsymbol{V_2} \times 1000}$$

$$\boldsymbol{N}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \boldsymbol{V_1} \times 14.01 \times 100}{\boldsymbol{Wt_2} \times \boldsymbol{V_2} \times 1000}$$

Where:

V = Volume of 0.01 *N* H_2SO_4 titrated for the sample (mL)
V_1 = Total volume of the plant digest (mL)
V_2 = Volume of plant digest used for distillation (mL)
B = Digested blank titration volume (mL)
N = Normality of H_2SO_4 solution
14.01 = Atomic weight of N
Wt_1 = Weight of EDTA (g)
Wt_2 = Weight of dry plant (g)
186.1 = Equivalent weight of the EDTA

7.3. Phosphorus

Phosphorous (P) is essential to crop production; it stimulates early root formation and growth, gives a rapid and vigorous start to plants, and promotes flower and seed production. Phosphorous is needed in the genetic coding material which controls cell division. The P content in plants is usually between 0.1 to 0.5 % of the dry matter. Total P in plant material can be attained either by ***wet-digestion*** *procedure or by* ***dry-ashing*** *procedure (given in Section 7.4.1). 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 is measured colorimetrically.*

Apparatus

Spectrophotometer or colorimeter
Block-digester
Vortex tube stirrer

Reagents

A. Ammonium Heptamolybdate-Ammonium Vanadate in Nitric Acid (HNO_3)

- 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 both dissolved Reagent (**a**) and (**b**) to a 1-L flask, and let the mixture cool to room temperature.
- Slowly add 250 mL *concentrated* 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 KH_2PO_4 in DI water, and bring to 1-L volume. 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 numbered flaks by adding DI water, and then bring to volume. These solutions contain 0.5, 1.0, 1.5, 2.0, and 2.5 ppm P, respectively.

Procedure (Wet Digestion)

A. Pre-treatment

1. Mix and spread finely ground plant sample in a thin layer, on a sheet of paper or plastic until the sample looks uniform.
2. Take a representative sub-sample of about 1 g by systematically withdrawing at least 10-small portions from all parts of the sample with a spatula, and put them into a plastic vial.
3. Dry the sub-sample at 60 °C in an oven (overnight), and then cool in a desiccator.

B. Digestion

1. Weigh 0.25 g (grain) or 0.50 g (straw) of dry plant material, and transfer quantitatively into a 100-mL digestion tube.
2. Add a few **pumice boiling granules**, and add about 3 g **catalyst mixtures** using a calibrated spoon.
3. Add 10 mL **concentrated H_2SO_4** using a dispenser, and stir with Vortex tube stirrer until mixed well.
4. Place tubes in a block-digester set at 100 °C for 20 minutes, and remove the tubes to wash down any material adhering to the neck of the tube with the same **concentrated H_2SO_4**.
5. Thoroughly agitate the tube contents, and then place the tubes back on the block-digester set at 380 °C for 2 hours after clearing.
6. After digestion is complete, remove tubes, cool, and bring to 100-mL volume with DI water.
7. Each batch of samples for digestion should contain at least one reagent blank (no plant), and one chemical standard (weigh 0.1 g EDTA standard digest), and one standard plant sample (internal reference).

C. Measurement

1. Pipette 10 mL clear filter into a 100-mL flask.
2. Add 10 mL **ammonium-vanadomolybdate** reagent, and dilute the solution to volume with DI water.
3. Prepare a standard curve as follows:
   - Pipette 1, 2, 3, 4, and 5 mL standard stock solution, and proceed as for the samples.
   - Make a blank with 10 mL ammonium-vanadomolybdate reagent, proceed as for the samples.
4. Read the absorbance of the blank, standards, and samples after 30 minutes on the **Spectrophotometer** at **410-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

$$\boldsymbol{P}\ (\%) = \boldsymbol{ppm\ P\ (from\ calibration\ curve)} \times \frac{\boldsymbol{V_1}}{\boldsymbol{Wt}} \times \frac{\boldsymbol{100}}{\boldsymbol{V2}} \times \frac{\boldsymbol{1}}{\boldsymbol{10000}}$$

Where:

V_1 = Total volume of the plant digest (mL)

V_2 = Volume of plant digest used for measurement (mL)

Wt= Weight of dry plant (g)

7.4. Macronutrients and Micronutrients

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 procedure is that of Chapman and Pratt (1961) with slight modifications. The HNO_3-$HClO_4$* ***wet digestion*** *(given in Section 7.4.3) is required for full recovery of micronutrient cations in high-silica plant tissues (e.g., wheat, barley, rice, and sugarcane, etc.).*

7.4.1. Macronutrients (Dry Ashing)

Dry ashing is carried out usually at an ignition temperature of 550 ^{0}C to 600 ^{0}C followed by its extraction in diluted HCl or H_2SO_4 for determining various elements. ***Ashing leads to considerable volatile loss of P, K, S and Cl****. For this reason, and being more time-taking, dry-ashing is only occasionally adopted.*

Critical factors in dry-ashing procedures include selection of ashing vessel, sample number, placement in furnace, ashing temperature, time, selection of acid to solubilise the ash, and final volume. Placement of vessels and ashing time are dependent on the type and number of samples. Selection of an ashing vessel, the solubilising acid, and final volume are dependent on the elements of interest and subsequent analytical procedures. A number of combinations of these factors have been used successfully.

Phosphorus, K, Ca, and Mg can be determined in a range of plant tissue concentrations from 0.1 to 0.7 % P, 0.5 to 5 % K, 0.2 to 2 % Ca, and 0.1 to 1 % Mg. Manganese and Fe can be determined in the range from 25 to 500 ppm. Copper and Zn can be determined in the range from 5 to 50 ppm. The ranges may be extended by dilution, or calibrating the instrument for higher concentrations.

Apparatus

Spectrophotometer or colorimeter
Flame photometer
Atomic absorption spectrophotometer
Porcelain crucibles or Pyrex glass beakers

Reagent

Hydrochloric Acid (HCl), 2*N*

Dilute165.6 mL *concentrated HCl* (37%, sp.gr.1.19) in DI water, mix well, let it cool, and bring to 1-L volume.

Procedure

1. Weigh 0.5 – 1.0 g dry and 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* HCl** and mix with a plastic rod.
7. After 15 – 20 minutes, bring to the volume (usually to 50-mL) using DI water.
8. Mix thoroughly, allow standing 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***, for Ca, Mg by **titration** with **EDTA**, and for Zn, Cu, Fe, and Mn by ***Atomic Absorption Spectroscopy***.

$$\boldsymbol{Macronutrients\ (ppm) = ppm\ Macronutrients\ (from\ calibration\ curve) \times \frac{V}{Wt}}$$

Where:

V= Total volume of the extract (mL)

Wt= Weight of dry plant (g)

Technical Remarks

1. 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 a similar La concentration to overcome anionic interference.
2. If a clean white ash remains after muffling, oxidation is complete and ashing aids are not required.
3. Plant materials with high sugar or oil content (highly carbonaceous) may require an ashing aid such as 10 % H_2SO_4, concentrated HNO_3, and 7% Mg $(NO_3)_2.6H_2O$.
4. Dry-ashing is not recommended for plant materials that are high in Si. Dry-ashing these materials results in low micronutrient values, especially for Zn.
5. Dry ashing results in lower Fe and Al values than wet-ashing.
6. The sensitivity varies depending on the instrument used and the wavelength selected for each element.
7. *This method has very little interference*, except due to spectral overlap which occurs when an element emits a wavelength that is very close to the wavelength of an element being analyzed and the optical system of the instrument is incapable of resolving the two wavelengths.
8. Samples to be analyzed after storage should be placed in an oven at 30 °C for 4 hours before weighing.
9. The crucible used for ashing should be rinsed first in DI water and later in several portions of 25 % HCl and then rinsed several times with DI water and dried in an oven.
10. When organic solution is required for determination of some of the micronutrients such as Cu, special attention must be paid to the cleaning of glassware or separator funnels by shaking with the reagent and organic solution.
11. There are no reported interferences for Mn, Cu, and Zn.
12. The presence of HNO_3 and Ni, or Si may depress the sensitivity of Fe. Using a very hot, lean-burning, air/acetylene flame appears to overcome these interferences.

7.4.2. Boron (Dry Ashing)

The content of boron (B) in plant dry matter ranges between 10 and 100-200 ppm. *Boron helps in the manufacture of sugars and carbohydrates in crops, which is essential for fruit development, and translocation of sugars, and development of seed and seed quality in some crops like mungbeans. Boron aids in the utilization of Ca, N and P, also B is important in the development of young roots and shoots. 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

Polypropylene test tubes

Spectrophotometer or colorimeter

Reagents

A. Sulfuric Acid (H_2SO_4), 0.36 *N*

Dilute 10.08 mL *concentrated H_2SO_4* (98%, sp.gr.1.84) in DI water, mix well, let it cool, and bring to 1-L volume.

B. 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.

C. Azomethine-H

Dissolve 0.45 g *Azomethine-H* and 1 g *L-ascorbic acid* in 100 mL DI water. 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. 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 numbered flasks by adding DI water, and then bring to volume. These solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm, respectively.

Procedure

A. Ashing

1. Weigh 1 g dry and 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* H_2SO_4** solution into the porcelain crucibles.
5. Heat on a steam bath for 20 minutes.

6. Let stand at room temperature for 1 hour, stirring occasionally with a plastic rod to break up ash.
7. Filter through Whatman No.1 filter paper into a 50-mL polypropylene flask and bring to volume. Filtrate is ready for B determination.

B. Measurement

1. Pipette 1-mL an 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), proceed as for the samples.
   - Make a blank with 1 mL DI water, proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 30 minutes on the **Spectrophotometer** at **420-nm wavelength**.
6. Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations.
7. Read B concentration in the unknown samples from the calibration curve.

Calculation

$$\boldsymbol{B\ (ppm) = ppm\ B\ (from\ calibration\ curve) \times \frac{V}{Wt}}$$

Where:
V= Total volume of the extract (mL)
Wt= Weight of dry plant (g)

Technical Remarks

1. Avoid the use of borosilicate glassware, and always use concentrated HCl-treated glassware (for a week), where absolutely essential. The main source of contamination during B determination may not always be the glassware, but rather, the impurities contained in the chemical reagents. Consequently, special care should care be taken to ensure that chemical reagents are purified before use.
2. Where possible interferences occur, NH_4 can be overcome by the use of NH_4OAc, K by use of KOAc; and Al, Cu, and Fe by use of EDTA.
3. The treatment of DI water with charcoal would remove organic substances that may interfere with the colour development.
4. The possible interference of residual H_2O_2 with color development is eliminated by a heating time of 40 minutes following the last H_2O_2 addition.
5. The use of photometric tubes for color development does eliminate the use of additional plastic or glassware; however, the importance of immediate and thorough mixing is stressed.

7.4.3. Micronutrient (Wet Digestion)

Full recovery of micronutrient cations (Zn, Fe, Mn, and Cu) in high-silica containing plant tissues (such as wheat, barley, rice, sugarcane, etc.) is not possible by the dry-ashing procedure. Therefore, this kind of plant material should be wet-digested using HNO_3-$HClO_4$. The digestion procedure is adapted from Rashid (1986). Many other elements (e.g., P, K, Ca, Mg, and Na) can also be determined in the same digest.

Apparatus

Block-digester
Vortex tube stirrer
Atomic absorption spectrophotometer
Flame photometer

Reagent

Nitric Acid-Perchloric Acid (HNO_3-$HClO_4$), 2:1 ratio

Add 1-L concentrated HNO_3 to 500 mL concentrated $HClO_4$.

Procedure

A. Digestion

1. Weigh 1 g dry and ground plant material, and then transfer quantitatively into a 100-mL Pyrex digestion tube.
2. Add 10 mL (**2:1 ratio) nitric acid-perchloric acid** mixture, and allow to stand overnight or until the vigorous reaction phase is over.
3. Place small and 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 HNO_3 disappear, and then remove U shaped glass rods.
7. Raise temperature to 235 °C. When the dense white fumes of $HClO_4$ appear in the tubes, continue digestion for 30 minutes more.
8. Lifts the tubes rack out of the block-digester, cool a few minutes, and add a few drops DI water carefully through the funnel.
9. After vapors condense, add DI water in small increments for washing down walls of tubes and funnels.
10. Bring to volume, mix the solution of each tube, and then leave undisturbed for a few hour.
11. Each batch of samples for digestion should contain at least one reagent blank (no plant material).

B. Measurement

1. Operate **Atomic Absorption Spectrophotometer** or **Flame Photometer** according to the instructions provided for the equipment.
2. Run a series of suitable standards, and draw a calibration curve.
3. Decant the supernatant liquid and analyze Zn, Fe, Mn, and Cu in the aliquots by **Atomic Absorption Spectrophotometer**, Ca and Mg by **Titration** with **EDTA**, and K and Na by **Flame Photometer**.
4. Calculate the supernatant liquid concentrations according to the calibration curve.

Calculations

$$\boldsymbol{Zn\ Fe, Cu\ or\ Mn\ (ppm) = ppm\ Zn\ Fe, Cu\ or\ Mn\ (from\ calibration\ curve) \times \frac{V}{Wt}}$$

$$\boldsymbol{Na\ or\ K\ (ppm) = ppm\ Na\ or\ K\ (from\ calibration\ curve)\ \times \frac{V}{Wt}}$$

Where:

V = Total volume of the plant digest (mL)
Wt = Weight of dry plant (g)

Technical Remarks

1. **Heated mixtures of $HClO_4$ and organic matter (OM) may explode violently**. Avoid this hazard by taking the following precaution: (a) do not add $HClO_4$ to a hot solution containing organic matter; (b) always pre-treat samples containing organic matter with HNO_3 before adding $HClO_4$.
2. Filtrate may be capped and refrigerated overnight.
3. Samples should be allowed to equilibrate to room temperature before analyses.

7.5. Heavy Metals

For the release of mineral elements from plant tissues, ***dry-ashing*** *and* ***wet oxidation*** *are the two widely adopted methods. Critical factors in wet digestion procedures include selection of the digestion vessel, temperature and its control, time, the digestion mixture, and final volume. Selection of a digestion vessel is dependent on the elements of interest and the heat source. Time and temperature are interrelated and are dependent on the digestion mixture. Wet oxidation employs oxidizing acids such as HNO_3-H_2SO_4-$HClO_4$ (tri-acid mixture) or HNO_3-$HClO_4$ (di-acid).* ***Use of $HClO_4$ avoids volatilization losses of P, K and S and provides a clear solution, while H_2SO_4 helps completing oxidation.*** *The wet oxidation method being easier, less time-consuming and convenien, is given below.*

7.5.1. Tri-Acid (HNO3-H2SO4-HClO4), Block Digester

Apparatus

Digestion tube

Block-digester

Atomic Absorption Spectrophotometer

Reagent

Tri-acid mixtures, (HNO_3-H_2SO_4-$HClO_4$), concentrated

Mix grade concentration *HNO_3, H_2SO_4* and *$HClO_4$* in 10:1:4 ratio and let the mixture to cool.

Procedure

1. Weigh 0.5-1.0 g dry and ground plant material (0.15 mm) into a 300-mL calibrated digestion tube (or into a 150-ml conical flask).
2. Add 5 mL **concentrated H_2SO_4** (in the fume hood), and swirl carefully and then place tubes in the rack.
3. Place the tubes rack in the block-digester (or hot plate), and then place a glass funnel in the neck of the tubes. Slowly increase temperature setting to about 145 °C for 1 hour.
4. Add 5 mL **tri-acid mixtures** and heat it to 240 ^{o} C for to further 1 hour, and if possible, keep overnight to prevent excessive foaming.
5. Lift the tubes rack out of the block-digester, carefully place on a rack holder, and let tubes cool to room temperature.
6. Filter through Whatman No. 42 filter paper and bring to 50-mL volume.
7. Determine Fe, Mn, Cu, Zn, Cd, Ni, Pb, Co, and Cr by **Atomic Absorption Spectrophotometer**.
8. Each batch of samples for digestion should contain at least one reagent blank (no plant).

Notes

- The first stage of destruction should take at least 40 minutes, preferably more, so the HNO_3 can destroy all the easily oxidizable materials. **If this is not done explosions might take place.**
- As a second precaution, H_2SO_4 is present to dilute the $HClO_4$ in the final stage.
- Wash fume hood and chimney regularly. **Condensed $HClO_4$ might start a fire**. The ventilator should be of the centrifugal type.
- Sodium-nitrite reduces insoluble higher Mn oxides that might have formed.

7.5.2. Di-Acid (HNO3-HClO4) , Block Digester

Apparatus

Digestion tube

Atomic Absorption Spectrophotometer

Block-digester

Reagent

Di-acid digestion (HNO_3-$HClO_4$)

Mix grade concentration HNO_3 and $HClO_4$ in 9:4 ratios, and let it cool.

Procedure

1. Weigh 0.5-1.0 g dry and ground plant material into a 300-mL calibrated digestion tube (or into a 150-ml conical flask).
2. Add 5 mL **concentrated HNO_3** (in the fume hood), and swirl carefully and then place tubes in the rack. .
3. Place a glass funnel on the calibrated digestion tube and leave for about 6-8 hours.
4. After pre-digestion, add 10 mL **di-acid mixture**, swirl carefully.
5. Place the tubes rack in the block-digester, and then place a glass funnel in the neck of the tubes. Slowly increase temperature setting to about 180-200 °C until the dense white fumes evolve and transparent white contents are left.

6. Lift the tubes rack out of the block-digester, carefully place on a rack holder, and let tubes cool to room temperature.
9. Filter through Whatman No. 1 filter paper, and bring to 50-mL volume.
10. Determine Fe, Mn, Cu, Zn, Cd, Ni, Pb, Co, and Cr by **Atomic Absorption Spectrophotometer**.
11. Each batch of samples for digestion should contain at least one reagent blank (no plant).

7.5.3. Tri-Acid (HNO3-H2O2-HCl), Microwave digestion

Apparatus

Vessels, MF/HF

Atomic Absorption Spectrophotometer

Microwave

Reagent

Nitric Acid (HNO_3), concentrated

Hydrogen Peroxide (H_2O_2), concentrated (30%)

Hydrochloric Acid (HCl), concentrated

Procedure

1. Weigh 0.3-0.5 g dried and processed plant into MF/HF vessels of microwave.
2. Add 3 mL **concentrated HNO_3** (in the fume hood), and swirl carefully, 3 mL **concentrated H_2O_2** and then add 0.5 mL **concentrated HCl**. Insert the vessels into the rotor of microwave.
3. Set the microwave system as; temperature 100 °C/ vessel, ramp-10 minute, hold- 10 minute, and cool 30 minutes.
4. After cooling, filter through Whatman No. 42 filter paper, and bring to 50-mL volume.
5. Determine Fe, Mn, Cu, Zn, Cd, Ni, Pb, Co, and Cr, by **Atomic Absorption Spectrophotometer**.

Note

- Microwave digestion procedures require the use of specially designed ovens to handle acid fumes.
- The microwave exhaust should be removed to a scrubber before being released to a fume exhaust system.
- Special safety precautions are required for microwave digestion. Therefore, read the manufacturer's specifications for details.

Calculation

$$\boldsymbol{Trace\ elemental\ (ppm) = ppm\ Trace\ elemental\ \ (from\ calibration\ curve)\ \times \frac{V}{Wt}}$$

Where:

V = Total volume of the plant digest (mL)

Wt = Weight of dry plant (g)

Technical Remarks

1. Calcium and Mg can be determined in the di-acid digest of plant samples either using **AAS** or **Versenate titration**. When using AAS, lanthanum (La) must be added in the sample. Addition of 0.1-1.0% La prevent the interference of Al, Be, P, Si, Ti, V and Zn.
2. The Microwave oven should be checked routinely for leakage using an electromagnetic monitor.
3. The digestion vessel carousel should be rotated during digestion period. This ensures that all samples are subjected to the same microwave flux.
4. After HNO_3 digestion, samples must be cooled before adding H_2O_2. Otherwise, there is excessive frothing due to the reaction between H_2O_2 and hot acid digest.
5. Immediately before use, all glass, plastic ware and Teflon digestion vessels should be thoroughly rinsed first with dilute HCl (1:3/acid:DI water ratio) and then with double-DI water.
6. Screw caps are used to provide reflux action.
7. Reagents should be added to the samples in the fume hood.
8. Nitric acid is used in most wet-oxidation procedures. The addition of H_2SO_4 is to raise digestion temperature, while $HClO_4$ or 30% H_2O_2 are used to increase the speed of reaction and ensure complete digestion.
9. Most wet-digestion procedures can be completed using covered beakers on hot plates but digestion blocks are preferred due to enhanced temperature control.
10. **Wet-ashing** is recommended for plant materials that are high in Si.
11. **Wet-ashing results** in higher Fe and Al values than dry-ashing.

7.6. Silicon

***Silicon (Si)**, in the form of silicon dioxide (SiO_2), is extracted out of plant samples (and converted into silicic acid, H_3SiO_4) during oxidation of organic material, i.e., by elimination of carbon (C) by digestion with hydrogen peroxide (H_2O_2) under alkaline conditions. The H_3SiO_4 can be determined colorimetrically by reaction with molybdate; blue and yellow (this procedure) analyses methods are usually distinguished. The method presented follows the description provided by Korndörfer et al. (2004), who, in turn, expanded on the method of Elliot et al., (1991).*

Apparatus

Ventilated drying oven
Mechanical plant grinder
Plastic bottles/cups
Horizontal shaker
Magnetic stirrer
Water bath
Autoclave
Volumetric flasks
Spectrophotometer

Reagents

A. Hydrogen Peroxide Solution (H_2O_2), 500 g/ L

B. Sodium hydroxide (NaOH), 500 g /L

Dissolve 50 g granular *NaOH* in 50 mL DI water. Let the solution cool down to room temperature and store in plastic bottle.

C. Ammonium heptamolybdate tetrahydrate solution ($(NH_4)_6Mo_7O_{24}$. $4H_2O$)

- Dissolve 100 g $(NH_4)_6Mo_7O_{24}$. $4H_2O$ in 500 mL DI water.
- Adjust to pH 7 – 8 with 5 *N NaOH*, and bring to 1-L volume. Transfer solution into plastic bottle and store in fridge.

D. Hydrochloric acid solution (HCl), (37%, sp. Gr. 1.19), 12 *N*

Dilute 500 mL *concentrated HCl* in DI water, mix well, let it cool, and bring to 500-mL volume.

Transfer this solution into a plastic bottle (polyethylene).

E. Oxalic acid solution ($(COOH)_2 \cdot 2H_2O$), 1 *N*

Dissolve 75 g $(COOH)_2 \cdot 2H_2O$ in DI water, and bring to 1-L volume.

As an alternative to oxalic acid solution, use this solution in case oxalic acid is not available

Acetic acid solution (CH_3COOH), 200 g/L

Dissolve 20 g CH_3COOH in 100 mL of DI water.

F. Standard Stock Solution ($SiCl_4$ in 14 % NaOH), 50 ppm

- With Merck Titrisol® Silicon standard 1000 mg solution ($SiCl_4$ in 14% NaOH; Merck-I.N.: 109947): Open the capsule and let the Si-solution flow into a 1-L volume (to make sure that the 1000 mg of Si in the capsule is transferred completely to the flask, rinse the capsule thoroughly with DI water). Fill up with DI water (*Stock Solution*). *The Silicon standard 1000 mg solution is not a 1000 ppm solution*!
- Add 25 mL *stock solution* to a 500-mL volumetric flask and then bring to volume with DI water. This solution contains 50 ppm Si (*Diluted Stock Solution*).*This solution cannot be stored, and needs to be prepared fresh*!

Procedure

A. Pre-treatment

1. Mix and spread finely ground plant sample in a thin layer, on a sheet of paper or plastic until the sample looks uniform.
2. Take a representative sub-sample of about 1 g by systematically withdrawing at least 10 small portions from all parts of the sample with a spatula, and put them into a plastic vial.
3. Dry the sub-sample at 60 °C in an oven (overnight), and then cool in a desiccator.

B. Extraction

1. Weigh 0.1 g dry and ground plant material into 100-mL polypropylene tubes (e.g., round centrifuge tubes).

2. Add 2 mL **500 g/L H_2O_2** solution (under fume hood, using plastic gloves). Agitate for a few seconds with the magnetic stirrer.
3. Add 3 mL **500 g/L NaOH** solution and agitate again.

Note

Depending on the type of plant samples, the oxidation process could be quite intensive and the solution in the tube might spill/foam over. Thus, it is recommended to watch the reaction closely. The digestion can be controlled by agitation with magnetic stirrer.

4. Put the tubes in 85 °C water bath for 1 hour (**avoid potential spilling**).
5. Once the sample has stopped releasing gas, put the stoppers on the tubes and put them into an autoclave for 1 hour at 123 °C and 1.5 bar (= 150 kPa).
6. In case the plant material does not appear to be completely digested, add another 1 mL **500 g/L H_2O_2** solution, and put the sample(s) into the autoclave for another 1 hour.
7. Add 45 mL DI water.
8. Transfer the extracts into plastic cups (labeled). Leave the samples until the residues have settled at the bottom of the cup.

C. Measurement

1. Pipette a 1-mL aliquot of the supernatant extract-solution into a 50-mL plastic cup.
2. Add 19 mL **DI water** (2 mL of supernatant may be used for samples with low Si-content. Then only 18 mL DI water is added).
3. Prepare a series of standard solution from the *Diluted stock solution* 50 ppm as follows: Dilute 0, 2, 4, 6 and 8 mL into a 50-mL flask, and bring to volume with DI water.
4. Pipette 20-mL an aliquot of each standard into a 50-mL plastic cup.
5. Add to samples and standards 1 mL **500 g/L HCl** solution and 2 mL **ammonium heptamolybdate** solution. Shake carefully. Depending on the concentration of Si, a more or less intensive yellow color develops.
6. After 5-10 minutes, add 2 mL **oxalic acid** solution. Shake again carefully.

Note

The amount of added HCl should be proportional to the withdrawn amount of supernatant extract solution, because complexation of Si and development of a yellow color does not occur under alkaline conditions.

7. Read the absorbance of blank, standards, and samples on the **Spectrophotometer** at **410 nm wavelength**.
8. Prepare a calibration curve for standards, plotting absorbance against the respective Si concentration.
9. Read Si concentration in the unknown samples from the calibration curve.

Note

The yellow color is not very stable and will start disappearing already after only 15 minutes.

Calculation

$$\boldsymbol{Si\ (ppm) = ppm\ Si\ (from\ calibration\ curve)\ \times \frac{V}{Wt} \times Dilution\ Factor}$$

Where:

V = Total volume of the extract (mL)

Wt = Weight of dry plant (g)

Technical Remark

For quality control, a standard sample (such as wheat or rice chaff) might be analyzed every 20th sample, and analyses repeated if deviation from originally measured Si-content is >5 %.

8. Water Sampling and Processing

Water sampling and analysis *is a vital part in agricultural and environmental applications for studying the quality of water treatment process, distribution system, or source of water supply. Therefore, water sampling program starts with collections of samples which accurately represent the characteristics of the bulk material and handled conveniently in the laboratory while still providing test results. The major source of error in the whole process of obtaining water quality information often occurs during sampling.* ***Over 50 % of the faulty data that occur in laboratory test results are due to sampling error, rather than during laboratory analysis****. Much of what is presented here is based on standard methods for the examination of waters and wastewaters (APHA, 1998).*

8.1. Preparation for Sampling

Suitability of the containers

The correct sample container type and volume should be used for the required analysis in consultation with the laboratory requirements such as:

- Containers should be examined for cleanliness, ensuring it is strong and durable, so that it will not break in transit and that the cap does not leak once it is secured.
- Containers must **not** contain any of the compounds that samples are to be analyzed for.
- Containers must be suitable for sampling the water.
- Container must be of the appropriate size.
- Containers must be high density polyethylene or glass containers with Teflon® lid liners for most analyses.
- Rubber and cork stoppers must not be used so as to avoid the risk of contaminating the sample.

Decontamination of the container

Decontamination procedures involve washing and rinsing. The decontamination of the containers is of utmost importance, for most parameters:

- Rinse the sampling device and the bottle thoroughly with DI water or with the sample (discarding the rinse into the effluent).
- When some solid matter is adhered, use a brush with cleanser or something suitable to give it a pre-wash before taking the above action (Soaps are not suitable as they are liable to remain inside the container).
- The suitability of the cleaning procedure should be checked at regular intervals by filling a cleaned container with distilled or ion exchange water and then analyzing the water for the target analyses. If none are detected, then the cleaning procedure is shown to be suitable.
- For certain target analyses there are preferred cleaning procedures. **For example, for *nitrate* analysis the container should not be rinsed with nitric acid and for chloride analysis should not be rinsed with hydrochloric acid.**
- For most purposes, containers can be reused if they are cleaned with a non-phosphate detergent, rinsed with hot tap water, rinsed with dilute acid, and then re-rinsed with tap water, followed by distilled or ion exchange water and air drying.

Sampling techniques and equipment

Sampling techniques depend on the available equipment, the sampling site and the analyses to be performed. Some possible variables to be considered and decisions to be made include:

- Manual or automatic sampling equipment.
- Location of the sampling site, whether indoors, outdoors, or difficulties involved.
- Temporary or permanent sampler installation.
- Power availability, compressed air.
- Field equipment such as:
  - Multiple samplers or automatic composite samplers.
  - Chilled cool-boxes.
  - Meters.
  - Filtration apparatus.
  - Adequate trouble-shooting equipment for small repairs.

Recommended sampling procedure

The recommended procedure for collecting water samples is as follows:

- Water samples must be put in labeled suitable container or clear glass (label should be written in waterproof ink) depending on laboratory requirement. Ensure that the bottle is adequately labeled so it can be identified at all stages of the transport and analytical process.
- The laboratory requirements for analysis are a minimum of 500-mL depending on required analyses (one liter would be suitable).
- Rinse the bottle at least 4 times with the water to be sampled.
- Do not dispose of the rinse water where it may contaminate or mix with that which is to be sampled.
- Take 5 sub-samples (100-200 mL each) to make up the full sample.
- Fill the bottle to the top, leaving little or no air space and seal tightly with the cap.
- All information about water samples is recorded on the sample container and in the field log book, e.g., the number with the site code, time and date of collection, parameter to be tested, sampler's name, and other details to avoid mix-ups. Each sample is given a laboratory number.
- Sketch your field. Diagram it the way you sampled it. Be sure sampled areas are labeled the same as sample containers. (This is so you have a record of which recommendations apply to which areas – *do not rely on your memory*).
- Information sheet should be clearly written with copying pencil. Fill out the information sheets. The more information you can provide with each sample, the better your recommendation will be.
- Use plastic bottles for inorganic and metals, and glass bottles for organics.
- Sterile containers for bacteriological analysis
- Filtering for metals if a preservative is being used.
- Every time you collect a sample, you should record it on the field data sheet.
- Clearly mark and identify the separate sample containers from each location.
- Certain observations and measurements before sending it to the laboratory. These observations should include appearance (color, haziness, turbidity, oil on the surface, and odor). Also, some measurements are valid only on a fresh sample (temperature, dissolved oxygen and pH).
- Containers must be closed until ready to analysis.
- All water samples should be stored in chilled cool-boxes prior to dispatch to laboratory.

8.2. Water Sampling Processing

Representative sample

A representative sample is taken from a large body of water to make up a sample (sub-samples of 500 mL) which represents the whole body of water. To minimize the effect of natural variation in the data collected, the sample should be collected:

- From exactly the same location
- At approximately the same time each day
- In the same way
- Analyze them in the same way

Water samples can be taken directly from the body of water (stream or dam), or if a pump is used, from the first outlet along the supply line. Below, some instructions for taking a representative of water samples:

- ***Stream***: Sample taken from main stream flow.
- ***Stock drinking water***: Sample taken from near the edge.
- ***Dam***: If water is pumped from a dam, and the sample is not taken from the outlet side of the pump, take the sample from near where suction side of the pump draws water. If wishing to assess changes in quality throughout the dam, where the water may settle into well-defined layers, it may be necessary to sample the chosen layers separately. The practicality of sampling from specific depths needs to be considered. The sample is taken from upper layers first.
- ***Underground source***: When water is drawn from an underground source, i.e. a bore or well, this is often the only way to sample. Allow the pump to run for sufficient time to flush out water which has been in the pipe, then take samples at time intervals of 5-10 minutes, from the first off take point, e.g., tap, trough, or sprinkler head.
- ***Reticulated source*:** If the water is drawn from a reticulated source, e.g., irrigation scheme, city or town water supply, check with the local supplying authority, as they may have analytical data on the chemical and other characteristics of the water.
- ***Water irrigation***: Where the water is used for irrigation or piped for livestock or domestic purposes, the simplest approach is normally to let the pump do the sampling.

Type of water sampling

The two types of samples most commonly obtained are **grab samples** and **composite samples**:

- ***Grab samples*** are single samples collected at a specific spot at a site over a short period of time not exceeding 15 minutes. Thus, they represent only the composition of its source at the time and place of collection. A grab sample is required for test parameters which must be measured immediately after collection, and for tests which require the entire contents of a single sample container for analysis or for tests where the parameter will change over time. Examples of test parameters which require grab samples are *temperature, pH, residual chlorine, and dissolved oxygen.*
- ***Composite samples*** are obtained by taking an appropriate number of grab samples collected at equal intervals or proportional to flow. Flow proportional composite samples are collected when the flow and waste characteristics are continually changing. This single sample reflects the average conditions of a point-source during an interval. The nature of the composite sample requires the tested parameters to be stable in the container for the duration of sampling, often 24 hours (this is usually means that the samples are refrigerated to 4 °C).

Protecting and preserving samples

The physical and chemical characteristics of a water sample can change during transport and storage. Therefore, protecting and preservation methods only slow chemical and biological changes that inevitably occur after collecting sample. Some parameters change more quickly than others, and so need to be analyzed on site.

Protecting and preservation of samples is the main thing to obtain good quality result (see Appendix No. 18 for more details):

1. The most common preservative approach is **cooling to 4 °C**. This can be achieved by making slurry of ice and water and placing the collected sample in the slurry. This serves to slow biological activity in the sample and keep dissolved gases in solution. However, it can hasten certain physical processes such as precipitation of metals.
2. Other common preservatives (prevent known chemical reactions and maintain a target analyte in solution) include:

- Use of sodium thiosulfate (final concentration is 0.008 %) to remove chlorine.
- Use of copper sulfate or mercuric chloride to halt biological activity.
- Use of zinc acetate to trap sulfides.
- Appropriate pH adjustment to either, over 12 SU (Standard Units) with sodium hydroxide (NaOH) or less than 2 SU with hydrochloric acid (HCl), sulfuric acid (H_2SO_4) or nitric acid (HNO_3) (depending on the intended analyze) can inactivate biological processes.

3. The analyst must be aware that acidification of nitrate-nitrite samples with HNO_3 or preservation of SO_4 samples with H_2SO_4 will invalidate the analyses, so the sampler must make adequate notation.
4. Samples which are not preserved must be analyzed immediately.

Handling and storage of samples for analysis

- As soon as the water samples are received at the water analysis laboratory, they should be checked with the accompanying information list (including sample number and date of sampling should be written on the container. Information regarding samples should be entered in a register and each sample be given a *laboratory number*.
- The sample(s) should be kept refrigerated or on ice in a cooler (but not allowed to freeze) as soon as possible. Changes caused by growth of microorganisms are best slowed by cold and dark.
- In general, the shorter the time between collection and analysis, the more reliable the results will be.

Health and safety

- Laboratory personnel who are routinely exposed to water samples, that may contain live microorganisms, are encouraged to protect themselves from water-borne illnesses by wearing clean disposable gloves and washing their hands frequently.
- Care must be taken when handling hazardous reagents by using protective clothing and safety goggles.

9. Water Analysis

Water is essential for all aspects of life, domestic use, industrial processes, and agricultural production. Its suitability for a particular purpose can be determined by analysis. Obtaining good results depends to a great extent on the following factors:

- Ensuring that the sample taken is truly representative of the water under consideration
- Using proper sampling techniques
- Protecting and preserving the samples until they are analyzed (in a timely manner)

The water and wastewater should be checked for chemical, physical (turbidity and solid matter) and biological contamination before used it, to avoid environmental pollution (most of diseases can be spread to plant, animal, and human by water contaminated).

9.1. pH

pH *is a measure of how acidic or alkaline a solution is; or the power or potential of the hydrogen [H_3O^+] ion. pH stands for the negative logarithm of H_3O^+* - or in short *H^+ - ion activity, in mol/L.*

$$pH = -\log [H^+]$$

The underlying reason is the slight dissociation of water molecules:

$$2\ H_2O \rightleftarrows H_3O^+ + OH^-$$

In pure water at room temperature, the product of $[H^+] \times [OH^-]$ is equal to $1 \times 10^{-14}\ mol^2/L^2$.

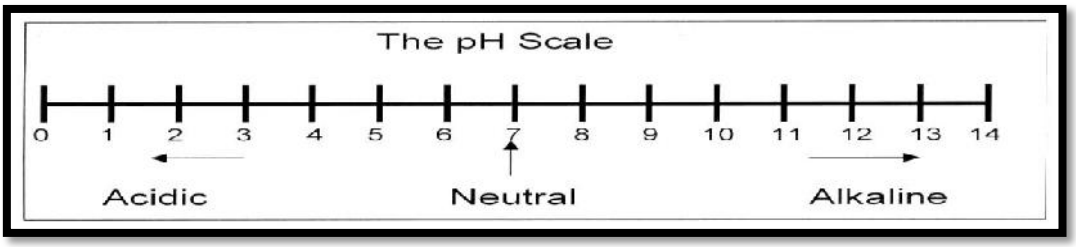
Thus, the pH of pure water is:

$$pH = -\log (1 \times 10^{-7}) = -(-7) = 7$$

Keep in mind that the logarithmic scale includes a factor of 10 in regard to changes in concentration of H ion. For example, a solution which has a pH of 3 contains 10 times as many H^+ ions as the same volume of a solution with a pH of 4, 100 times as many as one with a pH of 5, a thousand times as many as one of pH of 6, and so on.

The scale of measurement is from 1 to 14. However, on a pH scale:

- **Reading of 7 is neutral**
- **Reading of more than 7 is alkaline**
- **Reading of less than 7 is acidic**



Aqueous solutions with higher $[H^+]$ than $[OH^-]$ are acidic, and will have pH values <7; basic solutions have pH > 7, i.e. $[H^+] < 1 \times 10^{-7}$.

The pH of rainwater *is about 5.5-6.0. Typically, natural water has pH 6.5-8.5. Natural alkalinity is due to the dissolution of CO_2 either of atmospheric nature or from carbonate rocks such as limestone and dolomite.*

Alkalinity is increased by caustic substances from industry (KOH, NaOH), soil additives in agriculture such as lime $Ca(OH)_2$, superphosphate which is mixture of $Ca(H_2PO_4)_2$ and $CaSO_4$, and soaps and detergents. Natural acidity is due to CO_2 (g), HPO_4, H_2PO_4, H_2S, Fe, other acidic metal ions, proteins and organic acids. Increases in acidity can be due to acids used in industry, acid mine drainage, and acid rain.

Apparatus

pH meter with Combined Electrode

Beakers*:* Preferably use polyethylene or TFE beakers

Mechanical stirrer, with inert plastic coating

Wash bottle, plastic

Reagents

A. Potassium Chloride (KCl), 0.01 *M*

Dissolve 0.745 g *KCl* in DI water, and bring to 1-L volume.

B. Buffer solutions for pH 4 and 7

pH 4.0

- Dry about 15 g *potassium dihydrogen phthalate ($C_8H_5O_4K$)* in an oven at 110 °C for 3 hours.
- Dissolve 10.12 $C_8H_5O_4K$ in freshly DI water, and bring to 1-L volume.

pH 7.0

- Dry about 5 g *potassium dihydrogen phosphate* (KH_2PO_4) and *sodium hydrogen phosphate* (Na_2HPO_4) in an oven between 110 °C and 130 °C overnight.
- Dissolve 3.388 g KH_2PO_4 and 3.533 g Na_2HPO_4 in freshly DI water, and bring to 1-L volume.

Note

The DI water should be freshly boiled, cooled, and free of CO_2.

C. Distilled water (DI)

Boil and cool DI water having conductivity less than 2 µmhos/cm.

Procedure

1. Calibrate the pH meter (see Box No.3 for more details)
2. Take a 50-mL water sample in 100-mL flask, Put the **combined electrode** in the water sample (about 3-cm deep). Take the reading after 30 seconds.
3. Remove the **combined electrode** from the sample, and rinse thoroughly with DI water in a separate beaker, and carefully dry excess water with a tissue.

Technical Remarks

1. Make sure that the combined electrode contains *saturated* KCl solution and some solid KCl.
2. 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. However, Keep electrodes wet by returning them to storage solution, generally have a conductivity >4000 µmhos/cm, such as tap water (better substitute than DI water), pH 4.01 buffer is best for the single-glass electrode, saturated KCl is preferred for a calomel and Ag/AgCl reference electrode, and saturated KCl is the preferred solution for a combination electrode.
3. If there is adequate amount of water available for testing, discard the sample used for pH determination as it may be affected by slight leakage from types of saturated potassium chloride-calomel reference electrodes. If there is a limited supply of liquid for analysis, determine EC first, then pH; the same sample may subsequently be used for cation-anion analyses except K and Cl.
4. ACS-grade chemicals are generally satisfactory for preparing buffer solutions.
5. In preparing buffer solutions from solid salts, ensure complete dissolution.

9.2. Electrical Conductivity

Electrical Conductivity *(EC) is the measure of the ability of a solution to carry an electric current or the concentration of soluble salts in the sample at any particular temperature. The EC measurement is affected by dissolved CO_2, turbidity, temperature and the nature of various ions, their relative concentration and the ionic of water.*

The EC is measured in the field or in the laboratory using a conductivity meter, which is basically a Wheatstone bridge that measures the resistance of the solution between two parallel platinum electrodes. Conductivities range from less than 0.02 dS/m for distilled water to more than 20 dS/m for highly saline waters.

Apparatus

Conductivity meter

Conductivity cell, pipette-type or dip-type

Thermometer, covering room temperature

Beaker, 50 or 100-mL

Reagents

Potassium Chloride Solution (KCl), 0.01N

- Dry about 2-3 g *KCl* in an oven at 110 ^{o}C for at least 2 hours.
- Dissolve 0.7456 g *KCl*, in DI water, and bring to 1-L volume. The solution should be transferred to a plastic flask. The stability of the solution is one year at most.

Procedure

A. Pre-treatment the conductivity cell

1. Calibrate the conductivity meter according to maker's instructions (see Box No.4 for more details).
2. Rinse the **conductivity cell** thoroughly with DI water, and carefully dry excess water with a tissue.
3. Rinse the **conductivity cell** thoroughly with measured solution a few times.

B. Measurement

1. Put about 75-mL water sample in a 100-mL glass beaker, and then put the clean and dried conductivity cell in the glass beaker.
2. Take the reading. The display will also need some time to stabilize before the reading.
3. Remove the conductivity cell from the glass beaker, rinse thoroughly with DI water, and carefully dry excess water with a tissue.

Technical Remarks

1. It is essential that the manufactures instructions be followed.
2. Cleaning of the **conductivity cell** is needed if contaminated.
3. The reference temperature should be 25 °C, and the result expressed in dS/m. If the measurement is carried out at a different temperature, the result should be corrected to 25 °C. 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 $^{\circ}$C**.
4. 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/cm = 1 mmho/cm**.

9.3. Total Dissolved Solids

Total Dissolved Solid *(TDS) is defined as the substances remaining after evaporation and drying of a water samples. The remaining fraction is approximately equivalent to the total content of the dissolved and suspended matter in the water sample. Non-filterable residue corresponds to the* ***Total Suspended Solids*** *(TSS) and the filterable residue is the TDS. Determination of TDS can be used to check the accuracy of analyses when relatively complete analysis has been made on a water sample. This is accomplished by comparing the value of calculated TDS with the measured value. Ion concentration, in mg/L of constituents, required to calculate the TDS are as follow*:

Calculated TDS= 0.6 (alkalinity) =Na+K+Ca+Mg+Cl+SO_4+SIO_3+ (NO_3-N) +F

The measured TDS concentration should be higher than the calculated value, because a significant contributor may not be included in the calculation. If the measured value is less than the calculated value, all values are suspect and probably in error. If the measured value is higher than the calculated value, the low ion sum is suspect and selected constituent should be reanalyzed. The acceptable ratio is:

$$\mathbf{10} < \frac{\boldsymbol{Measured\ TDS}}{\boldsymbol{Calculated\ TDS}} < 1.2$$

Principle

A well mixed, measured portion of a sample is filtered through a standard glass-fiber filter and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180 ^{o}C. The increase in dish weight represents the total dissolved solids.

Apparatus

Evaporating dishes made porcelain, platinum or high-silica glass

Steam bath

Desiccators provided with a desiccant containing a colour indicator for moisture concentration

Analytical balance capable of weighing to 0.001 g

Filtration apparatus

Glass-fiber filters

Suction flask

Drying oven

Procedure

A. Pre-treatment of filter and crucible

1. Put the evaporating dishes for at least 1 hour in an oven at 180 ^{o}C. Cool and store in desiccators until needed. Weigh immediately before use.
2. Prepare the glass-fiber disc by placing it, wrinkled side up, in the filtration apparatus. Apply vacuum and wash the disc with 3 successive 20-mL washings of DI water. Continue suction to remove all traces of water. Discard washings.

B. Determination

1. Put an aliquot of sample to yield between 2.5 and 200 mg dried residue.
2. Filter measured volume of well mixed water sample through the glass-fiber filter;
3. Wash with 3 successive 10-mL volumes of DI water, allowing complete draining between washing.
4. Continue suction for about 3 minutes after filtration is complete.
5. Transfer filtrate to a weighed evaporating dish (***Wt_d***) and evaporate to dryness on a steam bath.
6. Dry for at least 1 hour in an oven at 180 ^{o}C, cool in desiccators, and weigh (***Wt_{d+s}***).
7. Repeat the cycle of drying, desiccating and weighing until a constant weight is obtained or until weight loss between successive weighing is less than 4 % or 0.5 mg which is less.

Calculation

$$\boldsymbol{Total\ Dissolved\ Solids\ (mg/L)} = \frac{\boldsymbol{Wt_{d+s} - Wt_d}}{\boldsymbol{V}} \times \mathbf{1000}$$

Where:

Wt_{d+s} = Weight of dish plus solids (mg)

Wt_d = Weight of dish before use (mg)

V= Volume of water sample used for measurement (mL)

9.4. Total Suspended Solids

Total Suspended Solids *(TSS) apply to dry weight of the material that is removed from a measured volume of water sample by filtration with a standard filter. The test is basically empirical and is not subject to usual criteria of accuracy. To achieve reproducibility and comparability of results requires close attention to procedural details, especially filter characteristics and time and temperature of drying.*

Apparatus

- Filter holder
- Desiccators provided with a desiccant containing a colour indicator for moisture concentration
- Analytical balance capable of weighing to 0.001 g
- Glass-fiber filter disc, Whatman GF/C or equivalent
- Suction flask apparatus, 500 mL capacity
- Vacuum pump
- Crucible
- Drying oven
- Buchner funnels

Procedure

A. Pre-treatment of filter disc and crucible

1. Place a filter disc on the filter holder. Assemble filter holder in suction flask apparatus, connect to vacuum source and apply vacuum.
2. Wash the filter disc with 3 successive 20-mL portions of DI water. Continue to apply vacuum for 2-3 minutes after the water has passed through the filter. Discard the filtrate.
3. Remove the filter paper from the membrane filter funnel or the Buchner funnel and place it on a supporting surface in drying oven.
4. Place the crucible (s) in the drying oven. The oven should be maintained at 105 ^{o}C and drying should be continued for at least 1 hour.
5. Cool the filter (s) and crucible (s) in desiccators and weigh it on an analytical balance.
6. Repeat the cycle of drying, desiccating and weighing until the weight loss between two successive series operations is less than 0.5 mg.
7. Store filter (s) and crucible (s) in desiccators until required.

B. Determination

1. Remove the filter disc and crucible from the desiccators, and weigh ($\boldsymbol{Wt_{f+c}}$).
2. Place the filter in the filter holder and assemble the filter holder in the suction flask apparatus. Connect to the vacuum source and apply vacuum.
3. Wet the filter with a few drops of DI water to seat the filter.
4. Shake the sample vigorously and measure out 100 mL in a 100-mL graduated cylinder or volume flask. Pour this portion of the sample into the filter funnel (be careful not to disturb the placing of the filter disc).

5. Rinse out the measuring flask or cylinder with a small quantity of DI water. If the sample is very low in suspended material, a large volume of sample may be used.
6. When filtration is complete, carefully remove the filter disc from the filter holder with tweezers (or remove the crucible from its supporting socket with a pair of tongs), and place it in the drying oven.
7. Dry for at least 1 hour at 105 °C. Cool in desiccators, and weigh ($\boldsymbol{Wt_{f+c+s}}$).
8. Repeat the drying, desiccating and weighing cycle until the weight loss between 2 successive weighing is less than 0.5 mg.
9. Record the final weight obtained.

Calculation

$$\boldsymbol{Total\ Suspended\ Solids\ (mg/L)} = \frac{\boldsymbol{Wt_{f+c+s} - Wt_{f+c}}}{\boldsymbol{V}} \times \mathbf{1000}$$

Where:

Wt_{f+c+s} = Weight of filter and crucible plus solids (mg)

Wt_{f+c} = Weight of filter and crucible before use (mg)

V = Volume of water sample used for measurement (mL)

Technical Remarks

1. It is necessary to report the specifications followed, e.g., TSS at …°C, type of filter and pore size or number.
2. Non-homogeneous particulates such as leaves, sticks, and lumps of fecal matter should be excluded from the sample. Too much residue on the filter will retain water and may require prolonged drying.
3. Long-term preservation of a sample is not practical; analysis should therefore begin as soon as possible. Transportation and short-term storage of sample normally does not affect the results of the test.
4. Volatile material in a sample can distort results.

9.5. Nitrogen

9.5.1. Ammonium Nitrogen and Nitrate Nitrogen

In water and wastewater the forms of N of greatest interest are NO_3-N, NO_2-N, NH_3 and organic forms of N. These N forms are interchangeable, being components of the N cycle. Ammonia is present naturally in surface water and wastewater. Its concentration is generally low in ground waters because it is adsorbed by soil particles and clay and is not leached readily from soils. Nitrate in groundwater is a major environmental and public health concern. Nitrates are converted to nitrites in the intestines. Once absorbed into bloodstream, nitrites prevent hemoglobin from transporting oxygen (older children have an enzyme that restores hemoglobin). ***High NO_3-N levels in drinking water (>10 ppm) are linked with health problems*** *(i.e., methemoglobinemia). Thus, while using water with high NO_3-N. For human consumption should be avoided, such water is a suitable for irrigation as it is an additional source of nutrients.*

Principle

Ammonium nitrogen and NO_3-N plus NO_2-N are determined by steam distillation, using ***heavy MgO*** *for NH_4 and* ***Devarda's Alloy*** *for NO_3 .The distillate is collected in saturated H_3BO_3 and titrated to pH 5.0 with dilute H_2SO_4.*

Apparatus

Distillation unit

Stirrer

Automatic titrator connected to a pH-meter

Reagents

A. 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.

B. 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 (0.05 mm).

D. Boric Acid Solution (H_3BO_3), saturated

- Add 500 g H_3BO_3 into a 5-L volume.
- Add 3-L DI water, and swirl vigorously.
- Leave overnight.

Note

There should always be solid H_3BO_3 on the bottom of the flask.

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

- Dry reagent-grade *Tris* in an oven at 80 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle.

- Dissolve 1.2114 g *Tris* in DI water, and bring to 1-L volume.

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

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

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 h, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 5.6605 g $(NH_4)_2SO_4$ and 8.6624 g KNO_3 in DI water, and bring to 1-L volume. This solution contains (1.2 g NH_4-N, and 1.2 g NO_3-N)/L (*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 KCl* 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

A. Pre-treatment of the distillation unit

1. The distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7-8 mL distillate/minute.
2. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.

B. Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the **0.01 *N* H_2SO_4** in the **Auto-Titrator** by titrating three separate 10-mL aliquots of the primary standard, **0.01 *N* Tris** solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found. The **H_2SO_4** normality is:

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

3. Carry out distillations as follows:

To determine NH_4-N

- Pipette 20 mL water or wastewater sample into a 100-mL distillation flask.
- Pipette 1 mL **saturated H_3BO_3** solution and 1 mL DI water into a 50-mL beaker (duplicate beakers).
- Place the first beaker underneath the condenser tip, with the tip touching the solution surface.
- Add about 0.2 g **heavy MgO**, with a calibrated spoon, to the distillation flask.
- Immediately, attach the distillation flask to the distillation unit with a clamp.

- Start distillation, and continue for 3 minutes, then lower the dish to allow distillate to drain freely into the Pyrex evaporating dish or beaker.
- After 4 minutes, when 35-mL distillate or more is collected, turn off the steam supply and remove the distillation flask (***first distillate***).
- Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NH_4-N from *Diluted Stock Solution*) and two blanks (pipette 20 mL 2 KCl solution). Recovery of NH_4-N should be at least 96 %.

To determine NO_3-N and NO_2-N

- Place the second beaker underneath the condenser tip, with the tip touching the solution surface.
- Immediately, add 0.2 g **Devarda's alloy**, with a calibrated spoon, **to the same distillation flask**, then attach back to distillation unit with a clamp, and start distilling.
- After 4 minutes, when 35-mL distillate or more is collected, turn off the steam supply and remove the distillation flask (***second distillate***).
- Wash tip of the condenser into Pyrex evaporating dish or the beaker with a small amount of DI water.
- Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NO_3-N from *Diluted Stock Solution*) and two blanks (pipette 20 mL DI water). Recovery of NO_3-N should be at least 96 %.

C. Titration

Titrate the first distillate (for ammonia) and the second distillate (for nitrate), separately, to **pH 5** with standardized **0.01 *N* H_2SO_4** using an **Auto-Titrator**.

Notes

- After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish.
- Between different samples, steam out the distillations. Disconnect distillation flasks containing the water sample, 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 distillation for different samples, not between ammonium (MgO) and nitrate (Devarda's alloy) in the same sample.

Calculation

$$NH_4 - N \text{ or } NO_3 - \mathrm{N}\ (ppm) = \frac{(V - B) \times N \times 14.01 \times 1000}{V_1}$$

Where:

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

B = Distillate blank titration volume (mL)

N = Normality of H_2SO_4 solution

V_1 = Volume of water sample used for distillation (mL)

14.01 = Atomic weight of N

Technical Remarks

1. If possible, NH_4-N and NO_3-N should be determined immediately after sampling. If the analysis cannot be done immediately, water and wastewater samples may be kept refrigerated (4 °C).
2. All water for reagents and dilution should be NH_3-free. DI water is usually satisfactorily.
3. Filter paper may contain traces of NH_4, so the first 20-25 mL of filtrate should be discarded.
4. Never use acid preservation for samples to be analyzed for NO_3 or NH_4.

9.5.2. Total Nitrogen

The Kjeldahl procedure *is a good estimate of N content in the water or waste water samples. This procedure involves digestion and distillation. The water or waste water sample 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, which 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).*

Apparatus

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

Reagents

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

Grind reagent-grade chemicals separately and mix. If caked, grind the mixture in a porcelain pestle and mortar to pass a 60-mesh screen (0.250 mm), taking care not to 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 *NaOH* in DI water, transfer to a 1-L heavy-walled Pyrex flask, let it cool, and bring to volume.

D. Boric Acid Solution (H_3BO_3), saturated

- Add 500 g H_3BO_3, to a 5-L flask.
- Add 3 L DI water, and swirl vigorously.
- Leave overnight.

Note

There should always be solid H_3BO_3 on the bottom of the flask.

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

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

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

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

G. Standard Stock Solution

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

Procedure

A. Digestion

1. Pipette 20-mL aliquot of the water sample into a 100-mL calibrated digestion tube.
2. Add about 3.0 – 3.5 g **catalyst mixture,** a few **pumice boiling granules**, add 10 mL **concentrated H_2SO_4** (in the fume hood) and then swirl carefully. Place the tubes in the rack and put a glass funnel in the neck of the tube.
3. Place the tubes rack in the block-digester, and slowly increase temperature setting to about 370 - 380 °C. The H_2SO_4 should condense about half-way up the tube neck; 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.
6. Each batch of samples for digestion should contain at least one reagent blank (DI water sample), and one chemical standard (DI water sample, pipette 1 mL *Stock Solution*).

B. Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the **0.01 *N* H_2SO_4** in the **Auto-Titrator** by titrating three separate 10-mL aliquots of the primary standard, **0.01 *N* Tris** solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found. The **H_2SO_4** normality is:

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

3. Carry out distillations as follows:
   - Dispense 1 mL **saturated H_3BO_3** solution and 1 mL **DI water** into a 100-mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
   - Pipette 20-mL aliquot into a 100-mL distillation flask, and add 10 mL **10 *N* NaOH** 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 an **Auto-Titrator**.
   - Each distillation should contain at least two standards (pipette 10 mL digested water sample) and two blanks (pipette 10 mL digested water blank). Recovery of NH_4-N should be at least 96 %.

Notes

- After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish.
- Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100-mL empty distillation flask to distillation unit. Place a 100-mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds.

Calculations

$$\boldsymbol{Recovery}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \mathbf{14.01} \times \mathbf{100}}{\boldsymbol{V_3} \times \boldsymbol{C}}$$

$$\boldsymbol{N}\ (\%) = \frac{(\boldsymbol{V} - \boldsymbol{B}) \times \boldsymbol{N} \times \mathbf{V_1} \times \mathbf{14.01} \times \mathbf{100}}{\boldsymbol{V_2} \times \mathbf{1000}}$$

Where:

V = Volume of 0.01 *N* H_2SO_4 titrated for sample (mL)
V_1= Total volume of the digest (mL)
V_2= Volume of water digest used for distillation (mL)
B = Digested blank titration volume (mL)
N = Normality of H_2SO_4 solution
14.01 = Atomic weight of N
V_3 = Volume of NH_4-N standard solution (mL)
C = Concentration of NH_4-N standard solution (µg/mL)

9.6. Phosphorus

Phosphorus *compounds are present in fertilizers and in many detergents. If surprising a critical level in the soil, they are carried into surface waters, sewage, industrial wastes, and storm runoff. In contrast to N, inorganic P forms are highly reactive and do not readily leach through soils. High concentration of P compounds may produce a secondary problem in water bodies where algal growth is normally limited by phosphorus. In such situations, the presence of additional P compounds can stimulates algal productively and enhance eutrophication.*

Phosphorus occurs in natural waters and in wastewaters as phosphate. Groundwater rarely contains more than 0.1 mg/L phosphate if not polluted by organic matter. Phosphorus is essential to the growth of organisms and can be the nutrient that limits biological growth in water bodies.

Principle

Phosphorus can be determined by ***vanadomolybdo phosphoric acid*** *(colorimetric methods), where are the ammonium molybdate and potassium antimony tartrate react in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid- that is reduced to an intensely colored molybdenum blue by ascorbic acid.*

9.6.1. Available Phosphorus

Apparatus

Spectrophotometer or colorimeter

Standard laboratory glassware: beakers, volumetric flasks, pipettes, funnels

Reagents

A. Sulfuric Acid Solution (H_2SO_4), 5 *N*

Dilute 148 mL *concentrated* H_2SO_4 (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume.

B. *p*-nitrophenol Indicator, 0.25 % w/v

C. Reagent A

- Dissolve 12 g *ammonium heptamolybdate* $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250 mL DI water **(a)**.
- Dissolve 0.2908 g *antimony potassium tartrate* $(KSbO.C_4H_4O_6)$ in 100 mL DI water **(b)**.
- Add both dissolved Reagents **(a)** and **(b)** to a 2-L flask.
- Slowly add 1-L 5 *N* H_2SO_4 to the *mixture*. Mix thoroughly, and dilute to 2-L volume.
- Store in a dark Pyrex bottle, cool place.

D. Reagent B

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

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 KH_2PO_4 in DI water, and bring to 1-L volume .This solution contains *500 ppm P* (*Stock Solution*).
- Dilute 50 mL *Stock Solution* to 250 mL 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 numbered flasks by adding DI water, and then bring to volume. These solutions contain 1, 2, 3, 4, and 5 ppm P, respectively.

Procedure

1. Pipette a suitable aliquot of clear filter water sample (10 mL natural water sample) into a 50-mL Erlenmeyer volumetric flask.
2. Add the required acid to all the water samples to bring the solution pH to 5.0 (see Technical Remark No. 1**)**.
3. Add 8 mL **Reagent B**, and dilute to 50-mL volume with **DI water**, mix well.
4. 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 only DI water, and proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 10 minutes on the **Spectrophotometer** at **882 nm wavelength**.
6. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
7. Read P concentration in the unknown samples from the calibration curve.

Calculation

$$P\ (ppm) = ppm\ P\ (from\ calibration\ curve) \times \frac{V_1}{V}$$

Where:

V = Volume of water sample used for measurement (mL)

V_1 = Volume of flask used for measurement (mL)

Technical Remarks

1. The required acid amount to acidify the water sample to pH 5.0 (using 5 *N* H_2SO_4). This can be done by taking 10 mL DI water and adding 2-3 drops of phenolphthalein indicator, if red color appears, add 5*N* H_2SO_4 till the color disappear. Then add the required amount of acid to all the water samples (adding 1 mL 5 *N* H_2SO_4 is adequate to acidify each 10 mL water sample).
2. If the sample solutions are too dark-colored for measurement against the highest standard, smaller water should be taken, and the calculation modified accordingly. *Once the blue color has developed, the solution cannot be diluted*.
3. Glassware used in P analysis should not be washed with detergents containing P (Most detergents contain P).
4. As glass tube density may vary, it is best to use the same tube (cuvette) for each absorbance reading on a spectrophotometer.
5. ***Interference*:** Arsenates (As) react with the molybdate (Mo) reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium (Cr) and NO_2 interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

9.6.2. Total Phosphorus

Organically-bound P and all phosphates forms are first converted to orthophosphate by an acid-persulfate digestion. To release P from combination with organic matter, a digestion or wet oxidation is necessary.

Principle

The digestion sample is analyzed for orthophosphate based on its reaction with a combined reagent containing ammonium molybdate, antimony potassium tartrate, and ascorbic acid to form intensely-colored ***molybdenum blue****.*

Apparatus

Spectrophotometer or colorimeter
Block digester
Digestion tube, 250 mL capacity
Standard laboratory glassware: beakers, pipettes, funnels, volumetric flasks and cylinders,

Reagents

A. Sodium Hydroxide Solution (NaOH), 5 *N*

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

B. Sulfuric Acid Solution (H_2SO_4), 5 *N*

Dilute 140 mL *concentrated H_2SO_4* solution (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume.

C. Perchloric Acid ($HClO_4$), concentrated

D. Nitric Acid (HNO_3), concentrated

E. phenolphthalein Indicator, 1%

Dissolve 1 g *phenolphthalein* indicator in 100 mL *ethanol*.

F. Reagent A

- Dissolve 12 g *ammonium heptamolybdate* $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250 mL DI water **(a)**.
- Dissolve 0.2908 g *antimony potassium tartrate* $(KSbO.C_4H_4O_6)$ in 100 mL DI water **(b)**.
- Add both dissolved Reagents **(a)** and **(b)** to a 2-L flask.
- Slowly add 1-L 5 *N H_2SO_4* to the *mixture*. Mix thoroughly, and dilute to 2-L volume.
- Store in a dark Pyrex bottle, cool place.

G. Reagent B

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

H. 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 2.197 g dried *KH_2PO_4* 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 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 numbered flasks by adding DI water, and then bring to volume. These solutions contain 1, 2, 3, 4, and 5 ppm P, respectively.

Procedure

A. Digestion

1. Add a suitable aliquot of a well-mixed water sample (10 mL natural water sample), into a 250-mL calibrated digestion tube. Rinse the cylinder twice with DI water.
2. Add 5 mL **concentration HNO_3**, and a few **pumice-boiling granules**, mix well, and then place tubes in the rack.
3. Place the tubes rack in the block-digester and gently heat to evaporate to about 15-20 mL (it takes 1-1.5 hour).
4. Cool, and then add 10 mL (**2:1 ratio) concentration HNO_3** and **$HClO_4$ mixture.**
5. Again, place the tubes rack in the block-digester and slowly increase the block-digester temperature to 180 °C and digest the samples until dense white fumes of **$HClO_4$** appear (it takes 1.5-2 hours).
6. If solution is not clear, continue heating at the boiling temperature for 15-20 minutes longer. If necessary, add 10 mL more **concentration HNO_3**to aid oxidation.
7. Cool the mixture, and add small amount of DI water, mix well, and then bring to 50-mL volume with DI water. If necessary, filter through Whatman No. 1 filter paper.

B. Measurement

1. Pipette 5-10 mL of the sample digest into a 50-mL flask.
2. Add 0.5 mL **5 *M* NaOH** and 1 drop **phenolphthalein** solution to each sample and mix well.
3. Add 0.4 mL **6 *M* NaOH** until the solution just turns pink, and then add **6 *M* H_2SO_4** until the pink color just clears.
4. Add **DI water** to about 40-mL volume, add 8 mL **Reagent B**, and then bring to volume.
5. Prepare a standard curve as follows:
   - Pipette a suitable volume of each standard (0.2 – 2 ppm), and proceed as for the samples.
   - Also, make a blank, and proceed as for the samples.
6. Read the absorbance of blank, standards, and samples after 10 minutes on the **Spectrophotometer** 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

$$\boldsymbol{P\ (ppm)\ =\ ppm\ P\ (from\ calibration\ curve)\ \times \frac{V_1}{V} \times \frac{V_3}{V_2}}$$

Where:

V = Total volume of the water sample used for digestion (mL)
V_1 = Total Volume of the digest (mL)
V_2 = Volume of digest used for measurement (mL)
V_3 = Volume of flask used for measurement (mL)

Caution

$HClO_4$ is explosive in presence of easily oxidizable organic matter. Avoid this hazard by taking the following precaution: (a) do not add $HClO_4$ to a hot solution containing high organic matter; (b) always pre-treat samples containing organic matter with HNO_3 before adding $HClO_4$.

Technical Remarks

1. No interferences are normally observed for Cu, Fe, or Si. However, high concentrations of Fe can cause precipitation of, and subsequent loss, of P.
2. Arsenate may interfere when present at concentration higher than P.
3. Phosphate adsorbed on glass surface may affect measurements at low P levels. Use of acid-washed glassware dedicated to this analysis prevents this interference.

9.7. Potassium

*Although **potassium (K)** is a relatively abundant element, its concentration in natural fresh waters is usually less than 20 mg/L. Brines and seawater, however, may contain as much as 400 mg/L K or more. Potassium in water can be determined by **flame photometry**.*

Principle

The estimation of K is based on the emission spectroscopy, which deals with excitation of electrons from ground state to a higher energy state and coming back to its original state with the emission of light.

Apparatus

Flame photometer with accessories
Beakers
Pipettes and volumetric flasks, as required for dilution and tests of interference effects

Reagents

Standard Stock Solution

- Dry 3-5 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 *KCl* in DI water, and bring to 1-L volume. 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 numbered flasks by adding DI water, and then bring to volume. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm K, respectively.

Procedure

1. Filter a portion of water sample through Whatman no. 42 filter paper.
2. For operating the **Flame Photometer**, follow the maker's instructions.
3. Calibrate **Flame Photometer** with a series of suitable K standards with DI water as blank sample.
4. Measure the water samples, take the emission reading on the **Flame Photometer** at **767-nm wavelength**, and record the readings.
5. Switch off the Flame Photometer according to the maker's instructions.
6. Draw a calibration curve.
7. Calculate K concentrations according to the calibration curve.

Calculations

$$\boldsymbol{K\ (ppm)\ =\ ppm\ K\ (from\ calibration\ curve)}$$

$$\boldsymbol{K\ (meq/L) =\ ppm\ K\ (from\ calibration\ curve)/39.1}$$

Where:

39.1 = Atomic weight of K

Technical Remarks

1. Check the performance of the photometer at frequent intervals by spraying some of the standard solutions and adjust the sensitivity as necessary.
2. If K concentration is higher than the top standard, make an appropriate dilution. If this is a dilution of the original sample, multiply by the appropriate factor.

9.8. Sodium

Sodium (Na) *is a common element, the sixth most abundant, and present to some extent in most natural waters. Sodium is present in a number of minerals, the principal one being rock salt (sodium chloride). The increased pollution of surface and ground water during the past decade has resulted in a substantial in the Na content of drinking water in different regions of the world. Sewage, industrial effluents, sea water intrusion in coastal area, and the use of Na compounds for corrosion control and water-softening processes all contribute to Na concentration in water because of the high solubility of sodium salts and minerals.*

Sodium levels in groundwater vary widely but normally range between 6 and 130 mg/L. In surface water the Na concentration may be less than 1 mg/L or exceed 300 mg/L depending upon the geographical area. Sodium may present a problem in drinking water if the Na in water exceeds 20 mg/L.

Principle

Sodium in the water sample can be determined by flame photometry emitting a sparkling yellowish-red color.

Reagents

A. Lithium Chloride (LiCl), 1000 ppm

- Dissolve 6.109 g dry *LiCl* 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 volume. This solution contains 100 ppm LiCl (*Diluted Stock Solution*).

B. Standard Stock Solution

- Dry 5 g *sodium chloride* (*NaC*l) in an oven at 110 °C for 3 hours, cools in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 2.5418 g dried *NaCl* in DI water, and bring to 1-L volume. 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 numbered flask by adding DI water, and 25 mL LiCl *Diluted Stock Solution,* and then bring to volume. 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. Filter a portion of water sample through Whatman filter paper No. 42.
2. For operating the **Flame Photometer**, follow the maker's instructions.
3. Calibrate **Flame Photometer** with a series of suitable Na standards with DI water as blank sample.
4. Measure the water samples, take the emission reading on the **Flame Photometer** at **589-nm wavelength**, and record the readings.
5. Switch off the flame photometer according to the maker's instructions.
6. Draw a calibration curve.
7. Calculate Na concentrations according to the calibration curve.

Calculations

$$\boldsymbol{Na\ (ppm)\ =\ ppm\ Na\ (from\ calibration\ curve)}$$

$$\boldsymbol{Na\ (meq/L) =\ ppm\ Na\ (from\ calibration\ curve)/23}$$

Where:

23 = Atomic weight of Na

Technical Remarks

1. In the analysis of natural waters, the only ions which may cause serious interference to Na measurements is calcium; in some types of flames, this tends to enhance the Na emission and the effect may be measured for a range of Ca concentrations.
2. Sodium ratios and appropriate corrections applied or the interference may be neutralized by the addition of aluminum (Al) ion, usually as aluminum nitrate.

9.9. Calcium and Magnesium

Calcium (Ca) *is dissolved easily out of almost all rocks and is, consequently, detected in most waters.* ***Magnesium*** *(Mg) are relatively abundant in the earth's crust and hence a common constituent of natural water. Waters associated with granite or siliceous sand usually contain less than 10 mg of calcium per liter and less than 5 mg magnesium per liter.*

Many waters from limestone areas may contain 30-100 Ca/L, and those associated with gypsiferous shale may contain several hundred milligrams per liter. But for the water in contact with dolomite or Mg-rich limestone may content 10-50 mg/L and several hundred milligrams per liter may be present in water that has been in contact with deposits containing sulfates and chlorides of magnesium.

Calcium and Mg contribute to the total hardness of water*. It should be noted that the difference between total hardness and the Ca concentration can be used to calculate the magnesium concentration. However, some $CaCO_3$ is desirable for domestic waters because it provide a coating in the pipes which protects them against corrosion.*

Principle

EDTA-disodium salt solution *is used to chelate Ca + Mg. Calcium is separately estimated by the versenate method using ammonium purpuate (Murexide) indicator, when the pH is made sufficiently high that the Mg is largely precipitated as hydroxide and an indicator is used that combines with Ca only. Thus, Mg can be obtained by deduction of Ca from Ca+Mg content. Both cations can also estimated by atomic absorption spectrophotometer.*

Apparatus

Burette, 25 or 50-mL
Pipette
Stirring rods
Graduated cylinder
Beakers

Reagents

A. Buffer Solution (NH_4Cl-NH_4OH)

Dissolve 67.5 g *NH_4Cl* in 570 mL *concentrated NH_4OH*, and transfer the solution to a 1-L flask, let it cool, and bring to volume.

B. Eriochrome Black Indicator

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

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

Dissolve 2 g *EDTA*, and 0.05 g *magnesium chloride* (*$MgCl_2$*) in DI water, and bring to 1-L volume.

D. Sodium Hydroxide Solution (NaOH), 2 *N*

Dissolve 80 g *NaOH* in about 800 mL DI water, transfer the solution to a 1-L flask, cool, and bring to volume.

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*

- Dry about 3 g $CaCO_3$ in an oven at 100 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 0.5 g dried $CaCO_3$ in 10 mL 3 *N hydrochloric acid (HCL)* and bring to1-L volume with DI water.

An alternative preparation

Standard stock 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 a suitable aliquot of water sample (10 mL natural water sample) into a 250-mL Erlenmeyer flask.
2. Dilute to 20 – 30 mL with **DI water**, add 2 – 3 mL **2 *N* NaOH** 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.

B. Calcium plus Magnesium

1. Pipette a suitable aliquot of water sample (10 mL natural water sample), 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.
7. In order to standardize the EDTA solution used in the determination of Ca and Mg:

- Pipette 10 mL 0.01 *N calcium chloride* solution, and treat it as in determining Ca and Ca+Mg procedure, respectively.
- Take the reading, and calculate **EDTA** normality:

$$N_{EDTA} = \frac{10 \times N_{CaCl_2}}{V_{EDTA}}$$

Where:

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

Calculations

$$Ca\ or\ Ca + Mg\ (meq/L) = \frac{V_1 \times N \times 1000}{V}$$

$$Mg\ (meq/L) = Ca + Mg\ (meq/L) - Ca\ (meq/L)$$

Where:

V_1 = Volume of EDTA titrated for the sample (mL)

N = Normality of EDTA solution

V = Volume of water sample used for measurement (mL)

Technical Remarks

1. Normality with Ca determination usually is 3 to 5% higher than with Ca + Mg.
2. If an Atomic Absorption Spectrophotometer is used, a small aliquot of the water sample is sufficient to determine Ca and Mg.
3. Orthophosphate precipitates Ca at pH of the test. Strontium (Sr) and barium (Ba) interfere with the Ca determination, and alkalinity in excess of 300 mg/L may cause an indistinct end-point with hard waters. Under the conditions of the test, normal concentration of the following ions causes no interference with the Ca determination: Cu, Fe, Mn, Zn, Al, Pb, Cu, and Sn.
4. **Calcium:** Low Ca intake can be related to hypertension and cardiovascular disorders. There is a link between low Ca intake and osteoporosis. With a low level of Ca in the diet, drinking water may provide a significant portion of the daily Ca requirement.
5. **Magnesium:** An average adult ingests as much as 480 mg of Mg daily. Any excess amounts are quickly expelled by the body. No upper limit has been set for this metal in drinking water. It can, however, create a problem for people with kidney disease. They may develop toxic reactions to high levels of Mg, including muscle weakness, coma, hypertension, and confusion.
6. **Hardness:** In most water nearly all of the hardness is due to Ca and Mg, which react with soap to form precipitates .This in increases soap consumption, and react with certain constituents to form scale. As a general rule, a value less than 60 is considered soft, and values above 200 are considered very hard.

9.10. Carbonate and Bicarbonate

Carbonates *(CO_3) and* ***bicarbonates*** *(HCO_3) in water can be determined by titration a known volume of water against standard H_2SO_4 using* ***phenolphthalein*** *and* ***methyl orange*** *indicators, respectively.*

Principle

Carbonates

When the pH value of a sample of natural water is above 8.4, the CO_3 is present, normally as sodium carbonate. The carbonate ion is converted to HCO_3. The amount of acid used a measure of the carbonate present.

$$\mathbf{CO_3 + H = HCO_3}$$

Bicarbonate

Bicarbonate ions react with mineral acid and release carbon dioxide (CO_2) into the solution.

$$\mathbf{HCO_3 + H = H_2O + CO_2}$$

The pH value at complete neutralization being about 3.8, HCO_3 ions are present. Thus HCO_3 may be measuring by titration with mineral acid to a pH 3.8, either potentiometrically or using an indicator unaffected by CO_2. Methyl orange is suitable and gives a good color change from green through grey (end-point) to red, which avoids the need for a matching buffer solution.

Apparatus

Pipettes
Burette
Erlenmeyer flasks
Graduated pipette
Magnetic stirrer

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 volume with DI water.

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

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

C. Phenolphthalein Indicator, 1%

Dissolve 1 g *phenolphthalein* indicator in 100 mL *ethanol*.

Procedure

1. Pipette a suitable aliquot of water sample (10 mL natural water sample) in a wide-mouthed porcelain crucible or a 250-mL Erlenmeyer flask.
2. Add 1 drop **phenolphthalein** indicator. If pink color develops, add **0.01*N* H_2SO_4** by a burette, drop by drop, until the color disappears.
3. Take the reading, ***y***.

4. Continue the titration with **0.01 *N* H_2SO_4** after adding 2 drops **0.1% methyl orange** indicator until the color turns to orange.
5. Take the reading, ***t***.

Calculations

$$CO_3\ (meq/L) = \frac{2y \times N \times 1000}{V}$$

$$HCO_3\ (meq/L) = \frac{(t - 2y) \times N \times 1000}{V}$$

Where:

2 = Valance of carbonate
Y = Volume of titrant against phenolphthalein indicator (mL)
T = Volume of titrant against methyl orange indicator (mL)
V = Volume of water sample used for measurement (mL)
N = Normality of H_2SO_4 solution

Technical Remarks

1. Standard HCl is used because H_2SO_4 may give rise to turbidity from calcium sulfate with Ca-rich samples.

2. **The alkalinity of water** is a measure of its capacity to neutralize acids and is due primarily to the presence of bicarbonates. The acceptable alkalinity for municipal water supplies is generally between 30 and 500 mg/L as $CaCO_3$, but there are many water supplies above and below these limitations. Waters with alkalinity greater than 500 mg/L as $CaCO_3$ have objectionable tastes.

9.11. Chloride

Chloride *(Cl) anions are usually present in natural waters. A high Cl concentration occurs in waters that have been in contact with Cl-containing geological formations. Otherwise, high Cl content may indicate pollution by sewage or industrial wastes or by intrusion of seawater or saline water into a freshwater body or aquifer.* ***A salty taste in water dependents on the ions with which the Cl are associated****. With Na ions the taste is detectable at about 250 mg/L, but with Ca or Mg the taste may be undetectable at 1,000 mg/L. Chlorides being highly soluble is present in all waters but the amount is often very low in natural waters.* ***High Cl content has a corrosive effect on metal pipes and structures and is harmful to most trees and plants****.*

Principle

The determination of Cl is done by $AgNO_3$ *(Mohrs's titration) method, which is based upon the fact that in solution containing Cl and chromate (Cr). Silver reacts with all the Cl and precipitates before the reaction with chromate begins. The appearance of the brick-red colour of the silver chromate precipitate is the end-point of the titration.*

Apparatus

Burette and stand

Beakers

Volumetric flask

Reagents

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

- Dissolve 5 g K_2CrO_4 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 $AgNO_3$ in an oven at 105 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered and brown bottle.
- Dissolve 1.696 g dried $AgNO_3$ in DI water, and bring to 1-L volume.

C. Sodium Chloride Solution (NaCl), 0.01 *N*

- Dry about 3 g *NaCl* in an oven at 140 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered and brown bottle.
- Dissolve 0.585 g *NaCl* in DI water, and bring to 1-L volume.

Procedure

1. Pipette a suitable aliquot of water sample (10 mL natural water sample) into a 250-mL Erlenmeyer flask.
2. Add 4 drops **potassium chromate** solution.
3. Titrate against **$AgNO_3$** solution until a permanent reddish-brown color appears.
4. In order to standardize the $AgNO_3$ solution used in the determination of Cl:
   - Titrate 10 mL **0.01*N* *NaCl*** solution against **0.01 *N* $AgNO_3$** after adding 4 drops ***potassium chromate*** solution until a permanent reddish-brown color appears.
   - Take the reading, and calculate **$AgNO_3$** normality:

$$N_{AgNO_3} = \frac{10 \times N_{NaCl}}{V_{AgNO_3}}$$

Where:

N_{AgNO3} = Normality of $AgNO_3$ solution
V_{AgNO3} = Volume of $AgNO_3$ solution used (mL)
N_{NaCl} = Normality of NaCl solution

Calculation

$$Cl\,(meq/L) = \frac{V_1 \times N \times 1000}{V}$$

Where:

V_1 = Volume of 0.01 *N* $AgNO_3$ titrated for the sample (mL)
N = Normality of $AgNO_3$ solution
V = Volume of water sample used for measurement (mL)

Technical Remarks

1. Natural waters are often low in Cl, and 10 mL is a suitable aliquot in most cases.
2. Saline waters may be high in Cl and 5 mL (or even less than 5 mL) may then be more appropriate aliquots.
3. The Cl estimation has two different purposes:
   - If the test is done regularly on a water supply and there is a sudden increase, it may indicate pollution due to ingress of sewerage or other chloride-rich wastes.
   - Many groundwaters have Cl content high enough to be of objectionable taste. By using the Cl test, the well with the lowest amount of Cl can be identified. If several well are being pumped, it can be planned in such a way that the lowest Cl content is obtained.

9.12. Sulfate

Sulfate *(SO_4) is an abundant ion in the earth's crust and its concentration in water can range from a few milligrams to several thousand milligrams/L. Industrial wastes and mine drainage may contain high concentration of SO_4, which also results from breakdown of SO_4-containing organic compounds. The SO_4 is not a notably toxic anion; however, SO_4 in water containing Ca forms hard scale in steam boilers. In large amounts, SO_4 in combination with other constituents gives a bitter taste to water. Concentrations above 250 mg/L may have a laxative effect, but 500 mg/L is considered safe.* ***The SO_4 in water is determined normally by barium sulfate ($BaSO_4$) precipitation****.*

Apparatus

Muffle furnace

Porcelain crucible

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 *HCl* with *DI water*.

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

Dissolve 122 g *$BaCl_2.2H_2O$* in DI water, and bring to 1-L volume.

Procedure

1. Pipette a suitable aliquot of water sample in a 250-mL Erlenmeyer flask or a wide-mouthed Pyrex beaker (50-mL natural water sample)
2. Add 1 mL **1:1 HCl** solution and 2 – 3 drops **methyl orange**; if the color does not turn pink; add some more 1:1 HCl.
3. Put Pyrex beakers on a hotplate, heat to boiling, and then add 10 mL **1 *N* $BaCl_2.2H_2O$** solution in excess to precipitate SO_4-S as barium sulfate ($BaSO_4$).
4. Boil for 5 to 10 minutes, cover with a watch-glass, and leave to cool.
5. Filter solution through ashless filter paper, collect the $BaSO_4$ precipitate on the filter paper, and then wash it several times with warm DI water until no trace of Cl remains. The presence of Cl 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 (***Wt_1***), 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 (***Wt_2***).

Calculation

$$SO_4 - S\ (meq/L) = \frac{Wt_2 - Wt_1}{V} \times \frac{1000}{0.1165}$$

Where:

Wt_2 = Weight of crucible + $BaSO_4$ precipitate (g)

Wt_1 = Weight of empty crucible (g)

V = Volume of water sample used for measurement (mL)

Note: 0.1165 g $BaSO_4$ equal to 1 milli-equivalent of SO_4

9.13. Boron

*In most natural waters **boron** (B) is rarely found in concentrations greater than 1 mg/L, but even this low concentration can have deleterious the effects on certain agricultural products. Water having B concentrations in excess of 2 mg/L can adversely affect many common crops. However, where levels are greater than 5 mg/L, toxicity may occur. Groundwater may have a greater B concentration, particularly in areas where the water comes in content with igneous rocks or other B-containing strata.*

The B content in many waters has been increasing as a result of the introduction of industrial waste and of the use of boric acid (H_3BO_3) and its salts in cleaning compounds. Ingestion of B at concentrations usually found in natural water has no adverse effects on humans. Ingestion of large quantities of B, however, can affect the central nervous system, while extended consumption of water containing B can lead to a condition known as borism.

*The **hot-water procedure** is still the most popular method for measuring B, and it was introduced by Berger and Truog (1939), and was modified by later researchers. The B is measured calorimetrically using **Azomethine-H** (Bingham, 1982).*

Apparatus

Erlenmeyer flasks

Polypropylene test tubes

Spectrophotometer or colorimeter

Reagents

A. Buffer Solution

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

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

Dissolve1 g *L-ascorbic acid* in 100 mL DI water, and then add 0.45 g *Azomethine-H*, and mix well. Fresh reagent should be prepared weekly and stored in a refrigerator.

C. Standard Stock Solution

- Dissolve 0.114 g *boric acid* (H_3BO_3) in DI water, and bring to 1-L volume. 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 numbered flasks by adding DI water, and then bring to volume. These solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm, respectively.

Procedure

1. Pipette 2-mL aliquot of the natural water sample into a 10-mL polypropylene tube.
2. Add 4 mL **buffer solution**.
3. Add 4 mL **Azomethine-H** solution, and mix well.
4. Prepare a standard curve as follows:

- Pipette 2 mL of each standard (0.5 – 3.0 ppm), and proceed as for the samples.
- Also make a blank with 2 mL DI water, and proceed as for the samples.

5. Read the absorbance of blank, standards, and samples after 30 minutes on the **Spectrophotometer** at **420-nm wavelength**.
6. Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations.
7. Read B concentration in the unknown samples from the calibration curve.

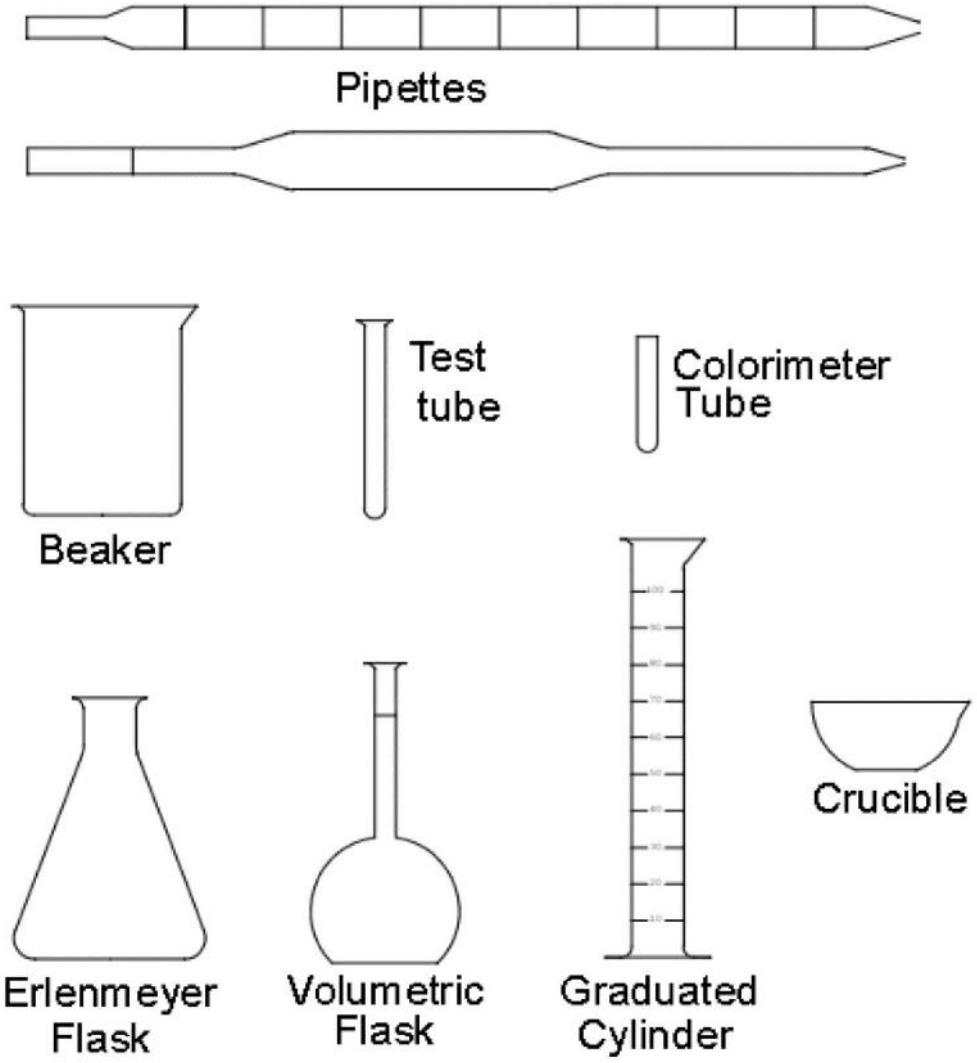
Calculation

$$\boldsymbol{B\ (ppm)\ = ppm\ B\ (from\ calibration\ curve)}$$

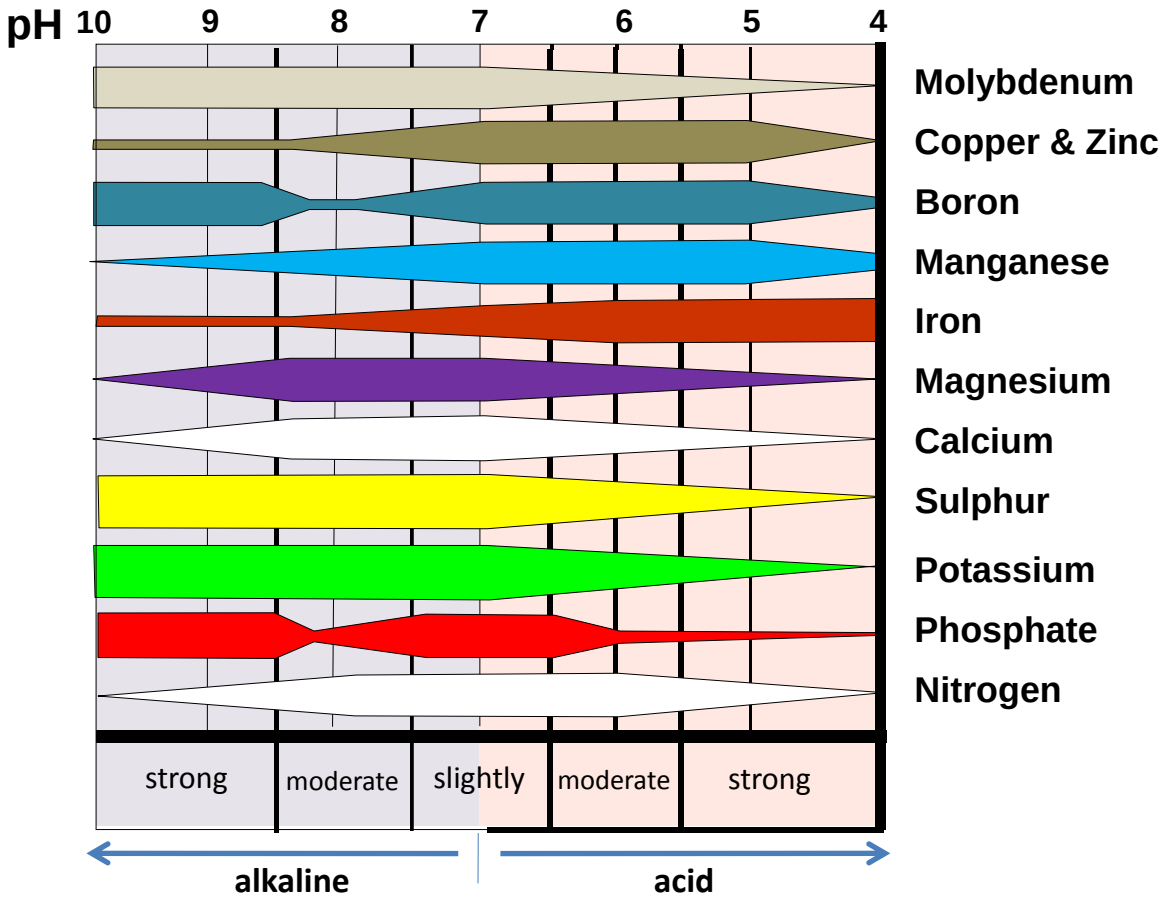
Technical Remark

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

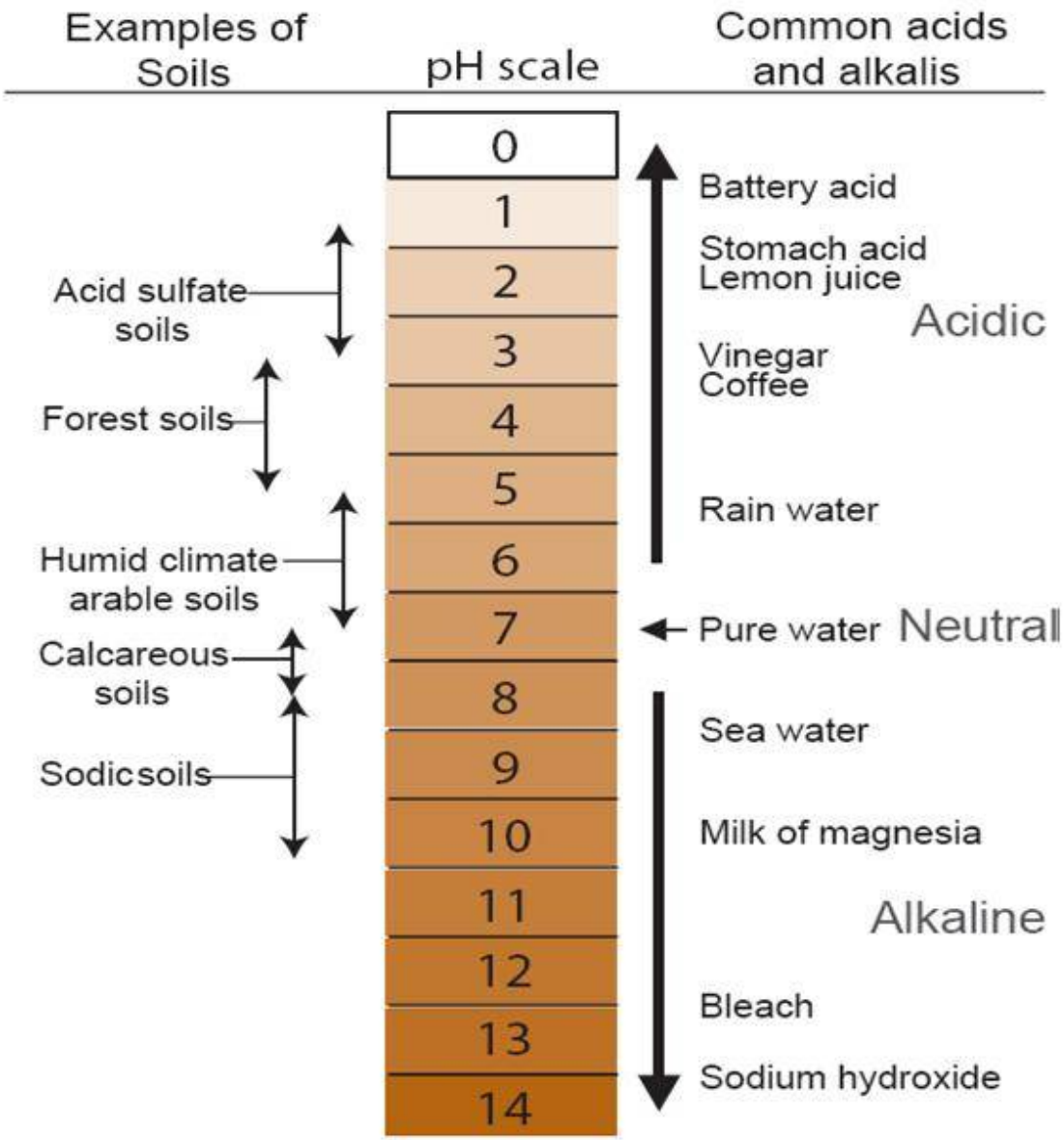
Box 1. Laboratory Equipments



Box 2. Influence of Soil pH on Plant Nutrient Availability



Box 3. Influence of pH on Soil and Common Acid and Alkalis



Box 4. pH Meter Calibration

Calibration pH meter

The pH meter should be calibrated daily. Use at least two buffer solutions of different pH values, usually 4.0 and 7.0, as follows:

1. Measure the temperature of the buffer used and adjusts the "temperature" knob for adjusting readings with temperature).
2. 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.
3. Repeat until pH meter gives correct reading of both buffer solutions (see below Table).
4. Wash the combined electrode thoroughly with DI water and carefully dry excess water with a tissue.

Relationship between pH values and temperature

T °C	10	20	30	37	40	50	60	70	80	90
pH 7.0	7.06	7.02	6.99	6.97	6.97	6.96	6.97	6.98	7.00	7.03
pH 4.01	4.00	4.00	4.02	4.03	4.04	4.06	4.09	4.13	4.16	4.30

General Instructions for pH Electrodes

a. Cleaning and maintenance

Cleaning the pH electrode that are new or that are providing erratic pH readings, as following:

1. Rinse the pH electrode with DI water, and then wipe the pH electrode with cloth or lens tissue.
2. Between measurements, leave the pH electrode in DI water.
3. Do not remove the electrode from solution until the meter is put on standby; otherwise, electrode polarization would occur.
4. Never allow sample solutions to dry on the electrode.
5. Do not rub electrode against the sides of the beaker.
6. Keep the pH buffer properly closed and never pour buffer solution back in the bottle.
7. Instrument should always be switched to standby or off position before removing electrode.

b. Storage

1. Keep the glass electrode in DI water or saturated KCl solution (saturated KCl solution is preferred) when not in use.
2. For short-term storage, store electrode in an electrode storage solution or a pH 4.0 to 7.0 buffer solution.
3. For long-term storage: clean the pH electrode with DI water and wipe it dry with soft paper or lens tissue.

Box 5. Electrical Conductivity Meter Calibration

Calibration EC meter

The EC meter should be calibrated daily as follows:

1. Measure the temperature of 0.01 *N* KCl.
2. Dip the platinum electrode in 0.01 *N* KCl, which should give a reading of 1.413 dS/m at 25 °C (See below table for adjusting readings with temperature).
3. Repeat until EC meter gives correct reading.
4. Wash the combined electrode thoroughly with DI water and carefully dry excess water with a tissue.

Relationship between EC values and temperature (using 0.01 *N* KCl)

T °C	20	21	22	23	24	25	26	27	28	29	30
EC	1.278	1.305	1.332	1.359	1.386	1.413	1.44	1.467	1.494	1.52	1.549

Conductivity of KCl solutions at 25 °C

Concentration of KCl (*N*)	0.001	0.01	0.02	0.05	0.1	0.2	0.5
Conductivity (dS/m)	0.147	1.413	2.767	6.668	12.9	24.82	58.64

General Instructions for Platinum Electrode

a. Cleaning and maintenance

Cleaning the platinum electrode that are new or that are providing erratic EC readings, as following:

1. Soak the platinum electrode in 32 mL of saturated sodium dichromate ($Na_2Cr_2O_7$) and then in 6 *M* H_2SO_4.
2. Rinse the platinum electrode three times with DI water.
3. Between the measurements leave the platinum electrode in DI water.

b. Storage

1. For short-term storage, immerse the platinum electrode in DI water.
2. For long-term storage, clean the electrode with DI water and wipe it dry with soft paper or lens tissue.

Box 6. Conductivity Reading and Soluble Salts

The general relationship between conductivity and soluble salts (solutes, solute conductivities, and equivalent weights) is:

Dissolved salt concentration (mg/L) = 640 X EC_e

Total cations or anions (mmolc/L or meq/L = 10 X EC_e

Osmotic potential ay 25 ^oC (KPa) = 0.39 X EC_e

Where:

ECe unit is dS/m; the factor for converting ECe to total dissolved salts (mg/L) ranges from 550 to 900 depending on the specific anions present and their concentration.

Box 7. Kjeldahl Method for Determining Nitrogen

The Kjeldahl method involves three main steps: **digestion**, **distillation**, and **titration**.

a. Digestion

Digestion is accomplished by boiling a homogeneous sample in concentrated sulfuric acid (H_2SO_4). The end result is an ammonium sulfate $(NH_4)_2SO_4$ solution. The general equation for the digestion of an organic sample is:

$$\textbf{Organic-N} + H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_4 + \textbf{other sample matrix by products}$$

b. Distillation

Excess base is added to the digestion product to convert ammonium (NH_4) to ammonia (NH_3) as indicated in the following equation. The NH_3 is recovered by distilling the reaction product.

$$(NH_4)_2SO_4 + 2\ NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2\ H_2O$$

Ammonia sulfate + heat → ammonia gas

c. Titration

Titration quantifies the amount of NH_3 in the receiving solution. The amount of N in a sample can be calculated from the quantified amount of NH_3 ion in the receiving solution. There are two types of titration:

In back titration (commonly used in macro Kjeldahl), the NH_3 is captured by a carefully measured excess of a standardized acid solution in the receiving flask. The excess of acid in the receiving solution keeps the pH low, and the pH does not change until the solution is "back titrated" with base.

$$2NH_3 + 2H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2SO_4$$

Ammonia + standard sulfuric acid → excess ammonium sulfate + sulfuric acid

$$(NH_4)_2SO_4 + H_2SO_4 + 2\ NaOH \rightarrow Na_2SO_4 + (NH_4)_2SO_4 + 2H_2O$$

Ammonia sulfate + measured excess acid + measured sodium hydroxide → sodium sulfate + ammonium sulfate

In direct titration, where boric acid is used as receiving solution instead of a standardized mineral acid, the chemical reaction is:

$$NH_3 + H_3BO_3 \rightarrow NH_4 + H_2BO^-_3 + H_3BO_3$$

Ammonia gas + boric acid → ammonium-borate complex + excess boric acid

The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the NH_3 collects, the pH of the receiving solutions changes.

$$2NH_4 + H_2BO^-_3 + H_2SO_4\ ((NH_4)_2SO_4 + 2H_3BO_3$$

Ammonium-borate complex + sulfuric acid + ammonium sulfate + boric acid

Box 8. 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., B or Cl). 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 WANA region can perform analyses for evaluating its quality for irrigation purposes. The EC of irrigation waters is usually expressed in units of deci-Siemens per meter (dS/m) at 25°C.

Calculations

$$\boldsymbol{Sodium\ Adsorption\ Ratio\ (SAR) = \frac{Na}{\sqrt{(Ca + Mg)/2}}}$$

Where:

Na, Ca and Mg represent the concentrations in meq/L of the respective ions in water (or solution).

$$\boldsymbol{Residual\ Sodium\ Carbonte(RSC) = (CO_3 + HCO_3) - (\ Ca + Mg)}$$

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	Residual Sodium 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

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.

Box 9. The "Feel" Method for Determining Soil Texture Class

Soil Texture Assessment Tests

Moist-Cast Test

Compress some moist soil by squeezing it in your hand. If the soil holds together (i.e., forms a cast), test the strength of the cast by losing it from hand to hand. The more durable it is, the higher the clay content.

Ribbon Test

Moist soil is rolled into a cylindrical shape and then squeezed out between the thumb and forefinger to form the longest and thinnest ribbon possible. Clay forms the longest, thinned ribbons.

Feel Tests

Graininess Test- Soil is rubbed between the thumb and forefinger to assess the sand percentage, where sand has a grainy feel. Silt, on the other hand, feels slippery.

Stickiness Test- Soil is moistened and compressed between the thumb and forefinger. The degree of stickiness is determined by noting how strongly it adheres to the thumb and forefinger upon release of pressure, and how much it stretches.

Shine Test

A small amount of moderately dry soil is rolled into a ball and rubbed once or twice against a hand, smooth object such as a knife blade or thumbnail. A shine on the ball indicates clay in the soil.

Texture	Moist Cast Test	Ribbon Test	Feel Test	Shine Test
Sand	No Cast	None	Very grainy	No shine
Loamy Sand	Very Weak Cast	None	Very grainy	No shine
Sandy Loam	Weak Cast	None	Grainy	No shine
Silt Loam	Weak Cast	Flakes (rather than ribbons)	Slippery, slightly grainy, slightly sticky	No shine
Loam	Moderate Cast	Thick, Short (< 1 cm)	Soft and smooth, slightly grainy, slightly sticky	No shine
Sand Clay Loam	Strong Cast	Thick, Short (< 3 cm)	Grainy, slightly to moderately sticky	Slight shine
Clay Loam	Strong Cast	Thin, barely supports own weight	Moderately grainy, sticky	Slight shine
Silty Clay	Very Strong Cast	Thin, long (5-7 cm), holds own weight	Smooth very sticky	Moderate shine
Clay	Very Strong Cast	Very thin, Very long	Smooth very sticky	Very shiny

Box 10. Salt and Sodium Hazard

SALINITY (Salt Hazard)

Class 1, **Low Salinity**: Good water with little or no likelihood of salt accumulation when normal irrigation practices are followed

Class 2, Medium Salinity: Can be used if a moderate amount of excess water is applied to provide some leaching. Plants with moderate salt tolerance can be grown without serious yield reduction when normal cropping practices are followed

Class 3, High Salinity: Cannot be used on clay soils or soils with poor drainage. With adequate drainage, considerable excess water should be applied to provide leaching. Irrigations must be more frequent than normal and soil should be maintained relatively wet. Crops with medium or high salt tolerance should be grown to maintain normal yields. This water is not recommended for use under sprinkler application methods unless leaching is practiced.

Class 4, Very High Salinity: Not suitable for irrigation under ordinary conditions. May be used occasionally on sandy soils with excellent drainage if considerable excess water is applied for leaching, and if crops with high salt tolerance are grown. Should not be used for continuous irrigation and is not suitable for use with sprinkler systems.

ALKALINITY (Sodium Hazard)

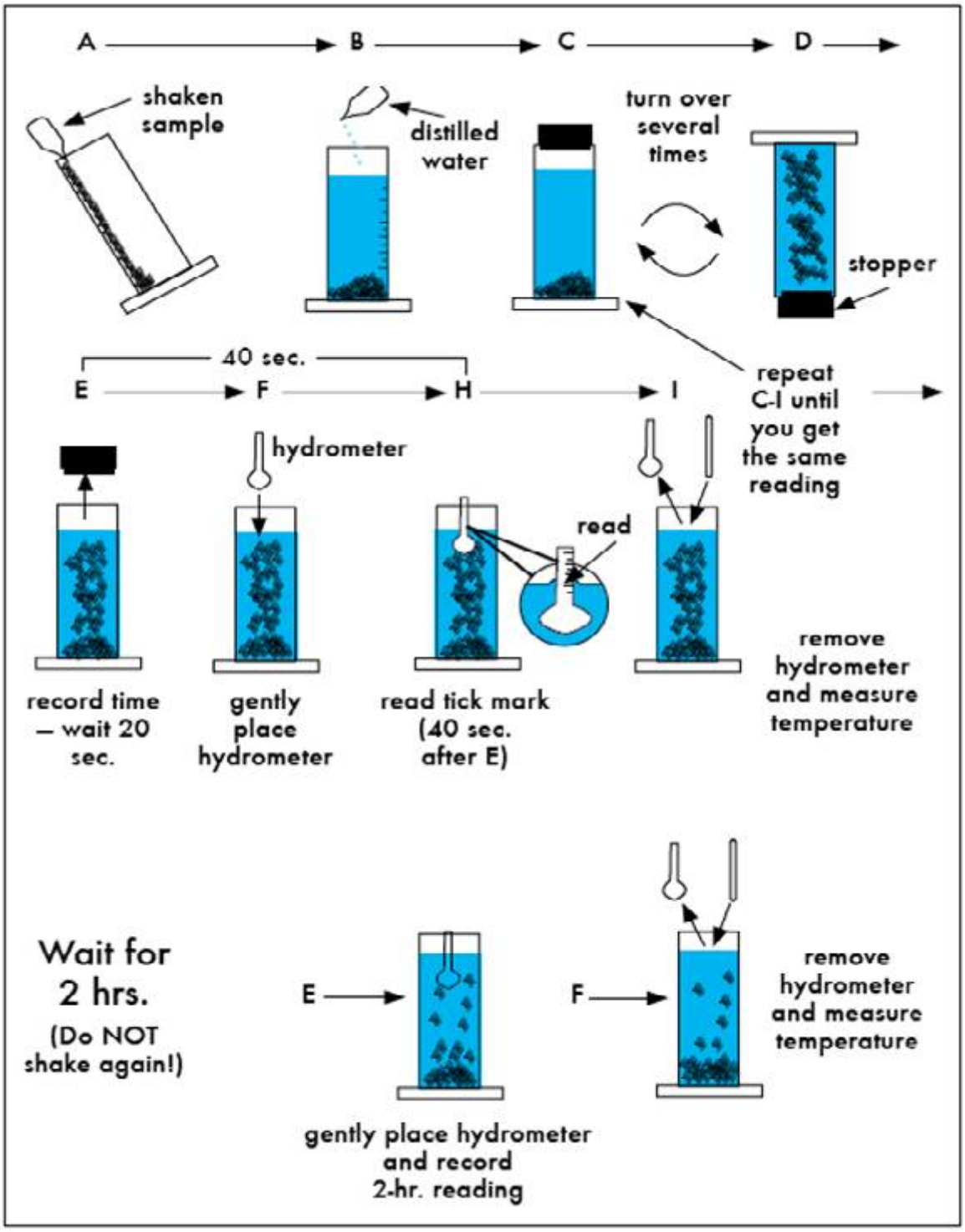
Class 1, Low Sodium: Good water that can be used safely on most soils in Wyoming

Class 2, Medium Sodium: Suitable for use on sandy and loamy soils if water moves through them readily. Can cause alkali problems on heavy clay soils, under low leaching conditions, unless gypsum (or equivalent soil amendments) are present or are added to the soil

Class 3, High Sodium: May produce harmful levels of exchangeable sodium in all soils and will require special management -- good drainage, high leaching and additions of organic matter. Soils containing natural gypsum or other chemical amendments may make the water usable, but it is not feasible if the water is also high in salinity (Class 3 or 4). This water will cause surface soil particles to puddle, thus limiting water penetration.

Class 4, Very High Sodium: Generally unsatisfactory for irrigation. Special conditions of low salinity water, favorable gypsum content of soils, high leaching and special management may permit use of this water. A complete soil analysis is recommended prior to any use of this water.

Box 11. Bouyoucos Hydrometer Method



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Appendix 1. Abbreviations

To convert column 1 into column 2, multiply by:	Column 1 SI Unit	Column 2 non-SI Unit	To convert column 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			
0.001 2.47	m hectare, ha	ha acre	10,000 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
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}	milliequivalents per 100 grams,	1
	(ion exchange capacity)	meq 100 g^{-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

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 column 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 (tone)	ton (U.S.), ton	0.907

1.102	tone, 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	tons 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
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}	milliequivalents per 100 grams,	1
	(ion exchange capacity)	meq 100 g^{-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

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

Element	Symbol	Atomic Number	Atomic Weight	Element	Symbol	Atomic Number	Atomic Weight
Actinium	Ac	89	227*	Mercury	Hg	80	200.59
Aluminum	Al	13	26.9815	Molybdenum	Mo	42	95.94
Americium	Am	95	243*	Neodymium	Nd	60	144.24
Antimony	Sb	51	121.75	Neon	Ne	10	20.183
Argon	Ar	18	39.948	Neptunium	Np	93	237*
Arsenic	As	33	74.9216	Nickel	Ni	28	58.71
Astatine	At	85	210*	Niobium	Nb	41	92.906
Barium	Ba	56	137.34	Nitrogen	N	7	14.0067
Berkelium	Bk	97	249	Nobelium	No	102	254*
Beryllium	Be	4	9.0122	Osmium	Os	76	190.2
Bismuth	BI	83	208.98	Oxygen	O	8	15.9994
Boron	B	5	10.81	Palladium	Pd	46	106.4
Bromine	Br	35	79.909	Phosphorus	P	15	30.9738
Cadmium	Cd	48	112.4	Platinum	Pt	78	195.09
Calcium	Ca	20	40.08	Plutonium	Pu	94	239*
Californium	Cf	98	251*	Polonium	Po	84	209*
Carbon	C	6	12.011	Potassium	K	19	39.102
Cerium	Ce	58	140.12	Praseodymium	Pr	59	140.907
Cesium	Cs	55	132.905	Promethium	Pm	61	145*
Chlorine	Cl	17	35.453	Protactinium	Pa	91	231*
Chromium	Cr	24	51.996	Radium	Ra	88	226*
Cobalt	Co	27	58.9332	Radon	Rn	86	222*
Copper	Cu	29	63.54	Rhenium	Re	75	186.2
Curium	Cm	96	247*	Rhodium	Rh	45	102.905
Dysprosium	Dy	66	162.5	Rubidium	Rb	37	85.47
Einsteinium	Es	99	254*	Ruthenium	Ru	44	101.07
Erbium	Er	68	167.26	Samarium	Sm	62	150.35
Europium	Eu	63	151.96	Scandium	Sc	21	44.956
Fermium	Fm	100	253*	Selenium	Se	34	78.96

Appendix 3 (Contd….)

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

Appendix 4. Solution Concentrations

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

Appendix 5. Some Useful Relationships

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

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

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

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

Appendix 7. Soil pH Levels and Associated Conditions

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

Source: Hach Company, USA (1992).

Appendix 8. Summarized Soil Test Methods for Fertility Evaluation

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

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

Appendix 9. Generalized Guidelines for Interpretation of Soil Analysis Data

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

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

$NaHCO_3$ = Sodium bicarbonate.

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

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

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

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

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

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

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

Source: Walsh and Beaton (1973).

Appendix 12. Classification Criteria for Salt-Affected Soils

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

[1]EC in saturated paste extract

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

Appendix 13. Soil Salinity Classification

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

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

Source: Hach Company (1992).

Appendix 14. Relative Salt-Tolerance Limits of Crops

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

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

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

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

Appendix 15. Relative Tolerance of Plant Species to Boron Toxicity

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

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

Source: Keren and Bingham (1985).

Appendix 16. Mesh Sizes of Standard Wire Sieves

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

Appendix 17. Equivalent Weights

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

Conversion of milliequivalents per liter to parts per million:

ppm = milliequivalents/liter × equivalent weight.

Appendix 18. Preservation Methods and Holding Times for Water Samples

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

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

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

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

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

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

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

About the authors

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

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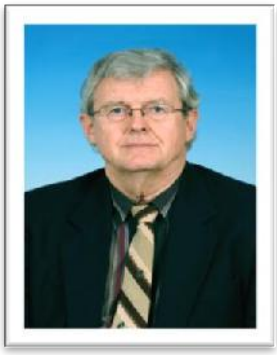

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