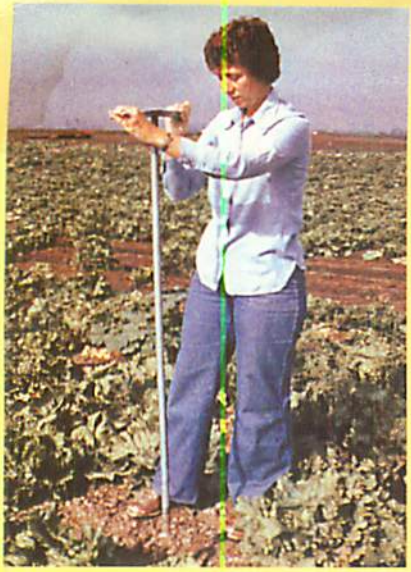

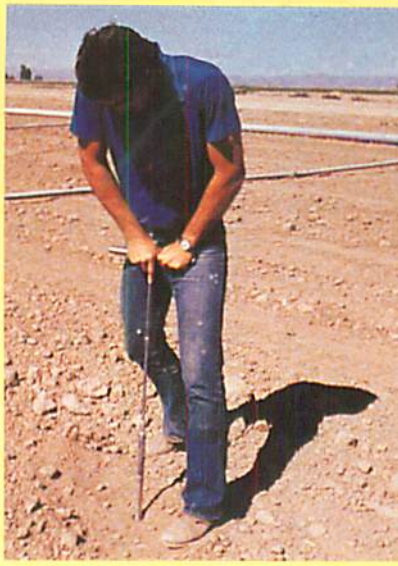

A Soil and Plant Analysis Manual Adapted for the West Asia and North Africa Region

By

John Ryan, Sonia Garabet, Karl Harmsen, and Abdul Rashid

ICARDA

International Center for Agricultural Research in the Dry Areas

About ICARDA

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The results of research are transferred through ICARDA's cooperation with national and regional research institutions, with universities and ministries of agriculture, and through the technical assistance and training that the Center provides. A range of training programs is offered extending from residential courses for groups to advanced research opportunities for individuals. These efforts are supported by seminars, publications, and specialized information services.

A SOIL AND PLANT ANALYSIS MANUAL ADAPTED FOR THE WEST ASIA AND NORTH AFRICA REGION

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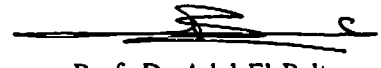
Foreword

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

Soils vary greatly throughout the world, they have inherent weakness, primarily deficiency in nutrients which 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; it is transient in nature and plants need a lot of it. In many cases, phosphorus 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 K, and usually don't need fertilization, especially for low-yielding rainfed crops. In recent years, a realization has grown that other elements, e.g., micronutrients, are deficient in some areas of the WANA region.

As no essential element will substitute for another, it is critically important to identify *where* and *when* such deficiencies occur. *That's where the role of soil and plant analysis comes in.* Techniques have been developed to evaluate soil fertility constraints based on soil chemical extraction and analysis of the plants that grow on such soils. Both are complimentary and when calibrated with field crop response to fertilizer, *provide a rational basis to identify what elements are missing, and how much fertilizer to apply.* Therefore, soil and plant analysis laboratories have a vital role in agricultural development of the 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 and plant analysis manual grew out of the perception that such conditions were less than ideal in the WANA region. As ICARDA had spearheaded the Soil Test Calibration program within the region's NARS, and laid the basis for sound fertilizer recommendations, it was only appropriate that ICARDA should, in collaboration with NARS, 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 training program and a vital link with the scientists of the WANA region.



Prof. Dr Adel El-Beltagy
Director General
ICARDA

Preface

The idea of a common laboratory manual for the WANA region is based on the fact that the soils in this arid to semi-arid area have a common suite of properties. In addition, scientists in the Soil Test Calibration Network in the Mediterranean zone have shown that a number of tests for nutrient availability have regional applicability.

A common soil analysis manual is fundamental for success of the WANA soil fertility network. The present manual, which was 2 years in the making, is designed to fill a gap that already exists.

The manual was endorsed by a sub-committee chaired by Dr. Abdul Rashid, National Agricultural Research Center, Islamabad, Pakistan, at the recent Soil Fertility Meeting in ICARDA (Nov. 19-23, 1995).

It is intended as a draft document for distribution to soil fertility scientists in charge of laboratories through the WANA region. It is not the *final word* in soil or plant analysis. Rather it is intended to be tried out by the various lab managers. We encourage each recipient to assess its suitability for his/her particular situation, and to make written suggestions on the manual for incorporation into a second, and hopefully improved, edition.

Acknowledgements

We would like to thank all past and present ICARDA staff associated with the Soils Laboratory who have contributed to the operation of the Lab and the development of the various procedures adapted.

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

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

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

We also extend our thanks to Dr. Azaeiz Gharbi, Ecole Superiore des Agriculture du Kef, Tunisia, for reviewing a draft of this manual.

Finally, we extend our appreciation to the Soil Fertility sub-group for laboratory procedure under the chairmanship of Dr. Abdul Rashid, NARC, Islamabad, Pakistan, who suggested some modifications and approved the dissementation of this draft manual in the WANA region.

Introduction

The idea that one could test or analyze a soil and obtain some information about its properties--especially its acidity or alkalinity and its nutrient status--is long established, and can be traced back to the beginning of scientific inquiry about the nature of soil. Analysis of the plant to reflect the fertility status of the soil in which it grew is more recent, although visual *crop* observations are as old as the ancient Greek 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 testing procedures have been developed, and are still evolving.

With the advent of chemical fertilizers, the need to know the nutrient status of a soil in order to more effectively use these expensive and limited inputs became all the more crucial. However, if soil testing is to be an effective means of evaluating the 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 (Walsh and Beaton, 1973):

1) field plot fertilizer trials,
2) greenhouse pot experiments,
3) crop deficiency symptoms,
4) plant analysis,
5) rapid tissue or sap analysis,
6) biological tests, such as growing microorganisms, and
7) rapid chemical analysis of the soil prior to cropping.

While all approaches can be used in research, the latter one is most amenable, and one upon which recommendations for farmers can be based. 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. 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 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), *boron* (B) and *molybdenum* (Mo) are often measured, since deficiencies of these elements are more frequently associated with semi-arid calcareous soils. Indeed such areas may also have excessive or toxic levels of some elements, e.g., B.

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

Soil testing involves four distinct phases:

1) *Sample collection*--this should be such that it reliably reflects the *average* status of a field, representative for the parameter considered.

2) *Extraction or digestion* and *nutrient determination*--the reagent used and the procedures adopted should identify 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.

4) *Fertilizer recommendation*--this is based upon the test being calibrated for field conditions, and considers other factors such as expected crop yield, crop characteristics, management of the crop, soil type, and method of fertilizer application, etc.

It should be emphasized, however, that a soil test, *reliable* or *accurate*, is only one *factor* in making decisions about the *need for fertilization.* Other factors are: soil type and environmental conditions, i.e., moisture, 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 to a lesser extent, N (Walsh and Beaton, 1973). Being mobile in soils and subject to mineralization - immobilization, N poses particular problems to establish a reliable test. Tests for K, pH, OM, and $CaCO_3$ are more straightforward. Since the development of the DTPA test of Lindsay and Norvell (1978), micronutrients tests (Fe, Mn, Zn, Cu) are more useful. On the contrary, methods for B are older but still useful (Richards, 1954). Though tests for gypsum are developed (Richards 1954; FAO, 1990), there are unique problems for CEC measurement in such soils (Rhoades and Polemio, 1977).

The research literature on soil testing is rich and varied, which is reflected in the manuals on soil testing. These include:

1. Monographs from the American Society of Agronomy for physical (Klute, 1986) and chemical analysis (Page, 1982) which give detailed descriptions of all available soil tests and their modifications.

2. Soil Science Society of America publications (Walsh and Beaton, 1973; Westerman, 1990) that take a broader look at the philosophy, procedures, and laboratory operations for soil and plant analysis, with criteria for specific crops.

3. Publications on soil testing with a textbook format (Hesse, 1971).

4. University publications which range from those that deal with all soil, water and plant tests (Chapman and Pratt, 1961) to more narrowly based ones (Reisenaur, 1983).

5. Publications that deal with theoretical considerations involved with sampling, correlation, and calibration, to interpretation (Brown, 1987).

6. Those that are commercially oriented and reflect "state of the art" instrumentation and computer-assisted data analysis and handling (Jones, 1991; Jones *et al.*, 1991).

7. Finally, publications that are written in "recipe-cookbook" style with little or no discussion; only listed are the 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, soil research pertaining to testing is often fragmentary. Statements (p.15) from Walsh and Beaton (1973) regarding the interdependence of background research and routine soil testing are illustrative: "Too often, especially in *underdeveloped countries,* soil testing programs are started without an *adequate research background*" and "it is perhaps no exaggeration to say that the success of a soil testing program is directly proportional to its research backing".

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

Central to the Network, and indeed ICARDA's operations, has been its Soil Fertility Laboratory. Though its facilities have been designed and developed without some of the constraints experienced by other governmental and educational labs in the region, some of the procedures adapted by the lab are based on *validated regional research* i.e., N and P. A key element in any worthwhile lab is a list of appropriate tests presented in such a manner that it can be readily followed routinely by those who do soil tests - *the lab technicians*. We are not aware of the existence of a simple manual designed for the regions' labs.

Therefore, the target audience for this proposed manual is the cadre of technical staff throughout the region. A brief introduction to each test is given; 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 in the region, and therefore more readily identify gross errors. We have attempted to select the most appropriate method for each test and present it in a clear stepwise manner.

While the manual primarily deals with soil testing, a number of essential plant tests are presented, since they may complement the soil tests and are needed for nutrient balance studies. Similarly, minimum emphasis is given to physical properties; only those tests routinely done prior to chemical analysis are described. We have also presented material on *lab organization* and *safety* - aspects which are often *overlooked* by technical staff, but which impinge greatly on their work output and its reliability.

Although the manual is written in English--the language of ICARDA's publications, as it deals with a multi-lingual diverse region--only an elementary knowledge of English is required to follow the procedures listed. Currently, many technician-level staff in the region can be expected to have this degree of competence. If necessary, it can be readily translated in whole or in part into French for the North Africa region and into Arabic for the Middle East in general or other languages such as Turkish or Persian, as need is perceived. *Upon distribution in the region, it is hoped that modifications based on our collaborators' experience can be incorporated into future editions.*

I. BACKGROUND CONSIDERATIONS

While the main focus of this manual is nutrient testing in the soil and, to a lesser extent, in the plant, there are other aspects related to such operations that are worthy of mention. A brief description of such aspects follows.

Soil Sampling And Processing

Introduction

While much attention is given to laboratory testing, 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 due to *sampling* is generally *much greater* than that due to chemical analysis. Therefore, obtaining a representative soil sample from a field is the most important aspect of soil analysis.

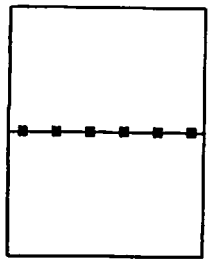
Sampling

A sample is normally composed of several sub-samples which represent a seemingly uniform area or field with similar cropping and management history. There is no *universally* accepted number of samples specified.

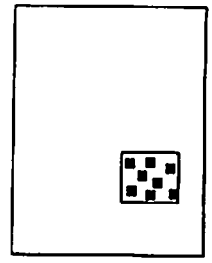
The following points can serve as guidelines:

Sampling Sites

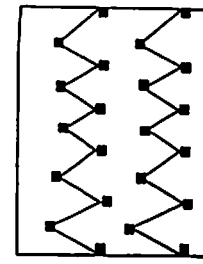
- At ICARDA, eight sub-samples are taken per hectare (ha) in a diagonal pattern.
- Other plans range from 5 to 25 borings or sub-samples per composite sample, with sample units varying from 2 to 8 ha.
- Fewer samples are needed where little or no fertilizer has been used. Sampling areas are often traversed in a zig-zag pattern to provide a uniform distribution of sampling sites. Some of these methods are represented in Fig. 1.
- Correspondingly more sub-samples are needed where fertility is variable due to hand broadcasting of fertilizers and with cropping-livestock systems. Indeed banding of fertilizer poses problems for reliable sampling.



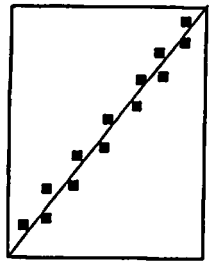
Cross strip sampling in a uniform field



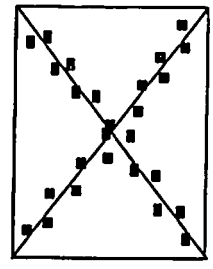
Test plot in a uniform field



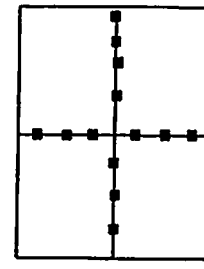
Zig zag method of field sampling



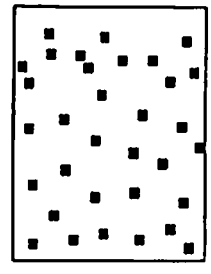
Diagonal sampling in a uniform field



Two way diagonal sampling in a uniform field



Two way cross strip sampling in a uniform field



Ideal way of field sampling (random method)

Fig. 1. Soil sampling methods for fertility evaluation.

- The number of sub-samples taken by farmers has to be *realistic.* Researchers tend to have more elaborate sampling strategies.

Time of Sampling

The correct time of sampling is not specified. Samples can be taken any time that soil conditions permit. However, sampling directly after fertilization should be avoided. Samples be *taken* during the crop growth period; in this way the nutrient content of the soil is determined under the conditions in which plants are drawing nutrients from the soil. 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 during the year to compare analysis at time intervals.

Depth of Sampling

For most purposes, the sample is taken to a depth of about 20 cm. Research in the WANA region has shown that available P and NO_3 in such samples are related to crop growth. In some cases, especially irrigated areas, sampling to a depth of 60-100 cm is sometimes desirable, especially for nitrate (NO_3). Depthwise samples should also be taken where there is a concern about B toxicity.

Sampling Tools

There are two important requirements of a sampling tool: first, that a uniform slice be taken from the surface to the depth of insertion of the tool, and second, that the same volume of soil be obtained from each area. Augers generally meet these requirements. In areas where the top-soil is dry, i.e., summer, the top-soil sampling can be done by a metal ring, by digging out the soil inside the ring, because it is almost impossible to sample dry top-soils with an auger. Soil samples for micronutrient analysis should be taken using a stainless steel, or at least ungalvanized auger.

- Researchers generally use augers for field sampling. Farmers or extension agents could use shovels or trowels with the same effect.

- Samples are put in plastic bags (tags and markers are required).

- Samples can be transported in cardboard boxes or sacks.

Laboratory Processing

- Samples are put in a freezer to reduce microbial activity.

- All information about samples is recorded; each is given a number.

- Samples are air-dried or in a forced-air oven at 30° C on a tray.
- When dry, samples are cleaned from stones and plant residues.
- Samples are ground in a stainless steel soil grinder and passed through a 2-mm sieve. Samples for micronutrients and particle-size distribution are ground with a mortar and pestle.
- The < 2 mm size fraction is collected, sub-sampled, and stored in plastic bottles (500 g). Some analyses call for use of a < 1 mm fraction.
- Sub-samples are made with a riffle-type sample splitter (see Fig. 2).
- Sub-samples are given a number and sent to the lab for all requested analyses.

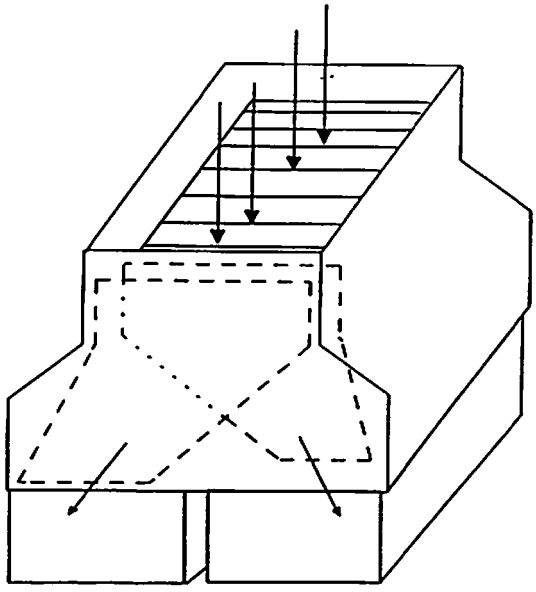


Fig. 2 Diagram of soil sample splitter

Plant Sampling and Processing

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

Fortunately, years of research in soil fertility - plant nutrition have produced reliable sampling criteria and procedures for most of the world's commercial crops: *leaves* are most commonly chosen, but petioles are selected in certain cases, e.g. cotton, sugarbeet. Seeds are rarely used for analysis, except in the case of B toxicity and nutrient deficiencies in certain grain crops. In some cases, i.e., cereals, the entire above-ground plant material is chosen. When leaves are involved, recently matured ones are taken; both new and old growth is avoided. However, young emerging leaves are sampled for diagnosing iron chlorosis by determining ferrous (Fe^{++}) content of fresh leaves (Katyal and Sharma, 1980). Damaged or diseased leaves are excluded, while plants should not be sampled when the crop is under moisture or temperature stress. *Standardization of sampling techniques is essential if meaningful interpretation of the resulting analyses is to be achieved.* Sampling procedures for important dryland crops of the WANA region are given in Appendix Table 7. Care should be taken to transport samples to the lab in properly labeled paper bags that allow for transpiration; this reduces the possibility of rotting. After sampling, four steps are followed:

1) 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, followed by water.

2) Drying in an oven to stop enzymatic activity - usually at 65°C for 24 hr.

3) Mechanical grinding to produce a material suitable for analysis - usually to pass a 60-mesh sieve; stainless steel mills are preferable.

4) Final drying at 65°C of ground tissue to obtain a constant weight upon which to base the analysis.

Since most analytical methods require grinding of a dry sample, careful attention must be given to avoiding contamination with the element being analyzed. Particular care is required for the micronutrients. While lab technicians do not normally sample plants, it is important that they be *aware* of the necessity of proper sampling. Otherwise, analyses that they are asked to perform on plant samples are *meaningless* and a *waste of their time.*

Laboratory Organization

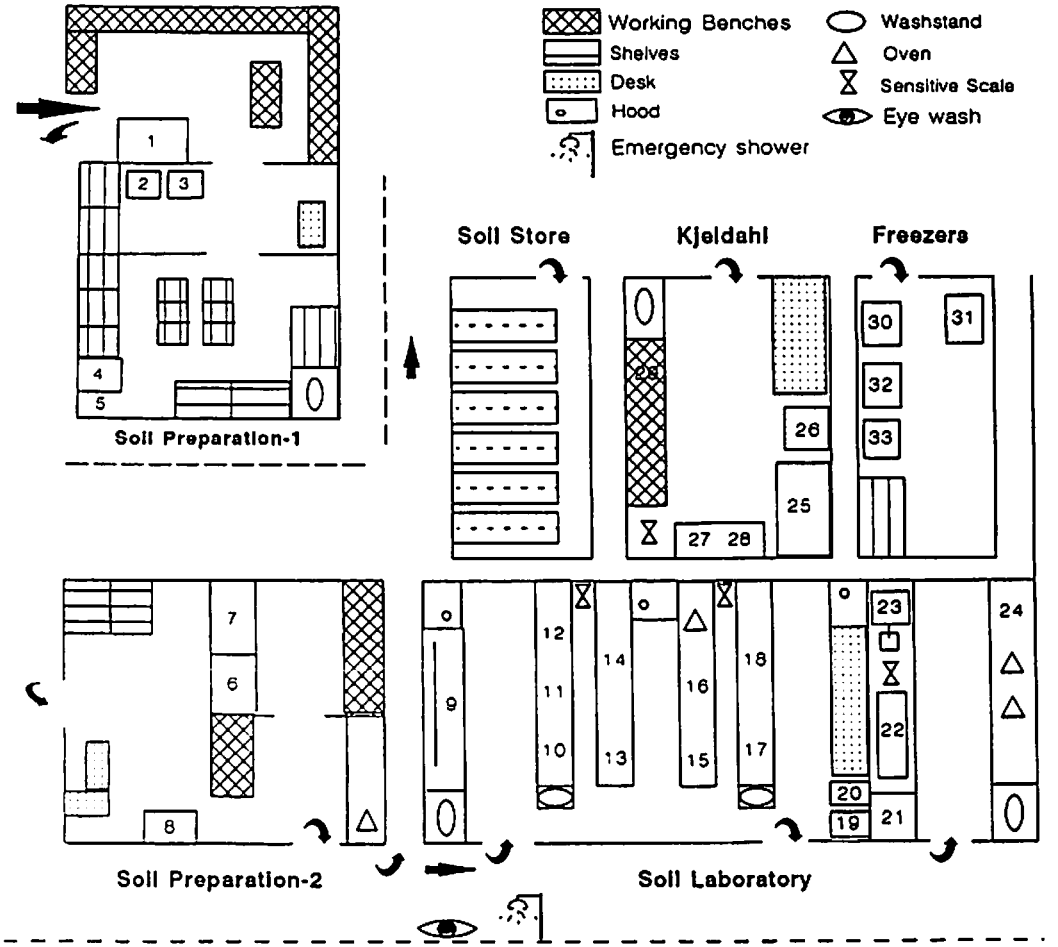
Soil and plant analysis are carried out by various institutions in the public or government sector and in the private domain. Labs are found in Ministries of Agriculture, national research and teaching institutes, international organizations, and in commercial companies. Analytical services should be closely linked to the extension/advisory services and should maintain a functional relationship with the universities, research stations, etc. The kind of facility for such analyses depends on the type of institution it serves, the nature of the clientele, and the volume of samples to be analyzed. Nevertheless, all labs, regardless of the size, should be designed in a manner to facilitate *operational efficiency*, *minimize contamination*, and produce *reliable* and *repeatable* results.

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

Soil and plant analysis, and water analysis should be located in the same building and be under one unified administration. The Soils Laboratory of ICARDA's Farm Resource Management Program (FRMP) was designed with these considerations in mind (Fig. 3). The various components reflect a logical activity framework. In Soil Preparation Room No 1, large bulk samples, transported by truck, are received, dried, sieved, and stored in bulk bins. An inventory or catalogue of all soil samples is maintained. All samples are retained in this area for at least two years after analysis; bulk samples of special soil types are kept indefinitely. This facility is complete with a large oven, freezer, soil grinder, exhaust hood, and a compressed air machine.

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

While no two labs are ever the same or have the same complement of equipment, the details presented for our lab will, hopefully, serve as a general guideline for lab arrangement and the type of equipment needed for routine service-oriented operations.



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

Fig. 3 Schematic layout of ICARDA soil laboratory facilities

Data Processing

A considerable amount of information is generated in any soil testing laboratory. In order to economically justify the existence of a lab, 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.
- more sophisticated quality control.
- automated invoicing and addressing.
- 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 to be offered. In general, laboratories with a large volume of samples, and which offer a range of analyses have a need for more computer sophistication and automation than laboratories with a small throughput.

In order to facilitate data processing, standard information sheets are required. These vary from lab to lab, 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, available P, etc) have to be calibrated with field crop response.

With a relatively large throughput of analyses associated with the large number of on-station and on-farm trials, analytical results from ICARDA's lab are stored in the computer program to generate fertilizer recommendations based on soil test values. Where soil maps and rainfall data are available it is possible, with the accumulation of test values of known locations to establish relationships with soil type, region, climatic zone, etc.

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 reproducability and adequate accuracy of results are important objectives.* Repeated measurement of an air-dried 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 to laboratory quality control and should be routinely included among the test samples. These precautions involve the use of *blanks*, *repeats*, and *internal references,* as mentioned below.

Whenever a new procedure is introduced to a laboratory, its accuracy should be evaluated and compared to current tests already in use. Both methods should be compared for a homogeneous test sample using ten-fold replicates, with the standard deviation calculated for each set. This provides a *measure of precision.* Then a known amount of reagent should be added to the homogeneous test sample, the procedures repeated, and the *mean* and *standard deviation* calculated. The agreement between the increase in the values obtained to the known increase in test sample concentration provides *a test of accuracy.* For procedures in which the test material is known to interact with the added reagent, as with phosphorus-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 but have no added test material. Blanks allow correction for any background contamination introduced from reagents, filter papers or other systemic sources of error. Provided the blank values are consistent, the mean value can be subtracted from the sample value. When blanks yield large values, this suggests excess extraneous contamination; in such cases, this requires that an 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 of 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 $\pm$ 3 standard deviation limits or if two successive values exceed the $\pm$ 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 these have been obtained using standardized methods. Collaboration between laboratories can be improved by exchanging reference materials and then comparing their results (Ryan and Garabet, 1994). Such materials are referred as "external references". An example of such standardization is the exchange network of ISRIC (International Soil Reference and Information Center) in Wageningen, The Netherlands operating international soil- and plant-analytical exchange programs.

Most external reference samples are costly, and frequent use increases the 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 the internal reference sample with occasional use of the external reference sample can reduce costs, while still providing acceptable quality assurance.

Laboratory Safety

Introduction

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

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

While rules pertaining to safety can be extensive, we have endeavored to concisely list the more important ones within different categories of concerns. These have been adapted from a laboratory safety guide 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, clean work environment.

4. Avoid working alone.

Instrument Operation

5. Follow the safety precautions provided by the manufacturer when operating instruments.

6. Monitor instruments while they are operating.

7. Atomic absorption spectrophotometer must be vented to the atmosphere. Ensure that the drain trap is filled with water prior to igniting the burner.

8. *Never* open a centrifuge cover until machine has completely stopped.

Accidents

9. Learn what to do in case of emergencies (e.g., fire, chemical spill etc.). Fire fighting equipment must be readily accessible in the event of fire. Periodic maintenance inspections must be conducted.

10. Learn emergency first aid. First aid supplies are a necessity and laboratory staff should be well trained in their use. Replacement of expended supplies must take place in a timely fashion.

11. Seek medical attention immediately if affected by chemicals, and use first aid until medical aid is available.

12. Access to eye-wash fountains and safety showers must not be locked. Fountains and showers should be checked periodically for proper operation.

Chemicals

13. Use fume hoods when handling concentrated acids, bases or other hazardous chemicals.

14. Do not pipette by mouth; *always* use a suction bulb.

15. When diluting, always add acid to water, *not* water to acid.

16. Many metal salts are *extremely toxic* and may be *fatal* if swallowed. Wash hands thoroughly after handling such salts. Chemical spills should be cleaned promptly and all waste bins regularly emptied.

17. All reagent bottles should be clearly labelled and must include information on any particular hazard. This applies particularly to poisonous, corrosive and inflammable substances.

Furnaces, etc.

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

Handling Gas

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

Maintenance

20. All electrical, plumbing, and instrument maintenance work should be done by qualified personnel. Fume hoods should be checked routinely.

21. As most 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.

Eating-Drinking

22. Do *not* eat, drink, or smoke in laboratory. These should be discouraged at all times within the working areas of the laboratory both for reasons of health and to reduce contamination. To effect these standards, specific areas should be designated for staff breaks.

23. Do *not* use laboratory glassware for eating or drinking.

24. Do *not* store food in the laboratory.

Protective Equipment

25. Use personnel safety equipment as follows:

- **Body Protection**

  Lab coat and chemical-resistant apron.

- **Hand Protection**

  Use gloves, particularly when handling concentrated acids, bases, and other hazardous chemicals.

- **Dust Mask**

  When grinding soil, plant samples, etc.

- **Eye Protection**

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

- **Full Face Shield**

  Wear face shields over safety glasses in experiments involving corrosive chemicals.

- **Foot Protection**

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

Waste Disposal

26. For chemicals cited for waste disposal, write down contents on the label. 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 sewage system.

27. Dispose of chipped or broken glassware in specially marked containers.

Continuing Education

- Display in a prominent place posters on "Laboratory Safety" which pictorially describe various phases of laboratory activities.

- Similarly, posters depicting First Aid after lab accidents should be prominently displayed.

- Such posters are *not* for ornamentation; they are for the *protection of lab personnel* who should be thoroughly conversant with all procedures and eventualities.

- If the lab is a part of a large institution, the lab staff should know the safety officer or person responsible for safety. If it is a small operation, one lab staff member should be responsible for safety.

Contamination

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

- External dust blown from the surrounding environment.

- Internal dust resulting from cleaning operations.

- Cross contamination deriving from handling many samples at the same time (i.e., handling plant and soil samples together).

- Reagents: failure to store volatile reagents well away from the samples.

- Washing materials, particularly soap scouring powder.

- Smoking in the laboratory.

II. SOIL PHYSICAL ANALYSIS

Any measurement or test of soil may be broadly categorized as either *physical, chemical, mineralogical,* or *microbiological.* While chemical analyses are more closely related to nutrient behavior in soils, some physical properties also need to be considered in the interpretation of chemical data in relation to crop production.

The physical measurements that can be made on soil are numerous, depending on the objective and whether agricultural or non-agricultural use is concerned. This range indicated in authorative monographs (Klute, 1986): includes *soil water content, infiltration* and *hydraulic conductivity, evapotranspiration, heat, temperature, reflectivity, porosity, particle size, bulk density, aggregate stability,* and *particle size distribution.* However, only a few physical measurements are normally conducted in fertility-oriented labs.

Soil moisture is routinely measured on field-moist samples, since all chemical analyses are expressed on an air - or oven dry basis. Since texture, whether sandy or clay, is 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.

A physical process which is especially common in irrigated soils is the preparation of a saturated paste. This provides an extract in which soluble anions and cations are measured; criteria for deficiency or toxicity of some elements are based on such an extract. For example, criteria for salinity tolerance, presented in Appendix Table 4, are based on a saturation extract. Similarly, standards for B in relation to plant growth, shown in Appendix Table 8, are based on this extract.

Soil Moisture Content

Introduction

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

Apparatus

Electric oven with thermostat and desiccator.

Procedure

1. Weigh 10.00 g air-dry soil (< 2 mm) into a previously dried (105°C) and weighed metal can with lid.

2. Dry in an oven, with the lid unfitted, at 105°C overnight.

3. Next day, remove from oven, fit the lid, cool in a desiccator for at least 30 min. and re-weigh.

CALCULATIONS

$$\%\ Moisture\ (\Theta) = \frac{Wet\ soil\ (g) - Dry\ soil\ (g)}{Dry\ soil\ (g)} \times 100 \qquad (1)$$

$$Dry\ soil\ (g) = \frac{1}{1 + \frac{\Theta}{100}} \times Wet\ soil\ (g) \qquad (2)$$

$$Moisture\ Correction\ Factor\ (MCF) = \frac{Wet\ soil}{Dry\ soil}\ or\ = \frac{100 + \%\Theta}{100} \qquad (3)$$

Particle Size Distribution

Introduction

Individual soil particles vary widely in any soil type. Similarly, as these particles are cemented together, a variety of larger shapes and sizes occur. For standard laboratory measurement, the fraction that passes a 2-mm sieve is considered. Procedures normally identify sand (0.05 - 2.0 mm), silt (0.002 - 0.05 mm), and clay (<0.002 mm) fractions.

As these primary particles are usually cemented together by organic matter, this has to be removed by H_2O_2 treatment. However, if substantial amounts of $CaCO_3$ are present, actual percentages of sand, silt or clay can only be determined by prior dissolution of the $CaCO_3$. The two common procedures for particle size analysis, or *mechanical analysis*, involves either the *hydrometer* or the *pipette-gravimetric* methods.

Because virtually all soil samples received in our laboratory are highly calcareous, it would be impractical to remove solid-phase $CaCO_3$. In using the relatively simple hydrometer method (Bouyoucos, 1927; 1936; 1962; Day, 1965; FAO, 1974), data are reported in size categories of actual soil rather than as sand, silt, or silicate clay. The hydrometer method of silt and clay measurement relies in the effect of particle size on the differential settling velocities within a water column. The settling velocity is also a function of liquid temperature, viscosity and specific gravity of the falling particle.

Theoretically the particles are assumed to be spherical and to have a specific gravity of 2.65. If all other factors are constant, then the settling velocity is proportional to the square of the radius of the particle (Stoke's law). In practice, therefore, we must know and make corrections for the temperature of the liquid. Greater temperatures result in reduced viscosity due to liquid expansion and a more rapid descent of falling particles.

Particle size distribution is an important parameter in soil classification and genesis and has implications for soil water, aeration, and soil fertility.

Apparatus

Soil dispersing stirrer: A high speed electric stirrer with a cup receptable.
Hydrometer with Bouyoucos scale in g/L (ASTM 152H).

Reagents

Dispersing Solution

Dissolve 40 g **sodium hexametaphosphate** $[(NaPO_3)_{13}]$ and 10 g **sodium carbonate** (Na_2CO_3) in 1 L de-ionized water. This solution deteriorates with time and should *not*

be kept for more than 1 to 2 weeks.

Amyl alcohol

Procedure

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

Determination of Blank

- Dilute 60 ml dispersing solution to 1 L in the hydrometer jar.
- Insert hydrometer and take blank reading, R_b.
- The blank reading must be re-determined for temperature changes of more than 2°C from 20°C.

A. Silt plus Clay

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

The percent silt plus clay follows from:

$$Silt + Clay\ (\%\ w/w) = (R_{sc} - R_b) \times \frac{100}{Oven\text{-}dry\ Soil\ (g)} \quad (4)$$

B. Clay

- Mix suspension in dry cylinder with paddle, and leave undisturbed.
- After 4 hr, insert hydrometer and take reading, R_C.

The percent clay follows from:

$$\%\ Clay\ (w/w) = (R_c - R_b) \times \frac{100}{Oven\text{-}dry\ Soil\ (g)} \quad (5)$$

The percent silt follows from:

$$\%\ Silt\ (w/w) = [Silt + Clay\ (\%\ w/w)] - [Clay\ (\%\ w/w)] \quad (6)$$

D. Sand

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

$$\%\ Sand\ (w/w) = Sand\ weight\ (g) \times \frac{100}{Oven\text{-}dry\ Soil\ (g)} \qquad (7)$$

Where weight of sand follows from:

$$Sand\ (g) = [Beaker + Sand\ (g)] - [Beaker\ (g)] \qquad (8)$$

Notes

1. If possible, all hydrometer jars should be placed in a water bath at constant temperature (20°C); in that case, temperature corrections are not needed.

2. For temperature correction use a value of 0.4. For each degree temperature difference from 20°C, add or subtract this factor if the temperature is more or less than 20°C, respectively.

3. All results of mechanical analysis should be expressed on the basis of oven-dry soil (24 hr 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 plus sand should be 100%. Deviation from 100 is an indication for the accuracy.

Soil texture

Once the sand, silt and clay distribution is measured, the soil may be assigned to a texture class on the soil textural triangle (Fig. 4). Within the textural triangle are various soil textures which depend on the relative proportions of soil particles. Users simply obtain the appropriate texture based on the particle size distribution.

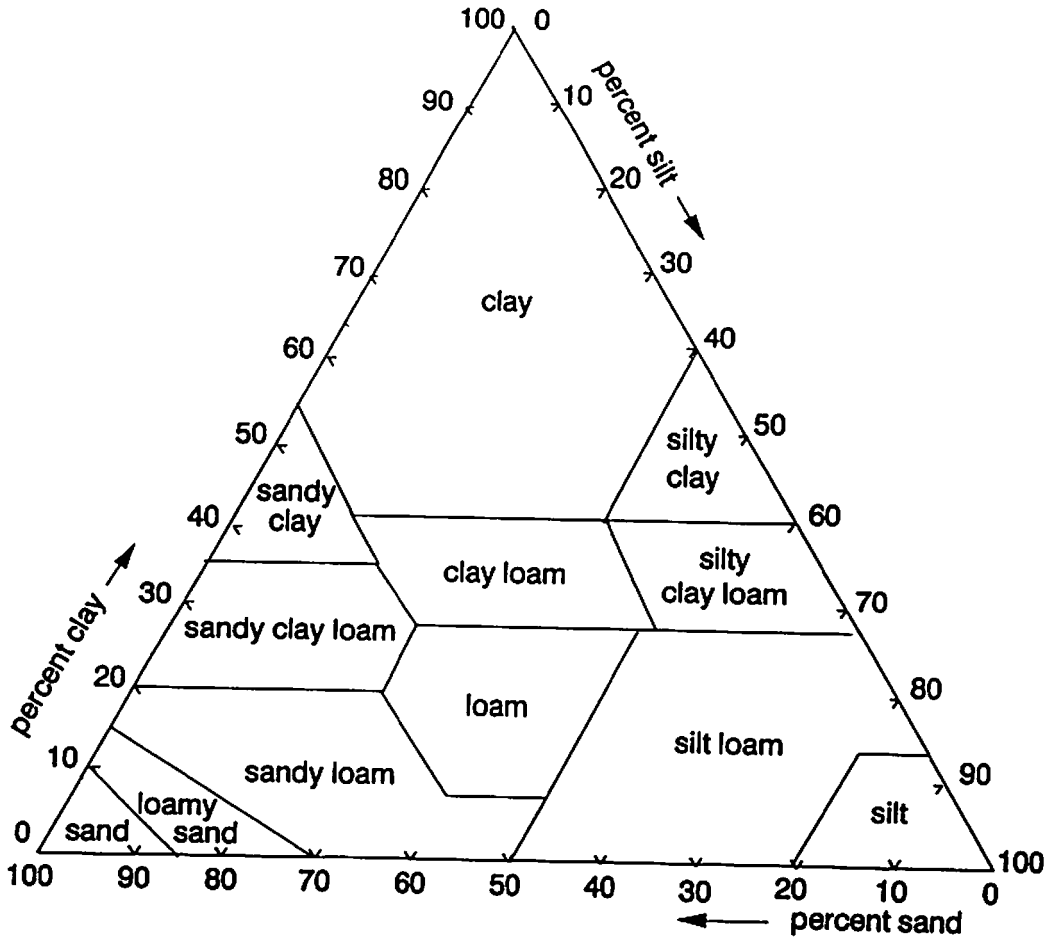


Fig. 4. The soil texture triangle.

Saturated Paste

Introduction

The use of an extract from a saturated paste is advantageous for 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 important parameters such as sodium adsorption ratio (SAR) which, in turn, predicts exchangeable sodium percentage (ESP). Criteria for boron (B) toxicity have been developed for such an extract.

Thus, a saturation extract is routinely used where salinity is a concern (Richards, 1954). However, in dryland areas, which constitute the major part of the WANA region, it is seldom used. Nevertheless, with encroachment of supplementary irrigation in traditionally dry areas, increased use is likely to be made of saturation extracts in soil analysis.

The cations likely to be analyzed are Ca^{++}, Mg^{++}, K^{+}, and Na^{+}, while SO_4^{-}, CO_3^{-}, HCO_3^{-} and Cl^{-} are the major anions. Boron is often measured where toxicity might be expected.

Apparatus

1. Porcelain dishes
2. Spatulas
3. Vacuum filtration system.

Reagents

De-ionized water only.

Procedure

1. Place 200-300 g (< 2 mm) air-dry soil in a porcelain dish.

2. Slowly add de-ionized water and mix with a spatula until the paste glistens and flows slightly as the porcelain dish is tipped; it should slide off the spatula without collection of any free water on the surface of the paste.

3. Allow the paste to stand for 1 hr, then re-check the criteria for saturation by adding more water or soil as needed.

4. Leave the paste for 6 to 16 hr, and then filter with the vacuum filtration system using a Buchner funnel 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, refilter.

III. SOIL CHEMICAL CHARACTERIZATION

Plant nutrition is the biochemical process of absorption, assimilation and utilization of nutrients essential for growth and reproduction. Balanced plant nutrition contributes to high crop yields at the most economical return of crop investment costs. Three major factors contribute to plant nutrition:

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

The essential nutrients in plant nutrition are classified into four groups:

1) *Major non-mineral macronutrients*: these are 90-95% of dry plant weight, and are supplied to the plant by water absorption and photosynthesis, i.e., C,H,O.

2) *Primary macronutrients*, i.e., N, P, K.

3) *Secondary macronutrients*: i.e., Ca, Mg, S, and

4) *Micronutrients*: i.e., B, Cu, Fe, Mn, Mo, and Zn.

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 (Page, 1982). For any one element, numerous procedures or variations of procedures can be found in the literature (Walsh and Beaton, 1973). We have endeavored to select procedures which, in our experience, are appropriate for soils of the WANA region, i.e, where a good relationship exists between the test value and crop growth. Where alternative methods are appropriate, we have presented the salient features of these methods. A bibliography of soil testing information is provided for users who may need to expand or modify their range of soil testing procedures.

We initially presented analyses which are routinely done to characterize a soil sample or soil type in terms of background information, i.e., pH, EC, $CaCO_3$, organic matter, cation exchange capacity, and gypsum. With regard to N, the dominant fertility factor in WANA soils, we have dealt with the most convenient methods for measuring the different forms or fractions of N in soils. This is subsequently followed by procedures for available P, soluble and exchangeable cations, soluble anions, and extractable micronutrients. Where appropriate, we have given the user of this manual guidelines for interpreting the data produced with the analytical procedures listed.

pH

Introduction

Soil pH is one of the most common measurements in soil laboratories. It reflects whether a soil is *acid, neutral, basic or alkaline*. It is more correctly defined as the negative log of the hydrogen ion activity. Since the pH is logarithmic, the H-ion concentration in solution increases *ten times* when the pH is lowered one unit. The pH range normally found in soils varies from 3 to 9. The various categories 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 the availability of soil nutrients, the solubility of toxic nutrients in the soil, physical breakdown of root cells, the cation exchange capacity in soils whose colloids (clay/humus) are pH dependent, and biological activity. Both tend to be maximum around neutrality. At high pH values, availability of P and most micronutrients, except boron (B) and molybdenum (Mo), tends to decrease.

Acid soils are not commonly found in semi-arid dryland areas of the world; they tend to occur in *temperate and tropical areas where rainfall is substantial*; conversely, *soils of drier areas are generally alkaline*, i.e., above pH 7.0, as a result of the presence of calcium carbonate ($CaCO_3$), and will visibly effervesce (fizz) when 10% hydrochloric acid is added dropwise to the soil. Most soils in the West Asia-North Africa region have pH values of 8.0-8.2. Calcareous soils with gypsum have somewhat lower pH values, while those with excess Na have values over 8.5 (sodic soils).

This procedure may be used for the determination of soil pH in a 1:1 (soil:water) suspensions (Mckeague, 1978; Mclean, 1982).

Procedure

1. Weigh 50 g air-dry soil (< 2mm) into a 100-mL glass beaker.
2. Add 50 mL water using a graduated cylinder or 50-mL vol. flask.
3. Mix well with a glass rod and allow to stand for 30 min.
4. Stir suspension every 10 min. during this period.
5. After 1 hr, stir the suspension.
6. Put the combined electrode in suspension (about 3 cm deep). Take the reading after 30 sec.

7. Remove the electrode from the suspension, clean with DI in a separate beaker, and dry excess water with a tissue.

Notes

1. If a combined electrode is used, make sure that it contains *saturated* KCl solution and some solid KCl.

2. Calibrate the pH meter using at least two buffer solutions of different pH values, usually 7.0 and 9.0. First, measure the temperature of the solution and adjust the "temperature" knob. Dip the electrode in pH 9 buffer solution, check for actual pH at measured temperature, and adjust with the "buffer" knob. Dip the electrode in the pH 7 buffer solution and adjust with "sensitivity" knob. Repeat until both buffer solutions give correct readings.

3. At ICARDA, pH is measured in a 1:1 (w/v) soil-water suspension. For special purposes, pH can be measured in a saturated soil paste, or in more dilute suspensions. In some laboratories, pH is measured in a suspension of soil and 1 *N* KCl or 0.01 *M* $CaCl_2$. The main advantage of the measurement of soil pH in salt solution is the tendency to eliminate interferences from suspension effects and from variable salt contents, such as fertilizer residues.

4. Air-dry soils may be stored several months in closed containers without affecting the pH measurement.

5. If the pH meter and electrodes are not to be used for extended periods of time, the instructions for storage published by the instrument manufacturer should be consulted.

6. For samples high in organic matter, use 1:2 or a 1:4 soil:water ratio.

Electrical Conductivity (EC)

Introduction

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

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

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

Procedure

1. Prepare a 1:1 (w/v) soil-water suspension, as for pH determination.

2. Filter the suspension using suction. First, put a round Whatman No. 42 (dia 9 cm) filter paper in the Buchner funnel. Moisten the filter paper with water and make sure that it is tightly attached to the bottom of the funnel and that all holes are covered.

3. Start the vacuum pump.

4. Open the suction, and add suspension to Buchner funnel.

5. Continue the filtration until the soil on the filter starts cracking.

6. If the filtrate is not clear, the procedure has to be repeated.

7. Transfer the clear filtrate into a 50-mL bottle, immerse the conductivity cell in the solution, and take the reading.

8. Remove the cell, and clean thoroughly with distilled water, and let it dry.

Notes

1. Readings are recorded in milli-mhos per centimeter (mmhos/cm) or milli-Siemens per centimeter (mS/cm). The use of the unit milli-Siemens is preferred over the unit milli-mhos. Both units are equal, that is, 1 mS = 1 mmho.

2. Check the accuracy of the EC meter, using a 0.01 *N* KCl solution, which should give a reading of 1.413 mS/cm at 25°C.

Calcium Carbonate

Introduction

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.* Thus, soils of the WANA region may contain up to 50% $CaCO_3$-equivalent or more.

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

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

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

Principle

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

Reagents

A. HCl Solution, 1 *N*

Dilute 82.8 mL **conc. HCl** (37%, sp.gr. 1.19) in 1 L distilled water. Standardize with 1 *N* Na_2CO_3 solution using **methyl-orange indicator**, and determine the exact normality of the HCl solution.

B. NaOH Solution, 1 *N*

Dissolve 40.0 g **NaOH** in 1 L distilled water. Standardize with Reagent A.

C. Phenolphthalein Indicator

Dissolve 0.500 g **phenolphthalein** in 100 mL ethanol (ethyl alcohol).

Procedure

1. Grind 1 to 2 g soil to pass a 150-mesh sieve, using a porcelain mortar and pestle.

2. Weigh 1.00 g soil in a 250-mL Erlenmeyer flask and add 10 mL of **Reagent A** to the flask with a vol. pipette.

3. Swirl and leave the flask overnight, or heat to 50-60°C, and let the flask cool.

4. Add 50-100 mL distilled water using a graduated cylinder and 2-3 drops **phenolphthalein** indicator (Reagent C).

5. Titrate with **Reagent B** while swirling the flask. Continue the titration until a faint pink color develops, and take the reading (R).

CALCULATIONS

$$\% \ CaCO_3 = [(10 \times N_A) - (R \times N_B)] \times 0.05 \times \frac{100}{wt} \qquad (9)$$

Where: N_A = Normality of HCl solution (A)
R = Amount of NaOH solution used (mL)
N_B = Normality of NaOH solution (B)
w = Weight of soil (g)

Notes

1. It requires some experience to accurately determine the **color change of the suspension from colorless to pink.**

2. 10 mL of 1 *N* **HCl** would dissolve up to 0.5 g $CaCO_3$. That is, if a soil contains 50% $CaCO_3$ or more, 10 mL 1*N* **HCl** would not be sufficient. In that case, 15 or 20 mL

would have to be added.

3. When a soil is reacted with acid to dissolve carbonates, other soil components may also consume acid. Most of the latter reactions are assumed to be reversible, i.e, if the suspension is back-titrated, the acid is released again. For this reason it is *not recommended* to filter the suspension and titrate the clear filtrate. The color change is easier to determine in a clear solution, but the titration value may overestimate the actual $CaCO_3$ content of a soil.

4. Not all reactions involving acid and soil components are completely reversible, and therefore the acid titration of the soil suspension may also slightly overestimate the actual soil carbonate content. The acid titration method can be calibrated against the calcimeter method.

Preparation of Standard Solutions

1. **Sodium Carbonate, 1 *N***

   Dissolve 53.0 g **anhydrous sodium carbonate** (Na_2CO_3) in 1 L distilled water.

2. **Hydrochloric Acid, 1 *N***

   - Add about 300 mL DI water to a 1-L vol. flask.
   - Slowly add 82.8 mL conc. **HCl** (37%, sp. gr. 1.19) using a graduated cylinder.
   - Bring to volume (1 L) with de-ionized water.
   - Pipette 10 mL 1 *N* **Na_2CO_3** solution into a 100-mL Erlenmeyer flask, add 2 drops of **methyl orange** (0.1% in DI water) as indicator, and titrate this solution against HCl (in the burette). The solution changes from light to dark orange.

The exact **HCl** normality is:

$$N_{HCl} = \frac{10 \; x \; N_{Na_2CO_3}}{X_{HCl}} \qquad (10)$$

Where: N_{HCl} = Normality of hydrochloric acid.
X_{HCl} = Hydrochloric acid used, mL.

3. **Sodium Hydroxide, 1 *N***

   - Weigh 40 g **NaOH** in a 500-mL beaker.
   - Add about 250 mL DI water.

- When all **NaOH** is dissolved, transfer the solution to a 1-L vol. flask.
- Wash the beaker with DI water. Let the solution in the flask cool, and bring to volume with DI water.
- Pipette 10 mL standardized 1 *N* **HCl** into a 100-mL Erlenmeyer flask, add 2 drops **phenolphthalein** (0.5 g in 100 mL ethanol) as indicator, and titrate against NaOH solution. The solution changes from colorless to pink.

The exact **NaOH** normality is:

$$N_{NaOH} = \frac{10 \times N_{HCl}}{V_{NaOH}} \qquad (11)$$

Where: N_{NaOH} = **NaOH** solution normality
N_{HCl} = **HCl** solution normality
V_{NaOH} = **NaOH** solution used (mL)

Organic Matter

Introduction

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

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

Most laboratories in the region perform this analysis. 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 sulfate (Walkley and Black, 1934; 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.

Reagents

A. Potassium dichromate solution ($K_2Cr_2O_7$) (0.167 *M*).

- Dry for 2 hr in an oven at 105°C and cool in a desiccator (silica gel).
- Weigh 49.04 g in a 500-mL beaker and dissolve in DI water.
- Transfer to a 1-L vol. flask and bring to volume.

B. Sulfuric Acid (H_2SO_4), conc.

C. Orthophosphoric acid (H_3PO_4), conc.

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

- Weigh 196 g in a 500-mL beaker and dissolve in DI water.
- Add 5 mL conc. H_2SO_4, and transfer to a vol. flask and dilute to 1L.

E. Diphenylamine Indicator

Dissolve 1 g **diphenylamine** in 100 mL conc. H_2SO_4.

Procedure

1. Weigh 1.00 g <150 mesh soil into a 500-mL beaker.

2. Add 10 mL **potassium dichromate** solution (Reagent A) using a pipette, and 20 ml conc. H_2SO_4 using a dispenser, and swirl the beaker to mix the suspension.

3. Allow to stand for 30 min.

4. Add about 200 mL DI water and 10 mL H_3PO_4 acid (Reagent C) using a dispenser, and allow the mixture to cool.

5. Add 10-15 drops **diphenylamine** indicator solution (Reagent E), add a teflon-coated magnetic stirring bar, and place the beaker on a magnetic stirrer.

6. Titrate with **ferrous ammonium sulfate** solution (Reagent D), 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

$$M = \frac{10}{\text{mL (blank)}} \qquad (12)$$

Where:

M = molarity of FAS solution (approx. 0.5 *M*)
mL (blank) = FAS solution required to titrate 10 mL **potassium dichromate** solution (approx. 0.167 *M*).

$$OOC\ (\%\ w/w) = \frac{[\text{mL (blank)} - \text{mL (sample)}]\ x\ 0.3\ x\ M}{\text{g soil}} \qquad (13)$$

Where: OOC = oxidizable organic carbon
mL (sample) = FAS required to titrate the sample solution

$$\textit{Total Organic Carbon (\% w/w)} = 1.334 \times \textit{OOC} \quad (14)$$

$$\textit{Organic Matter (\% w/w)} = 1.724 \times \textit{TOC} = 2.3 \times \textit{OOC} \quad (15)$$

Notes

1. For soils that are high in organic matter (1% Oxidizable Organic Carbon or more), more than 10 mL **potassium dichromate** is needed.

2. The factors 1.334 and 1.724 used to calculate TOC and OM are approximate; they may vary with soil depth and between soils.

3. Soils containing large quantities of chloride (Cl^-), manganous (Mn^-) and ferrous (Fe^{++}) ions will give higher results. The chloride interference can be eliminated by adding **silver sulfate** (Ag_2SO_4) to the oxidizing reagent. No known procedure is available to compensate for the other interferences.

4. The presence of $CaCO_3$ up to 50% causes no interferences.

Cation Exchange Capacity

Introduction

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

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

Several methods are available for CEC determination (Rhoades, 1982). Most involve saturation of the soil with an index cation (ammonium), removal by washing of excess cation, and subsequent replacement of the adsorbed index cation by another cation (sodium) and measurement 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).

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

Reagents

A. Sodium Acetate Solution, 1.0 *N*

- Dissolve 136 g **NaOAc** (trihydrate) in DI water, and dilute to 1 L.
- The pH value of the solution should be about 8.2. This could be adjusted by either **acetic acid** or **NaOH**.

B. Ethanol, 95%

C. Ammonium Acetate Solution, 1.0 *N*.

- To 700-800 mL water, add 57 mL conc. **acetic acid** and cool.
- Add 68 mL conc. **ammonium hydroxide** (NH_4OH) and cool.
- Adjust pH to 7.0 by adding more acetic acid or NH_4OH, and dilute to 1 L with DI water.

Procedure

1. Weigh 4 g (medium to fine textured) or 6 g (coarse textured) soil samples into centrifuge tubes, and add 33 mL **Reagent A**, stopper tube, and shake for 5 minutes.

2. Unstopper tube and centrifuge at 3000 rpm until supernatant liquid is clear. Decant the supernatant as completely as possible, and discard.

3. Repeat with 33-mL portions of **Reagent A** four times, discarding the supernatant liquid each time. Then add 33 mL **Reagent B**, stopper, shake for 5 minutes, unstopper, and centrifuge until the supernatant is clear and decant.

4. Wash the sample with 33-mL portions of **Reagent B** three times; the electrical conductivity (EC) of the supernatant from the third washing should be less than 400 μS/cm.

5. Replace the adsorbed Na from the sample by extraction with three 33-mL portions of **Reagent C**. (Each time shake for 5 min. and centrifuge until supernatant is clear).

6. Decant the three supernatant liquids as completely as possible into a 100-mL vol. flask, and dilute to volume, and mix.

7. Determine the Na concentration of the combined extracts by a flame photometer.

CALCULATIONS

$$CEC\ (meq/100g) = Na\ (meq/L)\ in\ extract\ x\ \frac{100}{W\ x\ 10} \qquad (16)$$

Where:

W = weight of the oven-dry soil sample used.

Note

Though it is laborious, for soils containing carbonates, gypsum, and zeolite, the method of Rhoades and Polemio (1977) is particularly suited.

Gypsum

Introduction

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

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

Apparatus

Centrifuge, 50-mL conical centrifuge tubes, conductivity cell, and Wheatstone bridge.

Reagent

Acetone

Procedure

1. Weigh 10 to 20 g air-dried soil into an 250 mL bottle, and add a measured volume of water sufficient to dissolve the gypsum present.

2. Stopper the bottle and shake by hand six times at 15 min. intervals or agitate for 15 min. in a mechanical shaker.

3. Filter extract through 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, and let stand until precipitate is flocculated. This usually requires 5 to 10 min.

5. Centrifuge at 4000 rpm for 3 minutes, decant supernatant liquid, invert tube, and drain on filter paper for 5 min.

6. Disperse precipitate and rinse tube wall with a stream of 10 mL acetone blown from a pipette.

7. Again, centrifuge for 3 min., decant supernatant liquid, invert tube, and drain on filter paper for 5 min.

8. Add exactly 40 mL DI water to tube, stopper, and shake until the precipitate is completely dissolved. Measure electrical conductivity of solution, and correct conductivity reading to 25°C.

9. Determine gypsum concentration in the solution by reference to a graph showing the relationship between the concentration and EC constructed by means of the following data from the International Critical Tables (Richards, 1954, Fig.2, p.10).

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

CALCULATIONS

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

Gypsum (meq/100 g soil) = 100 x meq $CaSO_4$ in aliquot / soil:water ratio x mL soil-water extract used.

Notes

1. Sodium and potassium sulfates, when present in sufficiently high concentrations, are also precipitated by acetone. The maximum concentrations of sodium sulfate and of potassium that may be tolerated are 50 and 10 meq/L, respectively.

2. At a 1:5 soil:water ratio, water will dissolve approximately 15 meq gypsum per 100 g soil. If it is found that the gypsum content of the soil approaches 15 meq/100 g using a 1:5 soil:water extract, the determination should be repeated, using a diluted extract.

3. In some soils from the Euphrates, gypsum may be well over 25%, in which case dilutions of 1:500, or 1:1000 have to be used.

IV. SOIL NUTRIENTS

Nitrogen

Introduction

In terms of crop requirements for N and the low levels of available N in virtually all soils, *N* is the most important nutrient element in agriculture. Increasingly, it is becoming more important from the environmental perspective, mainly in a negative sense, i.e., pollution.

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

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

The NH_4^+ and NO_3^- N forms are routinely measured in soil laboratories, as they reflect the extent of mineralization, and are the forms of N taken up by plants. In the WANA region, NO_3^--N in soils was shown to be a good indicator of the need for N fertilization. The organic N fraction is a measure of the soils reserve of N or its capacity to release N for crop needs through *mineralization*. Thus, methods of N analysis vary depending on the N fraction or forms of interest.

Total soil N -mainly organic- is generally measured after wet digestion using the established Kjeldahl procedure. Total inorganic N (NH_4^+, NO_3^-, NO_2^-) is usually determined by distillation of a KCl soil extract. In addition to distillation, NO_3^--N can be determined by a procedure involving *chromotropic acid* or by an *ion selective electrode*.

Kjeldahl Nitrogen

This procedure is essentially one of *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. Ammonium in the digest is determined by steam distillation, using excess NaOH to raise the pH. The distillate is collected in saturated H_3BO_3 and titrated to pH 5 with dilute H_2SO_4 (Bremner and Mulvaney, 1982).

The method determines NH_4^+-N, most of the organic N forms, and a variable fraction of NO_3^--N in soil. For most soils, the *Kjeldahl procedure is a good estimate of total soil N content.*

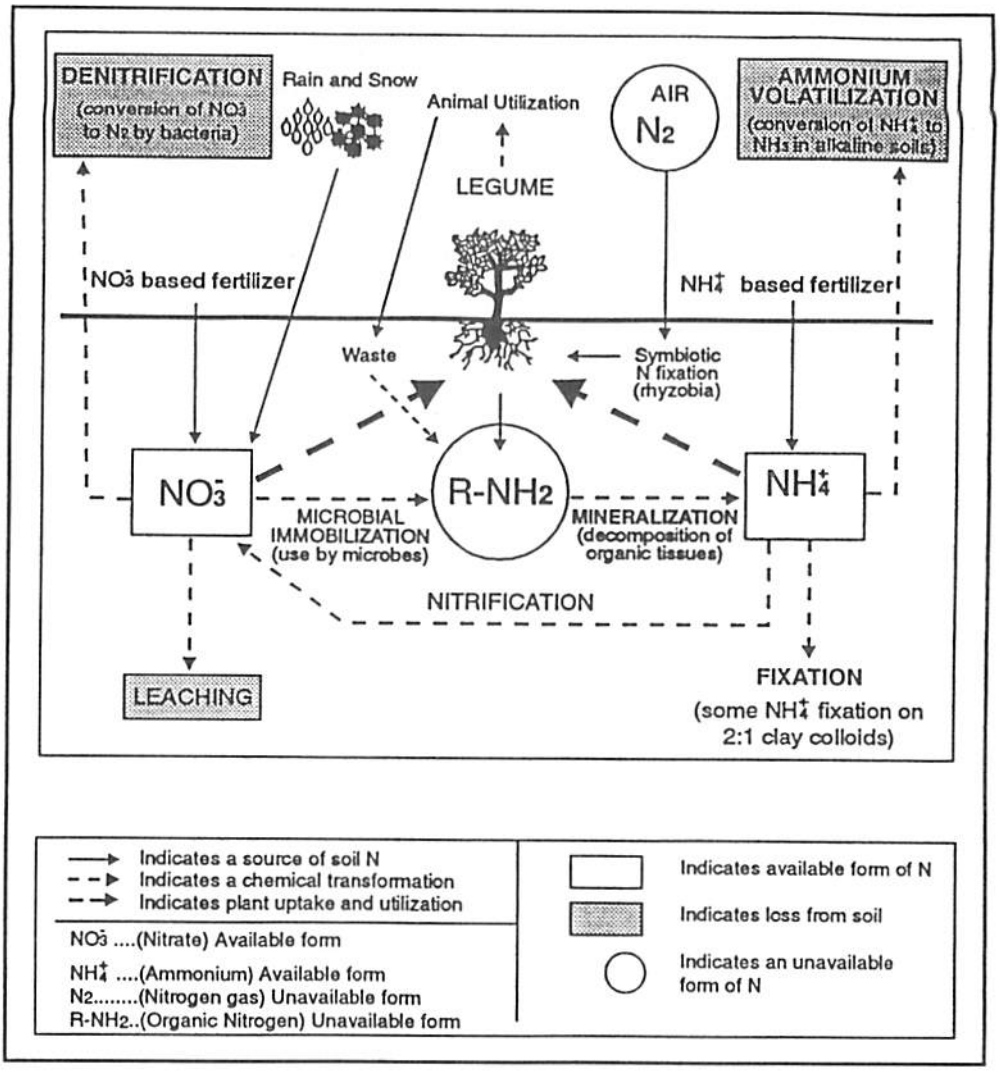


Fig. 5. The nitrogen cycle (Hach Company, 1992).

If desired, NO_3^--N can be included through the reduced iron or salicylic acid modifications of the Kjeldahl procedure (see following section).

Apparatus

- **Block digester**
- **Distillation unit**
- **Automatic titrator connected to a pH-meter.**

Reagents

A. Catalyst Mixture, K_2SO_4 - $CuSO_4.5H_2O$ - Se in 100:10:1 w/w ratio. Grind reagents separately before mixing, and, if caked, grind the mixture in a mortar to pass a 60-mesh screen (0.250 mm).

B. Sulfuric Acid, Conc.

C. NaOH Solution, (10 *N*)

- Transfer 2.0 kg NaOH into a heavy-walled 5-L Pyrex flask, add 2 L DI water, and swirl the flask until dissolved.
- Close the bottle, let it cool, and bring to vol. with DI water.

D. Standard Solution

1.2 mg NH_4-N per L: Dissolve 5.6605 g of dry reagent grade $(NH_4)_2SO_4$ in 1 L DI water.

E. Saturated Boric Acid

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

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

- Dry the reagent at 80°C for 3 hr.
- Cool in a desiccator and store in a tightly stoppered bottle.
- Dissolve 1.2114 g Tris in 1 L DI water.

G. Dilute Sulfuric Acid, 0.01 *N*

- Add approximately 500 mL DI water to a 1-L calibrated flask.

- Add 28 mL conc.H_2SO_4 to the flask (in the fume hood!) and dilute with DI water to volume; this solution is about 1 *N*.
- Then dilute 100 times (10 mL to 1 L) to obtain a 0.01 *N* H_2SO_4 solution.

Procedure

1. Grind soil with a porcelain mortar and pestle to pass a 100-mesh sieve (0.150 mm) and weigh 1.0 g samples into a 100-mL calibrated digestion tube.

2. Add 5.55 g catalyst mixture and 15 mL H_2SO_4 (when in the fume hood). Place a glass funnel in the neck of the tube and swirl carefully, then place tube in the rack, and leave overnight.

3. Pre-heat the block digester to about 370°C for about 45 min. and place the tube rack in the block digester.

4. After the solution clears (about 15 min.), continue heating for about 3 hr. The H_2SO_4 should condense about half-way up the tube neck. Lift the tube rack out of the block digester and (carefully) place on rack holder, place heat shield on block digester, and let tubes cool to room temperature.

5. Slowly add 15 mL DI water to the tubes, cool and bring to volume with DI water. If tube contents are solidified and do not dissolve, heat tubes again until the precipitate (gypsum) dissolves, and cool with tap water.

6. Each batch of samples for digestion should contain at least one blank (no soil) and one standard (no soil; 1 mL Standard Solution D).

Distillation

7. Before starting a batch for distillation, 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 normality of the acid (N) is given by:

$$N = \frac{10 \times 0.01}{mL\ acid} \qquad (17)$$

9. Distillations are carried out as follows: (A diagram of the distillation unit is shown in Fig.6).

   - 1 mL saturated H_3BO_4 solution and 1 mL DI water are dispensed into a 100-ml Pyrex evaporating dish, which is placed underneath the condenser tip, with the tip touching the solution surface.

   - Pipette a 10-mL aliquot into a 100-mL distillation flask, add 10 mL of 10 *N* NaOH solution, and start distillation.

   - A 10-mL aliquot of the digested solution is pipetted into a 100-mL distillation flask.

   - The flask is attached to the still with a clamp, and distillation is started and continued for 3 min. After 2 min., the dish is lowered to allow distillate to drain freely into the dish.

   - After 4 min., when about 35 mL distillate is collected, the steam supply is turned off, and the tip of the condenser is washed into the evaporating dish with a small amount of DI water.

   - The distillate is then titrated to pH 5 with standardized 0.01 *N* H_2SO_4 on the auto titrator.

   - After titration, the teflon-coated magnetic stirring bar, the burette, and the combined electrode should be washed into the dish.

   - Between distillation of different samples, the stills should be steamed out for 90 seconds.

   - Place 100-mL beakers underneath the condenser tips, turn off cooling water supply (drain the water in the condenser jacket), and steam out for 90 seconds. Steaming out is done only between different samples.

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

Total Nitrogen

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

Reagents

A. Sulfuric Acid (H_2SO_4), conc. reagent grade

B. Potassium Permanganate Solution

Dissolve 50 g $KMnO_4$ in 1 L DI water, and store the solution in an amber bottle.

C. Sulfuric Acid, 50% V/V

Slowly add 1 L conc. H_2SO_4 with continuous stirring to 1 L DI water in a 4-L flask.

D. Reduced Iron

Grind in a ball mill and sieve to remove any material which does not pass a 0.15-mm screen.

E. N-Octyl Alcohol

F. Digestion Catalyst Mixture. Prepare as in Kjeldahl-N.

G. EDTA, reagent - grade disodium salt (M.W. = 372.2). Store in a desiccator.

H. NaOH, 10 *N* : as in Kjeldahl-N.

I. Saturated Boric Acid: as in Kjeldahl-N.

J. TRIS: as in Kjeldahl-N.

K. Sulfuric Acid 0.01 *N*: as in Kjeldahl-N.

Procedure

1. Mix and spread the soil sample in a thin layer on a sheet of paper, ensuring that root material is uniformly mixed in the soil.

2. Take a representative soil sample, which contains about 3 to 8 mg N, by withdrawing

10 small portions from the soil sample, e.g., 10 g.

3. Weigh the sample to 0.01 g and place in a 250-mL vol. digestion tube.

4. At the same time, take a sample for moisture determination (105°C).

5. Add 10 mL DI water to each tube and swirl thoroughly to wet the soil. Allow wet soil to stand for 30 min.

6. Prepare a blank digest and an **EDTA** standard digest (0.1 g EDTA accurately weighed to 0.1 mg) with each batch.

7. Add 10 mL **$KMnO_4$** solution, swirl, allow 30 sec., 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.

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

8. Allow 15 min., then swirl.

9. Add 2 drops **N-octyl alcohol**.

10. Add a few pumice granules to the blank and EDTA digests.

11. 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.

12. Excessive frothing at this stage may be halted by pouring 5 mL water through the 5-cm, funnel; do *not* swirl.

13. Allow the tubes to stand overnight.

14. Pre-digest the samples by placing them on the cold block and heating at 100°C for 1 hr. The block comes to 100°C within 15 min.; therefore, total time on the block will be approximately 1 hr and 15 min.

15. Samples should be swirled at 45 min.

16. Remove tubes from the block and cool. Rapid cooling may be effected in tap water.

17. Leave overnight.

18. Add 5 g **catalyst mixture** through a long stem funnel. Then add 25 mL **H_2SO_4** to each

Mineral Nitrogen

Nitrogen is used by plants in two forms, ammonium (NH_4^+-N) and nitrate (NO_3^--N). Ammonium ions are present in soils through breakdown of organic tissue or manure application. Nitrate is the final form of N breakdown, but can also be supplied by fertilizers and irrigation.

Available N is 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 on the surface of clay and humus particles. However, when NH_4^+ is transformed to NO_3^-, the (+) charge is lost and the soil no longer attracts the available N. Water percolating through a soil profile leaches and depletes the mobile NO_3^- from the upper layers to the lower layers, and even into the groundwater if leaching is excessive. *Excessive nitrate leaching is most likely in fields where over-fertilization has occurred.*

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

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

Reagents

A. Potassium Chloride Solution, 2 *M*

- Dissolve 1500 g reagent-grade KCl in 8 L water
- Dilute to 10 L.

B. Magnesium Oxide

- Heat heavy MgO in a muffle furnace at 600-700° C for 2 hr.
- 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 alloy until the product will pass a 100-mesh screen (0.150 mm) and at least 75% will pass a 300-mesh screen (0.050 mm).

D. Saturated Boric Acid Solution, about 1 *M* : As in Kjeldahl-N.

E. Tris Solution (hydroxymethyl aminomethane) $C_4H_{11}NO_3$, 0.010 N as in Kjeldahl-N.

F. Buffer Solutions, pH 4 and 7.

G. Dilute Sulfuric Acid, 0.01 *N*: As in Kjeldahl-N.

H. Standard Solution

Prepare a *stock solution* containing 1.2 mg NH_4-N and 1.2 mg NO_3-N per L.

- Dry reagent-grade $(NH_4)_2SO_4$, and KNO_3 at 100°C for 2 hr.
- Cool in a desiccator and store in a tightly stoppered bottle.
- Weigh 5.6605 g $(NH_4)_2SO_4$ and 8.6624 g KNO_3, and add to a 1-L vol. flask.
- Bring to the mark with distilled water. This will be the stock solution.
- Prepare a *standard solution* by diluting the stock solution 20 times with 2 *M* KCl solution *(50 mL to 1 L).*
- A 20-mL aliquot of this solution contains 1.2 mg NH_4-N and 1.2 mg NO_3-N.

Procedure

1. Weigh 30 g soil in 250-mL Erlenmeyer flask, add 150 mL 2 *M* KCl solution .

2. Stopper flask and shake for 1 hr on an orbital shaker at 200-300 rpm, and filter suspensions using Whatman No. 42 filter paper.

3. Calibrate pH-meter with buffer solutions of pH 7 (buffer) and 4 (sensitivity), and set temperature.

4. Before starting distillation, the still should be steamed out for at least 10 minutes. Adjust steam rate to 7-8 mL distillate per min.

5. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22°C.

6. Distillations are carried out as follows:

   - 1 mL saturated H_3BO_4 solution and 1 mL DI water are dispensed into a 100-ml Pyrex evaporating dish, which is placed underneath the condenser tip, with the tip touching the solution surface.

   - A 20-mL aliquot of the clear supernatant solution is pipetted into a 100-ml distillation flask.

- To determine NH_4^+-N in solution, 0.2 g heavy **MgO** is added to the distillation flask with a calibrated spoon.

- The flask is attached to the still with a clamp, and distillation is started and continued for 3 min. After 2 min., the dish is lowered to allow distillate to drain freely into the dish.

- After 4 min., when about 35 mL distillate is collected, the steam supply is turned off, and the tip of the condenser is washed into the evaporating dish with a small amount of DI water.

- The distillate is then titrated to pH 5 with standardized 0.01 *N* H_2SO_4 on the auto titrator.

- After titration, the teflon-coated magnetic stirring bar, the burette, and the combined electrode should be washed into the dish.

- To determine NO_3^--N (plus NO_2^--N) in the same extract, 0.2 g **Devarda's alloy** is added to the same distillation flask with a calibrated spoon.

- Attach flask to still with a clamp, and start distilling. Further proceed as for ammonium.

- Between distillation of different samples, the stills should be steamed out for 90 seconds. Disconnect distillation flasks containing the **KCl** extracts, and attach empty flasks to stills.

- Place 100-mL beakers underneath the condenser tips, turn off cooling water supply (drain the water in the condenser jacket), and steam out for 90 seconds. Steaming out is done only between different samples, not between ammonium (MgO) and nitrate (Devarda's alloy) in the same sample.

- Each distillation should contain at least two standards and two blanks, i.e., KCl extracts with no soil added (reagent blanks).

CALCULATIONS

For N in Air-Dry Soil:

$$NH_4 - N\ (ppm) = (V - B)\ x\ N\ x\ 14.01\ x\ \frac{150}{20}\ x\ \frac{1}{30}\ x\ 1000 \qquad (22)$$

For N in Oven-Dry Soil:

$$NH_4 - N\ (ppm) = (V - B)\ x\ N\ x\ 14.01\ x\ \frac{150}{20}\ x\ \frac{1}{30 - c}\ x\ 1000 \qquad (23)$$

Where:

V = Volume of acid titrated (mL)
B = MgO blank in KCl solution (mL)
N = Normality of acid
14.01 = Atomic weight of N
150/20 = Ratio of extractant volume-to-volume distilled
30 = Weight of air-dry soil
c = weight of water (g) per 30 g air-dry soil.

Notes

1. The concentration of NO_3^--N (ppm) is calculated in the same way as for NH_4^+-N, except that the Devarda's Alloy blank has to be inserted in the formula.

2. In some laboratories, a 1:3 (w/v) soil-solution extract is used for Mineral-N determination. For soils in northwestern Syria, a 1:5 extract gives a higher recovery of NH_4^+-N than a 1:3 extract.

3. For determination of NO_3^--N in calcareous soils, we recommend using **de-ionized water** as the extracting solution, because carbonates dissolve in the KCl solution and some CO_2 may be collected in the H_3BO_3 during distillation. This causes a negative interference with NO_3^--N determinations in KCl extracts.

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

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

6. There is much confusion about the relationship between NO_3^- and NO_3^--N. The nitrate ion is a combination of 1 nitrogen atom and 3 oxygen atoms. The total mass of NO_3^- is 14+48=62. So, in 62 g of NO_3^-, 14 g of N and 48 g of oxygen are present. This relationship can be expressed in two ways, either as 62 g of NO_3^-, or as 14 g of NO_3^--N. Either expression is 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 x 4.43, or 44.3 ppm NO_3^-. Both values indicate the same concentration in a different format.

Nitrate Nitrogen

Nitrate N can be measured rapidly either by a spectrophotometric method (using chromotropic acid), or by specific ion electrode.

Chromotropic Acid: Use of chromotropic acid is a rapid spectrophotometric method used originally for water and later for soil analysis (Sims and Jackson, 1971; Hadjidemetriou, 1982). It can be used as an alternative for NO_3-N determination by the distillation method. A close relationship exists between NO_3 by chromotropic acid and distillation.

Reagents

A. Copper Sulfate Solution, 0.02 *N*

Dissolve 4.9936 g $CuSO_4.5H_2O$ in 2 L DI water.

B. Chromotropic Acid, 0.1 %

Dissolve 0.368 g $C_{10}H_6Na_2O_8S_2.2H_2O$ in 200 mL conc. H_2SO_4.
Keep solution in a dark bottle for 2 weeks.

C. Sulfuric Acid, Concentrated Analytical Reagent

Procedure

1. Weigh 10.0 g air dry soil in an Erlenmeyer flask, and add 50 mL **$CuSO_4.5H_2O$** solution (A).

2. Shake for 15 min. and filter through a double Whatman No. 42 filter paper.

3. Transfer 3 mL filtrate in 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 min. to cool.

5. Mix solution, and add 6 mL conc. **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 develops after 45 min.

7. Read the percent transmittance at 430 nm using a 1-cm cuvette.

Standard Solutions

- Dissolve 3.6092 g KNO_3 (dried at 105°C for 2 hr) in 500 mL 0.02 *N* $CuSO_4.5H_2O$ solution (Solution A).
- Dilute 10 mL Solution A to 200 mL by adding 0.02 *N* $CuSO_4.5H_2O$ solution to give Solution B, which has 50 ppm NO_3-N.
- Prepare series of Standard Solutions from Solution B:

  Dilute 1, 2, 3, 4, 5, 6 and 7 ml of solution B to 100 ml final volume of each by adding by 0.02 *N* $CuSO_4.5H_2O$ solution. This will give NO_3-N concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ppm NO_3-N.
- Take 3 mL of each standard and proceed as for the samples.
- Also make a blank with 0.02 *N* $CuSO_4.5H_2O$ solution.
- Read the percent transmittance of blank and standards.
- Plot standards on a graph paper (ppm against transmittance).
- Read the unknown samples from the graph.

CALCULATIONS

$$NO_3 - N\ (ppm) = C \times R \times \frac{10}{V} \qquad (24)$$

Where:

C = NO_3-N conc. from the graph (ppm).
R = Soil/solution ratio.
V = Aliquot used for measurement (3 mL)

Notes

1. Where soils contain (> 1 ppm) NO_2-N , add 0.1 ml sulphamic acid (0.2% m/v in 0.1 *N* H_2SO_4) to the 3-mL sample solution.
2. If filter paper gives purple solutions, it must be washed with distilled water and dried before use.

Specific Ion Electrode: The NO_3 electrode consists of an electrode body and a replaceable pre-tested sensing module. The sensing module contains a liquid internal filling solution, a membrane saturated with a liquid ion exchanger, and a reservoir of liquid ion exchanger. When the membrane is in contact with a nitrate solution, an electrode potential develops across the membrane. This potential, which depends on the level of free nitrate ion in solution, is measured against a constant reference potential with a digital pH/mV meter or specific ion meter. The measured potential corresponding to the level of nitrate ion in solution is described by the Nernst equation. If the background ionic strength is high and constant relative to the sensed ion concentration, the activity coefficient is constant and activity is directly proportional to concentration.

Ionic Strength Adjustor (ISA) is added to all NO_3^--N standards and samples so that the background ionic strength is high and constant relative to the variable NO_3^--N concentrations (Mills, 1980; Keeney and Nelson, 1982).

Equipment

- pH/MV-meter (Orion Expandable Ion Analyzer EA-940)
- Nitrate electrode (Orion Model 93-07)
- Reference electrode (double junction 90-02)
- Magnetic stirrer

Solutions

A. Distilled or DI water: To prepare all solutions and standards.

B. 1000 ppm NO_3-N solution

Dissolve 7.22 g dried KNO_3 in 1 L distilled water to give a concentration of 1000 ppm. Prepare a standard series 5, 10, 20, 40, and 60 ppm NO_3-N.

C. Electrode Assembly

D. Ionic Strength Adjuster (ISA)

To keep a constant background ionic strength, prepare a 2 *M* ammonium sulfate solution:

- Add 26.4 g reagent-grade $(NH_4)_2SO_4$ to a 100-mL vol. flask. Dissolve and dilute to mark with DI water.

- Add 2 mL ISA to 100 mL of all samples and standards to bring the background ionic strength to 0.12 *M*.

E. Reference Electrode (outer chamber) Filling Solution

- Add 2 mL ISA to 100 mL DI water and fill outer chamber of reference electrode.

Do not use outer chamber filling solution shipped with the Reference Electrode.

F. Preservative Solution

- Prepare a 1 *M* H_3BO_3 preservative solution by dissolving 6.2 g reagent-grade H_3BO_3 in 100 mL boiling water. Let cool.

- Add 1 mL preservative H_3BO_3 solution to 100 mL of all standards and samples to prevent degradation of solutions.

Procedure

1. Weigh 20 g soil in 100-mL Erlenmeyer flask and add 100 mL DI water.

2. Shake for 2 hr, then filter with Whatman No 42 paper using suction. Keep filtrate for NO_3-N measurement.

3. Calibrate the Orion Ion Analyzer according to the Manual instructions, using at least two standards (20 and 40 ppm) so that concentration is read directly.

4. Check the slope of the electrode according to electrode use instructions.

5. After the instrument has been programmed and properly set, take 50 ml soil filtrate in a 100-mL beaker and add 1 mL **ISA solution** with one stirring bar.

6. Insert electrodes into the sample and stir gently on a magnetic stirrer (no vortex should be formed).

7. Read concentration directly in ppm (R).

CALCULATIONS

$$NO_3\text{-}N\ (ppm) = R \times 5\ (i.e.,\ Soil{:}Solution\ Ratio) \qquad (25)$$

Note

The NO_3 specific ion electrode offers a simple method of analysis in plants, soils and water. The disadvantages of this technique is that it is susceptible to interfering ions, particularly Cl^-. Nitrate electrode can give excellent results for advisory purposes where a high degree of accuracy is not warranted.

Microbial Biomass Carbon And Nitrogen

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).

Reagents

Chloroform (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 in a dark bottle.

Potassium Sulfate, 0.5 *M*

Prepare by dissolving 87.13 g K_2SO_4 in 1L DI water.

Procedure

1. Place 15 g of fresh soil samples into a 50 mL beaker. Conduct a moisture determination on soil sub-samples so that the results can be expressed on a dry weight basis.

2. Place the beakers into the two paired desiccators, place a 100-mL beaker containing 25 mL **chloroform** (alcohol free) into the center of the desiccator. Adding boiling chips to the chloroform assists in rapid volatilization of the chloroform. The second desiccator contains non-fumigated control samples which apart from fumigation-evacuation are to be handled in the same fashion. Close the lids of the desiccators paying particular attention that the sealant is uniformly distributed (Fig. 7).

3. Apply a vacuum to the fumigated treatment until the chloroform is rapidly boiling. Close the desiccator and store under darkened conditions for 72 hr at room temperature.

4. Evacuate the fumigated treatments using a vacuum pump repeatedly (8-12 times). Remember that the chloroform is being trapped by the oil in the vacuum pump so the oil must be changed more often than normal. Alternatively, chloroform can be trapped by a cooling finger to prevent contamination of the vacuum oil. It is not necessary to

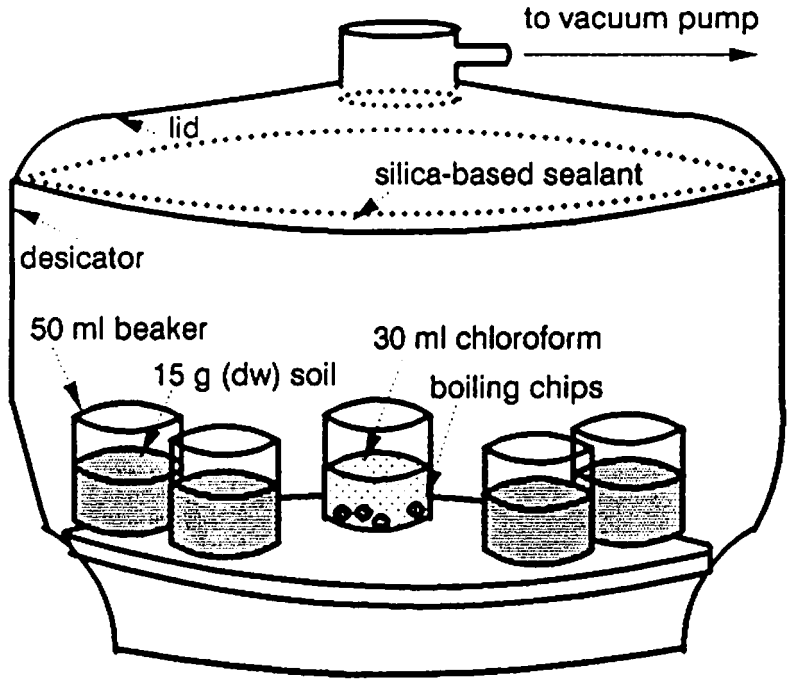


Fig. 7. Experimental apparatus and sample arrangement in the fumigation procedure. (Okalebo, et al., 1993)

evacuate the control desiccator.

5. Open the desiccators and transfer the soil samples to shaking bottles or flasks (125-250 mL). Add 50 mL of 0.5 M K_2SO_4 and shake on a wrist shaker for 25 min.

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

7. Digest the sample and analyze for N as described in total N. Also, analyze for C as described in organic carbon.

CALCULATIONS

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

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

Note

Some authors suggest that empirically derived correction factors should be applied to these results. These factors may be obtained by conducting the fumigation/extraction procedure on inert soils containing a known quantity of microbial biomass (e.g. mushrooms or washed bacterial cells). Vance et al. (1987) advocate a factor of 2.64 for microbial biomass, while Brooks et al. (1985) recommend a factor of 1.46 for biomass N. If these factors are applied, this should be clearly indicated when reporting the results. Because of the large variation in soil microbial (and microfaunal) populations in soils, it is suggested that these factors not be applied.

Phosphorus

Extractable Phosphorus

Because of its significance as a major element essential for plant growth, phosphorus (P) is measured in virtually all soil laboratories. Compared to N and most other elements, soil tests for P is generally a fairly reliable indicator of the need for P fertilizer for 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. A good test must be well correlated with crop P uptake and must be calibrated to fertilizer response of crops in the field. Tests for routine use should be *simple*, *quick*, *easy to execute*, and *inexpensive*.

The sodium bicarbonate procedure of Olsen *et al.* (1954) meets these criteria and is generally accepted as a suitable index of P "availability" for calcareous 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, at ICARDA, considerable emphasis has been placed in adopting this test for routine use as a basis for soil fertility evaluation.

The sodium bicarbonate extractant was first developed and described by Olsen *et al.* (1954). In the original method, carbon black was added in the extraction reagent to eliminate the color in the extract. This procedure was later modified so that the use of carbon black was eliminated (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.

Reagents

A. Sodium Hydroxide Solution, 5 *N*

Dissolve 200 g NaOH in about 1 L DI water. Bring to volume after cooling.

B. Sodium Bicarbonate Solution, 0.5 *M*

Dissolve 42 g $NaHCO_3$ in about 900 mL DI water, adjust to pH 8.5 with 5 *N* NaOH solution. Bring to 1 L with DI water. Keep the bottle closed and do not store over 1 month in a glass container, or use polyethylene container for periods > 1 month.

C. Sulfuric Acid, 5 *N*

Dilute 141 mL conc. H_2SO_4 to 1 L with DI water.

D. P-Nitrophenol Indicator, 0.25 % w/v

E. Standard Phosphorus Solution

- Dry about 2.5 g potassium dihydrogen phosphate (KH_2PO_4) for *1* hr at 105° C.
- Transfer to a desiccator (silica gel) and let cool.
- Dissolve 2.197 g dry KH_2PO_4 in DI water in 1-L vol. flask and bring to volume. This solution should contain *500 ppm P* (Stock solution).
- Pipette 50 mL stock solution into a 250-mL vol. flask, and bring to volume with DI water; this solution should contain *100 ppm P* (Diluted Stock solution).
- For working standards, dilute 5, 10, 15, 20 and 25 mL of the diluted stock solution to 500 mL. This should give solutions containing 1, 2, 3, 4, and 5 ppm P, respectively.

F. Reagent A

- Dissolve 12.0 g ammonium heptamolybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250 mL DI water.
- Dissolve 0.2908 g antimony potassium tartrate $(KSbO.C_4H_4O_6)$ in 100 mL DI water.
- Add both dissolved reagents to 1 L 5 *N* H_2SO_4 (148 mL conc. H_2SO_4 per liter). Mix thoroughly, and dilute to 2 L with DI water.

G. Reagent B

- Dissolve 1.056 g ascorbic acid in 200 mL Reagent A and mix. This reagent should be prepared as required because it does not keep more than 24 hr.

Procedure

1. Weigh 5.0 g 2 mm sieved soil into a 250-mL Erlenmeyer flask; add 100 mL **Solution B (0.5 *M* $NaHCO_3$)**.
2. Close the flask with a rubber stopper, and shake for 30 min. on an orbital shaker at 200-300 rpm. Include one flask containing all chemicals but no soil.
3. Filter the solution over a Whatman No. 40 filter paper, and pipette 10 mL clear filtrate into a 50-mL vol. flask.
4. Acidify with 5 *N* H_2SO_4 to pH 5.0. This can be done by taking 10 mL **$NaHCO_3$** solution and determining the amount of acid required to bring the solution to pH 5 using **P-nitrophenol** indicator (Solution D) (color change is from *yellow* to *colorless*). Then add

the required acid to all the unknowns. Adding 1.0 mL 5 *N* H_2SO_4 is adequate to acidify each 10 mL $NaHCO_3$ extract.

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

6. Read the transmittance after 10 minutes using a wavelength of 882 nm.

7. Prepare a standard curve as follows:

   - Pipette 2 mL of each standard (1-5 ppm) into a 50-mL vol. flask.
   - Add 10 mL $NaHCO_3$ solution
   - Acidify with 5 *N* H_2SO_4 solution
   - Add 8 mL of Reagent B
   - Bring to 50 mL volume and develop color.

8. Plot percent transmittance against P concentration.

CALCULATIONS

$$\textit{Extractable P concentration in soils (ppm)} = \textit{ppm P graph} \times \frac{100}{5} \times \frac{50}{10} \qquad (28)$$

Notes

1. The unit *ppm* (parts per million) is commonly used in soil and plant analysis. One ppm is exactly equal to 1 mg/L if the specific weight of the solution is exactly 1 kg/L. For dilute standard solutions in distilled water, 1 ppm is approximately equal to 1 mg/L at room temperature.

2. The amount of available P extracted from a soil depends on pre-treatment of samples, shaking frequency and time, and on temperature during extraction. Therefore, sample treatment and the conditions during extraction should be standardized.

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

4. Glassware used in P analysis should not be washed with detergents containing P.

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

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 or minerals. This measurement is usually employed only for soil genesis or mineralogical studies (Olsen and Sommers, 1982).

Reagents

A. Perchloric Acid, (60%)

B. Ammonium Heptamolybdate-Vanadate

- Dissolve 25 g of ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}.\ _4H_2O$] in 400 mL DI water.
- Dissolve 1.25 g of ammonium metavanadate (NH_4VO_3) in 300 mL of boiling DI water. Cool the solution and add 250 mL of conc. nitric acid (HNO_3). Cool the solution to room temperature.
- Pour the ammonium heptamolybdate solution into the NH_4VO_3-HNO_3 solution, and dilute to 1L with DI water.

C. Standard Phosphorus Stock Solution

- Dry about 2.5 g potassium dihydrogen phosphate (KH_2PO_4) for one hr at 105°C.
- Transfer to a desiccator and let cool.
- Dissolve 0.4393 g dry KH_2PO_4 in DI water in a 1-L vol. flask and bring to volume. This solution should contain 100 ppm P.
- Prepare solutions containing 2-10 ppm P by diluting suitable aliquot of the solution with DI water.

Procedure

1. Weigh 2 g 0.5 mm soil into a 250-mL calibrated digestion tube.

2. Add 30 mL **$HClO_4$** (60%), and add few pumice stones. Mix well.

3. Pre-heat the block digester to about 100°C and place the tubes in the rack.

4. Increase temperature to 180°C and digest the samples until dense white fumes of acid appear. Use a little extra perchloric acid to wash down the sides of the digestion tube as necessary.

5. Continue heating at the boiling temperature 20 min. longer. At this stage the insoluble material becomes like white sand. The total digestion with $HClO_4$ acid usually requires

about 40 min.

6. Cool the mixture and add DI water to obtain a volume of 250 mL and mix the contents, and filter through Whatman No. 41 filter paper.

Note

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

Measurement

1. Pipette 5 mL of the sample digest into a 50-mL vol. flask.
2. Add 10 mL of the **vanadomolybdate** reagent.
3. Dilute the solution to 50 mL with DI water.
4. Measure absorbance on a spectrophotometer, after 10 min. at 410 nm wavelength.
5. Prepare a standard curve as follows:
   - Pipette 2, 4, 6, 8 and 10 mL of standard phosphorus stock solution (C) into a 100-mL vol. flask.
   - Add 10 mL of vanadomolybdate reagent.
   - Bring to 100 mL volume and develop color.
6. Plot a graph of absorbance against P concentration in standards.

CALCULATIONS

$$P\ (ppm) = P\ ppm\ (from\ standard\ calibration\ curve)\ x\ \frac{250}{V}\ x\ \frac{50}{W} \qquad (29)$$

Where: V = aliquot of soil digest measured (mL)
W = soil weight digested (g)

Potassium

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

Highly weathered soils (tropical and temperate regions) tend to be deficient in plant available K, whereas 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 with high K-demanding crops, i.e, sugarbeet and potatoes.

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

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

Extractable Potassium (exchangeable plus soluble)

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

Apparatus

A flame photometer with accessories.

Reagents

A. Ammonium Acetate Solution, 1.0 *N*

- Add 57 mL conc. acetic acid (CH_3COOH) to 700 or 800 mL DI. water, then add 68 mL conc. ammonium hydroxide (NH_4OH).
- Dilute to 1 L and adjust to pH 7.0 by adding more NH_4OH or acetic acid.

B. Standard Potassium (K) Solutions

- Dissolve 1.907 g KCl (dried 1-2 hr for 120°C) in DI water and dilute to 1L.

- This is the *stock solution* and containing *1000* ppm K.
- For working standards, dilute 2, 4, 6, 8, 10, 15 and 20 mL of stock solution (solution B) to 100 mL to give a range of *20 to 200* ppm K.

Note

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

Procedure

1. Weigh 5 g < 2 mm soil into a 50-mL centrifuge tube, add 33 mL **ammonium acetate** solution, and shake for 5 min. on a shaker. The tubes should be stoppered with clean rubber or polyethylene stopper, but not corks, which introduce errors.

2. Centrifuge until the supernatant liquid is clear and collect the extract in 100-mL vol. flask through a filter paper to exclude any soil particles. Repeat this process two more times and collect the extract each time.

3. Dilute the combined **ammonium acetate** extracts to 100 mL with ammonium acetate solution, and read the K concentration on a flame photometer.

4. Prepare a standard curve using standard solutions of K.

CALCULATIONS

$$A)\ \textit{Extractable K (ppm)} = \textit{K in the extract (ppm)} \times \frac{100}{5} \qquad (30)$$

Where:

5 = weight of soil taken (g).
100 = total volume of extract (mL).

Soluble Potassium

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

Procedure

1. Take 5 g soil (<2 mm) into a 250-mL Erlenmeyer flask, add 100 mL of DI water, and shake about 1 hr.

2. Filter and measure soluble K on a flame photometer.

CALCULATIONS

$$A)\ \textit{Soluble K (ppm)} = \textit{K in the extract (ppm)}\ x\ \frac{100}{5} \qquad (31)$$

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:

$$\textit{Exchangeable K (ppm)} = \textit{Extractable K - soluble K.} \qquad (32)$$

Notes

1. Exchangeable sodium (Na) could be measured in the same way as for exchangeable K. Where extractable-Na is measured in the ammonium acetate extract and soluble Na is measured in the water extract. The difference will represent exchangeable Na.
2. A range of 20 to 200 ppm of Na standards could be prepared in ammonium acetate solution for extractable-Na and in de-ionized water for soluble-Na.

3. Soils may be stored in an air-dry condition for several months with no effects on the exchangeable K, Mg, Ca and Na content.

4. After extraction, the filtrate containing K, Mg, Ca and Na should not be stored for longer than 24 h unless it is refrigerated or treated to prevent bacterial growth.

Soluble Calcium and Magnesium

This fraction of Ca and Mg involves extraction by water and measurement by titration of Ca and Mg in that extract (Richards, 1954). Calcium and Mg in the extracts can also be measured by an atomic absorption spectrophotometer.

Reagents

A. Buffer Solution (NH_4Cl-NH_4OH)

Dissolve 67.5 g NH_4Cl in 570 mL conc. ammonium hydroxide (NH_4OH) in a 1-L vol. flask, dissolve and bring to 1 L with DI water.

B. Eriochrome Black Indicator

Dissolve 0.5 g Eriochrome Black with 4.5 g **hydroxylamine hydrochloride** in 100 ml ethyl alcohol (95%). Prepare fresh monthly.

C. EDTA ($\approx$ 0.01 N)

Dissolve 2.0 g **ethylene diaminetetraacetic acid** (EDTA) and 0.05 g $Mgcl_2$ in 1 L DI water.

D. Standard Calcium Chloride Solution, 0.01 *N*

Dissolve 0.5 g pure $CaCO_3$ in 10 mL 3*N* HCl and dilute to 1 L.

E. Sodium Hydroxide, 2 *N*

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

F. Ammonium Purpurate Indicator

Mix 0.5 g ammonium purpurate with 100 g K_2SO_4.

Procedure

A. Calcium

1. Pipette 10-20 mL saturation extract, having not more than 1.0 meq Ca, into a 250-mL Erlenmeyer flask.

2. Dilute to 20-30 mL with DI water, add 2-3 mL 2 *N* NaOH solution (Reagent E) and about 50 mg **ammonium purpurate** indicator (Reagent F).

3. Titrate with **Reagent C** (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 sec. since the color change is not instantaneous.

4. Always run a blank containing **Reagents E** and **F**. One or two drops of **Reagent C** help to distinguish the end-point.

B. Calcium plus Magnesium

1. Pipette 10-20 mL of saturation extract into a 250-mL flask, dilute to 20-30 mL with DI water. Then add 3-5 mL **Buffer Solution** (Reagent A) and few drops of **Eriochrome Black** indicator (Reagent B).

2. Titrate with **Reagent C** until the color changes from red to blue.

CALCULATIONS

$$Ca \text{ or } Ca + Mg \text{ } (meq/L) = \frac{A \times N \times 1000}{V} \quad (33)$$

$$Mg \text{ } (meq/L) = Ca + Mg \text{ } (meq/L) - Ca \text{ } (meq/L) \quad (34)$$

Where:

A = EDTA used for titration (mL)
N = EDTA normality
V = Volume of extract titrated (mL)

EDTA Standardization

- Take 10 mL 0.01 *N* $CaCl_2$ (Solution D) and treat as in determining Ca and Ca+Mg procedure, respectively.

- Calculate EDTA normality:

$$EDTA \text{ } Normality = \frac{10 \times 0.01}{V} \quad (35)$$

Where:

10 = Aliquot of $CaCl_2$ titrated (mL).
0.01 = Normality of $CaCl_2$.
V = Volume of EDTA needed for titration (mL).

Notes

- Normality with Ca determination usually is 3 to 5% higher than with Ca + Mg.
- If there is not enough saturation extract, a 1:5 ratio soil/water suspension can be prepared. Shake for 30 min., filter, and use filtrate for analysis.
- If an atomic absorption spectrophotometer is used, a small aliquot of the saturation extract is sufficient to determine Ca and Mg.

Sodium

Sodium in extractable form can be extracted with ammonium acetate solution in the same way as K, while soluble Na may readily be obtained in a water extract or from a saturated paste as for E.C. Sodium readily lends itself to determination by a flame photometer. Many elements, such as Na, have the property that, when their salts are introduced into a flame, they emit light with a wavelength (color) specific to the element and of intensity proportional to the concentration (Richards, 1954).

Apparatus: Flame photometer with accessories.

Reagents

A. Standard NaCl solution, 1000 ppm

Dissolve 2.5418 g dried NaCl in DI water, and dilute to 1 L.

B. Lithium Chloride, 1000 ppm

Dissolve 6.109 g dry LiCl in DI water and dilute to 1 L.

Procedure

1. Using **Reagents A** and **B**, prepare a series of standard NaCl solutions in DI water ranging from 0, 25, 50, 75, 100, 150, and 200 ppm Na, with each containing the same concentration of LiCl (25 ppm /L).
2. Operate flame photometer according to instructions; set the filter on Na.
3. Run a series of suitable Na standards and draw a calibration curve.
4. Measure the samples (soil extract) and take the emission readings.
5. Calculate Na concentrations according to the calibration curve.

CALCULATIONS

$$Na\ (meq/L) = Na\ from\ calibration\ curve\ (meq/L) \times dilution\ factor\ (DF) \quad (36)$$

$$Na\ (ppm) = Na\ (meq/L) \times DF \times 23.0\ (atomic\ weight\ for\ Na) \quad (42)$$

Carbonate and Bicarbonate

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

Reagents

A. Methyl Orange Indicator, 0.01% in water.
B. Sulfuric Acid, 0.01 *N*
C. Phenolphthalein, 1% in ethanol.

Procedure

1. Pipette 10-15 mL soil extract in a wide-mouthed porcelain or 150-mL Erlenmeyer flask.

2. Add 1 drop **Reagent C**. If pink color occurs, add **Reagent B** by a burette, drop by drop, until the color disappears.

3. Take this reading, y.

4. Continue the titration with 0.01 *N* H_2SO_4 after adding 2 drops of **methyl orange** indicator until the color turns to orange.

5. Take the reading, t.

Note

Always use blanks for reagents, and subtract from the determination.

CALCULATIONS

$$CO_3^{--}\ (meq/L) = 2y \times N \times \frac{1000}{V} \qquad (38)$$

$$HCO_3^{-}\ (meq/L) = (t - 2y) \times N \times \frac{1000}{V} \qquad (39)$$

Where: N = H_2SO_4 Normality
V = mL aliquot used for titration

Chloride

Chloride, which is soluble in water, is determined by silver nitrate titration (Richards, 1954).

Reagents

A. Potassium Chromate, 5% in water

- Dissolve 5 g K_2CrO_4 in 50 mL water.
- Add 1 *N* $AgNO_3$ dropwise until a slight permanent red precipitate forms.
- Filter and dilute to 100 mL with water.

B. Silver Nitrate, 0.01 *N*

Dissolve 1.696 g dry $AgNO_3$ (105°C for 2 hr) in DI water, dilute to 1 L.

C. Sodium Chloride Solution, 0.01 *N*

- Dissolve 0.585 g dry NaCl in 1 L water.
- Titrate 10 mL of this solution against Reagent B after adding 4 drops of Reagent A until permanent reddish brown color appears.
- Take the reading as *a* and from this calculate the exact $AgNO_3$ normality.

Procedure

1. Take 5-10 mL soil saturation extract in a wide-mouth porcelain crucible or 150-ml Erlenmeyer flask.

2. Add 4 drops of **Reagent A.**

3. Titrate against **Reagent B** until permanent reddish brown color appears.

4. Take the reading *b*.

5. Run two blanks in the same manner using distilled water and take the reading as *c*.

CALCULATIONS

$$Cl^- \ (meq/L) = (b - c) \times N \times \frac{1000}{V} \qquad (40)$$

Where: N = normality of silver nitrate.
V = aliquot (mL) taken for titration.

Sulfate

The commonly used method of sulfur determination in alkaline soils is the extraction of SO_4-S with 0.15% $CaCl_2.2H_2O$ (Williams and Steinbergs, 1959) and measurement of SO_4-S concentration in the extracts by a turbidimetric procedure using barium chloride (Verma, 1977). A critical level of 10-13 mg/kg $CaCl_2$ extractable SO_4-S has commonly been reported for cereal (e.g. wheat, maize) and oilseed (e.g. mustard) crops (Tandon, 1991).

Apparatus

Reciprocal shaker
Spectrophotometer

Reagents

A. Calcium chloride dihydrate ($CaCl_2.2H_2O$), 0.15%

Dissolve 1.5 g of $CaCl_2.2H_2O$ in about 700 mL of DI water and make to 1 L with DI water.

B. HCl, 6 *M*

C. $BaCl_2.2H_2O$

D. Sorbitol, 70% aqueous solution

E. K_2SO_4

Standard Sulfate Solutions

- Dissolve 0.5434 g K_2SO_4 in 1 L of DI water. This contains 100 mg SO_4-S/L. Transfer 0, 5, 10, 30, 40, and 50 mL of this solution to 100 mL vol. flasks and make up the volume with 0.15% $CaCl_2$. These standards contain 0, 5, 10, 20, 30, 40 and 50 mg SO_4-S/L solution.

Procedure

Extraction

1. Transfer 5 g soil into an 150 mL Erlenmeyer flask.

2. Add 25 mL solution of 0.15% $CaCl_2$ to it (don't use 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 min. on a reciprocal shaker (180+ oscillations per min.).

4. Filter the suspension through Whatman no. 42 filter paper. This procedure yields almost colorless extracts.

Measurement

5. Transfer 10 mL aliquot of the extract to a 50-mL test tube, or a smaller aliquot diluted to 10 mL with DI water.

6. Add 1 mL 6 *M* **HCl** followed by 5 mL of 70% **sorbitol** solution from a pipette with an enlarged jet. Finally add about 1 g of $BaCl_2.2H_2O$ crystals (using a measuring spoon).

7. Shake vigorously (on a test tube shaker for 30 sec) to dissolve the barium chloride and obtain a homogeneous suspension.

8. Read the turbidity of the suspension on a spectrophotometer at 470 nm along with standards prepared in the same way and covering the SO_4-S concentration range 0-50 mg/L (ppm).

9. Preference standard curve and obtain SO_4-S concentration in the unknowns.

10. Calculate SO_4-S concentration in the soil using appropriate dilution factor.

CALCULATION

$$SO_4\text{-}S \text{ in soil (ppm)} = SO_4\text{-}S \text{ in the aliquot (ppm)} \times 5 \quad (41)$$

Where:

5 = dilution factor (5 g soil : 25 mL extractant)

Note

Don't let stand the standards and unknowns (soil extracts) for longer than 2-3 min, otherwise re-shake the suspension before spectrophotometric reading. Allow approximately the same time to standards and unknowns between shaking and turbidimetric reading.

Hot Water Extractable Boron

The hot-water procedure was introduced by Berger and Truog (1939), and was modified by later researchers, but is still the most appropriate method for measuring "available" soil B, or the fraction of B related to plant growth. 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. Boron in soil extracts is measured colorimetrically using Azomethine-H (John et al., 1975).

Apparatus

- 50-mL Erlenmeyer flasks (Pyrex) pre-treated with conc. HCl for one week.
- Spectrophotometer.
- Polypropylene test tubes, 10-mL capacity.

Reagents

A. De-ionized Water

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, and mix.

C. Activated Charcoal (Boron - free)

This can be prepared by giving repeated washings (8-9 times) of deionized distilled water (boiling of charcoal with water and in 1:5 ratio) and subsequent filtering. Boron in the filtered water is checked by azomethine-H color development.

D. Azomethine-H Reagent

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

E. Boron Standard Solutions

- Dissolve 0.114 g boric acid (H_3BO_3) in DI water, and adjust the volume to 1 L with DI water. This stock solution contains 20 ppm solution.

- Dilute 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mL of the stock solution to 100 mL

with deionized distilled water to have solutions with B concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm, respectively.

- Include a DI water sample for the B standard solution without B.

Procedure

1. Place 10 g soil in a 50-mL Erlenmeyer flask (Pyrex) pre-treated with conc. HCl for one week.
2. Add about 0.2 g of activated charcoal (B-free).
3. Add 20 mL DI water.
4. Boil on a hot plate for 5 min. with flasks covered by a watchglass.
5. Filter the suspension immediately through Whatman No. 40 filter paper. Filtrate is ready for B determination.

Measurement

1. Transfer a 1-mL aliquot of blank, diluted B standards, or sample solution into a 10-mL polypropylene tube.
2. Add 2 mL of buffer solution, and mix well.
3. Add 2 ml Azomethine-H reagent and mix.
4. After 30 minutes read the absorbance at 420 nm; refer these readings to that of a standard curve prepared with 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm of B in solutions for converting readings to B concentration in the test sample.

CALCULATION

$$\textit{Boron in soil (ppm)} = C \times 2 \qquad (42)$$

Where: C = ppm B from the standard calibration curve.
2 = Dilution factor (soil : extracting solution ratio).

Note

Use of glassware should be minimal and always use conc. HCl treated glassware, where essential.

Extractable Micronutrient Cations

Though occurring in soils and plants in smaller amounts than major plant nutrients, micronutrients are nevertheless equally essential for crop growth. In general, solubility of micronutrients, except Mo, decreases with an increase in soil pH and calcareousness. As most soils of the WANA region are calcareous, micronutrient deficiencies are becoming more frequent and widespread in fruit trees and agronomic crops with increasing intensification of cropping.

The DTPA test of Lindsay and Norvell (1978) is the commonly used method for evaluating fertility status with respect to micronutrient cations, i.e. Fe, Zn, Mn, and Cu; Boron is determined by hot water extraction; and deficiencies of Mo and Cl do not occur in alkaline soils.

Apparatus

- Reciprocal Shaker
- Atomic absorption spectrophotometer

Reagents

A. DTPA Extraction Solution

- Weigh 1.97 g diethylenetriaminepentaacetic acid (DTPA), 1.1 g $CaCl_2$ (or 1.47 g $CaCl_2.2H_2O$) into a beaker. Dissolve with water and then transfer to a 1-L volumetric flask.

- Into another beaker, weigh 14.92 g triethanolamine (TEA) and transfer with DI water into the 1-L vol. flask, and make up to about 900 mL with DI water.

- Adjust pH to exactly 7.30 with 6 *N* HCl and make to volume (1 L) with DI water. The final extractant solution is 0.005 *M* DTPA, 0.1 *M* $CaCl_2$, 0.1 *M* TEA.

B. Standard Solutions

Prepare working standard solutions for micronutrients in DTPA extracting solution: Fe, 0, 1, 2, 3, 4, 5, ppm; Zn, 0, 0.2, 0.4, 0.6, 0.8, 1.0, ppm; Cu, 0, 1, 2, 3, 4 ppm; Mn, 0, 1.0, 1.5, 2.0, 2.5 ppm.

Procedure

1. To 10 g soil placed in a 125-mL extraction flask, add 20 mL extractant and shake on a reciprocating shaker for 2 hr, and then filter.

2. Measure Zn, Fe, Cu, or Mn directly in filtrate by atomic absorption spectrophotometry.

3. Follow operating procedure for atomic absorption spectrophotometer using appropriate lamp for each element.

CALCULATION

$$Zn, Fe, Cu \text{ or } Mn \text{ in soil } (ppm) = (ppm \text{ in filtrate} - blank) \times 2 \quad (43)$$

Where:

2 = dilution factor (1:2 soil-extractant ratio).

Ammonium Bicarbonate-DTPA Extractable Nutrients

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 extraction reagent is 1 *M* in ammonium bicarbonate (NH_4HCO_3) and 0.005 *M DTPA* adjusted to pH 7.6. DTPA will chelate metals and ammonium will exchange with K bringing *the* latter into solution. The original pH of 7.6 allows DTPA to evolution of carbon dioxide. As pH rises, bicarbonate changes to carbonate. The carbonate ions precipitate calcium from calcium phosphates and thus increases P solubility.

This method is highly correlated with ammonium acetate method for K, sodium bicarbonate method for P, and DTPA method for Zn, Fe, Mn and Cu. The range and sensitivity is the same as those for DTPA test, sodium bicarbonate test and ammonium acetate test for *micronutrients*, *P*, and *K* respectively. Coefficient of variability for different determinations by this method ranges from 5 to 10 percent.

Apparatus

- Atomic absorption spectrophotometer
- Spectrophotometer suitable for measurement at 880 and 420 nm
- Accurate automatic dilutor
- Flame photometer

Reagents

A. Extracting Solution

A 0.005 *M* DTPA (diethylenetriaminepentaacetic acid) solution is obtained by adding 1.97 g DTPA to 800 mL DI water. Approximately 2 mL of 1:1 NH_4OH is added to facilitate dissolution and to prevent effervescence when bicarbonate is added. When most of DTPA is dissolved, 79.06 g NH_4OH are added and stirred gently until dissolved. The pH is adjusted to 7.6 with ammonium hydroxide. The solution is diluted with DI water, and is either used immediately or stored under mineral oil.

B. Mixed Reagent for Phosphorus

Dissolve 12.0 g ammonium molybdate $(NH_4)_6Mo_7.4H_2O$ in 250 mL DI water. Dissolve 0.2908 g antimony potassium tartrate [K(6SbO) $C_4H_4O_6$. $1/2H_2O$] in 1L of 5 *N* H_2SO_4 (148 ml conc. H_2SO_4 per liter), mix the two solutions together thoroughly, and make to 2 L volume with DI water. Store in a pyrex bottle in a dark, cool place.

C. Color Developing Reagent for Phosphorus

Add 0.739 g ascorbic acid to 140 mL of the mixed Reagent B. This reagent should be prepared as required, as it does not keep for more than 24 hours.

D. Hydrazine Stock Sulfate Solution

Dissolve 27.0 g $H_2N_2H_2.H_2SO_4$ (F.W. 130.12) in 750 mL *DI* water, make up the volume to 1 L and mix well.

Prepare **hydrazine sulfate working solution** by diluting 22.5 mL of stock solution to 1 L with DI water. This solution is stable for 6 months.

E. Copper Sulfate Stock Solution

Dissolve 3.9 g $CuSO_4.5H_2O$ (F.W. 249.68) in 800 mL DI water, make up the volume to 1 L and mix well.

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

F. NaOH Stock Solution (1.5 *N*)

Dissolve 60.0 g NaOH in 500 mL DI water, cool and make up the volume to 1 L.

Prepare **NaOH working solution** (0.3 *N*) by diluting 200 mL of the stock solution to 1 L with DI water.

G. Color Developing Reagent for Nitrates

Add 5.0 g sulfanilamide (F.W. 172.21) and 0.25 g N-(1-naphthyl)-ethylenediamine dihydrochloride to 300 mL DI water. Add 50 mL H_3PO_4 slowly with stirring and make the volume to 500 mL. This reagent should be prepared as required, as it can not be used after appearance of pink color.

H. Standard Solutions

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

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

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

Micronutrients: Prepare working standards for:

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

Procedure

- **Extracting Method**

  Place 10 g of soil (2-mm soil) in a 125-mL conical flask. Add 20 ml of **extracting solution** and shake on a reciprocal shaker for 15 min. at 180 cycles/minute with flasks kept open. The extracts are then filtered through Whatman No 42 filter paper.

- **Nitrates**

  Transfer 1 mL of the soil extract to 25-mL test tube, add 3.0 mL copper sulfate working solution, 2 mL hydrazine sulfate working solution, and 3 mL sodium hydroxide working solution. Mix and heat in water bath (38°C) for 20 min. Remove from water bath, add 3 mL color developing reagent for NO_3-N, mix and let stand at room temperature for 20 min. Read absorbance at 540 nm on a spectrophotometer (Kampshake et al., 1967).

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

- **Phosphorus**

  A 1.0 mL aliquot of the soil extract is diluted to 10.0 mL with DI water. Add 2.5 ml of **color developing reagent** carefully to prevent loss of sample due to excessive foaming. Stir, let stand 30 min., and measure color intensity at 880 nm. Standards are developed in 1 mL extract in exactly the same way as described above, and a standard calibration curve is obtained using absorbance values for standards.

- **Potassium**

  The potassium in the soil extract is determined directly by either a flame photometer, or by atomic absorption using a potassium hollow cathode lamp. The 404 nm wavelength is used to reduce sensitivity. Standard solutions are made in the extraction solution.

- **Micronutrients**

  Zinc, Fe, Cu, and Mn are determined by atomic absorption. The standard solutions of these metals are developed in the extracting solution.

CALCULATIONS

$$NO_3\text{-}N, ppm\ of\ soil = NO_3\text{-}N\ (ppm\ in\ extract) \times 2 \quad (44)$$

$$P, ppm\ of\ soil = P\ (ppm\ in\ extract) \times 2 \quad (45)$$

$$K, ppm\ of\ soil = K\ (ppm\ in\ extract) \times 2 \quad (46)$$

$$Zn, Fe, Mn, Cu, ppm\ of\ soil = Zn, Fe, Mn, Cu\ (ppm\ in\ extract) \times 2 \quad (47)$$

Effects of Storage

Air-drying and storage will not have any significant effect on the levels of nutrients. Extracting solution can be stored for 2 weeks under mineral oil and then the pH adjusted if necessary.

INTERPRETATION

Table 1. Index values and their interpretation for P, K, Zn, Fe, Cu, and Mn for ammonium bicarbonate-DTPA soil test.

	NH_4HCO_3-DTPA					
Status	**Zn**	**Fe**	**Cu**	**Mn**	**P**	**K**
	ppm					
Low	0-0.9	0-2.0	0.5	1.8	0-3	0-60
Medium	-	-	-	-	4-7	61-120
Marginal	1.0-1.5	2.1-4.0	-	-	-	-
Adequate	>1.5	>4.0	>0.5	>1.8	-	-
High	-	-	-	-	8-11	>120
Very High	-	-	-	-	>11	-

V. PLANT ANALYSIS

The concentration of nutrients in plants may be measured in a plant extract from fresh plant material e.g., *tissue analysis*, and in whole dried plant material. The former test is qualitative and is appropriate for quick measurements on a growing crop. Nitrate in plant sap is such a test which can give a reliable indicator of the N nutrition status.

However, total plant analysis or plant-part analysis is quantitative and more reliable. Criteria for nutrient concentration have been developed which are relatively reliable, given the many and variable factors which may influence such data (Walsh and Beaton, 1973).

Examples of ranges of *deficiency*, *adequacy*, and *excess* are given for the common range of nutrients in the case of cereals in the Appendix (Table 9). Of prime concern are forms of N, as well as P, B, and micronutrient cations.

While valuable in itself as a guide to a plant's nutritional well-being, plant analysis can complement soil analysis and thus lead to more effective diagnosis and recommendations. In this context, its use in the WANA region is, as yet, in its infancy.

Nitrogen

The most common form of plant analysis is that for N, whether a determination of the organically-bound forms or both organic and inorganic N (Buresh *et al.*, 1982). While Kjeldahl digestion is commonly used for plant N and P determination, wet ashing with H_2SO_4 and H_2O_2 is also used. This will eliminate the use of selenium in the former method (Linder, 1944; Van Schouwenberg and Walinge, 1973).

Kjeldahl Nitrogen

Apparatus

- **Block digester**
- **Distillation unit**
- **Automatic titrator connected to a pH-meter**

Reagents

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

A. Digestion Catalyst Mixture (K_2SO_4 containing 1% selenium).

Grind the mixture, taking care not to breathe Se dust or allow Se to come in contact with skin.

B. Sulfuric Acid, Concentrated.

C. EDTA, Reagent-Grade Disodium Salt (m.w. = 372.2).

D. Sodium Hydroxide Solution, 10 *N*

E. Saturated Boric Acid

F. Sulfuric Acid, 0.01 *N*

G. Ammonium Standard Solution: 1.2 mg N/L

Procedure

1. Mix and spread finely ground (Cyclone mill) plant sample in a thin layer on a sheet of paper until it looks uniform.

2. Select representative sub-samples of about 1 g by taking at least 10 small portions from all parts of the sample with a spatula, and put them into a plastic vial.

3. Dry the sub-sample at 60° C in an oven (overnight) and then cool in a desiccator.

4. Weigh 0.25 g (grain) or 0.50 g (straw) of dry plant material and transfer quantitatively into a 100-mL digestion tube.

5. Add 3 pumice boiling granules, and 3 g **catalyst mixture** using a calibrated spoon.

6. Add 10 mL conc. H_2SO_4 using a dispenser, and stir with Vortex tube stirrer until mixed well.

7. Place tubes in a block digester set at 380°C, and continue digestion for 2 hr after clearing.

8. After digestion is complete, remove tubes, cool, and bring to volume (100 mL) with DI water.

9. Include one reagent blank, one chemical standard (EDTA) 0.10 g, and one standard plant sample (internal reference) in each batch.

Determination of N by Distillation

1. Set distillation and titration apparatus as for soil Kjeldahl N and steam out the apparatus for at least 10 minutes.

2. Mix the plant digest in the tube and take 20 mL in a 100-mL distillation flask.

3. Carefully dispense 10 mL 10 *N* **NaOH** solution, and immediately connect flask to distillation unit to begin distillation.

4. Set timer at 4 min after distillation starts flowing into the collecting dish.

5. Remove distillation flask and connect an empty 100-mL distillation flask to the distillation unit. Drain water from the condenser jacket and steam out apparatus for 90 sec. before connecting the next sample.

6. Titrate the distillate to pH 5 with standardized 0.01 *N* H_2SO_4 using the auto-titrator; record titration volume of acid.

Notes

- Each batch of distillations should include a distillation of 10 mL ammonium-N standard with 0.2 g MgO and 10 mL DI water with 0.2 g MgO. Recovery of ammonium-N standards should be at least 98%. Recovery of EDTA, corrected for reagent blank, should be at least 97%.

- The plant digest by sulfuric acid and hydrogen peroxide may be distilled and N be measured as in the Kjeldahl Digest.

CALCULATIONS

Percentage recovery of ammonium-N standard:

$$\% \ Recovery = \frac{(V - B) \ x \ A \ x \ 14.01 \ x \ 100}{C \ x \ D} \qquad (48)$$

Where:

V = Sample titration volume (ml)
B = MgO distillation blank titration (ml)
A = Acid normality
C = Volume of NH_4-N standard solution (ml)
D = Concentration of NH_4-N standard solution ($\mu g \ ml^{-1}$)

Percentage recovery of EDTA standard:

$$\% \ Recovery = \frac{(V - B) \ x \ A \ x \ R \ x \ 186.1 \ x \ 100}{W \ x \ 1000} \qquad (49)$$

Where:

R = Ratio of total digest volume to distillation volume
B = Digestion blank titration volume (ml)
W = Weight of EDTA (g)

Percentage N in plant:

$$\% \ N = \frac{(V - B) \ x \ A \ x \ (14.01) \ x \ R \ x \ 100}{W \ x \ 1000} \qquad (50)$$

Where: W = dry plant weight (g).

Determination by Specific Ion Electrode

The Orion Ammonia electrode is a gas-sensing electrode which detects dissolved NH_3 in aqueous solutions in which the pH has been adjusted above pH 11 with NaOH. Above pH 11, virtually all NH_4^+ is converted to NH_3. The NH_4^+ electrode is used to determine ammonia concentration in soil and water samples, and in Kjeldahl digests of soils and plant tissues. Use of this electrode is *simple*, *rapid*, and *precise*, with results that correlate well with other ammonia determination methods (e.g. standard distillation method) from aqueous solutions (Eastin, 1976).

Apparatus

1. Orion Expandable Ion Analyzer EA-940
2. Orion Ammonia Electrode Model 95-12
3. Magnetic stirrer

Reagents

A. Sodium Hydroxide, 10 *N*

Dissolve 400 g NaOH in 1-L water.

B. Standard Solution: 1000 ppm $(NH_4)_2SO_4$ solution.

Dissolve 4.714 $(NH_4)_2SO_4$ in 1 L water (1000 ppm NH_4-N).
Dilute this solution 100 and 10 times to get 10 and 100 ppm NH_4-N.

Procedure

1. Prepare plant tissue digests as described for plant Kjeldahl N.

2. Calibrate the Orion Ion Analyzer EA-940 according to the manual instructions, using at least one standard. Calibrate to read concentration directly.

3. Check slope of electrode, according to electrode's use instructions.

4. Take 4 mL diluted plant digest in 100-mL vol. flask and dilute to 100 mL with DI water.

5. Mix, and pour solution into a 150-mL beaker on a magnetic stirrer at low speed.

6. Insert Orion Ammonia Electrode (Model 95-12) in solution positioning at a 20° angle with the tip about 1 cm below surface of test solution.

7. Add 2 mL 10 *N* NaOH solution, and read concentration directly on Ion Analyzer. After equilibration for at least 1 min, take the reading as R.

CALCULATIONS

$$Plant\ \%\ N = R \times \frac{100}{S} \times \frac{1}{W} \times \frac{100}{10000} \quad (51)$$

Where: S = Aliquot size (mL)
W = Plant sample weight (g)

Wet Ashing By H_2SO_4 and H_2O_2

The routine use of heavy metals as catalysts is not environmentally sound, and it should be replaced by less hazardous alternative procedures. From this standpoint, treatment with a mixture of H_2O_2/H_2SO_4 in the absence of metal catalysts has been recently proposed as an alternative digestion procedure for the routine analysis for soil and plant Kjeldahl-N (McGill and Figueiredo, 1993).

Reagents

A. Sulfuric Acid (s.g. 1.84)

B. Hydrogen Peroxide 30% (A.G.)

Procedure

1. Weigh out 0.5 g of plant material in a 100-mL digestion tube.
2. Add 3-4 pumice particles.
3. Add 5 mL conc. sulfuric acid and mix.
4. Keep overnight.
5. Heat on a block digester at a moderate temperature 100-150°C.
6. Swirl to restrict foaming.

7. If foaming enters the neck of the digestion tube, add 2 mL of H_2O_2.

8. Heat the tubes 30-60 min. in the block digester.

9. Cool the tubes, then add 2 mL of H_2O_2.

10. Raise the temperature of the block digester to 280°C.

11. Heat the tubes for 10 minutes at 280°C.

12. Cool, then add 2 mL of H_2O_2, and heat for 10 minutes.

13. Repeat 11 and 12 until solution remains clear after 10 min. of heating.

14. Cool and make up to the mark (100 mL) with DI water.

Note

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

Total Nitrogen

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

Reagents

A. **Sulfuric-Salicylic Acid Mixture (concentrated H_2SO_4 containing 2.5 % w/v salicylic acid)**

Dissolve 62.5g of reagent-grade salicylic acid in 2.5 L concentrated H_2SO_4.

B. **Digestion Catalyst** Mixture of 1000 g K_2SO_4 and 10 g selenium.

C. **Sodium Thiosulfate**: reagent-grade $Na_2S_2O_3.5H_2O$.

D. **EDTA, Reagent-Grade Disodium Salt** (molecular weight = 372.2).

Procedure

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.

3. Place them into a plastic or glass vial and dry the sample at 60°C in a forced draft oven overnight, and then cool it in a desiccator.

4. Weigh the sample and vial to 0.1 mg, then transfer the sample to a dry 250-ml digestion tube, with a circular scratch on the neck at 250 mL. Weigh the empty vial, and record the net sample weight.

5. Add 20 mL **sulfuric acid-salicylic acid** mixture while rotating the tube to wash down any sample adhering to the neck of the tube, and allow to stand 2 hr 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** (100 parts K_2SO_4: 1 part Se) and 3 to 4 boiling granules and place tubes on the aluminum digestion block 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 digestion block 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 digestion block and digest for 1 hr *after clearing*. No particular 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 the 250-mL mark.

Note

Each set of 20 digestion should contain one reagent blank and one EDTA standard (0.1 g accurately weighed to 0.1 mg).

Distillation

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

Procedure

1. Prior to distillation, shake the digestion tube to thoroughly mix its contents.

2. Immediately pipette an aliquot into a distillation flask (25 or 50 mL).

3. Add 7 mL 10 *N* NaOH for 25 mL aliquot or 15 mL 10 *N* NaOH for 50 mL digest.

4. Immediately connect the flask to the distillation unit and begin distillation.

5. Collect about 35 mL distillate in the evaporating dish, and proceed to titrate as for Kjeldahl-N in soils: record titration volume of acid.

CALCULATIONS

Percentage Recovery of EDTA Standard:

$$\% \ Recovery = \frac{(V - B) \ x \ A \ x \ R \ x \ 186.1 \ x \ 1000}{W \ x \ 1000} \quad (52)$$

Where:

R = Ratio of total digest volume to distillation volume
B = Digested blank titration volume (mL)
W = Weight of EDTA (g)
A = Acid normality

Percent Plant Nitrogen:

$$\% \ N = \frac{(V - B) \ x \ A \ x \ (14.01) \ x \ R \ x \ 100}{W \ x \ 1000} \quad (53)$$

Where: W = Dry plant weight (g)

Nitrate Nitrogen

This soluble N fraction is estimated for the plant after extraction with water or a $CuSO_4$ solution and measured by specific ion electrode (Mills, 1980; Orion Research, 1981; Soil and Plant Analysis Council, 1992).

Reagents

A. Extracting Solution: Copper Sulfate, 0.02 *N*

Dissolve 7.98 g $CuSO_4$ and 6.6 g Ag_2SO_4 in 5 L DI water, and adjust pH to 3.05 by adding 5 *N* H_2SO_4.

B. Standards

a. *Stock solution: 1000 ppm* NO_3-N

Dissolve 1.8043 g KNO_3 (dried for 2 hr at 105°C) in 250-mL Reagent A.

b. Dilute 25 mL of stock solution to 100 mL by Reagent A. This solution should have *250 ppm* NO_3-N

Prepare dilute standard solutions as follows:

5 mL (b)	→	250	mL	=	5	ppm NO_3-N
10 mL (b)	→	250	mL	=	10	ppm NO_3-N
5 mL (a)	→	250	mL	=	20	ppm NO_3-N
10 mL (a)	→	250	mL	=	40	ppm NO_3-N
15 mL (a)	→	250	mL	=	60	ppm NO_3-N
25 mL (a)	→	250	mL	=	100	ppm NO_3-N

Always bring to volume with **Reagent A**.

C. Reference electrode *outer* filling solution (Ionic Strength Adjuster or ISA)

a. Prepare 2 *M* **ammonium sulfate** $(NH_4)_2SO_4$ solution by dissolving 26.42 g in 100 mL DI water.

b. Dilute 2 mL ISA to 100 DI water and fill the outer chamber of the reference electrode.

Procedure

1. Weigh 0.4 g sample in an Erlenmeyer flask and add 40 mL **extracting solution**

(Reagent A).

2. Shake for 15 min. and filter through Whatman No. 42 filter paper (or any other nitrate-free filter paper).

3. Allow all standards and samples to come to room temperature before measurement (temperature of samples and standards should be the same). A 1°C difference in temperature will give rise to about a 2% error. Temperature must be less than 40°C.

4. Read millivolts of standards, samples and blank using a digital pH/mv meter.

5. Determine the unknown concentration from the calibration curve R. Recalibrate after one hour if needed.

D. Reference electrode *inner* filling solution:

Use the inner filling solution, 90-00-02 (ORION), to fill the inner chamber of reference electrode.

CALCULATIONS

$$NO_3\text{-}N = R \times \frac{40}{W} \qquad (54)$$

Where: R = concentration from the calibration curve (ppm).
W = sample weight (0.4 g)

Total Phosphorus

This is based on digestion of plant material and measurement of P colorimetrically. The P content is related to plant P nutritional status (Murphy and Riley, 1962).

Reagents

A. Ammonium Molybdate-Ammonium Vanadate in Nitric Acid:

- Dissolve 22.5 g ammonium molybdate in 300 ml water (a).
- Dissolve 1.25 g ammonium metavanadate in 400 ml hot water (b).
- Add (b) to (a) in 1 L vol. flask and let the mixture cool to room temperature.
- Slowly add 250 mL HNO_3 to mixture, cool to room temperature and bring to volume.

B. Phosphorus Standard Stock Solution:

Weigh 0.2197 g dried KH_2PO_4 (potassium dihydrogen phosphate) and transfer to 1 L vol. flask, dissolve with DI water and bring to volume. This solution should contain 50 ppm P.

C. Working Standards

Add 1, 2, 3, 4 and 5 mL 50 ppm stock solutions to 100-mL vol. flasks to obtain 0.5, 1.0, 1.5, 2.0, and 2.5 ppm P standard solutions.

Procedure

1. Digest plant material as described for Kjeldahl-N in plants.

2. Filter plant digest with Whatman No.1 filter paper and collect filtrate in a small bottle.

3. Take 10 mL filtrate in 100-mL vol. flask, add 10 mL Reagent A, bring to volume with DI water.

4. Leave for 30 min. for color to develop, and read the percent transmittance at 410 nm wavelength on a spectrophotometer.

5. For standards, use the required P stock solution and add 10 mL Reagent A, bring to volume with DI water, and leave for 30 min.

6. Plot standards on a graph paper (ppm against transmittance) and read unknown samples from the graph (R).

CALCULATIONS

$$Plant\ \%P = R\ (ppm) \times \frac{100}{10} \times \frac{100}{W} \times \frac{100}{1000} \qquad (55)$$

Where: W = dry plant weight (g).

Note

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

Dry Ashing for Macro- and Micronutrient Cations

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. However, micronutrient cations (Fe, Zn, Cu, and Mn) can only be analyzed in plant tissues low in silica. Therefore, HNO_3-$HClO_4$ wet digestion is required for analyzing micronutrient cations high silica plant tissues (like wheat & barley leaves etc.). In dry ashing for B, use of glassware should be avoided (use specific procedure given for B analysis in plant tissue).

Procedure

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

1. Place 0.5-1.0 g portions of ground plant material into 30 or 50 mL glass beakers.
2. Place beakers 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 beakers carefully.
6. Dissolve the cool ash in 5 mL portions of 2 *N* HCl and mix with a plastic rod.
7. After 15-20 min., make up the volume (usually 50 mL) using 0.1 *N* HCl.
8. Mix thoroughly, allow to stand for about 30 min., 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 yellow color method), for K and Na by flame photometry, and for Ca, Mg, Zn, Cu, Fe, and Mn by atomic absorption spectroscopy.

Note

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

Boron

Boron in plant samples is measured by dry ashing (Richards, 1954; Gaines and Mitchell, 1979) and subsequent measurement of B colorimetry using Azomethine-H (John et al., 1975).

Reagents

- **HCl acid (1:1).**
- **CaO powder.**
- **Buffer Solution:** same as for B analysis in soil.
- **Azomethine-H:** same as for B analysis in soil.
- **Boron Standard Solutions:** same as for B analysis in soil.

Dry Ashing Procedure

1. Weight 1.0 g dry sample, transfer to a porcelain crucible and mix with 1 g CaO powder.
2. Ignite in a muffle furnace by slowly raising the temperature to 500°C.
3. Continue ashing for 6 hrs. at 500°C.
4. Wet the ash with five drops of DI water and then pipet 3 mL HCl (1:1) solution into the crucible. Heat on a steam bath for 20 min.
5. Let stand at room temperature for 1 hr, stirring occasionally with a plastic rod to break up ash.
6. Filter through Whatman No.1 filter paper into a 50-mL polypropylene flask and bring to volume.

Measurement

Same as in hot water extractable B in soils.

CALCULATION

$$\textit{Boron in plant material (ppm)} = C \times 50 \qquad (56)$$

Where: C = ppm B from standard calibration curve
50 = Dilution factor (plant material: final digest volume ratio).

Ferrous Analysis in Fresh Plant Tissue

As total iron (Fe) content in plant tissue does not indicate Fe nutritional status of plants determination of ferrous iron (Fe^{2+}) in fresh tissue by o-phenathroline (Katyal and Sharma, 1980) extraction is suggested for the purpose. Ferrous iron content in the extracts can be measured by colorimetry or atomic absorption spectrophotometry.

Ferrous Extraction with o-phenathroline

Apparatus

- Spectrophotometer or Atomic Absorption Spectrophotometer

Reagents

- **1-10 o-phenathroline (o-Ph; $C_{12}H_8N_2$), 1.5% in HCl-buffer with pH 3.0:** Add 15 g of o-Ph to about 850 mL DI water. Dropwise add 1*N* HCl to continuously stirring solution until last traces of o-Ph are solubilized. Final pH of the solution will be around 3.0. Make volume to 1 L with DI water.

- **Stardard Solutions for Ferrous (Fe^{2+}):** Prepare working standards of iron containing 0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg Fe/L using o-Ph extraction solution.

Extraction Procedure

- Use carefully washed fresh plant tissues for ferrous analysis.

- Place 2 g of the fresh-chopped samples in a 50 mL Erlenmeyer flask.

- Add 20 mL o-Ph solution and stir gently to ensure that all the plant tissue is completely dipped in the solution.

- Close the flask using parafilm and allow to stand for about 16 hr at room temperature.
- Filter the contents through Whatman no. 1 filter paper.

Estimation of Ferrous

Ferrous content in the filtrate is determined by colorimetry at 510 nm or by atomic absorption spectrophotometry. Standards for Fe are run along with the plant extracts. Ferrous content in plant tissue is expressed on dry weight basis by determining moisture content of a fraction of fresh plant tissue.

CALCULATION

$$Fe^{2+} \text{ in fresh tissue (ppm)} = Fe^{2+} \text{ (ppm from the std. calib. curve)} \times 10 \quad (57)$$

Where: 10 = plant tissue : o-Ph extractant ratio.

Note

Fe^{2+} concentration on dry weight basis is estimated according to the moisture content in fresh plant tissue.

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VII. APPENDICES

Appendix Table 1. Soil pH Levels and Associated Conditions

Soil pH	Indications	Associated Condition
< 5.5	Soil is deficient in Ca and/or Mg and should be limed.	Poor crop growth due to low CEC and possible Al^{3+} toxicity. Expect P deficiency.
5.5-6.5	Soil is lime-free and should be closely monitored.	Satisfactory for most crops
6.5-7.5	Ideal range for crop production.	Soil CEC 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 micro nutrients are less available.
>8.4	Almost invariably indicates a sodic soil.	Very poor physical conditions. Infiltration and percolation of soil water is slow. Possible root deterioration and organic matter dissolution.

Source: Hach Company, USA (1992).

Appendix Table 2. Chemical Criteria for Calcareous and Salt-Affected Soils

Soil Type	pH	EC_e[1], mS/cm	Sodium Adsorption Ratio (SAR)
Calcareous	7.5-8.5	<2.0	<13
Saline	<8.5	>2.0	<13
Sodic	>8.5	<2.0	>13
Saline/Sodic	<8.5	>2.0	>13

[1] EC in saturated paste extract.
Source: Hach Company, USA (1992).

Appendix Table 3. Soil Salinity Classification

Texture	Degree of Salinity				
	None	Slightly	Moderate	Strongly	Very Strong
	EC[1] (mS/cm)				
Coarse sand to sandy loam	0-1.1	1.2-2.4	2.5-4.4	4.5-8.9	9.0+
Loamy fine sand to loam	0-1.2	1.3-2.4	2.5-4.7	4.8-9.4	9.5+
Silt loam to clay loam	0-1.3	1.4-2.5	2.6-5.0	5.1-10.0	10.1+
Silty clay loam to clay	0-1.4	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 Table 4 . Salinity Tolerance of Crops[1].

Crop	High	Medium	Low
Fruit	Date palm	Pomegranate, Fig, Olive, Grape, Cantaloupe	Pear, Apple, Orange, Grapefruit, Prune, Plum, Almond, Apricot, Peach, Strawberry, Lemon, Avocado
Vegetable	$EC_e \times 10^3 = 12$ Garden beets, Kale, Asparagus, Spinach $EC_e \times 10^3 = 10$	$EC_e \times 10^3 = 10$ Tomato, Broccoli, Cabbage, Bell pepper, Cauliflower, Lettuce, Sweet corn, Potatoes, Carrot, Onion, Peas, Squash, Cucumber $EC_e \times 10 = 4$	$EC_e \times 10^3 = 4$ Radish, Celery, Green beans $EC_e \times 10^3 = 3$
Forage	$EC_e \times 10^3 = 18$ Alkali sacaton, Saltgrass, Bermuda grass, Canada wildrye, Barley, Birdsfoot trefoil $EC_e \times 10^3 = 12$	$EC_e \times 10^3 = 12$ White sweetclover, Yellow sweetclover, Perennial ryegrass, Mountain brome, Sudan grass, Alfalfa, Tall fescue, Rye, Oats, Orchardgrass, Blue grama Meadow fescue, Big trefoil, Smooth brome, $EC_e \times 10^3 = 4$	$EC_e \times 10^3 = 4$ White Dutch, Clover, Meadow foxtail, Alsike Clover, Red Clover,
Field	$EC_e \times 10^3 = 16$ Barley (grain), Sugar beet, Rape, Cotton $EC_e \times 10^3 = 6$	$EC_e \times 10^3 = 10$ Rye (grain), Wheat (grain), Oats, Rice Sorghum, Corn, Flax, Sunflower, $EC_e \times 10^3 = 6$	$EC_e \times 10^3 = 4$ Field beans

[1] Numbers after $EC_e \times 10^3$ are the saturation extract electrical conductivity values (mS/cm) associated with 50% yield decrease. Within each group, crops are listed in order of decreasing salt tolerance.
Source: Richards, 1954.

Appendix Table 5. Summarized Soil Test Methodology for Fertility Evaluation of Alkaline Soils.

Parameter Property/ Nutrient(s)	Olsen P	AB-DTPA NO_3-N, P, K, Zn Cu, Fe, Mn	NH_4OAc K, Mg, Na, Ca	DTPA Zn, Cu, Fe, Mn	Hot water B
Sample size (g)	2.5	10	5	10	10
Vol. extractant (mL)	50	20	25	20	20
Extracting soln.	0.5 *M* $NaHCO_3$ at pH 8.5	1 *M* NH_4HCO_3 + 0.005 *M* DTPA (pH 7.6)	1 *N* NH_4OAc pH 7.0	0.005 *M* DTPA + H_2O, 0.01 *M* TEA+ *0.01 M* $CaCl_2$ (pH 7.3)	
Shake/boil (min.)	30	15	5	120	5
Shaking action and speed		All use reciprocating, 180+ oscillations/min., except for B			
Nutrient determination in extract	Colorimetry, 880 nm (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. no dilution (ppm)	P, 2-200	P, 2-100; K, 5-750; Zn, 0.5-35	K, 50-1000; Ca, 500-2000; Mg, 50-500; Na, 10-250	Zn, 0.5-20	B, 1-10
Primary reference	Olsen et al. (1954)	Soltanpour & Schawb (1977)	Schollenberger & Simon (1945)	Lindsay & Norvell (1978)	Berger & Truog (1939)

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

Appendix Table 6. 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 ----------		
Nitrogen	AB-DTPA	<11	11-20	>20
Phosphorus	$NaHCO_3$	<8	8-15	>15
	AB-DTPA	<4	4-7	>7
Potassium	NH_4OAc	<100	100-150	>150
	AB-DTPA	<60	60-120	>120
Zinc	DTPA	<0.5	0.5-1.0	>1.0
	AB-DTPA	<1.0	1.0-1.5	>1.5
Copper	DTPA	<0.2	0.2-0.5	>0.2
	AB-DTPA	<0.2		>0.5
Iron	DTPA	<4.5		>4.5
	AB-DTPA	≤2.0	2.1-4.0	>4.0
Manganese	DTPA	<1.0	1.0-2.0	>2.0
	AB-DTPA	<1.8		>1.8
Boron	Hot water	<0.5	0.5-1.0	>1.0

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

Appendix Table 7. Suggested Plant Tissue Sampling Procedures for Selected Dryland Crops[1].

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

[1] When specific guidelines are unknown, the general *rule of the thumb* is to sample *upper mature* leaves

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

Appendix Table 8. Relative Tolerance of Plants to Boron[1].

Tolerant[2]	*Semi-tolerant*	*Sensitive*
Athel *(Tamarix aphylla)*, Asparagus, Palm *(Phoenix canariensis)*, Date palm *(P. dactylifera)*, Sugar-beet, Alfalfa, Gladiolus, Broad bean, Onion, Turnip, Cabbage, Lettuce, Carrot	Sunflower, Potato, Cotton, Tomato, Sweetpea, Radish, Field pea, Olive, Barley, Wheat, Corn, Milo, Oat, Pumpkin, Bell pepper, Sweetpotato, Lima bean	Pecan, Jerusalem artichoke, Navy bean, Plum, Pear, Apple, Grape, Kadota fig, Persimmon, Cherry, Peach, Apricot, Thornless blackberry Orange, Avocado, Grapefruit, Lemon

1 In each column, the plants first named are considered as being more tolerant and the last named more sensitive.

2 Safe limit for sensitive crops = 0.7 ppm B in saturation extract. Boron-tolerant crops may grow satisfactorily, whereas sensitive crops may fail.

Source: Richards, 1954

Appendix Table 9. Interpretation of Nutrient Concentration in Cereal Plant Tissue 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
Phosphorus	<0.15	0.15-0.19	0.20-0.50	>0.50
Potassium	<1.25	1.25-1.49	1.50-3.00	>3.00
Calcium (wheat, oats)		<0.20	0.20-0.50	>0.50
(barley)		<0.30	0.30-1.20	>1.20
Magnesium		<0.15	0.15-0.50	>0.50
Sulfur		<0.15	0.15-0.40	>0.40
	----------	----------	ppm----------	----------
Manganese	<5	5-24	25-100	>100
Zinc		<15	15-70	> 70
Copper		<5	5-25	> 25

Source: Soil Testing and Plant Analysis (Walsh and Beaton, 1973)

Appendix Table 10. Concentration normality, amounts of concentrated acids and bases to make 1 liter of 1 N solution.

Acid or Base Solution	Chemical Properties				Solution needed for 1 liter of 1N (ml)
	Specific Gravity	Percent by weight	Grams per liter	Approximate Normality	
Acetic acid	1.05	99.0	1042.0	17.45	58
Ammonium hydroxide	0.90	28.33	255.0 (NH_3)	15.0	67
Hydrochloric acid	1.19	38.0	451.6	12.4	81
Hydrofluoric	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

Source: Tekalign et al. (1991).

Appendix Table 11. Conversion Factors of Concentrations.

To Convert	Into	Multiply By	To Convert	Into	Multiply By
μg(microgram)	g	1×10^{-6}	cmol/kg	me/100 g	1
me/l	mg %	0.1 x eq. wt	(centimol/kg)	mg/l	1000 x at. wt.
	μg %	100 x eq. wt		ppm	1000 x at. wt.
	mg/l	eq. wt			
ppm	mg/ml	1	Molar	ppm	1
	mg/l	1			
	μg/g	1	Mg/l	mg %	0.1
	μg	1		μg %	100
	g/l	0.001		me/l	1/at. wt.
	at wt.	1000		g/l	0.001
				at. wt.	0.001

- At. wt. = atomic weight; eq. wt. = equivalent weight = atomic weight/valence.
- 1 nm (nanometer) = 10^{-9} meter = 1000 pm (pico meter) = 1 mu (millimicron) = 10 Å (10 angstrom).
- Ultra-violet range of spectrum covers 185-400 nm; Visible range of spectrum covers 400-760 nm; Infra-red range of spectrum covers 760-1500 nm.

Source: Tekalign et al. (1991).

Appendix Table 12. Atomic Weights.

Element	Symbol	Atomic Number	Atomic Weight	Element	Symbol	Atomic Number	Atomic Weight
Actinum	Ac	89	227.0278	Gold	Au	79	196.9665
Aluminum	Al	13	26.9815	Hafnium	Hf	72	178.49
Americium	Am	95	243*	Helium	He	2	4.0026
Antimony	Sb	51	121.75	Holmium	Ho	67	164.9304
Argon	Ar	18	39.948	Hydrogen	H	1	1.0079
Arsenic	As	33	74.9216	Indium	In	49	114.82
Astatine	At	85	210	Iodine	I	53	126.9045
Barium	Ba	56	137.33	Iridium	Ir	77	192.22
Bekeliom	Bk	97	247	Iron	Fe	26	55.847
Beryllium	Be	4	9.01218	Krypton	Kr	36	83.80
Bismuth	BI	83	208.9804	Lanthanum	La	57	138.9055
Boron	B	5	10.8	Lawrencium	Lr	103	260*
Bromine	Br	35	79.04	Lead	Pb	82	207.2
Cadmium	Cd	48	112.41	Lithium	Li	3	6.941
Caesium	Cs	55	132.9054	Lutetium	Lu	71	174.967
Calcium	Ca	20	40.08	Magnesium	Mg	12	24.305
Californium	Cf	98	251*	Manganese	Mn	25	54.9380
Carbon	C	6	12.011	Mendelevium	Md	101	258*
Cerium	Ce	58	140.12	Mercury	Hg	80	200.59
Chlorine	Cl	17	35.453	Molybdenum	Mo	42	95.94
Chromium	Cr	24	51.996	Neodymium	Nd	60	144.24
Cobalt	Co	27	58.9332	Neon	Ne	10	20.179
Copper	Cu	29	63.546	Neptunium	Np	93	237.0482
Curium	Cm	96	247*	Nickel	Ni	28	58.69
Dysprosium	Dy	66	162.50	Niobium	Nb	41	92.9064
Einsteinium	Es	99	252*	Nitrogen	N	7	14.0067
Erbium	Er	68	167.26	Nobelium	No	102	259*
Europium	Eu	63	151.96	Osmium	Os	76	190.2
Fermium	Fm	100	257*	Oxygen	O	8	15.9994
Fluorine	F	9	18.9984	Palladium	Pd	46	106.42
Francium	Fr	87	233*	Phosphorus	P	15	30.9738

Appendix Table 12. (continued).

Element	Symbol	Atomic Number	Atomic Weight	Element	Symbol	Atomic Number	Atomic Weight
Gadolinium	Gd	64	157.25	Platinum	Pt	78	195.08
Gallium	Ga	31	69.72	Plutonium	Pu	94	244*
Germanium	Ge	32	72.59	Polonium	Po	84	209*
Potassium	K	19	39.0983	Tantalum	Ta	73	180.9479
Praseodymium	Pr	59	140.9077	Technetium	Tc	43	98*
Promethium	Pm	61	145*	Tellurium	Te	52	127.60
Protactinium	Pa	91	231.0359	Terbium	Tb	65	158.9254
Radium	Ra	88	226.0254	Thallium	Tl	81	204.383
Radon	Rn	86	222*	Thorium	Th	90	232.0381
Rhenium	Re	75	186.207	Thulium	Tm	69	168.9342
Rhodium	Rh	45	102.9055	Tin	Sn	50	118.69
Rubidium	Rb	37	85.4678	Titanium	Ti	22	47.88
Ruthenium	Ru	44	101.07	Tungsten	W	74	183.85
Samarium	Sm	62	150.36	Uranium	U	92	238.0289
Scandium	Sc	21	44.9559	Vanadium	V	23	50.9415
Selenium	Se	34	78.96	Xenon	Xe	54	131.29
Silicon	Si	14	28.08555	Ytterbium	Yb	70	173.04
Silver	Ag	47	107.868	Yttrium	Y	39	88.9059
Sodium	Na	11	22.9898	Zinc	Zn	30	65.38
Strontium	Sr	38	87.62	Zirconium	Zr	40	91.22
Sulphur	S	16	32.06				

Source: Tekalign et al. (1991).

Appendix Table 13. Solution Concentrations.

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

Source: Tekalign et al. (1991).

Appendix Table 14. Mesh Sizes of Standard Wire Sieves.

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

Source: Tekalign et al. (1991).

Appendix Table 15. Abbreviations.

1. Weight

g	gram
mg	milligram
μg	microgram

2. Length

m	meter
cm	centimeter
mm	millimeter

3. Time

wk	week
hr	hour
min	minute
sec	second

4. Salt Concentration

ms	milliSiemens
μs	microSiemens

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

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

5. Volume

l	liter
ml	milliliter

6. Concentration

mg/l	milligram solute per liter
meq/l	milliequivalent solute per liter

7. Temperature

°C	Degree Celsius
O.D.	oven dry
A.D.	air dry

8. Area

ha	hectare

9. Miscellaneous

Lab	laboratory	Conc.	concentrated
Vol.	volumetric	Soln.	solution
RCF.	Relative Centrifuge Force	ID.	internal diameter
Sp.gr.	specific gravity	DI.	De-ionized water
AAS	Atomic Absorption Spectrophotometer		

Appendix Table 16. 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, μ	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}
2.84×10^{-2}	liter, L (10^{-3} m^3)	bushel, bu	35.24
1.057	liter, L (10^{-3} m^3)	quart (liquid), qt	0.946
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

Table 16. Cont'd.

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
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
7.77×10^{-2}	kilogram per cubic meter, kg m^{-3}	pound per bushel, bu^{-1}	12.87
1.49×10^{-2}	kilogram per hectare, kg ha^{-1}	bushel per acre, 60 lb	67.19
1.59×10^{-2}	kilogram per hectare, kg ha^{-1}	bushel per acre, 56 lb	62.71
1.86×10^{-2}	kilogram per hectare, kg ha^{-1}	bushel per acre, 48 lb	53.75
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
2.24	meter per second, m s^{-1}	mile per hour	0.447
	Specific Surface		
10	square meter per kilogram, m^2 kg^{-1}	square centimeter per gram, cm^2 g^{-1}	0.1
1000	square meter per kilogram, m^2 kg^{-1}	square millimeter per gram, mm^2 g^{-1}	0.001
	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

Table 16. Cont'd.

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
		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)
		Energy, Work Quantity of Heat	
9.52×10^{-4}	joule, J	British thermal unit, Btu	1.05×10^{3}
0.239	joule, J	calorie, cal	4.19
10^{7}	joule, J	erg	10^{-7}
0.735	joule, J	foot-pound	1.36
2.387×10^{-5}	joule per square meter, J m^{-2}	calorie per square centimeter (langley)	4.19×10^{4}
10^{5}	newton, N	dyne	10^{-5}
1.43×10^{-3}	watt per square meter, W m^{-2}	calorie per square centimeter minute (irradiance), cal cm^{-2} min^{-1}	698
		Transpiration and Photosynthesis	
3.36×10^{-2}	milligram per square meter second, mg m^{-2} s^{-1}	gram per square decimeter hour, g dm^{-2} h^{-1}	27.8
5.56×10^{-3}	milligram (H_2O) per square meter second, mg m^{-2} s^{-1}	micromole (H_2O) per square centimeter second, μmol cm^{-2} s^{-1}	180
10^{-4}	milligram per square meter second, mg m^{-2} s^{-1}	milligram per square centimeter second, mg cm^{-2} s^{-1}	10^{4}
35.97	milligram per square meter second, mg m^{-2} s^{-1}	milligram per square decimeter hour, mg dm^{-2} h^{-1}	2.78×10^{-2}
		Plane Angle	
57.3	radian, rad	degrees (angle), °	1.75×10^{-2}

Table 16. Cont'd.

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
	Electrical Conductivity, Electricity, and Magnetism		
10	siemen per meter, S m^{-1}	millimho per centimeter, mmho cm^{-1}	0.1
10^4	tesla, T	gauss, G	10^{-4}
	Water Measurement		
9.73×10^{-3}	cubic meter, m^3	acre-inches, acre-in	102.8
9.81×10^{-3}	cubic meter per hour, $m^3\ h^{-1}$	cubic feet per second, $ft^3\ s^{-1}$	101.9
4.40	cubic meter per hour, $m^3\ h^{-1}$	U.S. gallons per minute, gal min^{-1}	0.227
8.11	hectare-meter, ha-m	acre-feet, acre-ft	0.123
97.28	hectare-meters, ha-m	acre-inches, acre-in	1.03×10^{-2}
8.1×10^{-2}	hectare-centimeters, ha-cm	acre-feet, acre-ft	12.33
	Concentrations		
1	centimole per kilogram, cmol kg^{-1} (ion exchange capacity)	milliequivalents per 100 grams, meq 100 g^{-1}	1
0.1	gram per kilogram, g kg^{-1}	percent, %	10
1	milligram per kilogram, mg kg^{-1}	parts per million, ppm	1
	Radioactivity		
2.7×10^{-11}	becquerel, Bq	curie, Ci	3.7×10^{10}
2.7×10^{-2}	becquerel per kilogram, Bq kg^{-1}	picocurie per gram, pCi g^{-1}	37
100	gray, Gy(absorbed dose)	rad, rd	0.01
	sievert, Sv (equivalent dose)	rem (roentgen equivalent man)	0.01
	Plant Nutrient Conversion		
	Elemental	*Oxide*	
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


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H –1B
W/F
H –7B
W/M