FAO
FERTILIZER
AND PLANT
NUTRITION
BULLETIN

19

ISSN 0259-2495

Guide to laboratory establishment for plant nutrient analysis

FAO
FIAT PANIS

Guide to laboratory establishment for plant nutrient analysis

FAO
FERTILIZER
AND PLANT
NUTRITION
BULLETIN

19

by

M.R. Motsara
New Delhi
India

R.N. Roy
Food and Agriculture
Organization
Rome
Italy

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2008

ISBN 978-92-5-105981-4

Contents

List of tables

List of figures

Acknowledgements

The contribution of R.N. Roy to the conceptualization, initiation and inputs in the preparation and finalization of this publication is duly acknowledged. Special thanks are due to M.R. Motsara, who assisted FAO in several field projects related to this subject and contributed to shaping this document. Thanks also go to R.P. Thomas and P. Bhattacharyya for peer reviewing the chapters on soil and biofertilizer, respectively, and for their suggestions.

Preface

This publication provides practical guidelines on establishing service laboratories for the analysis of soil, plants, water and fertilizers (mineral, organic and biofertilizers). A service laboratory needs information on a methodology that is widely acceptable, taking into consideration the ready availability of chemicals, reagents and instruments while ensuring a reasonable degree of accuracy, speed and reproducibility of results. The method needs to be easy to understand for practising technicians who are required to adopt it in a routine manner.

A manual, with simple procedural steps, is considered as providing the best help to the laboratory technicians. This publication provides various analytical methods for estimating soil constituents with the objective of assessing soil fertility and making nutrient recommendations. It describes methods for analysing plant constituents in order to determine the content of various nutrients and the need for their application. For assessing the quality of irrigation water, it presents standard methods for estimating the various parameters and constituents utilized, e.g. electrical conductivity, sodium adsorption ratio, residual sodium carbonate, the ratio of magnesium to calcium, and boron content. In providing the methodology for fertilizer analysis, special consideration has been given to the fact that fertilizers are often statutorily controlled commodities and are traded widely among countries.

This guide also examines biofertilizers. It discusses the bacterial cultures that serve either as a source of nitrogen, such as *Rhizobium*, *Azotobacter* and *Azospirillum*, or for improving the availability of soil phosphorus, such as phosphate-solubilizing microbes. It provides methods for their isolation, identification, multiplication and commercial production. The Reference section includes sources for further detailed information.

This guide details the equipment, chemicals and glassware required in order to establish a composite laboratory with facilities for soil, water and plant analysis. Similarly, it details the requirements for establishing a fertilizer testing laboratory and a biofertilizer testing/production laboratory. To save on the cost of some of the common equipment, facilities and supervision, the analytical facilities required for various materials can be combined. However, it is necessary to ensure that no contamination of the soil by the fertilizers or vice versa takes place. In view of this, even in a composite laboratory (which is otherwise desirable), it is necessary to keep rooms for processing and handling different types of samples separate from one another, while keeping them in close proximity in order to save on time for movement and supervision. The guide takes these considerations into account.

This publication should prove useful to administrators and planners in establishing laboratories, and to technicians through providing detailed and precise procedures for estimations.

List of acronyms, abbreviations and chemical symbols

AAS	Atomic absorption spectrophotometer
Al	Aluminium
AOAC	Association of Official Analytical Chemists, the United States of America
AR	Analytical reagent
As	Arsenic
B	Boron
BAC	Benzalkonium chloride
BGA	Blue-green algae
BOD	Biochemical oxygen demand
C	Carbon
Ca	Calcium
CEC	Cation exchange capacity
Cl	Chlorine
Co	Cobalt
COD	Chemical oxygen demand
CP	Chemically pure
CRYEMA	Congo red yeast extract mannitol agar
Cu	Copper
DAP	Di-ammonium phosphate
DDW	Double-distilled water
DTPA	Diethylenetriamine pentaacetic acid
EBT	Eriochrome Black T
EC	Electrical conductivity
EDTA	Ethylenediamine tetraacetic acid
Eq W	Equivalent weight
Fe	Iron
GLP	Good laboratory practice
H	Hydrogen
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HEPA	High-efficiency particulate air
Hg	Mercury

ICP	Inductively coupled plasma
ICP–AES	Inductively coupled plasma – atomic emission spectroscopy
INM	Integrated nutrient management
IPE	International Plant Analytical Exchange
ISE	International Soil Analytical Exchange
ISO	International Organization for Standardization
K	Potassium
LR	Laboratory reagent
M	Molar
MARSEP	International Manure and Refuse Sample Exchange Programme
mcf	Moisture correction factor
me	Milli-equivalent
mEq W	Milli-equivalent weight
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
MOP	Muriate of potash
N	Nitrogen
NaOH	Sodium hydroxide
Ni	Nickel
O	Oxygen
OM	Organic matter
P	Phosphorus
PSM	Phosphate-solubilizing micro-organism
RSC	Residual sodium carbonate
S	Sulphur
SAR	Sodium adsorption ratio
Se	Selenium
SETOC	International Sediment Exchange for Tests on Organic Contaminants
SOM	Soil organic matter
SOP	Standard operating procedure
SOP	Sulphate of potash
SSP	Single superphosphate
STPB	Sodium tetraphenyl boron
TEA	Triethanolamine
UV	Ultraviolet
WEPAL	Wageningen Evaluating Programme for Analytical Laboratories
WHC	Water holding capacity
YEMA	Yeast extract mannitol agar
Zn	Zinc

Chapter 1
Introduction

The role of plant nutrients in crop production is well established. There are 16 essential plant nutrients. These are carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), sulphur (S), zinc (Zn), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo) and chlorine (Cl). These nutrient elements have to be available to the crops in quantities as required for a yield target. Any limiting or deficient nutrient (or nutrients) will limit crop growth.

The required nutrients may come from various sources, such as the atmosphere, soil, irrigation water, mineral fertilizers, manures and biofertilizers. The combinations, quantities and integration of nutrients to be supplied from various sources (integrated plant nutrient supply) depend on various factors including the type of crop, soils, availability of various resources, and ultimately on economic considerations, such as the level of production and the costs of inputs and outputs.

Integrated nutrient management (INM) is a well-accepted approach for the sustainable management of soil productivity and increased crop production. To implement this approach successfully, well-equipped testing laboratories, among other things, are needed in order to evaluate the nutrient supplying capacities of various sources.

Accurate and timely analysis helps in determining the requirements of plant nutrients so as to arrange their supply through various sources. The analytical facilities required for chemical analysis of soils, plants, water and fertilizers are broadly identical in nature with a few specific requirements in terms of facilities and chemicals for certain estimations. The facilities for biofertilizer assay are of a highly specialized nature and are different from those required for chemical analysis. In view of this, it is possible to set up integrated facilities for soil, plant, water and fertilizer analysis, and a biofertilizer testing facility can be added (as appropriate) in an adjacent or expanded building. A common facility saves on supervision and other costs, such as common equipment and chemicals.

Depending on the need, different types of laboratories can be set up (Table 1). A soil, plant and water testing laboratory with an annual analysing capacity of about 10 000–12 000 samples requires a building space of about 370 m^2. For a fertilizer testing laboratory with an analysing capacity of 2 000 samples, the space requirement is about 185 m^2. A composite laboratory may require about 480 m^2. Annex 1 provides a laboratory floor plan.

A biofertilizer laboratory with an analysing capacity of 1 000 samples and a production of 25–100 tonnes of biofertilizer per year may require an area of about 270 m^2. Annex 2 provides a floor plan for such a laboratory.

Requirements in terms of equipment, glassware and chemicals have been determined separately for laboratories of types A (Annex 3), B (Annex 4) and D (Annex 5). For the setting up of type C and E laboratories, the requirements in terms of the relevant facilities can be added together. Under budget constraints, there would be advantages in ordering the same chemicals in one order, and in using the same glassware and costly equipment, such as a spectrophotometer and an atomic absorption spectrophotometer (AAS). An experienced chemist would be able to decide on the actual reduction and, thus, achieve cost savings in setting up a composite laboratory.

Some of the methods are common for estimating plant nutrients in soils, plants and fertilizers. Annex 6 summarizes the methods described in this publication.

Given the increasing need for analysis of larger numbers of soil, plant, water and fertilizer samples by the service laboratories to serve the farmers more rapidly and more effectively, various types of equipment/techniques capable of multinutrient analysis may be useful. Annex 7 describes some of them, e.g. autoanalysers and inductively coupled plasma – atomic emission spectroscopy (ICP–AES). However, for service laboratories in developing countries that are limited in terms of facilities, skilled personnel and financial resources, such equipment is not advocated.

TABLE 1
Laboratory types, with analysis capacity

Category	Laboratory type	Type of analysis	Capacity-samples per year
A	Soil, plant & water analysis	Soil	10 000
		Plant	1 000
		Water (irrigation)	500
B	Mineral & organic fertilizer analysis	Mineral fertilizers	1 500
		Organic fertilizers	500
C	Soil, plant, water, mineral & organic fertilizers analysis (A+B)	Soil	10 000
		Plant	1 000
		Water	500
		Mineral fertilizers	1 500
		Organic fertilizers	500
		Biofertilizer	1 000
D	Biofertilizer	Biofertilizer	1 000
E	Soil, plant, water, mineral, organic fertilizer & biofertilizer analysis (C+D)	Soil	10 000
		Plant	1 000
		Water (irrigation)	500
		Mineral fertilizers	1 500
		Organic fertilizers	500

Chapter 2

The basics of an analytical laboratory

In chemical laboratories, the use of acids, alkalis and some hazardous and explosive chemicals is unavoidable. In addition, some chemical reactions during the analysis process may release toxic gases and, if not handled well, may cause an explosion. Inflammable gases are also used as a fuel/heating source. Thus, work safety in a chemical laboratory calls for special care both in terms of the design and construction of the laboratory building, and in the handling and use of chemicals. For chemical operations, it is also necessary to provide special chambers.

The air temperature of the laboratory and work rooms should be maintained constant at 20–25 °C. Humidity should be kept at about 50 percent. Temperature and humidity often affect soil and fertilizer samples. Temperature also affects some chemical operations. Hence, maintaining the temperature and humidity as specified is critical.

Proper air circulation is also important in order to prevent hazardous and toxic fumes and gases from remaining in the laboratory for long. The release of gases and fumes in some specific analytical operations are controlled through fumehoods or trapped in acidic/alkaline solutions and washed through flowing water. The maintaining of a clean and hygienic environment in the laboratory is essential for the good health of the personnel.

Care is required in order to ensure that acids and hazardous chemicals are stored in separate and safe racks. An inventory of all the equipment, chemicals, glassware and miscellaneous items in a laboratory should be maintained (Annex 8 suggests a suitable format). A safe laboratory building should have suitable separate rooms for different purposes and for performing different operations as described below (with a floor plan in Annex 1):

- Room 1. Reception, sample receipt, and dispatch of reports.
- Room 2. Sample storage and preparation room (separate for soil/plant and fertilizers).
- Room 3. Nitrogen digestion/distillation room (with fumehood for digestion).
- Room 4. Instrument room to house:
  - atomic absorption spectrophotometer (AAS);
  - flame photometer;
  - spectrophotometer;
  - pH meter, conductivity meter;
  - ovens;
  - centrifuge;

- balances;
- water still.

➢ Room 5. Chemical analysis room (separate for soil/plant and fertilizers):
- to prepare reagents and chemicals, and to carry out their standardization;
- to carry out extraction of soil and fertilizer samples with appropriate chemicals/reagents;
- to carry out titration, colour development, precipitation, filtration, etc.;
- all other types of chemical work.

➢ Room 6. Storage room for chemicals and spare equipment.

➢ Room 7. Office room with computers for data processing and record keeping.

LABORATORY SAFETY MEASURES

Special care is required while operating equipment, handling chemicals and in waste disposal.

Equipment

Electrical cables, plugs and tubing need proper checking in order to avoid accidents. Various types of gas cylinders needed in the laboratory, such as acetylene, nitrous oxide and liquefied petroleum gas, must be kept under watch and properly sealed/capped, and they must be stored in ventilated cupboards.

Chemical reagents

Hazardous chemicals should be stored in plastic bottles. While working with chemicals, such as perchloric acid, a fumehood must be used. Chemicals must be labelled properly, indicating their hazardous nature.

Bottles with inflammable substances need to be stored in stainless-steel containers.

Waste disposal

Each country has special rules and methods for the disposal of hazardous waste.

Cyanides, chromates, arsenic (As), selenium (Se), cobalt (Co) and molybdate are commonly used but hazardous chemicals. They should never be disposed of in the laboratory sink but collected in a metal container for proper disposal at the specified places and in the manner described in national legislation for waste disposal.

General rules and requirements

General safety rules and requirements for personnel working in a laboratory are:

➢ Learn safety rules and the use of first-aid kits. Keep the first-aid kit handy in a conspicuous place in the laboratory.

➢ Personal safety aids, such as laboratory coats, protective gloves, safety glasses, face shields and proper footwear, should be used.

- Observe normal laboratory safety practice in connecting equipment to the power supply, in handling chemicals and in preparing solutions of reagents. All electrical work must be done by qualified personnel.
- Maintain an instrument manual and logbook for each item of equipment in order to avoid mishandling, accidents and damage to equipment.
- Keep work tables/spaces clean. Clean up spillage immediately.
- Wash hands after handling toxic/hazardous chemicals.
- Never suck the chemicals by mouth but use automatic pipetting devices.
- Use forceps/tongs to remove containers from hotplates/ovens/furnaces.
- Do not use laboratory glassware for eating/drinking.
- Never open a centrifuge cover until the machine has stopped.
- Add acid to water and not water to acid when diluting the acid.
- Always put labels on bottles, vessels and wash-bottles containing reagents, solutions, samples and water.
- Handle perchloric acid and hazardous chemicals in fumehoods.
- With the wet oxidation method of sample digestion, destroy organic matter (OM) first with nitric acid.
- Read the labels on the bottles before opening them.

LABORATORY QUALITY ASSURANCE/CONTROL

For uniformity of expression and understanding, this guide adopts the definitions of the terms quality, quality assurance and quality control as defined by the International Organization for Standardization (ISO) and also those compiled in FAO Soils Bulletin No. 74 (1998).

Quality

Quality is defined as the total features and characteristics of a product or service that bear on its ability to satisfy a stated and implied need. A product can be stated to possess good quality if it meets the predetermined parameters. In the case of an analytical laboratory, the quality of the laboratory may be considered adequate and acceptable if it has the capacity to deliver the analytical results on a product within the specified limits of errors and as per other agreed conditions of cost and time of analysis so as to enable an acceptable judgement on the product quality.

Quality assurance

As per the ISO, quality assurance means the assembly of all planned and systematic actions necessary to provide adequate confidence that a product, a process or a service will satisfy given quality requirements. Another independent laboratory/person checks the results of these actions in order to confirm the pronouncement on the quality of a product by a given laboratory.

Quality control

Quality control is an important part of quality assurance, and the ISO defines it as the operational techniques and activities that are used to satisfy quality

requirements. Quality assessment or evaluation is necessary to see whether the activities performed to verify quality are effective. Thus, an effective check on all the activities and processes in a laboratory can only ensure that the results pronounced on a product quality are within the acceptable parameters of accuracy.

In a quality control system, implementing the following steps properly ensures that the results delivered are acceptable and verifiable by another laboratory:

- check on the performance of the instruments;
- calibration or standardization of instruments and chemicals;
- adoption of sample check system as a batch control within the laboratory;
- external check: interlaboratory exchange programme.

To ensure the obtaining of accurate and acceptable results of analysis on a sample, the laboratory has to operate in a well-regulated manner, one where the equipment is properly calibrated and the methods and techniques employed are scientifically sound, which will give reproducible results. For ensuring high standards of quality, it is necessary to follow good laboratory practice (GLP). This can be defined as the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported. Thus, GLP expects a laboratory to work according to a system of procedures and protocols, while the procedures are also specified as the standard operating procedure (SOP).

STANDARD OPERATING PROCEDURE

As per FAO (1998), an SOP is a document that describes the regularly recurring operations relevant to the quality of the investigation. The purpose of an SOP is to carry out the operation correctly and always in the same manner. An SOP should be available at the place where the work is done. If, for justifiable reasons, any deviation is allowed from the SOP, the changed procedure should be documented fully.

In a laboratory, an SOP may be prepared for:

- safety precautions;
- procedures for operating instruments;
- analytical methods and preparation of reagents;
- registration of samples.

To sum up, all operations have to be documented properly in order to prevent any ad hoc approach to operations.

ERROR, PRECISION, ACCURACY AND DETECTION LIMIT

Error

Error is an important component of analysis. In any analysis, where the quantity is measured with the greatest exactness that the instrument, method and observer are capable of, it is found that the results of successive determinations differ to a greater or lesser extent. The average value is accepted as most probable. This may not always be the true value. In some cases, the difference in the successive values may be small, in some cases it may be large; the reliability of the result depends on

the magnitude of this difference. There could be a number of factors responsible for this difference, which is also referred to as error. In absolute terms, error is the difference between the observed or measured value and the true or most probable value of the quantity measured. The absolute error is a measure of the accuracy of the measurement. Therefore, the accuracy of a determination may be defined as the concordance between it and the true or most probable value. The relative error is the absolute error divided by the true or most probable value.

The error may be caused by any deviation from the prescribed steps to be taken in analysis. The purity of chemicals, their concentration/strength, the accuracy of the instruments and the skill of the technician are important factors.

Precision and accuracy

In analysis, other important terms are precision and accuracy. Precision is defined as the concordance of a series of measurements of the same quantity. The mean deviation or the relative mean deviation is a measure of precision.

Accuracy expresses the correctness of a measurement, while precision expresses the reproducibility of a measurement. Precision always accompanies accuracy, but a high degree of precision does not imply accuracy. In ensuring high accuracy in analysis, accurate preparation of reagents including their perfect standardization is critical. The purity of chemicals is also important. For all estimations where actual measurement of a constituent of the sample in terms of the "precipitate formation" or formation of "coloured compound" or "concentration in the solvent" is a part of the steps in estimation, chemical reagents involved in such aspects must always be of high purity, which is known as analytical-reagent (AR) grade.

Detection limits

In the analysis for elements, particularly trace elements in soils, plants and fertilizers and for environmental monitoring, the need arises to measure very low contents of analytes. Modern equipment is capable of such estimation. However, while selecting the equipment and the testing method for such a purpose, it is important to have information about the lowest limits to which analytes can be detected or determined with sufficient confidence. Such limits are called detection limits or lower limits of detection.

The capacity of the equipment and the method may be such that it can detect the traces of analyte in the sample. In quantitative terms, the lowest contents of such analytes may be decided through appropriate research as the values of interpretable significance. The service laboratories are generally provided with such limits.

QUALITY CONTROL OF ANALYTICAL PROCEDURES

Independent standards

The ultimate aim of the quality control measures is to ensure the production of analytical data with a minimum of error and with consistency. Once an appropriate

method has been selected, its execution has to be done with utmost care. To check and verify the accuracy of analysis, independent standards are used in the system. The extent of deviation of an analytical value on a standard sample indicates the accuracy of the analysis. Independent standards can be prepared in the laboratory from pure chemicals. When a new standard is prepared, the remainder of the old ones always has to be measured as a mutual check. If the results are not within acceptable levels of accuracy, the process of calibration, preparation of the standard curve and the preparation of reagents should be repeated until acceptable results are obtained on the standard sample. After ensuring this, analysis on an unknown sample can start.

Apart from independent standards, certified reference samples can also be used as "standard". Such samples are obtained from other selected laboratories where the analysis on a prepared standard is carried out by more than one laboratory. Such samples, along with the accompanying analytical values, are used as a check to ensure the accuracy of analysis.

Use of blank

A blank determination is an analysis without the analyte or attribute; in other words, it is an analysis without a sample, going through all steps of the procedure with the reagents only. The use of a blank accounts for any contamination in the chemicals used in actual analysis. The "estimate" of the blank is subtracted from the estimates of the samples. "Sequence control" samples are used in long batches in automated analysis. Generally, two samples, one with a low content and the other with a very high content of known analyte (but the contents falling within the working range of the method) are used as standards to monitor the accuracy of analysis.

Blind sample

A blind sample is one with a known content of analyte. The head of the laboratory inserts this sample in batches and at times unknown to the analyst. Various types of sample material may serve as blind samples, such as control samples or sufficiently large leftovers of test samples (analysed several times). It is essential that the analyst be aware of the possible presence of a blind sample but not be able to recognize the material as such.

Validation of analysis procedures

Validation is the process of determining the performance characteristics of a method/procedure. It is a prerequisite for assessing the suitability of produced analytical data for the intended use. This implies that a method may be valid in one situation but not in another. If a method is very precise and accurate but expensive for adoption, it may be used only when data with that order of precision are needed. The data may be inadequate if the method is less accurate than required. Two types of validation are followed.

Validation of own procedure

In-house validation of methods or procedures by individual user laboratories is a common practice. Many laboratories use their own version of even well-established methods for reasons of efficiency, cost and convenience. Changes in the liquid–solid ratio in extraction procedures for available soil nutrients, shaking time, etc., result in changed values, hence they need validating. Such changes are often introduced in order to take account of local conditions, the cost of analysis, and the required accuracy and efficiency.

Validation of such changes is a part of quality control in the laboratory. It is also a kind of research project, hence not all types of the laboratories may be in a position to modify the standard method. They should follow the given method as accepted and practised by most other laboratories.

Apart from validating methods, laboratories need to employ a system of internal quality control in order to ensure that they are capable of producing reliable analytical data with a minimum of error. This requires continuous monitoring of operations and systematic day-to-day checking of the data produced in order to decide whether these are reliable enough to be released.

Internal quality control requires implementing the following steps:

- Use a blank and a control (standard) sample of known composition along with the samples under analysis.
- Round off the analytical values to the second decimal place. The value of the third decimal place should be omitted if it is lower than 5. If it is higher than 5, the value of the second decimal should be raised by 1.

As quality control systems rely heavily on control samples, the sample preparation must be done with great care in order to ensure that:

- the sample is homogenous;
- the sample material is stable;
- the material has uniform and correct particle size as sieved through a standard sieve;
- relevant information, such as properties of the sample and the concentration of the analyte, is available.

The sample under analysis must also be processed/prepared in such a way that it has a similar particle size and homogeneity to that of the standard (control) sample.

As and when internal checks reveal an error in the analysis, corrective measures should be taken. The error could be one of calculation or typing. Where not, it requires thorough checks on sample identification, standards, chemicals, pipettes, dispensers, glassware, calibration procedure and equipment. The standard may be old or prepared incorrectly. A pipette may indicate the wrong volume. Glassware may not have been cleaned properly. The equipment may be defective, or the sample intake tube may be clogged in the case of a flame photometer or an AAS. The source of error must be detected and the samples analysed again.

Validation of the standard procedure

This refers to the validation of new or existing methods and procedures intended for use in many laboratories, including procedures accepted by national systems or the ISO. It involves an interlaboratory programme of testing the method by a number of selected renowned laboratories according to a protocol issued to all participants. Validation is relevant not only when non-standard procedures are used but also when validated standard procedures are used, and even more so when variants of standard procedures are introduced. The results of validation tests should be recorded in a validation report, from which the suitability of a method for a particular purpose can be deduced.

Interlaboratory sample and data exchange programme

Where an error is suspected in the procedure and the uncertainty cannot be resolved readily, it is not uncommon to have the sample analysed in another laboratory of the same system/organization. The results of the other laboratory may or may not be biased, hence, doubt may persist. It may be necessary for another accredited laboratory to check the sample in order to resolve the problem.

An accredited laboratory should participate in at least one interlaboratory exchange programme. Such programmes exist at local, regional, national and international level. Laboratory exchange programmes exist for method performance studies and laboratory performance studies.

In such exchange programmes, some laboratories or organizations have devised the system where samples of known composition are sent periodically to the participating laboratory without disclosing the results. The participating laboratory analyses the sample by a given method and obtains the results. This provides a possibility for assessing the accuracy of the method being used by a laboratory, and also information about the adoption of the method suggested by the lead laboratory. Some of these programmes are:

- International Plant Analytical Exchange (IPE) Programme;
- International Soil Analytical Exchange (ISE) Programme.

They come under the Wageningen Evaluating Programme for Analytical Laboratories (WEPAL) of Wageningen Agricultural University, the Netherlands. Other programmes run by Wageningen Agricultural University are:

- International Sediment Exchange for Tests on Organic Contaminants (SETOC);
- International Manure and Refuse Sample Exchange Programme (MARSEP).

Another international organization operating a laboratory and method evaluation programme is the Association of Official Analytical Chemists (AOAC) of the United States of America. One of its most popular programmes is for fertilizer quality control laboratories.

Every laboratory benefits if it becomes part of a sample/method checking and evaluation programme. The system of self-checking within the laboratory also has to be followed regularly.

PREPARATION AND STANDARDIZATION OF REAGENT SOLUTIONS

Chemical reagents are manufactured and marketed in different grades of purity. In general, the purest reagents are marketed as "analytical reagent" or AR-grade. Other labels are "LR", meaning laboratory reagent, and "CP", meaning chemically pure (details in Annex 9). The strength of chemicals is expressed as normality or molarity. Therefore, it is useful to have some information about the strength of the acids and alkalis most commonly used in chemical laboratories (Table 2).

Some important terms that are often used in a laboratory for chemical analysis are defined/explained below.

Molarity

A one-molar (M) solution contains one mole or one molecular weight in grams of a substance in each litre of the solution. The molar method of expressing concentration is useful because equal volumes of equimolar solutions contain equal number of molecules.

Normality

The normality of a solution is the number of gram equivalents of the solute per litre of the solution. It is usually designated by the letter N. Semi-normal, penti-normal, desi-normal, centi-normal and milli-normal solutions are often required; these are written as 0.5N, 0.2N, 0.1N, 0.01N and 0.001N, respectively. However, molar expression is preferred because "odd" normalities such as 0.121N are clumsily represented in fractional form.

The definition of normal solution uses the term "equivalent weight". This quantity varies with the type of reaction. Hence, it is difficult to give a clear definition of equivalent weight that covers all reactions. It often happens that the same compound possesses different equivalent weights in different chemical reactions. A situation may arise where a solution has a normal concentration when employed for one purpose and a different normality when used in another chemical reaction. Hence, the system of molarity is preferred.

TABLE 2
Strength of commonly used acids and alkalis

Reagent/ chemical	Normality (approx.)	Molarity (approx.)	Formula weight	% by weight (approx.)	Specific gravity (approx.)	Millilitres required for 1N/litre solution (approx.)	Millilitres required for 1M/litre solution (approx.)
Nitric acid	16.0	16.0	63.0	70	1.42	63.7	63.7
Sulphuric acid	35.0	17.5	98.0	98	1.84	28.0	56.0
Hydrochloric acid	11.6	11.6	36.5	37	1.19	82.6	82.6
Phosphoric acid	45.0	15.0	98.0	85	1.71	22.7	68.1
Perchloric acid	10.5	10.5	100.5	65	1.60	108.7	108.7
Ammonium hydroxide	15.0	15.0	35.0	28	0.90	67.6	67.6

Equivalent weight

The equivalent weight (Eq W) of a substance is the weight in grams that in its reaction corresponds to a gram atom of hydrogen or of hydroxyl, or half a gram atom of oxygen, or a gram atom of univalent ion. When one equivalent weight of a substance is dissolved in one litre, it gives 1N solution. Annex 10 gives the equivalent and molecular weights of some important compounds.

Milli-equivalent weight

Equivalent weight when expressed as milli-equivalent weight (mEq W) means the equivalent weight in grams divided by 1 000. It is commonly expressed as "me". It is the most convenient value because it is the weight of a substance contained in or equivalent to 1 ml of 1N solution. Therefore, it is a unit that is common to both volumes and weights, making it possible to convert the volume of a solution to its equivalent weight, and the weight of a substance to its equivalent volume of solution: number of mEq = volume × normality.

Buffer solutions

Solutions containing either a weak acid and its salt or a weak base and its salt (e.g. CH_3COOH + CH_3COONa and NH_4OH + NH_4Cl) possess the characteristic property of resisting changes in pH when some acid or base is added to them. Such solutions are called buffer solutions. The important properties of a buffer solution are:

- It has a definite pH value.
- Its pH value does not alter on keeping for a long time.
- Its pH value is only altered slightly when a strong base or strong acid is added.

Because of the above property, readily prepared buffer solutions of known pH are used in order to check the accuracy of pH meters used in the laboratory.

Titrations

Titration is a process of determining the volume of a substance required to just complete the reaction with a known amount of other substance. The solution of known strength used in the titration is called the titrant. The substance to be determined in the solution is called the titrate.

The completion of the reaction is assessed with the help of an appropriate indicator.

Indicators

A substance that indicates the end point on completion of the reaction is called an indicator. The most commonly used indicators in volumetric analysis are:

- internal indicators,
- external indicators,
- self-indicators.

Internal indicators

Indicators such as methyl red, methyl orange, phenolphthalein and diphenylamine that are added to the solution where reaction occurs are called internal indicators. On completion of the reaction of titrant on titrate, a colour change takes place owing to the presence of the indicator, which also helps in knowing that the titration is complete. The internal indicators used in acid–alkali neutralization solutions are methyl orange, phenolphthalein and bromothymol blue.

The indicator used in precipitation reactions such as titration of neutral solution of NaCl (or chlorine ion) with silver nitrate ($AgNO_3$) solution is $K_2CrO_4^-$. On completion of the titration reaction of $AgNO_3$ with Cl, when no more Cl is available for reaction with silver ions to form AgCl, the chromium ions combine with Ag^{2+} ions to form sparingly soluble Ag_2CrO_4, which is brick red in colour. This indicates that Cl has been titrated completely and that end point has occurred.

Redox indicators are also commonly used. These are substances that possess different colours in the oxidized and reduced forms. Diphenylamine has a blue-violet colour in the oxidation state and is colourless in reduced condition. Ferrocin gives a blue colour in the oxidation state and a red colour in reduced condition.

External indicators

Some indicators are used outside the titration mixture. Potassium ferricyanide is used as an external indicator in the titration of potassium dichromate and ferrous sulphate in acid medium. In this titration, a few drops of indicator are placed on a white porcelain tile. A glass rod dipped in the solution being titrated is taken out and brought into contact with the drops of indicator on the tile. First, a deep blue colour is noticed, which turns greenish on completion of titration.

Self-indicators

After completion of the reaction, the titrant leaves its own colour owing to its slight excess in minute quantities. In $KMnO_4$ titration with ferrous sulphate, the addition of $KMnO_4$ starts reacting with $FeSO_4$, which is colourless. On completion of titration, the slight excess presence of $KMnO_4$ gives a pink colour to the solution, which acts as a self-indicator and points to the completion of the titration.

Standard solutions

A solution of accurately known strength (or concentration) is called a standard solution. It contains a definite number of gram equivalent or gram mole per litre of solution. If it contains 1 g equivalent weight of a substance/compound, it is 1N solution. If it contains 2 g equivalent weights of the compound, it is 2N.

All titrimetric methods depend on standard solutions that contain known amounts of the reagents in unit volume of the solution. A solution is prepared, having about the desired concentration. This solution is then standardized by

titriting it with another substance that can be obtained in highly purified form. Thus, potassium permanganate solution can be standardized against sodium oxalate, which can be obtained in a high degree of purity as it is easily dried and is non-hygroscopic. Such a substance, whose weight and purity is stable, is called a "primary standard". A primary standard must have the following characteristics:

- It must be obtainable in a pure form or in a state of known purity.
- It must react in one way only under the condition of titration and there must be no side-reactions.
- It must be non-hygroscopic. Salt hydrates are generally not suitable as primary standards.
- Normally, it should have a large equivalent weight in order to reduce the error in weighing.
- An acid or a base should preferably be strong, that is, it should have a high dissociation constant for being used as standards.

A primary standard solution is one that can be prepared directly by weighing the material and with which other solutions of approximate strength can be titrated and standardized. Some primary standards are:

- acids:
  - potassium hydrogen phthalate,
  - benjoic acid;
- bases:
  - sodium carbonate,
  - borax;
- oxidizing agents:
  - potassium dichromate,
  - potassium bromate;
- reducing agents:
  - sodium oxalate,
  - potassium ferrocyanide;
- others:
  - sodium chloride,
  - potassium chloride.

Secondary standard solutions are those that are prepared by dissolving a little more than the gram equivalent weight of the substance per litre of the solution, and then their exact standardization is done with primary standard solution. Some secondary standards are:

- acids:
  - sulphuric acid,
  - hydrochloric acid;
- base:
  - sodium hydroxide.

Standard solutions of all the reagents required in a laboratory must be prepared and kept ready before commencing any analysis. However, their strength should be checked periodically, or fresh reagents should be prepared before analysis.

All titrations involving acidimetry and alkalimetry require standard solutions. These may be prepared either from standard substances by direct weighing, or by standardizing a solution of approximate normality of materials by titrating against a prepared standard. The methods for preparing standard solutions of some non-primary standard substances in common use are given below.

Standardization of hydrochloric acid

Concentrated hydrochloric acid (HCl) is about 11N. Therefore, to prepare a standard solution, say, decinormal (0.1N) of the acid, it is diluted about 100 times. Take 10 ml of acid and make about 1 litre by dilution with distilled water. Titrate this acid against 0.1N Na_2CO_3 (primary standard) using methyl orange as indicator. The colour changes from pink to yellow when the acid is neutralized. Suppose 10 ml of acid and 12 ml of Na_2CO_3 are consumed in the titration, then:

Acid		Alkali
$V_1 \times N_1$	=	$V_2 \times N_2$
$10 \times N_1$	=	12×0.1
$10\ N_1$	=	1.2
N_1	=	0.12

Thus, the normality of the acid is 0.12.

Standardization of sulphuric acid

Similar to above, the normality of sulphuric acid (H_2SO_4) can be determined. It needs to be diluted about 350 times in order to reach about 0.1N because it has a normality of about 35. Then, titrate against standard Na_2CO_3 to determine the exact normality of H_2SO_4.

Standardization of sodium hydroxide

As per the above method, the normality of HCl/H_2SO_4 has been fixed. Therefore, to determine the normality of sodium hydroxide (NaOH), titration is carried out by using either of these standard acids. To determine the molarity, molar standard solutions are used.

In the case of the standardization of NaOH or any other alkali, potassium hydrogen phthalate can also be used as a primary standard instead of titration with secondary standards.

Chapter 3
Soil analysis

Soil is the main source of nutrients for crops. Soil also provides support for plant growth in various ways. Knowledge about soil health and its maintenance is critical to sustaining crop productivity. The health of soils can be assessed by the quality and stand of the crops grown on them. However, this is a general assessment made by the farmers. A scientific assessment is possible through detailed physical, chemical and biological analysis of the soils.

Essential plant nutrients such as N, P, K, Ca, Mg and S are called macronutrients, while Fe, Zn, Cu, Mo, Mn, B and Cl are called micronutrients. It is necessary to assess the capacity of a soil to supply nutrients in order to supply the remaining amounts of needed plant nutrients (total crop requirement - soil supply). Thus, soil testing laboratories are considered nerve centres for nutrient management and crop production systems.

AVAILABLE NUTRIENT CONTENT OF SOILS

Soils may have large amounts of nutrient reserves in them. All or a part of these reserves may not be of any use to crops because they may not be in plant-available form. For the purpose of estimation or analysis of plant-available soil nutrients, such methods are to be used that have been tested/verified for the correlation of nutrients extracted and their plant availability. This guide describes internationally accepted and widely used methods.

Apart from nutrients, soil pH estimation is also critical in the assessment of soil health. Generally, plants prefer soils that are close to either side of neutrality. However, there are acid-loving crops and also crops that can withstand high soil alkalinity. Hence, good crop yields are possible in acid and alkali soils. With proper amendments, still higher yields can be obtained in acid and alkali soils. Soil pH also has a considerable influence on the activity of soil microflora and on the availability of soil nutrients to crops. It is also important to estimate physical properties such as soil texture and soil structure.

SOIL SAMPLING

The methods and procedures for obtaining soil samples vary according to the purpose of the sampling. Analysis of soil samples may be needed for engineering and agricultural purposes. This guide describes soil sampling for agricultural purposes, i.e. for soil fertility evaluation and fertilizer recommendations for crops.

The results of even very carefully conducted soil analyses can only be as good as the soil samples themselves. Thus, the efficiency of a soil testing service depends

on the care and skill with which soil samples are collected. Non-representative samples constitute the largest single source of error in a soil fertility programme. The most important phase of soil analysis takes place not in the laboratory but in the field where the soil is sampled.

Soils vary from place to place. In view of this, efforts should be made to take the samples in such a way that they are fully representative of the field. Only 1–10 g of soil is used for each chemical determination and this sample needs to represent as accurately as possible the entire surface 0–22 cm of soil, weighing about 2 million kg/ha.

Sampling tools and accessories

Depending on the purpose and precision required, the following tools may be needed for taking soil samples:

- a soil auger – it may be a tube, post-hole or screw-type auger or even a spade for taking sample;
- a clean bucket or a tray or a clean cloth – for mixing the soil and subsampling;
- cloth bags of a specific size;
- a copying pencil for markings, and tags for tying cloth bags;
- soil sample information sheet.

Selection of a sampling unit

A visual survey of the field should precede the actual sampling. Note the variation in slope, colour, texture, management and cropping pattern by traversing the field. Demarcate the field into uniform portions, each of which must be sampled separately. Where all these conditions are similar, one field can be treated as a single sampling unit. Such a unit should not exceed 1–2 ha, and it must be an area to which a farmer is willing to give separate attention. The sampling unit is a compromise between expenditure, labour and time on the one hand, and precision on the other.

Sampling procedure

Prepare a map of the area to be covered in a survey showing different sampling unit boundaries. Enter a plan of the number of samples and manner of composite sampling on the map, designating different fields by letters (A, B, C, etc.). Traverse each area separately. Cut a slice of the plough layer at intervals of 15–20 steps or according to the area to be covered. Generally, depending on the size of the field, 10–20 spots must be taken for one composite sample.

Scrape away surface litter to obtain a uniformly thick slice of soil from the surface to the plough depth from each spot. Make a V-shaped cut with a spade to remove a 1–2-cm slice of soil. Collect the sample on the blade of the spade and put it in a clean bucket. In this way, collect samples from all the spots marked for one sampling unit. In the case of hard soil, take samples with the help of an auger from the plough depth and collect them in the bucket.

Pour the soil from the bucket onto a piece of clean paper or cloth, and mix it thoroughly. Spread the soil evenly and divide it into quarters. Reject two opposite quarters and mix the rest of the soil again. Repeat the process until left with about 0.5 kg of the soil. Collect it and put in a clean cloth bag. Mark each bag clearly in order to identify the sample.

The bag used for sampling must always be clean and free from any contamination. If the same bag is to be used a second time, turn it inside out and remove the soil particles. Write the details of the sample on the information sheet (Annex 11 provides a suggested format). Put a copy of this information sheet in the bag. Tie the mouth of the bag carefully.

Precautions

When sampling a soil, bear in mind the following:

- Do not sample unusual areas, such as unevenly fertilized areas, marshy areas, old paths, old channels, old bunds, areas near trees, sites of previous compost piles, and other unrepresentative sites.
- For a soft and moist soil, the tube auger or spade is considered satisfactory. For harder soil, a screw auger may be more convenient.
- Where crops have been planted in rows, collect samples from the middle of the rows in order to avoid the area where fertilizer has been band placed.
- Avoid any type of contamination at all stages. Soil samples should never be stored with fertilizer materials and detergents. Contamination is likely when the soil samples are spread out to dry in the vicinity of stored fertilizers or on floor where fertilizers were stored previously.
- Before putting soil samples in bags, they should be examined for cleanliness as well as for strength.
- The information sheet should be filled in clearly with a copying pencil.

Sampling salt-affected soils

Salt-affected soils may be sampled in two ways. Surface samples should be taken in the same way as for soil fertility analysis. These samples are used to determine the gypsum requirement of the soil. For reclamation purpose, it is necessary to know also the characteristics of lower soil depth. Therefore, such soils are sampled down to a depth of 1 m. The samples may be removed from one to two spots per 0.4 ha where the soil is uniformly salt-affected. Where patches are conspicuous, then all large patches should be sampled separately. Soil is sampled separately for soil depths (about 0.5 kg from each depth) of 0–15, 15–30, 30–60 and 60–100 cm. If a stony layer is encountered during sampling, such a layer should be sampled separately and its depth noted. This is very important and must not be ignored.

Soil samples can be removed by a spade, or if the auger is used, then care should be taken to note the depth of "concretion" (stones) or other impermeable layer (hardpan). If the soil shows evidence of profile development or distinct stratification, samples should be taken by horizon. If a pit is dug and horizons are absent, then mark the vertical side of the pit at depths of 15, 30, 60 and 100 cm from

the surface and collect about 0.5 kg soil from each layer, cutting uniform slices of soil separately. In addition to the above sampling, one surface soil sample should be taken as in the case of normal soil sampling for fertilizer recommendation.

Pack the samples and label the bags in the same way as for normal soil sampling, giving additional information about the depth of the sample. The sheet accompanying the sample must include information on:

- nature of the soil;
- hardness and permeability of the soil;
- cause and source of salinity (where known);
- relief;
- seasonal rainfall;
- irrigation and frequency of waterlogging;
- water table;
- soil management history;
- crop species and conditions of plant cover;
- depth of the hardpan or concretion.

As the salt concentration may vary greatly with vertical or horizontal distance and with moisture and time, it is necessary to keep an account of the time of irrigation and of the amount of irrigation or rain received prior to sampling.

DISPATCH OF SOIL SAMPLES TO THE LABORATORY

Before sending soil samples to the testing laboratory, it is necessary to ensure that proper identification marks are present on the sample bags and labels placed in the bags. It is essential to use a copying pencil and not ink because ink can smudge and become illegible. The best system is to obtain soil sampling bags from the soil testing laboratory with most of the information printed or stencilled on them in indelible ink.

Compare the number and details on the bag with the dispatch list. The serial numbers of different places should be distinguished by putting the identification mark specific for each centre. This may be in letters, e.g. one for the district, another for the block/county, and a third for the village.

Pack the samples properly. Wooden boxes are most suitable for long transport. Sample bags should be packed only in clean bags never used for fertilizer or detergent packing.

Farmers may bring soil samples directly to the laboratory. However, most samples are sent to the laboratories through field extension staff. An organized assembly–processing–dispatch system is required in order to ensure prompt delivery of samples to the laboratory.

PREPARATION OF SOIL SAMPLES FOR ANALYSIS

Handling in the laboratory

As soon as the samples arrive at the soil testing laboratory, they should be checked against the accompanying information list. If the laboratory personnel have

collected the samples themselves, then adequate field notes should have been kept. All unidentifiable samples should be discarded. Information regarding samples should be recorded in a register, and each sample should be given a laboratory number, in addition to the sample number, to help to distinguish it where more than one source of samples is involved.

Drying of samples

Samples received in the laboratory may be moist. They should be dried in wooden or enamelled trays. Care should be taken to maintain the identity of each sample at all stages of preparation. During drying, the trays can be numbered or a plastic tag could be attached. The samples are allowed to dry in the air. Alternatively, the trays may be placed in racks in a hot-air cabinet, whose temperature should not exceed 35 °C and whose relative humidity should be 30–60 percent. Oven drying a soil can cause profound changes in the sample. This step is not recommended as a preparatory procedure despite its convenience. Drying has a negligible effect on total N content, but the nitrate content in the soil changes with time and temperature. Drying at a high temperature affects the microbial population. With excessive drying, soil K may be released or fixed depending on the original level of exchangeable K. Exchangeable K will increase if its original level was less than 1 me/100 g soil (1 cmol/kg) and vice versa, but the effect depends on the nature of clay minerals in the soil. In general, excessive drying, such as oven drying of the soil, affects the availability of most of the nutrients present in the sample and should be avoided. Only air drying is recommended.

Nitrate, nitrite and ammonium determinations must be carried out on samples brought straight from the field. These samples should not be dried. However, the results are expressed on an oven-dry basis by estimating separately the moisture content in the samples.

Post-drying care

After drying, the samples are taken to the preparation room. Air-dried samples are ground with a wooden pestle and mortar so that the soil aggregate is crushed but the soil particles do not break down. Samples of heavy clay soils may have to be ground with an end-runner grinding mill fitted with a pestle of hard wood and rubber lining to the mortar. Pebbles, concretions and stones should not be broken during grinding.

After grinding, the soil is screened through a 2-mm sieve. The practice of passing only a portion of the ground sample through the sieve and discarding the remainder is erroneous. This introduces a positive bias in the sample as the rejected part may include soil elements with differential fertility. Therefore, the entire sample should be passed through the sieve except for concretions and pebbles of more than 2 mm. The coarse portion on the sieve should be returned to the mortar for further grinding. Repeat sieving and grinding until all aggregate particles are fine enough to pass the sieve and only pebbles, organic residues and concretions remain.

If the soil is to be analysed for trace elements, containers made of copper, zinc and brass must be avoided during grinding and handling. Sieves of different sizes can be obtained in stainless steel. Aluminium or plastic sieves are useful alternative for general purposes.

After the sample has passed through the sieve, it must be mixed again thoroughly.

The soil samples should be stored in cardboard boxes in wooden drawers. These boxes should be numbered and arranged in rows in the wooden drawers, which are in turn fitted in a cabinet in the soil sample room.

ANALYTICAL METHODS

The following estimations are generally carried out in a service-oriented soil testing laboratory:

- soil texture,
- soil structure,
- cation exchange capacity (CEC),
- soil moisture,
- water holding capacity,
- pH,
- lime requirement,
- electrical conductivity,
- gypsum requirement,
- organic C,
- total N,
- mineralizable N,
- inorganic N,
- available P,
- available K,
- available S,
- calcium,
- calcium plus magnesium,
- micronutrients – available Zn, Cu, Fe, Mn, B and Mo.

Soil texture

Soil texture (or particle size distribution) is a stable soil characteristic that influences the physical and chemical properties of the soil. The sizes of the soil particles have a direct relationship with the surface area of the particles. Soil particles remain aggregated owing to various types of binding forces and factors. These include the content of OM, other colloidal substances present in the soil, oxides of Fe and aluminium (Al), and the hydration of clay particles. To estimate the content of various sizes of soil particles, the soil sample has to be brought into a dispersed state by removing the various types of binding forces.

In the dispersed soil samples, the soil particles settle down at a differential settling rate according to their size. In the estimation of soil texture, particles of less than 2 mm in diameter are determined separately and characterized as: coarse sand (2.0–0.2 mm); fine sand (0.2–0.02 mm); silt (0.02–0.002 mm); and clay (< 0.002 mm).

The soil sample is dispersed by removing the binding force in soil particles. The settling rate of dispersed particles in water is measured. Large particles are known to settle out of suspension more rapidly than do small particles. This is because larger particles have less specific area and, hence, less buoyancy than smaller particles. Stokes' law (1851) is used to express the relationship. It stipulates that the resistance offered by the liquid to the fall of the particle varies with the radius of the sphere and not with the surface. Accordingly, the formula is:

$$V = \frac{2}{9}\left(\frac{dp - d}{\eta}\right) gr^2$$

where:

- V is the velocity of the fall in centimetres per second;
- g is the acceleration due to gravity;
- dp is the density of the particle;
- d is the density of the liquid;
- r is the radius of the particle in centimetres,
- η is the absolute viscosity of the liquid.

The velocity of fall of the particles with the same density in a given liquid increases with the square of the radius.

With the above principle in view, the particle size distribution is estimated by measuring the amount of different sizes of soil particles present at different calibrated depths in the cylinder containing the suspended soil sample.

The two methods in general use for estimating particle size or soil texture are:

- the international pipette method;
- the Bouyoucos hydrometer method.

Hydrometer method

The hydrometer method is in more common use because it is less time-consuming and easier to follow in a service laboratory. Dispersion is obtained using sodium hexametaphosphate. It requires the following apparatus:

- a balance;
- cylinders – 1 litre and 1.5 litres;
- a glass beaker – 1 litre;
- a metal stirrer with a speed of 1 500 rpm;
- a Bouyoucos hydrometer;
- an oven;
- a thermometer (degrees Celsius).

The reagent is a sodium hexametaphosphate solution containing 50 g of salt per litre of water.

The procedure is:

1. Weigh 50 g of oven-dried, fine-textured soil (100 g for coarse-textured soil) into a baffled stirring cup. Half fill the cup with distilled water and add 10 ml of sodium hexametaphosphate solution.
2. Place the cup on the stirrer and stir until the soil aggregates are broken down. This usually takes 3–4 minutes for coarse-textured soils and 7–8 minutes for fine-textured clay.
3. Quantitatively transfer the stirred mixture to the settling cylinder by washing the cup with distilled water. Fill the cylinder to the lower mark with distilled water after placing the hydrometer in the liquid. Where 100 g of coarse-textured sample was used, fill to the upper mark on the settling cylinder.
4. Remove the hydrometer and shake the suspension vigorously in a back-and-forth manner. Avoid creating circular currents in the liquid as they influence the settling rate.
5. Place the cylinder on a table and record the time. After 20 seconds, carefully insert the hydrometer and read the hydrometer at the end of 40 seconds.
6. Repeat steps 4 and 5 to obtain hydrometer readings within 0.5-g differences of each other. The hydrometer is calibrated to read grams of soil material in suspension.
7. Record the hydrometer readings on the data sheet (Table 3).
8. Measure the temperature of the suspension. For each degree above 20 °C, add 0.36 to the hydrometer reading; for each degree below 20 °C, subtract 0.36 from the hydrometer reading. This is the corrected hydrometer reading.
9. Re-shake the suspension and place the cylinder on a table where it will not be disturbed. Take a hydrometer reading exactly 2 hours later. Correct for temperature as described above.
10. From the percentage of sand, silt and clay calculated on the data sheet, use the textural triangle diagram (Figure 1) to determine the texture class of the soil.

Soil structure

Soil structure is defined as the arrangement of the soil particles. With regard to structure, soil particles refer not only to sand, silt and clay but also to the aggregate or structural elements that have been formed by the aggregation of smaller mechanical fractions. Therefore, the word “particle” refers to any unit that is part of the make-up of the soil, whether a primary unit (sand, silt or clay fraction) or a secondary (aggregate) particle.

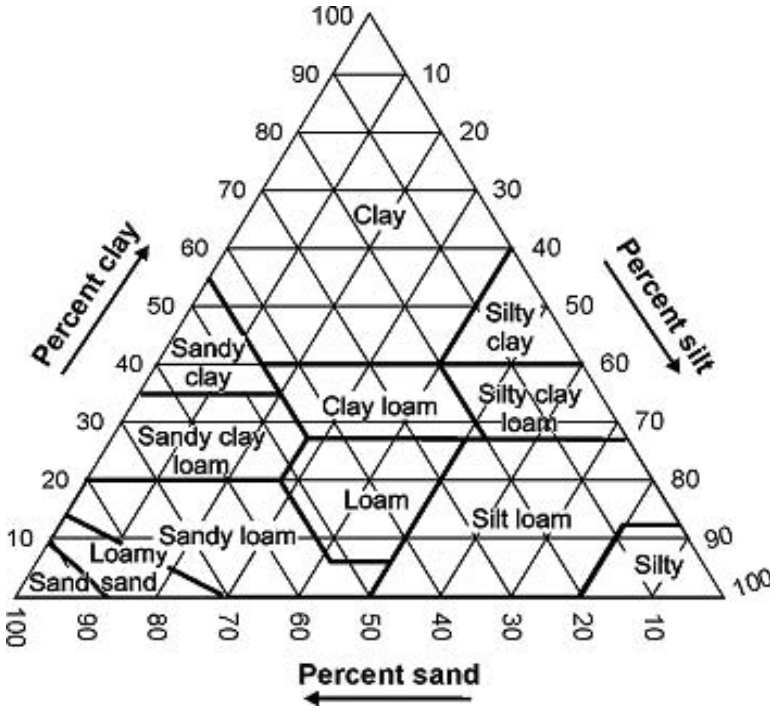
The size, shape and character of the soil structure varies (e.g. cube-like, prism-like or platter-like). On the basis of size, the soil structure is classified as:

- very coarse: > 10 mm;
- coarse: 5–10 mm;
- medium: 2–5 mm;

TABLE 3
Data sheet for recording hydrometer readings

1	Soil sample identification number
2	Soil weight (g)
3	40-second hydrometer reading (g)
4	Temperature of suspension (°C)
5	Corrected 40-second hydrometer reading (g)
6	2-hour hydrometer reading (g)
7	Temperature of suspension (°C)
8	Corrected 2-hour hydrometer reading (g)
9	Grams of sand (the sand settles to the bottom of the cylinder within 40 seconds, therefore, the 40-second corrected hydrometer reading actually gives the grams of silt and clay in suspension. The weight of sand in the sample is obtained by subtracting line 5 from line 2).
10	Grams of clay (the corrected hydrometer reading at the end of 2 hours represents grams of clay in the suspension as all sand and silt has already settled by this time).
11	Percent sand (line 9 ÷ line 2) × 100
12	Percent clay (line 10 ÷ line 2) × 100
13	Percent silt (find the silt by difference. Subtract the sum of the percent sand and clay from 100).
14	Soil class (as per Figure 1)

FIGURE 1
Soil texture classes according to proportions of sand, silt and clay



- fine: 1–2 mm;
- very fine: < 1 mm.

Depending on the stability of the aggregate and the ease of separation, the structure is characterized as:

- poorly developed;
- weakly developed;
- moderately developed;
- well developed;
- highly developed.

The soil structure or aggregate consists of an intermediate grouping of a number of primary particles into a secondary unit. The important factors that facilitate the aggregation of soil particles are:

- clay particles and types of clay minerals;
- cations such as Ca;
- OM;
- colloidal matter such as oxides of Fe and Al;
- plant roots;
- soil microbes and their types (fungi being most effective).

Soil structure influences the extent of pore space in the soil, water holding capacity (WHC), aeration, root movement and nutrient availability. The better and more stable soil aggregates are considered a desirable soil property with regard to plant growth. Therefore, the determination of soil structure is an important exercise in a soil fertility evaluation programme. An aggregate analysis aims to measure the percentage of water-stable secondary particles in the soil and the extent to which the finer mechanical separates are aggregated into coarser fractions.

The determination of aggregate or clod-size distribution involves procedures that depend on the disintegration of soil into clods and aggregates. The resulting aggregate-size distribution depends on the manner and condition in which the disintegration is brought about. For the measurements to have practical significance, the disruptive forces causing disintegration should compare closely with the forces expected in the field. The field condition, particularly with respect to soil moisture, should be compared with the moisture condition adopted for soil disintegration in the laboratory. The sampling of soil and the subsequent disintegration of clods in regard to seed bed preparation for upland crops should be carried out under air-dry conditions for dry-sieve analysis. A rotary sieve shaker is ideal for dry sieving. Similarly, the processes of wetting, disruption of dry aggregates, and screening of aggregates should be compared with the disruptive actions of water and mechanical forces of tillage under wetland conditions. Vacuum wetting of dry soil largely simulates the process of wetting *in situ*, particularly in the subsurface layers. However, the surface soil clods experience large-scale disruption when they are immersed in water at atmospheric pressure. The reproducibility of the size distribution of clods should be the criterion for

deciding the method of wetting by either vacuum wetting or immersion in water. Immersion wetting is closer to wetting of surface soil by irrigation.

After wetting, aggregates of different sizes can be obtained through several methods, e.g. sedimentation, elutriation and sieving. However, sieving under water compares more closely with the disruptive actions of water and other mechanical forces as experienced during wetland rice field preparation.

Dry aggregate analysis

The size distribution of dry clods is measured by dry sieving analysis performed on an air-dried bulk soil sample, either manually or with the help of a rotary sieve shaker (Gupta and Ghil Dyal, 1998).

The apparatus required consists of:

- a nest of sieves, 20 cm in diameter and 5 cm in height, with screens having 25.0, 10.0, 5.0, 2.0, 1.0, 0.5 and 0.25-mm-diameter round openings, with a pan and a lid;
- a rotary sieve shaker;
- some aluminium cans;
- a balance;
- a spade;
- a brush;
- some polyethylene bags;
- some labels.

Under this procedure, collect a bulk soil sample from the tilled field with the help of a 20-cm-diameter and 10-cm-height ring. Place the ring on the tilled soil and press until level with the surface. Remove the loose soil within the ring and collect it in a polyethylene bag.

Put one label indicating the depth and soil profile inside the bag, and tie another label with the bag. Take the soil sample to the laboratory and air-dry it.

Spread the soil on a sheet of paper and prepare the subsamples by "quartering". Cone the mixed soil material in the centre of the mixing sheet with care to make it symmetrical with respect to fine and coarse soil material. Flatten the cone and divide it through the centre with a flat metal spatula or metal sheet, moving one-half to one side. Further divide each half into halves; separating the four portions into separate piles or "quarters". Weigh the subsamples from two of these quarters and use them for clod-size and aggregate distribution analysis as duplicates. Transfer the weighed soil sample to the top sieve of the nest of sieves with the 5.0, 2.0, 1.0, 0.5 and 0.25-mm-diameter round openings and a pan at the bottom. Cover the top sieve with the lid, and place the nest of sieves on a rotary shaker. Switch on the shaker for 10 minutes, and then remove the sieves. Collect the soil retained on each screen in the pre-weighed aluminium cans (with the help of a small brush), and weigh the cans with the soil.

If the percentage of dry aggregates on the 5-mm sieve exceeds 25 percent, transfer these aggregates to a nest of sieves with 25.0, 10.0 and 5.0-mm sieves along with a pan. Cover the top sieve containing the aggregates with a lid, and place

the nest of sieves on the rotary sieve shaker. Switch on the motor for 10 minutes, and proceed as above for the estimation of aggregate-size distribution. Analyse the duplicate sample following the same procedure, and calculate the percentage distribution of dry aggregates retained on each sieve.

Dry the duplicate 100-g sample in an oven for 24 hours at 105 °C to calculate the oven-dry weight of the soil sample. The steps in the calculation are:

- Weight of aggregates in each sieve group = (Wt. of aggregates + Can) – Wt. of can
- Percent distribution of aggregates in each size group:

$$\frac{\text{Weight of Aggregates in each size group}}{\text{Total weight of soil}} \times 100$$

- Oven-dry weight:

$$\text{Oven-dry wt. of Aggregate (\%)} = \frac{\text{Air-dry wt.(\%)} \times 100}{100 + \text{Moisture \%}}$$

Wet aggregate analysis

For wet aggregate analysis (Gupta and Ghil Dyal, 1998), the apparatus required consists of:

- a mechanical oscillator powered by a gear-reduction motor with an amplitude of oscillation of 3.8 cm and a frequency of 30–35 cycles per minute;
- two sets of sieves, each 20 cm in diameter and 5 cm high, with screen openings of 5.0, 2.0, 1.0, 0.5, 0.25 and 0.1 mm in diameter,
- two Büchner funnels, 15 cm in diameter, with rubber stoppers;
- two vacuum flasks of 1-litre capacity;
- a suction pump or aspirator;
- a rubber policeman;
- twelve aluminium cans;
- some perforated cans;
- a sand bath;
- some filter papers.

The reagents required are:

- 5-percent sodium hexametaphosphate;
- 4-percent sodium hydroxide.

Among the different procedures adopted, wetting the samples under vacuum is suggested because the rate of wetting influences the slaking of crumbs. The time of sieving ranges from 10 minutes to 30 minutes depending on the type of wetting. Baver and Rhodes (1932) suggest 10 minutes pre-shaking of the soil sample in a reciprocating shaker or end-to-end shaker for fine-textured soil.

The technique used by Yoder (1936) and subsequently improved by the Soil Science Society of America's Committee on Physical Analysis is generally used for determining the size distribution of water-stable aggregates. Take the soil sample when it is moist and friable. By applying mild stress, break it into smaller

aggregates that can pass through an 8-mm screen. Put the sieved soil sample on a watch glass for wetting by either vacuum soaking or the immersion method. For vacuum wetting, place the sample in a vacuum desiccator containing de-aerated water at the bottom. Evacuate the desiccator until the pressure inside drops to about 3 mm and water starts boiling. Now, allow water to enter through the top of the desiccator and to flow into the watch glass holding the sample. Add enough water to cover the soil sample. Then, take the soil sample out of the desiccator.

Prepare four soil samples of 25 g each. Place a set of duplicate samples in an oven in order to determine the moisture content. Transfer another set of saturated duplicate soil samples to the top sieve of the nest of sieves (5.0, 2.0, 1.0, 0.5, 0.25 and 0.1 mm), and spread with the help of a glass rod and a slow jet of water. Remove the bottom pan, and attach the nest of sieves to the Yoder-type, wet-sieve shaker. Fill the drum (which holds the set of sieves) with salt-free water at 20–25 °C to a level somewhat below that of the screen in the top sieve of the nest of sieves, when the sieves are in the highest position. Then, lower the nest of sieves to wet the soil for 10 minutes. Bring the nest of sieves to the initial position, and adjust the level of water so that the screen in the top sieve is covered with water in its highest position. Now, switch on the mechanical oscillator to move the nest of sieves up and down with a frequency of 30–35 cycles per minute and a stroke of 3.8 cm. Sieve for 10 minutes. Remove the nest of screens from the water and allow it to drain for some time. Transfer the soil resting on each screen with a stream of distilled water, and brush it into a Büchner funnel having a pre-weighed filter paper and connected to a suction pump. Transfer the soil along with the filter paper into an aluminium can and dry at 105 °C for 24 hours. Weigh the soil to the nearest 0.01 g.

Transfer the oven-dry soil aggregates from all the cans of a set into the dispersion cup. Add dispersing agent (10 ml of 5 percent solution of sodium hexametaphosphate for normal and Ca-saturated soils, or 10 ml of 4 percent solution of sodium hydroxide for acid soil) and enough distilled water to fill the cup to within 4 cm of the rim, and then stir the suspension for 10 minutes. Wash the suspension on an identical set of sieves as used previously by means of a stream of tap-water and a brush, and transfer it to aluminium cans. Weigh and oven-dry the sand in each can in the same manner as above. Calculate the percentage distribution of soil particles (aggregates and the sand) and the sand particles retained on each sieve as follows:

Size distribution of soil particles (aggregate + sand):

- Soil particles in each size group (%) =

$$\frac{W_{od}\ (ag\ +\ s)\ i \times 100}{W_{od}}$$

- Sand particle in each size group (%) =

$$\frac{W_{od}\,(s)\ i \times 100}{W_{od}}$$

where W_{od} is the oven dried weight of the aggregates (*ag*) and sand (*s*), and *i* is the size group.

Cation exchange capacity

The total number of exchangeable cations a soil can hold is called its cation exchange capacity (CEC). The higher the CEC, the more cations it can retain. It can be expressed in terms of milli-equivalents per 100 g of soil (me/100 g) or in centimoles of positive charge per kilogram of soil (cmol/kg), which is numerically equal to me/100 g. The CEC of the soil depends on the kind of clay and OM present.

The apparatus required in order to determine the CEC consists of:

- a centrifuge;
- some 50-ml round-bottom centrifuge tubes;
- a mechanical shaker;
- a flame photometer and accessories that include propane, lithium and sodium standards.

The reagents required are:

- Sodium acetate (NaOAc) 1.0M: Dissolve 136.08 g of sodium acetate trihydrate in distilled water and bring the volume to 1 litre. Adjust the pH to about 8.2.
- Ethanol 95 percent.
- Ammonium acetate (NH_4OAc) 1.0M: Dissolve 77.09 g of ammonium acetate in distilled water and dilute to about 900 ml. Adjust the pH to 7.0 with dilute ammonium hydroxide or acetic acid as required, and make the volume up to 1 litre.
- Standard solution of NaCl: Dissolve 5.845 g of AR-grade NaCl in 1.0M ammonium acetate and make the volume up to 1 litre. It will give 100 me/litre of sodium in stock solution. From this solution take 0, 1, 2, 5, 7.5 and 10 ml and make the volume up to 100 ml each with the ammonium acetate. It will give 0, 1, 2, 5, 7.5 and 10 me/litre of sodium.

The procedure for determining the CEC is:

1. Weigh accurately 5 g of soil, and transfer the sample to a 50-ml centrifuge tube.
2. Add 25 ml of 1.0M sodium acetate solution to the tube, insert the stopper and shake in a mechanical shaker for 5 minutes.
3. Centrifuge at 2 000 rpm for 5 minutes or until the supernatant liquid is clear.
4. Decant the liquid completely and repeat the extraction three more times. Discard the decants.
5. Repeat steps 2–4 with ethanol or isopropyl alcohol until the electrical conductivity (EC) of the decant reads less than 40 mS/cm (it usually takes 4–5 washings).
6. To displace the adsorbed Na, repeat steps 2–4 using the ammonium acetate solution. Collect the decant in a 100-ml volumetric flask fitted with a funnel and filter paper. Make up to volume with ammonium acetate solution.

7. To determine the sodium concentration by flame photometry, prepare a series of Na standard solutions in the range of 0–10 me/litre of Na. Prepare a standard curve by plotting Na concentration on the x-axis and the flamephotometric readings on the y-axis. Feed an unknown sample extract onto the flamephotometer and take the reading, corresponding to which the concentration of Na is read from the standard curve. For better results, add lithium chloride (LiCl) in each standard to yield a final concentration of about 5 me/litre of LiCl.

The ammonium acetate extractable Na that is exchangeable Na in me/100 g soil =

$$\frac{\text{Na conc. of extract in meq/litre (Y)} \times 100}{\text{Wt. of soil in g (5)}} \times \frac{\text{Vol. of extract in ml (100)}}{1\,000} = \frac{Y \times 10}{5} = 2Y$$

This displaced Na is actually a measure of the CEC of the soil. Therefore, the me Na/100 g soil is actually me exchangeable cations (Ca, Mg, Na and K)/100 g soil.

Soil moisture

The gravimetric method of moisture estimation is most widely used where the soil sample is placed in an oven at 105 °C and dried to a constant weight. The difference in weight is considered to be the water present in the soil sample.

The apparatus required in order to determine the soil moisture consists of:

- an aluminium moisture box;
- an oven;
- a desiccator.

The procedure for determining the soil moisture is:

1. Put 100 g of soil sample in the aluminium moisture box and place in the oven after removing the lid of the box.
2. Keep the sample at 105 °C until it attains a constant weight. This may take 24–36 hours.
3. Cool the sample, first in the switched-off oven and then in a desiccator.
4. Weigh the cooled sample. The loss in weight is equal to the moisture contained in 100-g soil sample.

The percentage of moisture is calculated as:

$$\text{Moisture percent} = \frac{\text{Loss in wt.}}{\text{Oven - dry wt. of soil}} \times 100$$

The corresponding moisture correction factor (mcf) for analytical results or the multiplication factor for the amount of sample to be weighed for analysis is:

$$\text{Moisture correction factor} = \frac{100 + \%\ \text{moisture}}{100}$$

Water holding capacity

Veihmeyer and Hendrickson (1931) defined the field capacity or the water holding capacity (WHC) as the amount of water held in the soil after the excess gravitational water has drained away and after the rate of downward movement of water has materially ceased. The stage of field capacity is attained in the field after 48–72 hours of saturation. It is the upper limit of plant-available soil moisture.

The apparatus required in order to determine WHC consists of:

- some polyethylene sheets;
- a spade;
- a soil auger;
- some moisture boxes/cans;
- a balance;
- an oven.

The procedure for determining WHC is:

1. Select a uniform plot measuring 5 m × 5 m.
2. Remove weeds, pebbles, etc., and make bunds around the plot.
3. Fill the plot with sufficient water to saturate the soil completely.
4. Cover the plot area with a polyethylene sheet in order to check evaporation.
5. Take a soil sample from the centre of the plot from the desired layer, starting after 24 hours of saturation, and determine the moisture content daily until the values of successive days are nearly equal.
6. Record the weight as below:
   - weight of empty moisture box = X;
   - weight of moisture box + moist soil = Y;
   - weight of moisture box + oven-dry soil = Z;
   - repeat the above on the next day and so on until a constant Z value is reached.

The calculations are:

- Moisture content in soil = $Y - Z$;
- Weight of oven-dry soil = $Z - X$.

Thus, the percentage of moisture in the soil is given by:

$$\text{Percentage of moisture in soil (1st day)} = \left(\frac{Y - Z}{Z - X}\right) \times 100 = a$$

The percentage of moisture on succeeding days = a_1, a_2, etc.

Plot the daily readings on a graph paper. The lowest reading is taken as a value of field capacity of the soil.

Soil pH

The soil pH is the negative logarithm of the active hydrogen ion (H^+) concentration in the soil solution. It is the measure of soil sodicity, acidity or neutrality. It is a simple but very important estimation for soils as soil pH has a considerable

influence on the availability of nutrients to crops. It also affects microbial population in soils. Most nutrient elements are available in the pH range of 5.5–6.5.

In various chemical estimations, pH regulation is critical. Annex 12 details the specific colours as observed in the presence of various pH indicators and the colour changes due to pH change. The apparatus required in order to measure soil pH consists of:

- a pH meter with a range of 0–14 pH;
- a pipette/dispenser;
- some beakers;
- a glass rod.

The reagents required are:

- Buffer solutions of pH 4, 7 and 9.
- Calcium chloride solution (0.01M): dissolve 14.7 g of $CaCl_2.2H_2O$ in 10 litres of water to obtain 0.01M solution.

The procedure for measuring soil pH is:

1. Calibrate the pH meter, using two buffer solutions, one should be the buffer with neutral pH (7.0) and the other should be chosen based on the range of pH in the soil. Put the buffer solutions in the beakers. Insert the electrode alternately in the beakers containing the two buffer solutions, and adjust the pH. The instrument indicating pH as per the buffers is ready to test the samples.
2. Place 10.0 g of soil sample into a 50-ml or 100-ml beaker, add 20 ml of $CaCl_2$ solution (use water instead of $CaCl_2$ solution throughout the procedure where water is used as a suspension medium).
3. Allow the soil to absorb the $CaCl_2$ solution without stirring, then stir thoroughly for 10 seconds using a glass rod.
4. Stir the suspension for 30 minutes, and record the pH on the calibrated pH meter.

Based on soil pH values, soil reactions are distinguished as per Table 4.

Acid soils need to be limed before they can be put to normal agricultural production. Alkali soils need to be treated with gypsum in order to remove the excessive content of Na.

TABLE 4
Soil reaction ratings

pH range	Soil reaction rating
< 4.6	Extremely acidic
4.6–5.5	Strongly acidic
5.6–6.5	Moderately acidic
6.6–6.9	Slightly acidic
7.0	Neutral
7.1–8.5	Moderately alkaline
> 8.5	Strongly alkaline

Lime requirement

Crop yields are normally high in soils with pH values between 6.0 and 7.5. Lime is added to raise the pH of acid soils, and the amount of lime required to raise the pH to an optimal level is called lime requirement. Various methods are available for determining the lime requirement. The methods discussed here are based on the use of a buffer solution, whose pH undergoes change when treated with acid soils. The pH of the buffer solution decreases gradually as H^+ ion concentration increases. When H^+ increases by 1 me in 100 ml of buffer solution, the pH value decrease by 0.1 unit.

It is necessary to prepare fresh buffer solutions. A 0.05M solution of AR-grade potassium hydrogen phthalate (molecular weight 204.22) gives a pH of 4.0 at 25 °C, and it can be used as a buffer.

Woodruff method

For the method developed by Woodruff (1948), the apparatus required consists of:

- a pH meter;
- some automatic pipettes.

To prepare the reagent (Woodruff's buffer solution), dissolve 10 g of calcium acetate [$Ca(CH_3COOH)_2$], 12 g of paranitrophenol, 10 g of salicylic acid and 1.2 g of sodium hydroxide in distilled water. Adjust the pH to 7.0 with acetic acid or sodium hydroxide, transfer to a 1-litre volumetric flask, and make the volume up to the mark with distilled water.

The procedure for determining lime requirement is:

1. Place 10 g of soil sample in a clean 50-ml beaker.
2. Add 10 ml of distilled water, stir, and wait for 30 minutes.
3. Determine the pH value in soil suspension.
4. If the pH value is less than 5.0 (average of 4.5 and 5.5 to have one value), add 10 ml of Woodruff's buffer solution, stir, and wait for 30 minutes before determining the new pH value.

Table 5 shows the amount of lime required to raise the pH for agricultural purposes.

TABLE 5
Lime required to reduce soil acidity

pH (after buffer)	$CaCO_3$	$Ca(OH)_2$	Marl	Limestone	Dolomite
			(tonnes/ha)		
6.5	6.00	4.68	7.20	9.00	6.54
6.4	7.20	5.62	8.64	10.80	7.85
6.3	8.40	6.55	10.08	12.60	9.16
6.2	9.60	7.49	11.52	14.40	10.46
6.1	10.80	8.42	12.96	16.20	11.77
6.0	12.00	9.36	14.40	18.00	13.08
5.9	13.20	10.30	15.84	19.80	14.39
5.8	14.40	11.23	17.28	21.60	15.70
5.7	15.60	12.17	18.72	23.40	17.00
5.6	16.80	13.10	20.16	25.20	18.31

For practical purposes, the soils with a pH value of 6.6–7.5 are considered nearly neutral. Such soils do not need treating with lime or gypsum. With soils that are acidic and alkaline beyond these limits, the growing of acid-loving and salt-tolerant crops may be considered. As soil amendment is expensive process, only highly acidic soils and the soils with high alkalinity need to be treated with chemical amendments.

Shoemaker method

For the method developed by Shoemaker, McLean and Pratt (1961), the apparatus required consists of:

- a pH meter;
- some automatic pipettes (10 and 20 ml).

To prepare the reagent (extractant buffer), dissolve 1.8 g of nitrophenol, 2.5 ml of triethanolamine, 3.0 g of potassium chromate (K_2CrO_4), 2.0 g of calcium acetate and 53.1 g of calcium chloride in 1 litre of water. Adjust the pH to 7.5 with NaOH.

The procedure for determining lime requirement is:

1. Place 5.0 g of soil sample in a 50-ml beaker.
2. Add 5 ml of distilled water and 10 ml of extractant buffer.
3. Shake continuously for 10 minutes (or intermittently for 20 minutes), and read the pH of the soil buffer suspension with a glass electrode. The pH of the buffer solution is reduced, depending on the extent of soil acidity.

For various levels of measured pH of soil buffer suspension, Table 6 shows the amount of lime required in terms of $CaCO_3$ in order to raise the soil pH to 6.0, 6.4 and 6.8. Lime requirement varies with the type of soils and its CEC.

For practical purposes, the pH of acid soils should not be raised beyond 6.4–6.5.

TABLE 6
Lime requirement for different pH targets

Measured pH of soil buffer suspension	Lime requirement as $CaCO_3$ for bringing soil pH to different levels		
	6.0	6.4	6.8
		(tonnes/ha)	
6.7	2.43	2.92	3.40
6.6	3.40	4.13	4.62
6.5	4.37	5.35	6.07
6.4	5.59	6.56	7.53
6.3	6.65	7.78	8.99
6.2	7.52	8.93	10.21
6.1	8.50	10.21	11.66
6.0	9.48	11.42	13.12

Soil electrical conductivity and gypsum requirement

Soils with a pH value higher than 8.0–8.5 may have the following special features:

- presence of excessive amounts of soluble salts;
- presence of excessive amounts of Na on the exchange complex.

Table 7 summarizes the chemical properties of salt-affected soils.

Such soils are generally not considered suitable for growing most crops unless treated with suitable amendment materials. However, there are salt-tolerant crops that can grow on these soils.

To determine the quality of these soils, the following estimations are required:

- pH (as described above);
- salt content or EC;
- exchangeable Na or gypsum requirement.

Electrical conductivity

Electrical conductivity is a measure of the ionic transport in a solution between the anode and cathode. This means, EC is normally considered to be a measurement of the dissolved salts in a solution. Similar to a metallic conductor, they obey Ohm's law.

As EC depends on the number of ions in the solution, it is important to know the soil/water ratio used. The EC of a soil is conventionally based on the measurement of the EC in the soil solution extract from a saturated soil paste, as it has been found that the ratio of the soil solution in saturated soil paste is about 2–3 times higher than that at field capacity.

As determining the EC of soil solution from a saturated soil paste is cumbersome and requires 400–500 g of soil sample for the determination, a less complex method is normally used. Generally, a 1:2 soil/water suspension is used.

The apparatus required in order to determine EC consists of:

- an EC meter;
- some beakers (25 ml), Erlenmeyer flasks (250 ml) and pipettes;
- filter paper.

To prepare the reagent (0.01M potassium chloride solution), dry a small quantity of AR-grade potassium chloride at 60 °C for 2 hours. Weigh 0.7456 g of it, dissolve it in freshly prepared distilled water, and make the volume up to 1 litre.

TABLE 7
Chemical characteristics of saline, non-saline sodic and saline sodic soils

Soil	EC	Exchangeable sodium percentage	pH
	(dS/m)		
Saline	> 4.0	< 15	< 8.5
Sodic (non-saline)	< 4.0	> 15	> 8.5
Saline sodic	> 4.0	> 15	< 8.5

Source: Richards (1954).

This solution gives an EC of 1 411.8 × 10^{-3}, i.e. 1.412 mS/cm at 25 °C. For best results, select a conductivity standard (KCl solution) close to the sample value.

The procedure for determining EC is:

1. Place 40 g of soil in a 250-ml Erlenmeyer flask, add 80 ml of distilled water, stopper the flask, and shake on a reciprocating shaker for 1 hour. Filter through No. 1 filter paper.
2. Wash the conductivity electrode with distilled water, and rinse with standard KCl solution.
3. Pour some KCl solution into a 25-ml beaker, and dip the electrode in the solution. Adjust the conductivity meter to read 1.412 mS/cm, corrected to 25 °C.
4. Wash the electrode, and dip it into the soil extract.
5. Record the digital display corrected to 25 °C. The EC reading is a measure of the soluble salt content in the extract, and an indication of salinity status of the soil sample (Table 8).

Gypsum requirement

In the estimation of gypsum requirement of saline-sodic/sodic soils (Schoonover, 1952), the attempt is to measure the quantity of gypsum (calcium sulphate) required to replace the Na from the exchange complex. The Na so replaced with the Ca of the gypsum is removed through leaching of the soil. The soils treated with gypsum become dominated with Ca in the exchange complex.

When the Ca of the gypsum is exchanged with Na, there is a reduction in the Ca concentration in the solution. The quantity of Ca reduced is equivalent to the Ca exchanged with Na. It is equivalent to the gypsum requirement of the soil when "Ca" is expressed as $CaSO_4$.

To determine the gypsum requirement, the apparatus required consists of:

- a mechanical shaker;
- a burette (50 ml);
- some pipettes (100 ml and 5 ml).

The reagents required are:

- Saturated gypsum (calcium sulphate) solution: Add 5 g of chemically pure $CaSO_4.2H_2O$ to 1 litre of distilled water. Shake vigorously for 10 minutes using a mechanical shaker and filter through No. 1 filter paper.

TABLE 8
General interpretation of EC values

Soil	EC	Total salt content	Crop reaction
	(mS/cm)	*(%)*	
Salt free	0–2	< 0.15	Salinity effect negligible, except for more sensitive crops
Slightly saline	4–8	0.15–0.35	Yield of many crops restricted
Moderately saline	8–15	0.35–0.65	Only tolerant crops yield satisfactorily
Highly saline	> 15	> 0.65	Only very tolerant crops yield satisfactorily

- 0.01N $CaCl_2$ solution: Dissolve exactly 0.5 g of AR-grade $CaCO_3$ powder in about 10 ml of 1:3 diluted HCl. When completely dissolved, transfer to a 1-litre volumetric flask and dilute to the mark with distilled water. Do not use $CaCl_2$ salt as it is highly hygroscopic.
- 0.01N versenate solution: Dissolve 2.0 g of pure ethylenediamine tetraacetic acid (EDTA) – disodium salt and 0.05 g of magnesium chloride (AR-grade) in about 50 ml of water and dilute to 1 litre. Titrate a portion of this against 0.01N of $CaCl_2$ solution to standardize.
- Eriochrome Black T (EBT) indicator: Dissolve 0.5 g of EBT dye and 4.5 g of hydroxylamine hydrochloride in 100 ml of 95-percent ethanol. Store in a stoppered bottle or flask.
- Ammonium hydroxide-ammonium chloride buffer: Dissolve 67.5 g of pure ammonium chloride in 570 ml of concentrated ammonium hydroxide and dilute to 1 litre. Adjust the pH to 10 using dilute HCl or dilute NH_4OH.

The procedure for determining the gypsum requirement is:

1. Weigh 5 g of air-dry soil in a 250-ml conical flask.
2. Add 100 ml of the saturated gypsum solution. Firmly insert a rubber stopper, and shake for 5 minutes.
3. Filter the contents through No. 1 filter paper.
4. Transfer 5 ml of aliquot of the clear filtrate into a 100 or 150-ml porcelain dish.
5. Add 1 ml of the ammonium hydroxide-ammonium chloride buffer solution and 2–3 drops of EBT indicator.
6. Place 0.01N versenate solution in a 50-ml burette and titrate the contents in the dish until the wine red colour starts to change to sky blue. Volume of versenate used = B.
7. Run a blank using 5 ml of saturated gypsum solution in place of sample aliquot. Volume of versenate solution used = A.

The gypsum requirement (in tonnes per hectare) is given by: $(A - B) \times N \times 382$; where:

- A = millilitres of EDTA (versenate) used for blank titration;
- B = millilitres of ETDA used for soil extract;
- N = normality of EDTA solution.

Organic carbon / organic matter

There are various methods for estimating OM in soil. Loss of weight on ignition can be used as a direct measure of the OM contained in the soil. It can also be expressed as the content of organic C in the soil. It is generally assumed that, on average, OM contains about 58 percent organic C. Organic matter / organic C can also be estimated by volumetric and colorimetric methods. However, the use of potassium dichromate ($K_2Cr_2O_7$) involved in these estimations is considered a limitation because of its hazardous nature. Soil organic matter (SOM) content can be used as an index of N availability (potential of a soil to supply N to plants) because the N content in SOM is relatively constant.

Loss of weight on ignition

The apparatus required using this method consists of:

- a sieve;
- a beaker;
- an oven;
- a muffle furnace.

The procedure is:

1. Weigh 5.0–10.0 g (to the nearest 0.01 g) of sieved (2 mm) soil into an ashing vessel (50-ml beaker or other suitable vessel).
2. Place the ashing vessel with soil in a drying oven set at 105 °C and dry for 4 hours. Remove the ashing vessel from the drying oven and place in a dry atmosphere. When cooled, weigh to the nearest 0.01 g. Place the ashing vessel with soil into a muffle furnace, and bring the temperature to 400 °C. Ash in the furnace for 4 hours. Remove the ashing vessel from the muffle furnace, cool in a dry atmosphere, and weigh to the nearest 0.01 g.

The percentage of OM is given by:

$$\text{Percent organic matter (OM)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

where:

- W_1 is the weight of soil at 105 °C;
- W_2 is the weight of soil at 400 °C.

The percent of organic C is given by: % OM × 0.58.

Volumetric method

The apparatus required for the volumetric method (Walkley and Black, 1934) consists of:

- a conical flask (500 ml);
- some pipettes (2, 10 and 20 ml);
- a burette (50 ml).

The reagents required are:

- Phosphoric acid – 85 percent.
- Sodium fluoride solution – 2 percent.
- Sulphuric acid – 96 percent containing 1.25 percent of Ag_2SO_4.
- Standard 0.1667M $K_2Cr_2O_7$: Dissolve 49.04 g of $K_2Cr_2O_7$ in water and dilute to 1 litre.
- Standard 0.5M $FeSO_4$ solution: Dissolve 140 g of ferrous sulphate or 196.1 g of $FeSO_4.(NH_4)_2.6H_2O$ in 800 ml of water, add 20 ml of concentrated H_2SO_4 and make the volume up to 1 litre.
- Diphenylamine indicator: Dissolve 0.5 g of reagent-grade diphenylamine in 20 ml of water and 100 ml of concentrated H_2SO_4.

The procedure is:

1. Weigh 1.0 g of the prepared soil sample in a 500-ml conical flask.
2. Add 10 ml of 0.1667M $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4 containing Ag_2SO_4.

3. Mix thoroughly and allow the reaction to complete for 30 minutes.
4. Dilute the reaction mixture with 200 ml of water and 10 ml of H_3PO_4.
5. Add 10 ml of NaF solution and 2 ml of diphenylamine indicator.
6. Titrate the solution with standard 0.5M $FeSO_4$ solution to a brilliant green colour.
7. Run a blank without sample simultaneously.

The percentage of organic C is given by:

$$\frac{10\,(S - T) \times 0.003}{S} \times \frac{100}{\text{Wt. of soil}}$$

As 1 g of soil is used, this equation simplifies to:

$$\frac{3\,(S - T)}{S}$$

where:

- S = millilitres of $FeSO_4$ solution required for blank;
- T = millilitres of $FeSO_4$ solution required for soil sample;
- 0.003 = weight of C (1 000 ml 0.1667M $K_2Cr_2O_7$ = 3 g C. Thus, 1 ml 0.1667M $K_2Cr_2O_7$ = 0.003 g C).

Organic C recovery is estimated to be about 77 percent. Therefore, the actual amount of organic C (Y) will be:

$$\text{Percent value of organic carbon obtained} \times \frac{100}{77}$$

Or: percentage value of organic C × 1.3.

Percent OM = Y × 1.724 (organic matter contains 58 percent organic C, hence 100/58 = 1.724).

Published organic C to total OM conversion factors for surface soils vary from 1.724 to 2.0. A value of 1.724 is commonly used. However, where possible, the appropriate factor should be determined experimentally for each type of soil.

Colorimetric method

The apparatus required for the colorimetric method (Datta, Khera and Saini, 1962) consists of:

- a spectrophotometer;
- some conical flasks (100 ml);
- some pipettes (2, 5 and 10 ml).

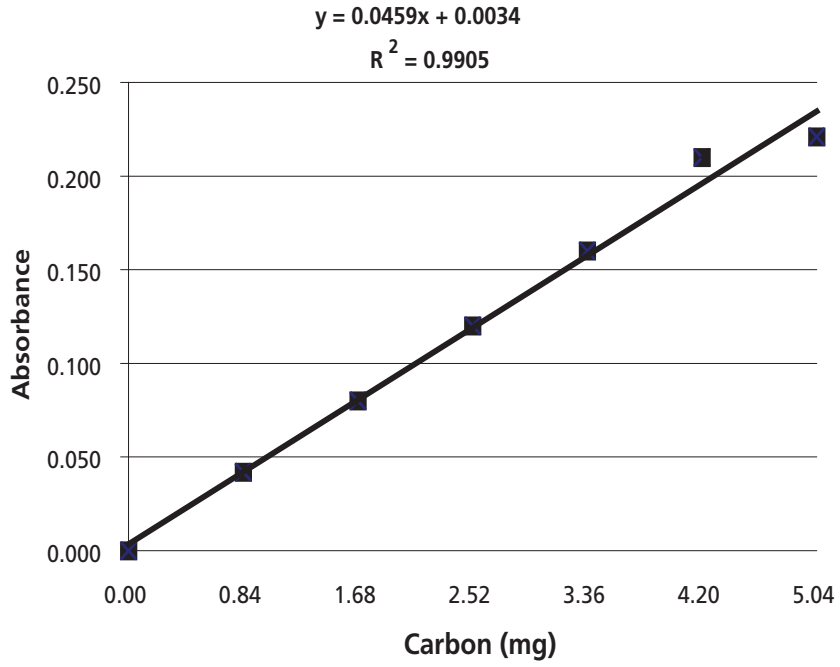
The reagents required are:

- Standard potassium dichromate 0.1667M.
- Concentrated sulphuric acid containing 1.25 percent of Ag_2SO_4.
- Sucrose (AR-grade).

The procedure is:

1. Preparation of standard curve: Sucrose is used as a primary standard C source. Place different quantities of sucrose (1–20 mg) in 100-ml flasks. Add 10 ml of standard $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 in each flask. Swirl the flasks, and leave for 30 minutes. Prepare a blank in the same way without adding sucrose. A green colour develops, which is read on spectrophotometer at 660 nm, after adjusting the blank to zero. Plot the reading so obtained against milligrams of sucrose as C source (C = weight of sucrose × 0.42 – because the C content of sucrose is 42 percent) or against milligrams of C directly. As an example, Figure 2 shows a standard curve as prepared for estimating organic C by the authors of this publication while setting up a soil testing laboratory. It shows the accuracy of the method (r^2 is as high as 0.991). For convenience, the curve is shown directly against C content, which has been derived from milligrams of sucrose used in preparing the standard curve.
2. Place 1 g of soil in a 100-ml conical flask.
3. Add 10 ml of 0.1667M $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 containing 1.25 percent of Ag_2SO_4.

FIGURE 2
Standard curve for organic carbon on spectrophotometer



4. Stir the reaction mixture and allow it to stand for 30 minutes.
5. The green colour of chromium sulphate so developed is read on a spectrophotometer at 660 nm after setting the blank, prepared in the similar manner, at zero.

The C content of the sample is found from the standard curve, which shows the C content (milligrams of C vs spectrophotometer readings as absorbance):

- Percent C = milligrams of C observed × 100 / 1 000 (observed reading is for 1 g soil, expressed as milligrams).
- Percent OM = %C × 1.724.

Total nitrogen

Total N includes all forms of inorganic N, such as NH_4, NO_3 and NH_2 (urea), and the organic N compounds such as proteins, amino acids and other derivatives. Depending on the form of N present in a particular sample, a specific method is to be adopted for determining the total N value. While organic N materials can be converted into simple inorganic ammoniacal salt by digestion with sulphuric acid, for reducing nitrates into ammoniacal form, the modified Kjeldahl method is adopted with the use of salicylic acid or Devarda's alloy. At the end of digestion, all organic and inorganic salts are converted into ammonium form, which is distilled and estimated by using standard acid.

As the precision of the method depends on complete conversion of organic N into NH_4-N, the digestion temperature and time, the solid–acid ratio and the type of catalyst used have an important bearing on the method. The ideal temperature for digestion is 320–370 °C. At a lower temperature, the digestion may not be complete, while above 410 °C, loss of NH_3 may occur. The salt–acid (weight–volume) ratio should not be less than 1:1 at the end of digestion. Commonly used catalysts to accelerate the digestion process are $CuSO_4$ and mercury (Hg). Potassium sulphate is added to raise the boiling point of the acid so that loss of acid by volatilization is prevented.

The apparatus required for this method consists of:

- a Kjeldahl digestion and distillation unit;
- some conical flasks;
- some burettes;
- some pipettes.

The reagents required are:

- Sulphuric acid (93–98 percent).
- Copper sulphate ($CuSO_4.H_2O$) (AR-grade).
- Potassium sulphate or anhydrous sodium sulphate (AR-grade).
- 35-percent sodium hydroxide solution: Dissolve 350 g of solid NaOH in water and dilute to 1 litre.
- 0.1M NaOH: Prepare 0.1M NaOH by dissolving 4.0 g of NaOH in water and make the volume up to 1 litre. Standardize against 0.1N potassium hydrogen phthalate or standard H_2SO_4.

- 0.1M HCl or 0.05M H_2SO_4: Prepare approximately the standard acid solution and standardize against 0.1M sodium carbonate.
- Methyl red indicator.
- Salicylic acid for reducing NO_3 to NH_4, if present in the sample.
- Devarda's alloy for reducing NO_3 to NH_4, if present in the sample.

The procedure is

1. Weigh 1 g of soil sample. Place in a Kjeldahl flask.
2. Add 0.7 g of copper sulphate, 1.5 g of K_2SO_4 and 30 ml of H_2SO_4.
3. Heat gently until frothing ceases. If necessary, add a small amount of paraffin or glass beads to reduce frothing.
4. Boil briskly until the solution is clear and then continue digestion for at least 30 minutes.
5. Remove the flask from the heater and cool, add 50 ml of water, and transfer to a distilling flask.
6. Place accurately 20–25 ml of standard acid (0.1M HCl or 0.05M H_2SO_4) in the receiving conical flask so that there will be an excess of at least 5 ml of the acid. Add 2–3 drops of methyl red indicator. Add enough water to cover the end of the condenser outlet tubes.
7. Run tap-water through the condenser.
8. Add 30 ml of 35-percent NaOH in the distilling flask in such a way that the contents do not mix.
9. Heat the contents to distil the ammonia for about 30–40 minutes.
10. Remove the receiving flask and rinse the outlet tube into the receiving flask with a small amount of distilled water.
11. Titrate excess acid in the distillate with 0.1M NaOH.
12. Determine blank on reagents using the same quantity of standard acid in a receiving conical flask.

The calculation is:

$$\text{Percent N} = \frac{1.401\,[\,(V_1 M_1 - V_2 M_2) - (V_3 M_1 - V_4 M_2)\,]}{W} \times df$$

where:

- V_1 – millilitres of standard acid put in receiving flask for samples;
- V_2 – millilitres of standard NaOH used in titration;
- V_3 – millilitres of standard acid put in receiving flask for blank;
- V_4 – millilitres of standard NaOH used in titrating blank;
- M_1 – molarity of standard acid;
- M_2 – molarity of standard NaOH;
- W – weight of sample taken (1 g);
- df – dilution factor of sample (if 1 g was taken for estimation, the dilution factor will be 100).

Note: 1 000 ml of 0.1M HCl or 0.05M H_2SO_4 corresponds to 1.401 g of N.

The following precautions should be observed:

- The material should not solidify after digestion.

- No NH_4 should be lost during distillation.
- If the indicator changes colour during distillation, determination must be repeated using either a smaller sample weight or a larger volume of standard acid.

Mineralizable nitrogen

In the case of soils, mineralizable N (also organic C) is estimated as an index of available N content and not the total N content (Subbiah and Asija, 1956). The easily mineralizable N is estimated using alkaline $KMnO_4$, which oxidizes and hydrolyses the OM present in the soil. The liberated ammonia is condensed and absorbed in boric acid, which is titrated against standard acid. Because of its rapidity and reproducibility, the method has been widely adopted. However, as the process of oxidative hydrolysis is a progressive one, a uniform time and heating temperature should be respected for best results. The use of glass beads checks bumping, while liquid paraffin checks frothing during heating (as recommended in total N estimation by the Kjeldahl method).

The apparatus required in order to estimate mineralizable N consists of:

- a nitrogen distillation unit, preferably with six regulating heating elements;
- some conical flasks, pipettes, burettes, etc.

The reagents required are:

- 0.32 percent $KMnO_4$: Dissolve 3.2 g of $KMnO_4$ in water and make the volume up to 1 litre.
- 2.5 percent NaOH: Dissolve 25 g of sodium hydroxide pellets in water and make the volume up to 1 litre.
- 2 percent boric acid: Dissolve 20 g of boric acid powder in warm water by stirring and dilute to 1 litre.
- Mixed indicator: Dissolve 0.066 g of methyl red and 0.099 g of bromocresol green in 100 ml of ethyl alcohol. Add 20 ml of this mixed indicator to each litre of 2 percent boric acid solution.
- 0.1M potassium hydrogen phthalate: Dissolve 20.422 g of the salt in water and dilute to 1 litre. This is a primary standard and does not require standardization.
- 0.02M H_2SO_4: Prepare approximately 0.1M H_2SO_4 by adding 5.6 ml of concentrated H_2SO_4 to about 1 litre of distilled water. From this, prepare 0.02M H_2SO_4 by diluting a suitable volume (20 ml made to 100 ml) with distilled water. Standardize it against 0.1M NaOH solution.
- 0.1M NaOH. Dissolve 4 g of NaOH in 100 ml of distilled water. Standardize against potassium hydrogen phthalate.

The procedure is:

1. Weigh 20 g of soil sample in an 800-ml Kjeldahl flask.
2. Moisten the soil with about 10 ml of distilled water, wash down the soil, if any, adhering to the neck of the flask.
3. Add 100 ml of 0.32 percent $KMnO_4$ solution.
4. Add a few glass beads or broken pieces of glass rod.

5. Add 2–3 ml of paraffin liquid, avoiding contact with the upper part of the neck of the flask.
6. Measure 20 ml of 2 percent boric acid containing mixed indicator in a 250-ml conical flask and place it under the receiver tube. Dip the receiver tube in the boric acid.
7. Run tap-water through the condenser.
8. Add 100 ml of 2.5 percent NaOH solution, and immediately attach to the rubber stopper fitted in the alkali trap.
9. Switch the heaters on and continue distillation until about 100 ml of distillate is collected.
10. First, remove the conical flask containing distillate and then switch off the heaters to avoid back suction.
11. Titrate the distillate against 0.02M H_2SO_4 in a burette until a pink colour starts to appear.
12. Run a blank without soil.
13. Carefully remove the Kjeldahl flask after cooling and drain the contents in the sink.

The calculation for estimating mineralizable N is:

Volume of acid used to neutralize ammonia in the sample = $A - B$ ml

N content in the test sample = $(A - B) \times 0.56$ mg

Percent N = $(A - B) \times 0.56 \times 5$

where:

- A = volume of 0.02M H_2SO_4 used in titration against ammonia absorbed in boric acid;
- B = volume of 0.02M sulphuric acid used in blank titration.

1 000 ml of 1M H_2SO_4 = 28 g N; thus, 1 ml of 0.02M sulphuric acid = 0.56 mg N. The weight of the soil sample is 20 g, thus, the factor for converting into percent N = 100/20 = 5.

It is important to remember that:

- All the joints of the Kjeldahl apparatus should be checked in order to prevent any leakage and loss of ammonia.
- Hot Kjeldahl flasks should neither be washed immediately with cold water nor allowed to cool for long (to avoid deposits from settling at the bottom, which are difficult to remove).
- If frothing occurs and passes through to the boric acid, such samples should be discarded and fresh distillation done.
- The opening of ammonia bottles in the laboratory should be strictly prohibited while distillation is on. The titration should be carried out in an ammonia-free atmosphere.
- If the titration is not to be carried out immediately, the distillate should be stored in ammonia-free cupboards after tightly stoppering the flasks.

Inorganic N – NO_3^- and NH_4^+

Inorganic N in soil is present predominantly as NO_3^--N and NH_4^+-N. Nitrite is seldom present in detectable amounts, and its determination is normally unwarranted except in neutral to alkali soils following the application of NH_4 or NH_4-forming fertilizers.

Nitrate is highly soluble in water, and a number of extractants including water have been used. These include saturated 0.35 percent $CaSO_4$ $2H_2O$ solution, 0.03M NH_4F, 0.015M H_2SO_4, 0.01M $CaCl_2$, 0.5M $NaHCO_3$ (pH 8.5), 0.01M $CuSO_4$, 0.01M $CuSO_4$ containing Ag_2SO_4, and 2.0M KCl.

Exchangeable NH_4 is defined as NH_4 that can be extracted at room temperature with a neutral K-salt solution. Various molarities have been used, such as 0.05M K_2SO_4, 0.1M KCl, 1.0M KCl, and 2.0M KCl.

The potential of a soil to mineralize N as measured by N-availability indices (OM, organic C and even total N) is fairly constant from year to year, making it unnecessary to conduct this type of determination each year. However, it is still necessary to take into consideration the initial amount of available N (inorganic N: NO_3-N and/or NH_4-N) in the rootzone at or near planting time for better prediction of N fertilizer needs. This type of test must be conducted each year, especially where there is the possibility of residual inorganic N remaining from a previous application or fallow period.

The methods for the determination of NO_3-N and NH_4-N are more diverse than the methods of extraction (Keeney and Nelson, 1982). They range from specific ion electrode to colorimetric techniques, microdiffusion, steam distillation, and flow injection analysis. Steam distillation is still a preferred method when using ^{15}N. However, for routine analysis, this guide details the phenoldisulphonic acid method for NO_3 and the indophenol blue method for NH_4 estimation.

Nitrate by phenoldisulphonic acid method

A major difficulty in estimating NO_3 in soils by colorimetric methods is obtaining a clear colourless extract with low contents of organic and inorganic substances, which interfere with the colorimetric method. In arid and salt-affected soils, chloride is the major anion that interferes with colour development of the phenoldisulphonic acid method. Therefore, if the chloride concentration is more than 15 µg/g soil, it should be removed before analysis by the use of Ag_2SO_4 to precipitate chloride as AgCl. The Ag_2SO_4 is added to the extract or to the reagent used for extraction, and the AgCl is removed by filtration or centrifugation after precipitation of the excess Ag_2SO_4 by a basic reagent such as $Ca(OH)_2$ or $MgCO_3$. It is necessary to remove the excess Ag^+ before analysis of the extract because it also interferes with the phenoldisulphonic acid method of determining NO_3.

The apparatus required using the method consists of:

- a reciprocating shaker;
- a heavy-duty hotplate;
- a spectrophotometer;

- a dispenser;
- an Erlenmeyer flask;
- some beakers;
- a glass rod.

The reagents required are:

- Phenoldisulphonic acid (phenol 2,4-disulphonic acid): Transfer 70 ml of pure liquid phenol (carbolic acid) to an 800-ml Kjeldahl flask. Add 450 ml of concentrated H_2SO_4 while shaking. Add 225 ml of fuming H_2SO_4 (13–15 percent SO_3). Mix well. Place the Kjeldahl flask (loosely stoppered) in a beaker containing boiling water and heat for 2 hours. Store the resulting phenoldisulphonic acid [$C_6H_3OH(HSO_3)_2$] solution in a glass-stoppered bottle.
- Dilute ammonium hydroxide solution (about 7.5M NH_4OH): Mix one part NH_4OH (specific gravity 0.90) with one part H_2O.
- Copper sulphate solution (0.5M): Dissolve 125 g of $CuSO_4.5H_2O$ in 1 litre of distilled water.
- Silver sulphate solution (0.6 percent): Dissolve 6.0 g of Ag_2SO_4 in 1 litre of distilled water. Heat or shake well until all salt is dissolved.
- Nitrate-extracting solution: Mix 200 ml of 0.5M copper sulphate solution and 1 litre of 0.6 percent silver sulphate solution and dilute to 10 litres with water. Mix well.
- Standard nitrate solution (100 µg NO_3-N/ml, stock solution): Dissolve 0.7221 g of KNO_3 (oven dried at 105 °C) in water and dilute to 1 litre. Mix thoroughly.
- Standard nitrate solution (10 µg NO_3-N/ml, working solution): Dilute 100 ml of 100 µg NO_3-N/ml stock solution to 1 litre with water. Mix well.
- Calcium hydroxide: AR-grade powder (free of NO_3).
- Magnesium carbonate: AR-grade powder (free of NO_3).

The procedure is:

1. Place about 5 g of soil in an Erlenmeyer flask.
2. Add 25 ml of nitrate-extracting solution.
3. Shake contents for 10 minutes.
4. Add about 0.2 g of $Ca(OH)_2$ and shake for 5 minutes.
5. Add about 0.5 g of $MgCO_3$ and shake for 10–15 minutes.
6. Allow to settle for a few minutes.
7. Filter through No. 42 filter paper.
8. Pipette 10 ml of clear filtrate into a 100-ml beaker. Evaporate to dryness on a hotplate at low heat in a fumehood (free of HNO_3 fumes). Do not continue heating beyond dryness.
9. When completely dry, cool residue, add 2 ml of phenoldisulphonic acid rapidly (from a burette having the tip cut off), covering the residue quickly. Rotate the beaker so that the reagent comes into contact with all residual salt. Allow to stand for 10–15 minutes.

10. Add 16.5 ml of cold water. Rotate the beaker to dissolve residue (or stir with a glass rod until all residue is in solution).
11. Once the beaker is cool, add 15 ml of dilute NH_4OH slowly until the solution is distinctly alkaline as indicated by the development of a stable yellow colour.
12. Add 16.5 ml of water (volume becomes 50 ml). Mix thoroughly.
13. Read the concentration of NO_3-N at 415 nm, using the standard curve.
14. Preparation of standard curve: Place 0, 2, 5, 8, and 10 ml of the 10 µg NO_3/ml working solution in separate 100-ml beakers, add 10 ml NO_3-extracting solution and evaporate to dryness. Follow steps 9–13, using these standard solutions with 0, 0.4, 1.0, 1.6 and 2.0 µg NO_3-N/ml. Prepare a standard curve to be used for estimation of NO_3 in the sample.

The calculation is:

$$NO_3^- - N \text{ in test soln.}(\mu g/m) = \frac{\text{Vol. after colour develop. (ml)}}{\text{Vol. evaporated (ml)}} \times \frac{\text{Vol. of extracting soln. (ml)}}{\text{Wt. of oven} - \text{dried soil (g)}}$$

Ammonium by indophenol blue method

Phenol reacts with NH_4 in the presence of an oxidizing agent such as hypochlorite to form a coloured complex in alkaline condition. The addition of sodium nitroprusside as a catalyst in the reaction between phenol and NH_4 increases the sensitivity of the method considerably. The addition of EDTA is necessary in order to complex divalent and trivalent cations present in the extract. Otherwise, it forms precipitate at a pH of 11.4–12 used for colour development, and this turbidity would interfere with formation of the phenol–NH_4 complex.

The first step is the extraction of exchangeable ammonium.

The apparatus required consists of:

- an Erlenmeyer flask;
- a volumetric flask;
- a shaker;
- a spectrophotometer.

The reagents required are:

- Potassium chloride (KCl) solution, 2M: Dissolve 150 g of AR-grade KCl in 1 litre of distilled water.
- Standard ammonium (NH_4^+) solution: Dissolve 0.4717 g of ammonium sulphate $(NH_4)_2SO_4$ in water, and dilute to a volume of 1 litre. If pure dry $(NH_4)_2SO_4$ is used, the solution contains 100 µg of NH_4-N/ml. Store the solution in a refrigerator. Immediately before use, dilute 4 ml of this stock NH_4^+ solution to 200 ml. The resulting working solution contains 2 µg of NH_4-N/ml. Accordingly, for the standard curve, prepare various concentrations of standard solution.
- Phenol-nitroprusside reagent: Dissolve 7 g of phenol and 34 mg of sodium nitroprusside [disodium pentacyanonitrosylferrate, $Na_2Fe(CN)_5NO.2H_2O$]

in 80 ml of NH_4^+-free water and dilute to 100 ml. Mix well, and store in a dark-coloured bottle in the refrigerator.

- Buffered hypochlorite reagent: Dissolve 1.480 g of sodium hydroxide (NaOH) in 70 ml of NH_4^+-free water, add 4.98 g of sodium monohydrogen phosphate (Na_2HPO_4) and 20 ml of sodium hypochlorite (NaOCl) solution (5–5.25 percent NaOCl). Use less or more hypochlorite solution if the concentration is higher or lower, respectively, than that indicated above. Check the pH to ensure a value between 11.4 and 12.2. Add a small amount of additional NaOH if required to raise the pH. Dilute to a final volume of 100 ml.
- EDTA reagent: Dissolve 6 g of EDTA disodium salt in 80 ml of deionized water, adjust to pH 7, mix well, and dilute to a final volume of 100 ml.

The procedure for the extraction is:

1. Place 10 g of soil in a 250-ml wide-mouth Erlenmeyer flask and add 100 ml of 2M KCl.
2. Insert stopper, and shake the flask on a mechanical shaker for 1 hour.
3. Allow the soil–KCl suspension to settle (about 30 minutes) until the supernatant is clear.
4. If the KCl extract cannot be analysed within 24 hours, then filter the soil–KCl suspension (No. 42 filter paper) and store in the refrigerator.

Aliquots from this extract are used for the NH_4 estimation.

The procedure for the estimation is:

1. Pipette an aliquot (not more than 5 ml) of the filtered 2M KCl extract containing between 0.5 and 12 µg of NH_4-N into a 25-ml volumetric flask. Aliquots of ≤ 3 ml normally contain sufficient NH_4-N for quantification.
2. Add 1 ml of the EDTA reagent, and mix the contents of the flask.
3. Allow the contents to stand for 1 minute, then add 2 ml of the phenol-nitroprusside reagent, followed by 4 ml of the buffered hypochlorite reagent, and immediately dilute the flask to volume (25 ml) with NH_4^+-free water, and mix well.
4. Place the flask in a water-bath maintained at 40 °C for 30 minutes.
5. Remove the flask from the bath, cool to room temperature, and determine the absorbance of the coloured complex at a wavelength of 636 nm against a reagent blank solution.
6. Determine the NH_4-N concentration of the sample by reference to a calibration curve plotted from the results obtained with 25-ml standard samples containing 0, 2, 4, 6, 8, 10, and 12 µg of NH_4-N/ml.
7. To prepare this curve, add an appropriate amount of 2M KCl solution (same volume as that used for aliquots of soil extract, i.e. about 5 ml) to a series of 25-ml volumetric flasks. Add 0, 1, 2, 3, 4, 5 and 6 ml of the 2 µg NH_4-N/ml solution to the flasks, and measure the intensity of blue colour developed with these standards by the procedure described above for the analysis of unknown extracts.

The calculation is (NH_4-N in the sample as noted from the standard curve = *A* [µg/ml]):

$$\mu g \text{ of } NH_4\text{- N in 1 g soil} = \frac{A \times 100 (\text{total vol. of extract})}{5 (\text{vol. of extract estimated})} \times \frac{1}{10 (\text{wt. of soil})} = 2A$$

where:

- weight of the soil taken for estimation = 10 g;
- total volume of extract = 100 ml;
- volume of extract taken for estimation = 5 ml.

Available phosphorus

The two methods most commonly used for determining the available P in soils are: Bray's Method No. 1 for acid soils; and Olsen's method for neutral and alkali soils.

In these methods, specific coloured compounds are formed with the addition of appropriate reagents in the solution, the intensity of which is proportionate to the concentration of the element being estimated. The colour intensity is measured spectrophotometrically. In spectrophotometric analysis, light of definite wavelength (not exceeding, say, 0.1–1.0 nm in bandwidth) extending to the ultraviolet region of the spectrum constitutes the light source. The photoelectric cells in the spectrophotometer measure the light transmitted by the solution.

A spectrophotometer, as its name implies, is really two instruments in one cabinet – a spectrometer and a photometer. A spectrometer is a device for producing coloured light of any selected colour (or wavelength) and, when employed as part of a spectrophotometer, it is usually termed a monochromator and is generally calibrated in wavelengths. A photometer is a device for measuring the intensity of the light. When incorporated in a spectrophotometer, it is used to measure the intensity of the monochromatic beam produced by the associated monochromator. Generally, the photometric measurement is made first with a reference liquid and then with a coloured sample contained in similar cells interposed in the light beam. The ratio of the two intensity measurements is a measure of the opacity of the sample at the wavelength of the test.

Table 9 lists the approximate wavelength ranges of complementary colours. White light covers the entire visible spectrum (400–760 nm).

Bray's Method No. 1

The apparatus required for Bray's Method No. 1 (Bray and Kurtz, 1945) for acid soils consists of:

- a spectrophotometer;
- some pipettes (2, 5, 10 and 20 ml);
- some beakers/flasks (25, 50, 100 and 500 ml).

The reagents required are:

- Bray's Extractant No. 1 (0.03M NH_4F in 0.025M HCL): Dissolve 2.22 g of NH_4F in 200 ml of distilled water, filter, and add to the filtrate 1.8 litres of

TABLE 9
Wavelengths and corresponding colour ranges

Wavelength	Hue (transmitted)*	Complementary hue of the solution
(nm)		
< 400	Ultraviolet	
400–435	Violet	Yellow green
435–480	Blue	Yellow
480–490	Greenish blue	Orange
490–500	Bluish green	Red
500–560	Green	Purple
560–580	Yellowish green	Violet
580–595	Yellow	Blue
595–610	Orange	Greenish blue
610–750	Red	Bluish green
> 760	Infrared	

* Hue is one of the three main attributes of perceived colour.
Source: Vogel, 1961.

water containing 4 ml of concentrated HCl, make the volume up to 2 litres with distilled water.

- Molybdate reagent: Dissolve 1.50 g of $(NH_4)_2MoO_4$ in 300 ml of distilled water. Add the solution to 350 ml of 10M HCl solution gradually with stirring. Dilute to 1 litre with distilled water.
- Stannous chloride solution (stock solution): Dissolve 10 g of $SnCl_2\,2H_2O$ in 25 ml of concentrated HCl. Add a piece of pure metallic tin, and store the solution in a glass stoppered bottle.
- Stannous chloride solution (working solution): Dilute 1 ml of the stock solution of stannous chloride to 66.0 ml with distilled water just before use. Prepare fresh dilute solution every working day.

The procedure is:

1. Preparation of the standard curve: Dissolve 0.2195 g of pure dry KH_2PO_4 in 1 litre of distilled water. This solution contains 50 µg P/ml. Preserve this as a stock standard solution of phosphate. Take 10 ml of this solution and dilute it to 0.5 litres with distilled water. This solution contains 1 µg P/ml (0.001 mg P/ml). Put 0, 1, 2, 4, 6 and 10 ml of this solution in separate 25-ml flasks. Add to each flask, 5 ml of the extractant solution, 5 ml of the molybdate reagent; and dilute with distilled water to about 20 ml. Add 1 ml of dilute $SnCl_2$ solution, shake again and dilute to the 25-ml mark. After 10 minutes, read the blue colour of the solution on the spectrophotometer at a wavelength of 660 nm. Plot the absorbance reading against "µg P" and connect the points.
2. Extraction: Add 50 ml of the Bray's Extractant No. 1 to a 100-ml conical flask containing 5 g of soil sample. Shake for 5 minutes and filter.
3. Development of colour: Take 5 ml of the filtered soil extract with a bulb pipette in a 25-ml measuring flask; deliver 5 ml of the molybdate reagent with an automatic pipette, dilute to about 20 ml with distilled water, shake

and add 1 ml of the dilute $SnCl_2$ solution with a bulb pipette. Fill to the 25-ml mark and shake thoroughly. Read the blue colour after 10 minutes on the spectrophotometer at 660 nm after setting the instrument to zero with the blank prepared similarly but without the soil.

The calculation is:

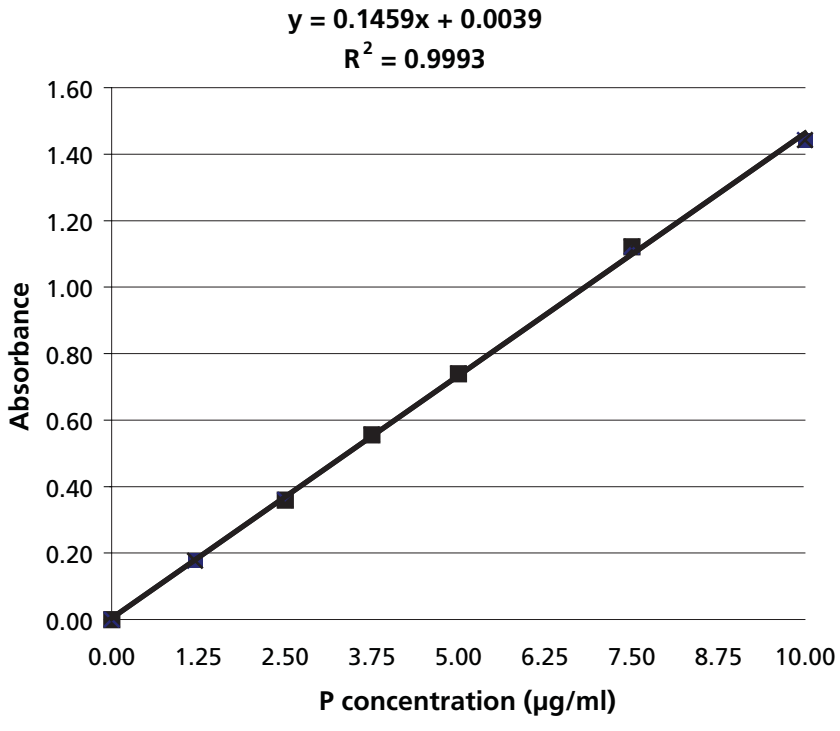
$$P\,(kg/ha) = \frac{A}{1\,000\,000} \times \frac{50}{5} \times \frac{2\,000\,000}{5} = 4A$$

where:

- weight of the soil taken = 5 g;
- volume of the extract = 50 ml;
- volume of the extract taken for estimation = 5 ml;
- amount of P observed in the sample on the standard curve = *A* (µg);
- weight of 1 ha of soil down to a depth of 22 cm is taken as 2 million kg.

As an example, Figure 3 shows a standard curve prepared by the authors of this publication for estimation of available P by Bray's Method No. 1 while establishing a soil testing laboratory.

FIGURE 3
Standard curve for P on spectrophotometer



Olsen's method

The apparatus required for Olsen's method (Olsen *et al*., 1954) for alkali soils is the same as that for Bray's Method No. 1 (above).

The reagents required are:

- Bicarbonate extractant: Dissolve 42 g of sodium bicarbonate in 1 litre of distilled water and adjust the pH to 8.5 by addition of dilute NaOH or HCl. Filter as necessary.
- Activated P-free carbon.
- Molybdate reagent: Same as for the Bray's Method No. 1 (above).
- Stannous chloride solution: Same as for Bray's Method No. 1 (above).

The procedure is:

1. Preparation of the standard curve: proceed as for Bray's Method No. 1 (above).
2. Extraction: Add 50 ml of the bicarbonate extractant to a 100-ml conical flask containing 2.5 g of soil sample. Add 1 g of activated carbon. Shake for 30 minutes on the mechanical shaker, and filter.
3. Development of colour: proceed as for Bray's Method No. 1 (above).

The calculation is the same as described for Bray's Method No. 1 (above).

In spite of all precautions, the intensity of blue colour changes slightly with every batch of molybdate reagent. It is imperative to check the standard curve every day by using 2–3 dilutions of the standard phosphate solution. If the standard curve does not tally, draw a new standard curve with fresh molybdate reagent.

Available potassium

Potassium present in the soil is extracted with neutral ammonium acetate of 1 molarity. This is considered as plant-available K in the soils. It is estimated with the help of a flame photometer (Toth and Prince, 1949).

The apparatus required consists of:

- a multiple dispenser or automatic pipette (25 ml);
- some flasks and beakers (100 ml);
- a flame photometer.

The reagents required are:

- Molar neutral ammonium acetate solution: Dissolve 77 g of ammonium acetate ($NH_4C_2H_3O_2$) in 1 litre of water. Check the pH with bromothymol blue or with a pH meter. If not neutral, add either ammonium hydroxide or acetic acid as per the need in order to neutralize it to pH 7.0.
- Standard potassium solution: Dissolve 1.908 g of pure KCl in 1 litre of distilled water. This solution contains 1 mg K/ml. Take 100 ml of this solution and dilute to 1 litre with ammonium acetate solution. This gives 0.1 mg K/ml as a stock solution.
- Working potassium standard solutions: Take 0, 5, 10, 15 and 20 ml of the stock solution and dilute each volume separately to 100 ml with the molar ammonium acetate solution. These solutions contain 0, 5, 10, 15 and 20 µg K/ml, respectively.

The procedure is:

1. Preparation of the standard curve: Set up the flame photometer by atomizing 0 and 20 µg K/ml solutions alternatively to readings of 0 and 100. Atomize intermediate working standard solutions and record the readings. Plot these readings against the respective K contents and connect the points with a straight line to obtain a standard curve.
2. Extraction: Add 25 ml of the ammonium acetate extractant to a conical flask fixed in a wooden rack containing 5 g of soil sample. Shake for 5 minutes and filter.
3. Determine the potash in the filtrate with the flame photometer.

The calculation is:

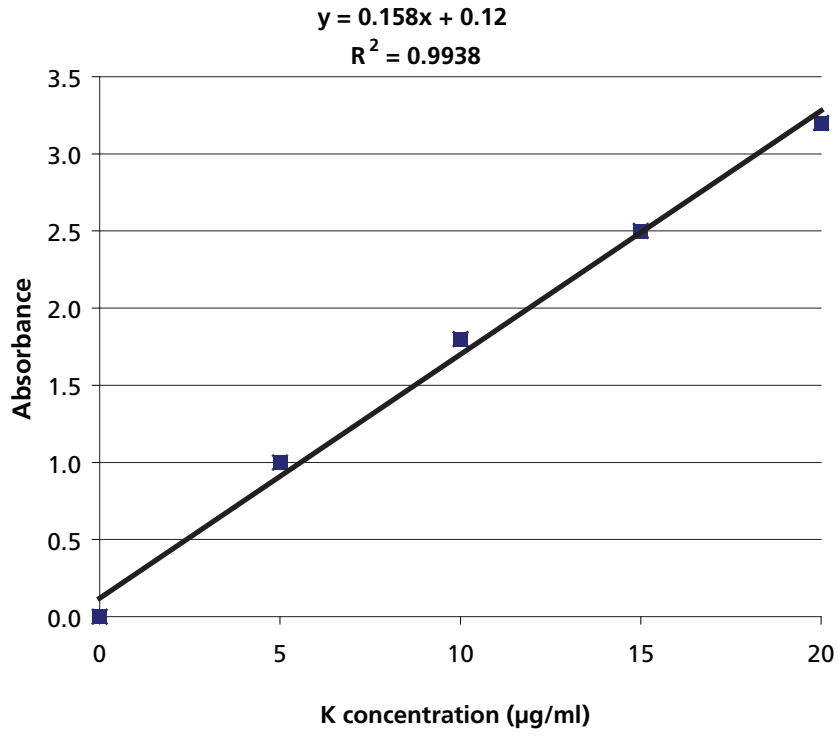
$$\text{K (kg/ha)} = \frac{A}{1\,000\,000} \times 25 \times \frac{2\,000\,000}{5}$$

where:

- A = content of K (µg) in the sample, as read from the standard curve;
- volume of the extract = 25 ml;
- weight of the soil taken = 5 g;
- weight of 1 ha of soil down to a plough depth of 22 cm is taken as 2 million kg.

Figure 4 shows an example of a standard curve for estimating K using the flame photometer method.

FIGURE 4
Standard curve for K on flame photometer



Available sulphur

Available S in mineral soils occurs mainly as adsorbed SO_4 ions. Phosphate ions (as monocalcium phosphate) are generally preferred for replacement of the adsorbed SO_4 ions. The extraction is also carried out using $CaCl_2$ solution. However, the former is considered to be better for more efficient replacement of SO_4 ions. The use of Ca salts has a distinct advantage over those of Na or K as Ca prevents deflocculation in heavy-textured soils and leads to easy filtration. The SO_4 in the extract can be estimated turbidimetrically using a spectrophotometer. A major problem arises when the amount of S extracted is too low to be measured. To overcome this problem, a seed solution of known S concentration is added to the extract in order to raise the concentration to a readily detectable level.

The apparatus required for the barium sulphate precipitation method of estimating available S (adapted from Singh, Chhonkar and Pandey, 1999) consists of:

- a spectrophotometer;
- a mechanical shaker;
- a volumetric flask.

The reagents required are:

- Monocalcium phosphate extracting solution (500 mg P/litre): Dissolve 2.035 g of $Ca(H_2PO_4)_2.H_2O$ in 1 litre of water.
- Gum acacia – acetic acid solution: Dissolve 5 g of chemically pure gum acacia powder in 500 ml of hot water and filter in hot condition through No. 42 filter paper. Cool and dilute to 1 litre with dilute acetic acid.
- Barium chloride: Pass AR-grade $BaCl_2$ salt through a 1-mm sieve and store for use.
- Standard stock solution (2 000 mg S/litre): Dissolve 10.89 g of oven-dried AR-grade potassium sulphate in 1 litre of water.
- Standard working solution (10 mg S/litre): Measure exactly 2.5 ml of the stock solution and dilute to 500 ml.
- Barium sulphate seed suspension: Dissolve 18 g of AR-grade $BaCl_2$ in 44 ml of hot water and add 0.5 ml of the standard stock solution. Heat the content to boiling and then cool quickly. Add 4 ml of gum acacia – acetic acid solution to it. Prepare a fresh seed suspension for estimation every day.
- Dilute nitric acid (about 25 percent): Dilute 250 ml of AR-grade concentrated HNO_3 to 1 litre.
- Acetic-phosphoric acid: Mix 900 ml of AR-grade glacial acetic acid with 300 ml of H_3PO_4 (AR-grade).

The procedure is:

1. Weigh 20 g of soil sample in a 250-ml conical flask. Add 100 ml of the monocalcium phosphate extracting solution (500 mg P/litre) and shake for 1 hour. Filter through No. 42 filter paper.
2. Put 10 ml of the clear filtrate in a 25-ml volumetric flask.
3. Add 2.5 ml of 25 percent HNO_3 and 2 ml of acetic-phosphoric acid. Dilute to about 22 ml, stopper the flask and shake well (if required).

4. Shake the $BaSO_4$ seed suspension and then add 0.5 ml of it, and 0.2 g of $BaCl_2$ crystals. Stopper the flask, invert 3 times, and keep.
5. After 10 minutes, invert 10 times. After another 5 minutes, invert 5 times.
6. Allow to stand for 15 minutes and then add 1 ml of gum acacia-acetic acid solution.
7. Make the volume up to 25 ml, invert 3 times and set aside for 90 minutes.
8. Invert 10 times and measure the turbidity intensity at 440 nm (blue filter).
9. Run a blank side by side.
10. Preparation of standard curve:
    - Put 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ml of the working standard solution (10 mg S/litre) into a series of 25-ml volumetric flasks in order to obtain 25, 50, 75, 100, 125 and 150 µg of S.
    - Proceed to develop turbidity as described above for sample aliquots.
    - Read the turbidity intensity and prepare the curve by plotting readings against S concentrations (in micrograms in the final volume of 25 ml).

The calculation is:

$$\text{Available sulphur } (SO_4^- - S) \text{ in soil (mg/kg)} = \frac{W \times 100}{10 \times 20} = \frac{W}{2}$$

where:

- W stands for the quantity of S (in milligrams) as obtained on the x-axis against an absorbance reading (y-axis) on the standard curve;
- 20 is the weight of the soil sample (in grams);
- 100 is the volume of the extractant (in millilitres);
- 10 is the volume of extractant (in millilitres) in which turbidity is developed.

Exchangeable calcium and magnesium

Exchangeable cations are usually determined in a neutral normal ammonium acetate extract of soil. Extraction is carried out by shaking the soil–extractant mixture, followed by filtration or centrifugation. Calcium and Mg are determined either by the EDTA titration method or by using an AAS after the removal of ammonium acetate and OM.

Appreciable amounts of soluble Calcium and Mg may be present is soils. Hence, these water-soluble cations are estimated in the 1:2 soil–water extract and deducted from ammonium acetate extractable Ca and Mg (as ammonium acetate also extracts water-soluble cations) in order to obtain exchangeable Ca and Mg. Generally, to obtain the soil–water extract, 25 g soil and 50 ml of water suspension is shaken for 30 minutes on a mechanical shaker and filtered. The method of estimation in the water extract (water-soluble cations) and ammonium acetate extract (exchangeable cations) is same.

The EDTA titration method developed by Cheng and Bray (1951) is preferred on account of its accuracy, simplicity and speed.

The method is based on the principle that Ca, Mg and a number of other cations form stable complexes with versenate (EDTA disodium salt) at different pH values. The interference of Cu, Zn, Fe and Mn is prevented by the use of 2 percent NaCN solution or carbamate. Usually, in irrigation waters and water extracts of soil, the quantities of interfering ions are negligible and can be ignored.

A known volume of a standard Ca solution is titrated with standard versenate 0.01N solution using muroxide (ammonium purpurate) indicator in the presence of NaOH solution. The end point is a change in colour from orange-red to purple at pH 12 when all the Ca forms a complex with EDTA.

Calcium by the versenate (EDTA) method

The apparatus required consists of:

- a shaker;
- a porcelain dish;
- some beakers;
- a volumetric/conical flask.

The reagents required are:

- Ammonium chloride – ammonium hydroxide buffer solution: Dissolve 67.5 g of ammonium chloride in 570 ml of concentrated ammonium hydroxide, and make up to 1 litre.
- Standard 0.01N Ca solution: Take accurately 0.5 g of pure calcium carbonate and dissolve it in 10 ml of 3N HCl. Boil to expel CO_2 and then make the volume up to 1 litre with distilled water.
- EDTA solution (0.01N): Take 2.0 g of versenate, dissolve in distilled water and make the volume up to 1 litre. Titrate it with 0.01N Ca solution and make the necessary dilution so that its normality is exactly equal to 0.01N.
- Muroxide indicator powder: Take 0.2 g of muroxide and mix it with 40 g of powdered potassium sulphate. This indicator should not be stored in the form of solution, otherwise it oxidizes.
- Sodium diethyl dithiocarbamate crystals: These are used to remove interference by other metal ions.
- Sodium hydroxide 4N: Prepare 16 percent soda solution by dissolving 160 g of pure sodium hydroxide in water, and make the volume up to 1 litre. This will give pH 12.

The procedure is:

1. Put 5 g of air-dried soil sample in a 150-ml conical flask and add 25 ml of neutral normal ammonium acetate. Shake on a mechanical shaker for 5 minutes and filter through No. 1 filter paper.
2. Take a suitable aliquot (5 or 10 ml) and add 2–3 crystals of carbamate and 5 ml of 16 percent NaOH solution.
3. Add 40–50 mg of the indicator powder. Titrate it with 0.01N EDTA solution until the colour changes gradually from orange-red to reddish-violet (purple). Add a drop of EDTA solution at intervals of 5–10 seconds, as the change of colour is not instantaneous.

4. The end point must be compared with a blank reading. If the solution is overtitrated, it should be backtitrated with standard Ca solution; thus, the exact volume used is found.
5. Note the volume of EDTA used for titration.

The calculation is:

If N_1 is normality of Ca^{2+} and V_1 is volume of aliquot taken and N_2V_2 are the normality and volume of EDTA used, respectively, then:

$$N_1V_1 = N_2V_2$$

$$N_1 = \frac{N_2V_2}{V_1} = \frac{\text{Normality of EDTA x Vol. of EDTA}}{\text{ml of aliquot taken}}$$

Here, N_1 (normality) = equivalent of Ca^{2+} present in 1 litre of aliquot. Hence, Ca^{2+} me/litre is:

$$\frac{\text{Normality of EDTA x Vol. of EDTA x 1000}}{\text{ml of aliquot taken}}$$

When expressed on soil weight basis, Ca^{2+} me/100 g soil is:

$$\frac{100}{\text{wt. of soil}} \text{ x } \frac{\text{extract volume}}{1000} \text{ x Ca as me/litre}$$

Calcium plus magnesium by the versenate (EDTA) method

Magnesium in solution can be titrated with 0.01N EDTA using EBT dye as indicator at pH 10 in the presence of ammonium chloride and ammonium hydroxide buffer. At the end point, the colour changes from wine-red to blue or green. Where Ca is also present in the solution, this titration will estimate both Ca and Mg. Beyond pH 10, Mg is not bound strongly to EBT indicator to give a distinct end point.

The apparatus required consists of:

- a shaker;
- a porcelain dish;
- a beaker;
- a volumetric/conical flask.

The reagents required are:

- EDTA or versenate solution (0.01N): Same as for Ca determination (above).
- Ammonium chloride – ammonium hydroxide buffer solution: Same as for Ca determination (above).
- EBT indicator: Take 100 ml of ethanol, and dissolve 4.5 g of hydroxylamine hydrochloride in it. Add 0.5 g of the indicator and prepare the solution. Hydroxylamine hydrochloride removes the interference of Mn by keeping it in a lower valency state (Mn^{2+}).

- Sodium cyanide solution (2 percent) or sodium diethyl dithiocarbamate crystals: This is used to remove the interference of Cu, Co and nickel (Ni).

The procedure is:

1. Put 5 g of air-dried soil in a 150-ml flask, add 25 ml of neutral normal ammonium acetate solution, shake on a mechanical shaker for 5 minutes, and filter through No. 1 filter paper.
2. Pipette out 5 ml of aliquot containing not more than 0.1 me of Ca plus Mg. If the solution has a higher concentration, it should be diluted.
3. Add 2–5 crystals of carbamate and 5 ml of ammonium chloride – ammonium hydroxide buffer solution. Add 3–4 drops of EBT indicator.
4. Titrate this solution with 0.01N versenate until the colour changes to bright blue or green and no tinge of wine-red colour remains.

For the calculation, if N_1 and V_1 are normality (concentration of Ca^{2+} + Mg^{2+}) and volume of aliquot taken, and N_2V_2 are the normality and volume of EDTA used, respectively, then, $N_1V_1 = N_2V_2$; or N_1 =

$$\frac{N_2V_2}{V_1} = \frac{\text{Normality of EDTA x Vol. of EDTA}}{\text{ml of aliquot taken}}$$

Here, N_1 (normality) = equivalents of Ca^{2+} plus Mg^{2+} present in 1 litre of aliquot. Hence:

$$Ca^{2+}\text{plus}\,Mg^{2+}\ \text{me/litre} = \frac{\text{Normality of EDTA x Vol. of EDTA x 1000}}{\text{ml of aliquot taken}}$$

Milli-equivalent (me) of Mg^{2+} = me (Ca^{2+} + Mg^{2+}) – me of Ca^{2+}

When expressed on a soil weight basis, Ca^{2+} + Mg^{2+} me/100 g soil =

$$\frac{100}{\text{wt. of soil}} \times \frac{\text{extract volume}}{1000} \times Ca^{++} + Mg^{++} \quad \text{me/litre}$$

Micronutrients

For the estimation of micronutrients in soils, it is the plant-available form that is critical and not the total content. The major objective of soil testing for micronutrients, as with macronutrients, is to determine whether a soil can supply adequate micronutrients for optimal crop production or whether nutrient deficiencies are expected in crops grown on such soils. The most commonly studied micronutrients are Zn, Cu, Fe, Mn, B and Mo, and the same are dealt with here.

Micronutrients are present in different forms in the soil. Among the most deficient ones is Zn, which is present as the divalent cation Zn^{2+}. Maize, citrus, legumes, cotton and rice are especially sensitive to Zn deficiency. Iron is present mostly in sparingly soluble ferric oxide forms, which occur as coatings of aggregate or as separate constituents of the clay fraction. Soil redox potential and

pH affect Fe availability. The Fe form that is predominantly taken up by plants is Fe^{2+}. The uptake of Fe is inhibited by phosphate levels caused by the formation of insoluble iron phosphate. Chemically, Mn behaves in soil in the same way as Fe. Soil Mn originates primarily from the decomposition of ferromagnesian rocks. It is taken up by the plants as Mn^{2+} ions although it exists in many oxidation states. Manganese and phosphate are mutually antagonistic. Copper, as zinc, exists in soils mainly as divalent ions, Cu^{2+}. It is usually adsorbed by clay minerals or associated with OM although they have little or no effect on its availability to crops. High phosphate fertilization can induce Cu deficiency. Molybdenum usually occurs as MoO_3, MoO_5 and MoO_2. These oxides are transformed slowly to soluble molybdates (MoO_4), which is the form taken up by plants. Boron deficiency occurs mostly in the light-textured acid soils when they are leached heavily through irrigation or heavy rainfall.

There are different extractants for assessing plant-available nutrient (element) content in soils. The elements so extracted can be estimated quantitatively through chemical methods or instrumental techniques. Table 10 lists commonly used extractants for different elements.

Zinc, copper, iron and manganese

Ethylenediamine tetraacetic acid (EDTA) with ammonium acetate is commonly used for the extraction of many elements. Diethylenetriamine pentaacetic acid (DTPA) is another common (universal) extractant and it is widely used for the simultaneous extraction of elements such as Zn, Cu, Fe and Mn (Lindsay and Norvell, 1978). Although a specific extractant for an element that has a higher correlation with plant availability may be preferred, the universal or common extractant saves on the cost of chemicals and the time involved in estimation, especially in a service laboratory where a large number of samples need to be analysed in a short period.

The estimation of elements in the extract is done with the help of an AAS. Table 11 details the critical limits for DTPA-extractable micronutrient elements as proposed by Lindsay and Norvell (1978).

Diethylenetriamine pentaacetic acid is an important and widely used chelating agent that combines with free metal ions in the solution to form soluble complexes

TABLE 10
Commonly used extractants for micronutrients

Element	Extractants
Zinc	EDTA + ammonium acetate, EDTA + ammonium carbonate, DTPA + $CaCl_2$, HCl, HNO_3 and dithiozone + ammonium acetate
Copper	EDTA, EDTA + ammonium acetate, ammonium bicarbonate + DTPA, HCl and HNO_3
Iron	EDTA, DTPA, EDTA + ammonium acetate, HCl and HNO_3
Manganese	Hydroquinone, ammonium phosphate, DTPA and EDTA + ammonium acetate
Boron	Hot water and mannitol + $CaCl_2$
Molybdenum	Ammonium oxalate, ammonium acetate, ammonium fluoride and water

TABLE 11
Critical limits for DTPA-extractable micronutrients

Availability	Micronutrients			
	Zn	Cu	Fe	Mn
		(µg/g soil)		
Very low	0–0.5	0–0.1	0–2	0–0.5
Low	0.5–1	0.1–0.3	2–4	0.5–1.2
Medium	1–3	0.3–0.8	4–6	1.2–3.5
High	3–5	0.8–3	6–10	3.5–6
Very high	> 5	> 3	> 10	> 6

of elements. To avoid excessive dissolution of CaCO3, which may release occluded micronutrients that are not available to crops in calcareous soils and may give erroneous results, the extractant is buffered in slightly alkaline pH. Triethanolamine (TEA) is used as buffer because it burns cleanly during atomization of extractant solution while estimating on an AAS. The DTPA has a capacity to complex each of the micronutrient cations as 10 times of its atomic weight. The capacity ranges from 550 to 650 mg/kg depending on the micronutrient cations.

To prepare DTPA 0.005M, 0.01M $CaCl_2.2H_2O$ and 0.1M TEA extractant:

1. Add 1.967 g of DTPA and 13.3 ml of TEA in 400 ml of distilled water in a 500-ml flask.
2. Put 1.47 g of $CaCl_2.2H_2O$ in a separate 1 000-ml flask. Add 500 ml of distilled water and shake to dissolve.
3. Add DTPA+TEA mixture to the $CaCl_2$ solution and make the volume up to 1 litre. Adjust the pH to 7.3 by using 1M HCl before making the volume.

The extracted elements can be estimated by various methods, including volumetric analysis, spectrometry and atomic absorption spectroscopy. Volumetric methods such as EDTA and $KMnO_4$ titrations are used for estimating Zn and Mn, and Fe, respectively. Copper can be estimated by titration with $Na_2S_2O_3$. Spectrometric methods are used in the estimation of a specific colour developed because of the presence of an element that forms coloured compounds in the presence of specific chemicals under a definite set of conditions. The colour intensity has to be linear with the concentration of the element in question. The interference caused by any other element has to be eliminated. Such methods are:

- the dithiozone method for Zn;
- the orthophenonthroline method for Fe;
- the potassium periodate method for Mn;
- the carbamate method for Cu.

These methods are generally cumbersome and time-consuming. Therefore, the most commonly employed method is atomic absorption spectrometry. Here, the interference by other elements is almost nil or negligible because the estimation is carried out for an element at a specific emission spectraline. In fact, in atomic absorption spectrometry, traces of one element can be determined accurately in the presence of a high concentration of other elements.

The procedure is based on flame absorption rather than flame emission and on the fact that metal atoms absorb strongly at discrete characteristic wavelengths that coincide with the emission spectralines of a particular element. The liquid sample is atomized. A hollow cathode lamp (which precedes the atomizer) emits the spectrum of the metal used to make the cathode. This beam traverses the flame and is focused on the entrance slit of a monochromator, which is set to read the intensity of the chosen spectraline. Light with this wavelength is absorbed by the metal in the flame, and the degree of absorption being the function of the concentration of the metal in the flame, the concentration of the atoms in the dissolved material is determined. For elemental analysis, a working curve or a standard curve is prepared by measuring the signal or absorbance of a series of standards of known concentration of the element under estimation. From such a curve, the concentration of the element in the unknown sample is estimated.

Atomic absorption spectroscopy can be applied successfully for estimating Zn, Cu, Fe and Mn. For specific estimation with an AAS, hollow cathode lamps specific to specific elements are used. Table 12 lists the relevant parameters.

The software provided with the equipment manual details the operating parameters that are specific to a particular model. Accordingly, the current supply, wavelength of hollow cathode lamp, integration time and anticipated estimation ranges are fixed. Hollow cathode and deuterium lamps need to be aligned properly before starting the equipment. After proper alignment and adjustment, standard curves are prepared to ensure that the concentration of the elements in solutions relates perfectly to the absorbance.

Ready-made standard solutions of 1 000 µg/ml or 1 mg/ml (1 000 ppm) of dependable accuracy are supplied with the AAS and are also available from suppliers of chemical reagents. Where the standard solutions are to be prepared in the laboratory, either metal element foils of 100-percent purity or the standard chemical salts can be used. Table 13 details the quantities of chemical required to make 1 litre of standard solution of 100 µg/ml for different elements.

In the case of Zn, Cu and Fe, 1 000 µg/ml (1 000 ppm) standard solutions are preferably prepared by dissolving 1.0 g of pure metal wire and making the volume up to 1 litre as per the method described under each element. The solution is diluted to obtain the required concentration. In the case of Mn, $MnSO_4.H_2O$ is preferred.

TABLE 12
Parameters for estimation of micronutrients using an AAS

Specifications	Zn	Cu	Fe	Mn
Lamp current (m A^o)	5	3	7	5
Wavelength (nm)	213.9	324.8	248.3	279.5
Linear range (mg/litre)	0.4–1.5	1.0–5.0	2.0–9.0	1.0–3.6
Slit width (nm)	0.5	0.52	0.2	0.2
Integration time (seconds)	2.0	2.0	2.0	2.0
Flame	Air acetylene			

TABLE 13
Specifications for preparing micronutrient standard solutions

Element	Concentration of stock solution	Salt to be used	Quantity of salt required
	(µg/ml)		*(g/litre)*
Zn	100	Zinc sulphate ($ZnSO_4.7H_2O$)	0.4398
Cu	100	Copper sulphate ($CuSO_4.5H_2O$)	0.3928
Fe	100	Ferrous sulphate ($FeSO_4.7H_2O$) or Ferrous ammonium sulphate	0.4964 0.7028
Mn	100	Manganese sulphate ($MnSO_4.H_2O$)	0.3075

Preparation of standard curve for zinc

The reagents required are:

- Standard Zn solution: Weigh 1.0 g of pure zinc metal in a beaker. Add 20 ml of HCl (1:1). Keep for a few hours, allowing the metal to dissolve completely. Transfer the solution to a 1-litre volumetric flask. Make up the volume with glass-distilled water. This is 1 000 µg/ml Zn solution. For preparing the standard curve, refer to the 1 000 µg/ml solution as solution A. Dilute 1 ml of standard A to 100 ml in order to obtain a 10 µg/ml solution, to be designated standard B.
- Glass-distilled or demineralized acidified water of pH 2.5 ± 0.5: Dilute 1 ml of 10 percent sulphuric acid to 1 litre with glass-distilled or mineralized water and adjust the pH to 2.5 with a pH meter using 10 percent H_2SO_4 or NaOH. This solution is called acidified water.
- Working Zn standard solutions: Pipette 1, 2, 4, 6, 8 and 10 ml of standard B solution in 50-ml numbered volumetric flasks and make the volume up with DTPA solution to obtain 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 µg/ml zinc. Stopper the flasks and shake them well. Fresh standards should be prepared every time when a fresh lot of acidified water is prepared.

The procedure is:

1. Flaming the solutions: Atomize the standards on an AAS at a wavelength of 213.8 nm (Zn line of the instrument).
2. Prepare a standard curve of known concentrations of Zn solution by plotting the absorbance values on the y-axis against their respective Zn concentration on the x-axis.

Special points to note are:

- Weighing must be done on an electronic balance.
- All the glass apparatus to be used should be washed first with dilute hydrochloric acid (1:4) and then with distilled water.
- The pipette should be rinsed with the same solution to be measured.
- The outer surface of the pipette should be wiped with filter paper after use.

- After using the pipette, place it on a clean dry filter paper in order to prevent contamination.

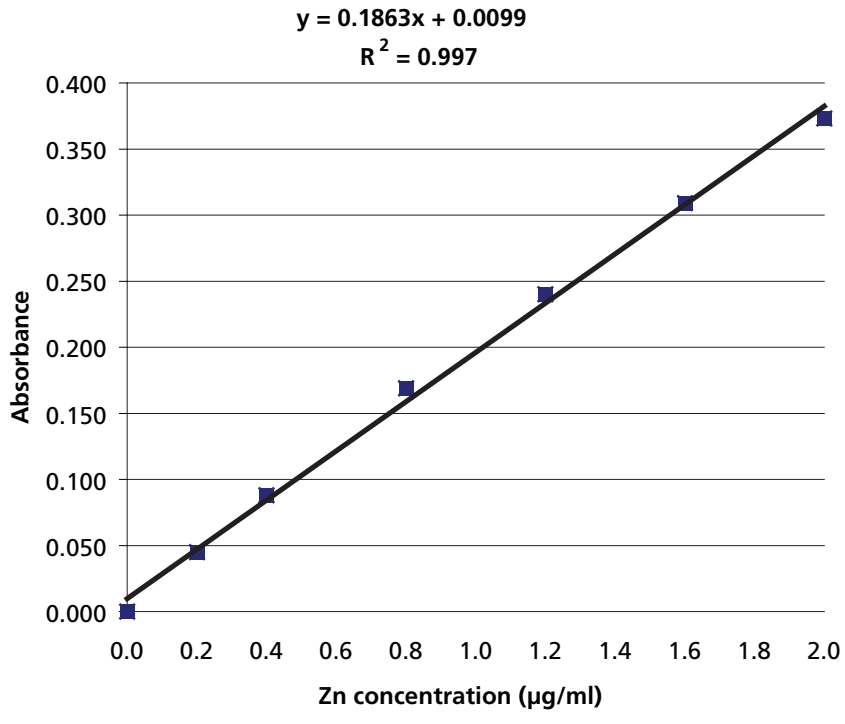
As an example, Figure 5 shows a standard curve prepared by the authors for Zn estimation while establishing a soil testing laboratory.

Preparation of standard curve for copper

The reagents required are:

- Standard Cu solution: Weigh 1 g of pure copper wire on a clean watch glass and transfer it to a 1-litre flask. Add 30 ml of HNO_3 (1:1) and make up to the mark by glass-distilled water. Stopper the flask and shake the solution well. This is 1 000 µg/ml Cu solution and it should be stored in a clean bottle for further use. Dilute 1 ml of 1 000 µg/ml solution of Cu to 100 ml to obtain 10 µg/ml of standard Cu solution.
- Glass-distilled or demineralized acidified water of pH 2.5 ± 0.5: Same as that for Zn (above).

FIGURE 5
Standard curve for Zn on an AAS



- Working Cu standard solutions: Pipette 2, 3, 4, 5, 6 and 7 ml of 10 µg/ml of standard Cu solution in 50-ml numbered volumetric flasks and make the volume up with DTPA solution to obtain 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 µg/ml Cu. Stopper the flasks and shake them well. Prepare fresh standards every fortnight.

The procedure is:

1. Flame the standards on an AAS at a wavelength of 324.8 nm (Cu line of the instrument).
2. Prepare the standard curve with the known concentration of Cu on the x-axis by plotting against the absorbance value on the y-axis.

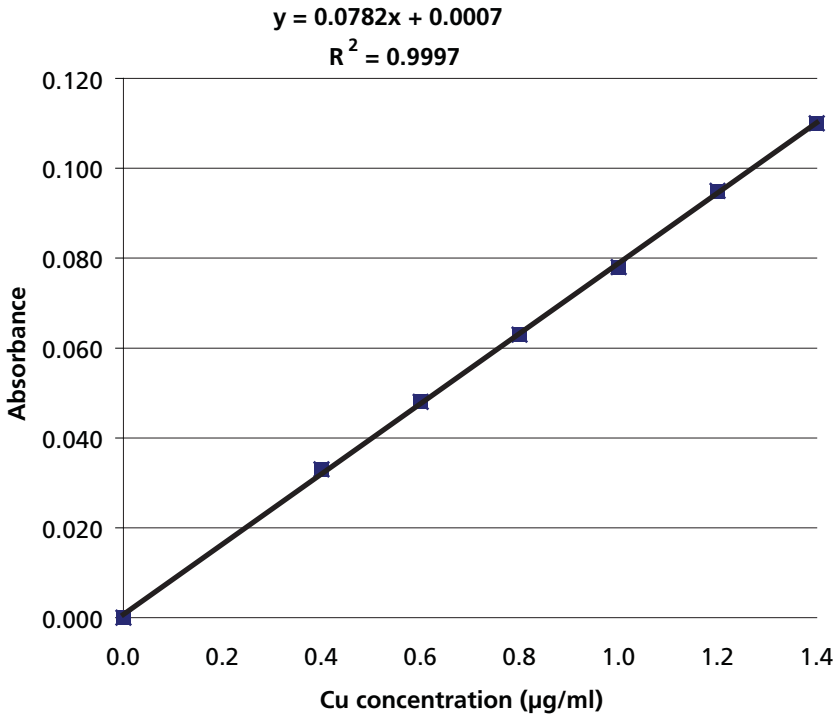
As an example, Figure 6 shows a standard curve prepared by the authors for Cu estimation while establishing a soil testing laboratory.

Preparation of standard curve for iron

The reagents required are:

- Standard Fe solution: Weigh accurately 1 g of pure iron wire, put it in a beaker, and add about 30 ml of 6M HCl and boil. Transfer it to a 1 litre

FIGURE 6
Standard curve for Cu on an AAS



volumetric flask through a funnel, giving several washings to the beaker and funnel with glass-distilled water. Make the volume up to the mark. Stopper the flask and shake the solution well. This is 1 000 µg/ml iron solution.

- Glass-distilled or demineralized acidified water of pH 2.5 ± 0.5: Same as that for Zn (above).
- Working Fe standard solutions: Pipette 10 ml of Fe stock solution in a 100-ml volumetric flask, and dilute to volume with DTPA solution. This is 100 µg/ml iron solution. Take 2, 4, 8, 12 and 16 ml of 100 µg/ml solution and dilute each to 100 ml to obtain 2, 3, 8, 12 and 16 µg/ml of Fe solution.

The procedure is:

1. Flame the standards on an AAS at a wavelength of 248.3 nm (Fe line of the instrument).
2. Prepare the standard curve with the known concentration of Cu on x-axis by plotting against the absorbance value on the y-axis.

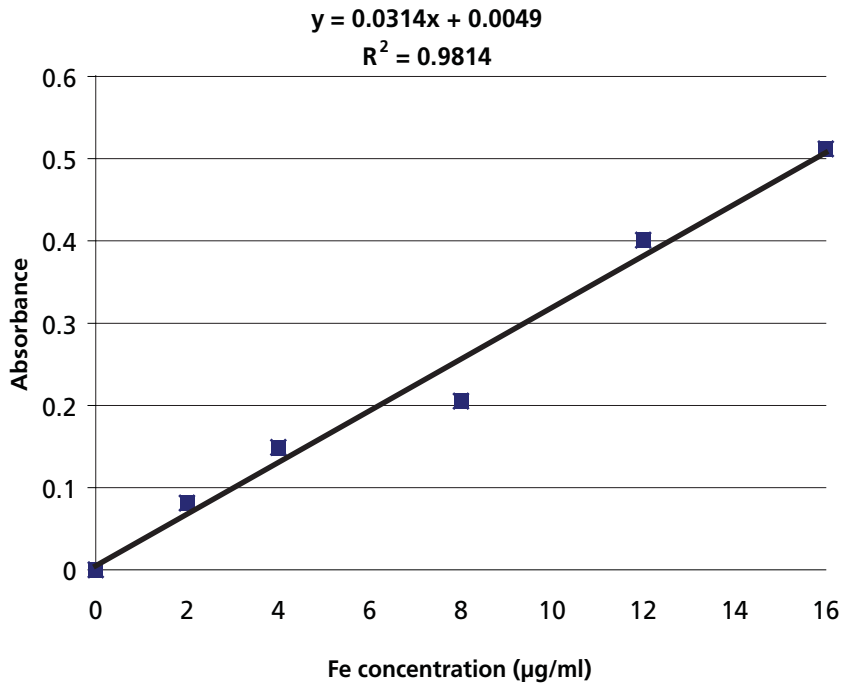
As an example, Figure 7 shows a standard curve prepared by the authors for Fe estimation while establishing a soil testing laboratory.

Preparation of standard curve for manganese

The reagents required are:

- Standard Mn solution: Weigh 3.0751 g of AR-grade manganese sulphate ($MnSO_4.H_2O$) on a clean watch glass and transfer it to a 1-litre flask through

FIGURE 7
Standard curve for Fe on an AAS



a funnel, giving several washings to the watch glass and funnel with acidified water, and make the volume up to the mark. This solution will be 1 000 µg/ml Mn. A secondary dilution of 5 ml to 100 ml with acidified water gives a 50 µg/ml solution.

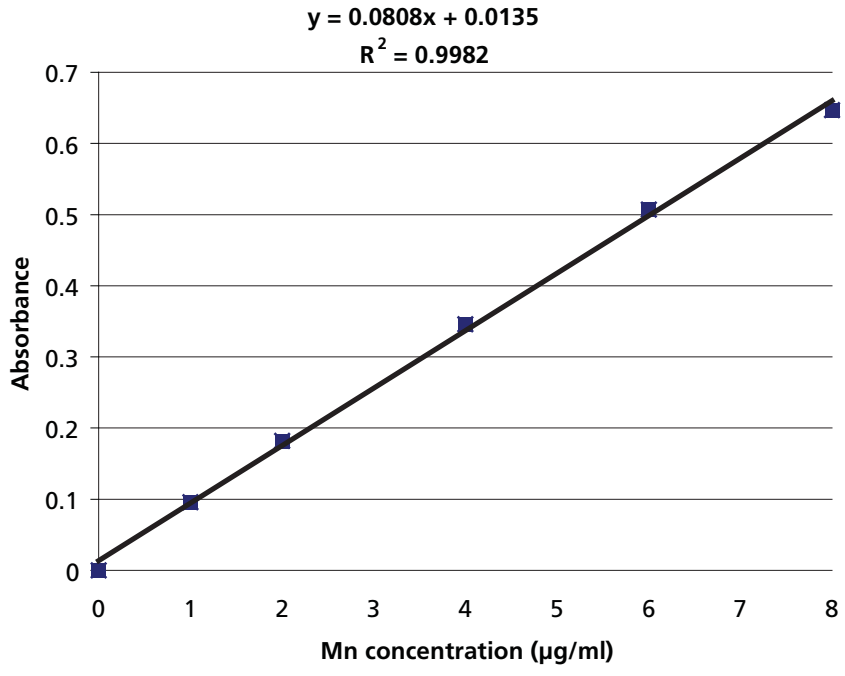
- Glass-distilled or demineralized acidified water of pH 2.5 ± 0.2: Same as that for Zn (above).
- Working Mn standard solutions: The standard curve is prepared by taking lower concentrations of Mn in the range of 0–10 µg/ml. Take 1, 2, 4, 6 and 8 ml of 50 µg/ml solution, and make the volume up with DTPA solution to 50 ml to obtain 1, 2, 4, 6 and 8 µg/ml working standards.

The procedure is:

1. Flame the standards on an AAS at a wavelength of 279.5 nm (Mn line of the instrument).
2. Prepare the standard curve with the known concentration of Mn on the x-axis by plotting against the absorbance value on the y-axis.

As an example, Figure 8 shows a standard curve prepared by the authors for Mn estimation while establishing a soil testing laboratory.

FIGURE 8
Standard curve for Mn on an AAS



Procedure for extraction by DTPA

Once standard curves have been prepared, proceed for extraction by DTPA as follows:

1. Put 10 g of the soil sample in a 100-ml narrow-mouthed polypropylene bottle.
2. Add 20 ml of DTPA extracting solution.
3. Stopper the bottle, and shake for 2 hours at room temperature (25 °C).
4. Filter the content using filter paper No. 1 or No. 42, and collect the filtrate in polypropylene bottles.
5. Prepare a blank following all steps except taking a soil sample.

The extract so obtained is used for estimation of different micronutrients. For extraction of a more accurate quantity of an element that has a higher degree of correlation with plant availability, there are element-specific extractants. An extractant standardized/recommended for a given situation in a country may be used. However, the estimation procedure on an AAS remains unchanged.

Estimation on an AAS

The procedure is:

1. Select an element-specific hollow cathode lamp and mount it on the AAS.
2. Start the flame.
3. Set the instrument at zero by using blank solution.
4. Aspirate the standard solutions of different concentrations one by one and record the readings.
5. Prepare the standard curve, plotting the concentration of the element concerned and the corresponding absorbance in different standard samples (as described above).
6. When the operation is performed accurately, a straight line relationship is obtained between the concentration of the element and the absorbance on the AAS with a correlation coefficient that may be nearly as high as 1.0.
7. Aspirate the soil extractant obtained for estimation of the nutrient element in the given soil sample and observe the readings.
8. Determine the content of the element in the soil extract by observing its concentration on the standard curve against its absorbance

The relevant calculation is:

Content of micronutrient in the sample (mg/kg) = C µg/ml × 2 (dilution factor)

where:

- dilution factor = 2.0 (soil sample taken = 10.0 g and DTPA used = 20 ml);
- absorbance reading on AAS of the soil extract being estimated for a particular element = X
- concentration of micronutrient as read from the standard curve for the given absorbance (X) = C µg/ml.

Available boron

The most commonly used method for available B is hot water extraction of soil as developed by Berger and Truog (1939). A number of modified versions of this method have been proposed but the basic procedure remains the same.

Water-soluble B is the available form of B. It is extracted from the soil by water suspension. In the extract, B can be analysed by colorimetric methods using reagents such as carmine, azomethine-H, and, most recently, by inductively coupled plasma (ICP) and atomic emission spectrometry. However, the colorimetric method is preferable owing to the fact that as B is a non-metal, the use of an AAS for its estimation poses some limitations.

The extraction procedure for the methods presented below is:

- Put 25 g of soil in a quartz flask or beaker.
- Add about 50 ml of double-distilled water (DDW) and about 0.5 g of activated charcoal.
- Boil the mixture for about 5 minutes, and filter through No. 42 filter paper.

Estimation by AAS

The specifications/relevant parameters for estimation of B on an AAS are:

- lamp current: 20 m A°;
- wavelength: 249.7 nm;
- linear range: 1–10 µg/ml;
- slit width: 0.2 nm;
- integration time: 2.0 seconds;
- flame: acetylene nitrous oxide.

The software provided with the equipment manual gives the operating parameters that are specific to a particular model. Accordingly, the current supply, wavelength of hollow cathode lamp, integration time and anticipated estimation ranges are fixed. Hollow cathode and deuterium lamps need to be aligned properly before starting the equipment. After proper alignment and adjustment, standard curves are prepared in order to ensure that the concentration of the element in solutions relates perfectly to the absorbance.

The reagents required are:

- Standard B solution: Dissolve 8.819 g of $Na_2B_4O_7.10H_2O$ in warm water. Dilute to 1 litre to obtain 1 000 µg/ml B stock solution. Dilute 1 ml of standard to 100 ml to obtain 10 µg/ml B.
- Working standards: Take 1, 2, 3, 4, 5, 6, 7 and 10 ml of 10 µg/ml solution and dilute each to 50 ml to obtain 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 2.0 µg/ml B.

The procedure is:

1. Atomize the working standards on an AAS using acetylene nitrous oxide as fuel instead of air acetylene fuel (as used for other micronutrients) at a wavelength of 249.7 nm.

2. Prepare a standard curve of known concentration of B by plotting the absorbance values on the y-axis against their respective B concentration on the x-axis. Measure the absorbance of the soil sample extract and determine the B content in the soil from the standard curve.

The relevant calculation is:

Content of B in the sample (mg/kg) = *C* µg/ml × 2 (dilution factor)

where:

- *C* = concentration of B in the sample, as read from the standard curve for the given absorbance;
- dilution factor = 2.0 (soil sample taken = 25 g and water used = 50 ml).

Estimation by colorimetric method

The extracted B in the filtered extract is determined by the azomethine-H colorimetric method.

The apparatus required consists of:

- an analytical balance;
- a flask or beaker;
- a volumetric flask;
- some funnels;
- some No. 42 filter paper;
- a spectrophotometer.

The reagents required are:

- Azomethine-H: Dissolve 0.45 g of azomethine-H and 1.0 g of L-ascorbic acid in about 100 ml of deionized or double-distilled water. If the solution is not clear, it should be heated gently in a water-bath or under a hot water tap at about 30 °C until it dissolves. Every week, a fresh solution should be prepared and kept in a refrigerator.
- Buffer solution: Dissolve 250 g of ammonium acetate in 500 ml of deionized or double-distilled water, and adjust the pH to about 5.5 by adding slowly about 100 ml of glacial acetic acid, stirring continuously.
- EDTA solution (0.025 M): Dissolve 9.3 g of EDTA in deionized or double-distilled water, and make the volume up to 1 litre.
- Standard stock solution: Dissolve 0.8819 g of $Na_2B_4O_7.10H_2O$ (AR-grade) in a small volume of deionized water, and make the volume up to 1 000 ml to obtain a stock solution of 100 µg B/ml.
- Working standard solution: Put 5 ml of stock solution in a 100-ml volumetric flask and dilute it to the mark. This solution contains 5 µg B/ml.

The procedure is:

1. Put 5 ml of the clear filtered extract in a 25-ml volumetric flask and add 2 ml of buffer solution, 2 ml of EDTA solution and 2 ml of azomethine-H solution.
2. Mix the contents thoroughly after the addition of each reagent.
3. Let the solution stand for 1 hour to allow colour development. Then, make the volume up to the mark.

4. Measure the intensity of colour at 420 nm. The colour thus developed has been found to be stable for 3–4 hours.
5. Preparation of the standard curve: Put 0, 0.25, 0.50, 1.0, 2.0 and 4.0 ml of 5 µg B/ml solution (working standard) in a series of 25-ml volumetric flasks. Add 2 ml each of buffer reagent, EDTA solution and azomethine-H solution. Mix the contents after each addition and allow to stand at room temperature for 30 minutes. Make the volume up to 25 ml with deionized or double-distilled water, and measure absorbance at 420 nm. This will give reading for standard solution with B concentration of 0, 0.05, 0.10, 0.20, 0.40 and 0.80 µg B/ml.

The relevant calculation is:

Content of B in the soil (µg/g or mg/kg) = C × dilution factor (10)

where:

- C (µg/ml) = concentration of B as read from the standard curve against the absorbance reading of the soil solution on the spectrophotometer;
- dilution factor = 10, which is calculated as follows:
  - weight of the soil taken = 25 g;
  - volume of extractant (water) added = 50 ml;
  - first dilution = 2 times;
  - volume of the filtrate taken = 5 ml;
  - final volume of filtrate after colour development = 25 ml;
  - second dilution = 5 times;
  - total dilution = 2 × 5 = 10 times.

Important points to note are:

- The use of azomethine-H is an improvement over that of carmine, quinalizarin and curcumin because the procedure involving this chemical does not require the use of concentrated acid.
- The amount of charcoal added may vary with the OM content of the soil, and it should be just sufficient to produce a colourless extract after 5 minutes of boiling on a hotplate. Excess amounts of charcoal can result in a loss of extractable B from soils.

Available molybdenum

Molybdenum is a rare element in soils, and it is present only in very small amounts in igneous and sedimentary rocks. The major inorganic source of Mo is molybdenite (MoS_2). The total Mo content in soils is perhaps the lowest of all the micronutrient elements.

In the soil solution, Mo exists mainly as $HMoO_4$ ion under acidic condition, and as MoO_4^{2-} ion under neutral to alkaline conditions. Because of the anionic nature of Mo, its anions will not be attracted much by the negatively charged colloids, and therefore, tend to be leached from the soils in humid region.

Molybdenum can be toxic owing to greater solubility in alkali soils of the arid and semi-arid regions, and deficient in acid soils of the humid regions.

In plants, a deficiency of Mo is common at levels of 0.1 µg/g soil or less. Molybdenum toxicity (molybdenosis) is common where cattle graze forage plants with 10–20 µg Mo/g.

The extraction of Mo usually uses ammonium acetate and/or ammonium oxalate. Estimations can be done both by the AAS and colorimetric methods, with preference for the latter owing to the formation of oxide in the flame in the case of estimation by AAS. Ammonium oxalate is considered a better extractant. However, for estimation on an AAS, ammonium acetate is preferred as the oxalates pose a limitation on the AAS unless removed by digesting with di-acid (below).

Estimation by AAS

The specifications/relevant parameters for estimation of Mo on an AAS are:

- lamp current: 7 m A°;
- wavelength: 313.3 nm;
- linear range: 1–4 µg/ml;
- slit width: 0.2 nm;
- integration time: 2.0 seconds;
- flame: acetylene nitrous oxide.

The software provided with the equipment manual gives the operating parameters that are specific to a particular model. Accordingly, the current supply, wavelength of hollow cathode lamp, integration time and anticipated estimation ranges are fixed. Hollow cathode and deuterium lamps need to be aligned properly before starting the equipment. After proper alignment and adjustment, standard curves are prepared in order to ensure that the concentration of the element in solutions relates perfectly to the absorbance.

The apparatus required consists of:

- a centrifuge and some 50-ml centrifuge tubes;
- an automatic shaker;
- an AAS.

The reagents required are:

- Ammonium acetate solution (NH_4OAc) 1.0M: Dissolve 77.09 g of ammonium acetate in 1 litre of distilled water, and adjust the pH to 7.0.
- Glass-distilled acidified water of pH 2.5: Same as that for Zn estimation (above).
- Standard molybdenum solution: Dissolve 0.15 g of MoO_3 (molybdenum trioxide) in 100 ml of 0.1M NaOH. Dilute to 1 litre to obtain 100 µg/ml Mo stock solution. Dilute 10 ml of the standard to 100 ml to obtain 10 µg/ml Mo.
- Working standard solutions: Take 1, 2, 3, 4, 5, 6, 7 and 10 ml of 10 µg/ml Mo standard solution and dilute each to 50 ml. This will give 0.2, 0.4, 0.6,0.8, 1.0, 1.2, 1.4 and 2.0 µg/ml Mo, respectively.

The procedure is:

1. Weigh accurately 5 g of soil, and transfer it to a 50-ml centrifuge tube.
2. Add 33 ml of 1M ammonium acetate solution to the tube, stopper, and shake in a mechanical shaker for 5 minutes.
3. Centrifuge at 2 000 rpm for 5 minutes or until the supernatant is clear.
4. Decant the solution into a 100-ml volumetric flask.

5. Repeat steps 2–4.
6. Make up the volume to 100 ml with ammonium acetate.
7. Atomize the working standards on an AAS at a wavelength of 313.5 nm. Prepare a standard curve of known concentration of Mo by plotting the absorbance values on the y-axis against their respective Mo concentration on the x-axis.
8. Measure the absorbance of the soil sample extract and determine the Mo content in the soil from the standard curve.

The relevant calculation is:

Content of Mo in the sample (mg/kg) = *C* µg/ml × 20 (dilution factor).

where:

- *C* = concentration of Mo in the sample, as read from the standard curve for the given absorbance;
- dilution factor = 20.0 (soil sample taken = 5 g and volume made to 100 ml).

Estimation by colorimetric method

The apparatus required consists of:

- a spectrophotometer;
- a hotplate;
- a refrigerator;
- a water-bath.

The reagents required are:

- 50 percent potassium iodide solution: Dissolve 50 g in 100 ml of DDW.
- 50 percent ascorbic acid solution: Dissolve 50 g in 100 ml of DDW.
- 10 percent sodium hydroxide solution: Dissolve 10 g of NaOH in 100 ml of DDW.
- 10 percent thiourea solution: Dissolve 10 g in 100 ml of DDW and filter. Prepare a fresh solution on the same day of use.
- Toluene-3,4-dithiol solution (commonly called dithiol): Place 1.0 g of AR-grade melted dithiol (51 °C) in a 250-ml glass beaker. Add 100 ml of the 10 percent NaOH solution and warm the content up to 51 °C with frequent stirring for 15 minutes. Add 1.8 ml of thioglycolic acid, and store in a refrigerator.
- 10 percent tartaric acid: Dissolve 10 g in 100 ml of DDW.
- Iso-amyl acetate.
- Ethyl alcohol.
- Ferrous ammonium sulphate solution: Dissolve 63 g of the salt in about 500 ml of DDW and then make the volume up to 1 litre.
- Nitric–perchloric acid mixture (4:1).
- Extracting reagent: Dissolve 24.9 g of AR-grade ammonium oxalate and 12.6 g of oxalic acid in water, and make the volume up to 1 litre.
- Standard stock solution (100 µg/ml Mo): Dissolve 0.150 g of AR-grade MoO_3 in 100 ml of 0.1M NaOH, make slightly acidic with dilute HCl, and make the volume up to 1 litre.

- Working standard solution (1 µg/ml Mo): Dilute 10 ml of the stock solution to 1 litre.

The procedure is:

1. Weigh 25 g of air-dry soil sample in a 500-ml conical flask. Add 250 ml of the extracting solution (1:10 ratio) and shake for 10 hours.
2. Filter through No. 50 filter paper. Collect 200 ml of the clear filtrate in a 250-ml glass beaker and evaporate to dryness in a water-bath.
3. Heat the contents in the beaker at 500 °C in a furnace for 5 hours to destroy OM and oxalates. Keep overnight.
4. Digest the contents with 5 ml of HNO_3–$HClO_4$ mixture (4:1), followed by 10 ml of 4M H_2SO_4 and then with H_2O_2, each time bringing to dryness.
5. Add 10 ml of 0.1M HCl and filter. Wash the filter paper, first with 10 ml of 0.1M HCl and then with 10 ml of DDW, until the volume of the filtrate is 100 ml.
6. Run a blank side by side (without soil).
7. Put 50 ml of the filtrate in 250-ml separatory funnels, add 0.25 ml of ferrous ammonium sulphate solution and 20 ml of DDW, and shake vigorously.
8. Add excess of potassium iodide solution and clear the liberated iodine by adding ascorbic acid drop by drop while shaking vigorously.
9. Add 1 ml of tartaric acid and 2 ml of thiourea solution, and shake vigorously.
10. Add 5 drops of dithiol solution, and allow the mixture to stand for 30 minutes.
11. Add 10 ml of iso-amyl acetate, and separate out the contents (green colour) in colorimeter tubes/cuvettes.
12. Read the colour intensity at 680 nm (red filter).
13. Preparation of standard curve: Measure 0, 2, 5, 10, 15 and 20 ml of the working standard Mo solution containing 1 mg/litre Mo in a series of 250-ml separatory funnels. Proceed for colour development as described above for sample aliquots. Read the colour intensity and prepare the standard curve by plotting Mo concentration against readings.

The relevant calculation is.

Content of Mo in the soil (µg/g or mg/kg) = *C* µg/ml × dilution factor (0.5)

where:

- C (µg/ml) = concentration of Mo as read from the standard curve against the absorbance reading of the soil solution on the spectrophotometer;
- dilution factor = 0.5, which is calculated as follows:
  - weight of the soil taken = 25 g;
  - volume of extract = 250 ml;
  - first dilution = 10 times;
  - volume of the filtrate taken = 200 ml;
  - filtrate digested (concentrated) to 100 ml;
  - volume of concentrated filtrate taken = 50 ml;
  - second dilution = 0.25 times;

- volume of solvent (iso-amyl acetate) used for extraction = 10 ml;
- third dilution (50 ml extracted by 10 ml) = 0.2 times;
- total dilution = 10 × 0.25 × 0.2 = 0.5 times.

Chapter 4
Plant analysis

Plant and soil testing enables scientific assessment of the needs of the plant for nutrient elements and of the capacity of the soil to supply them. The nutrient elements enter the plant in ionic form from the soil solution. Ion transport to the root surface may take place through ion diffusion and bulk transport (mass flow). Mass flow is the sweeping along of ions as water moves to the root. It is particularly important for ions that are not absorbed on soil colloids such as nitrates and sulphates. Because of mass flow, a plant deficient in N can extract all the nitrates from the soil. Potassium, phosphate and the micronutrient cations are absorbed on soil colloids with various degrees of affinity and are greatly retarded in movement with the soil water. Diffusion (movement along a concentration gradient) is the main mode of transport from the solid phase to the root surface for these non-mobile ions. There is also a possibility that some of them are absorbed by a direct exchange of an ion, usually hydrogen, between the root surface and ions absorbed on soil colloids. Most ions are sorbed by the root against a concentration gradient and, thus, the process involves the use of metabolic energy. The fact that nutrient uptake is an active process explains some of its peculiarities. Plants not only accumulate nutrients against a concentration gradient, they are also able to select from the nutrients at the root surface according to their requirements (preferential uptake).

The understanding of the mechanism of nutrient uptake by plants explains why a chemical solvent for non-mobile elements does not extract the same amount of nutrient as does the root, and any attempt to match the value is futile. The root can contact only a part of the soil, while a chemical solvent can contact all but some internal surfaces. Plant analysis generally provides more current plant-based information than soil testing but it is more costly and entails more effort in terms of sampling, sample handling and analysis. Ideally, both tools should be used as they complement each other.

Unlike soil analysis where the amount of available nutrients are of importance, in plant analysis, the total content of nutrients is relevant. The available nutrients taken from the soil or fertilizer source are assimilated by the plants and, thus, they become part of crop composition.

Knowledge of nutrient concentration in growing plants can serve as a tool for correcting any deficiencies where carried out early enough to safeguard yield. The nutrient uptake by a healthy crop, which has attained its growth and yield potential, is taken as the effective requirement for the crop. It can also be used to evaluate the efficacy of a recent application. The information it provides can help to plan nutrient application in subsequent crops on that field or to compute nutrient removals in relation to productivity and nutrient balance sheets.

Although the nutrient contents vary from one species to another, the composition of a particular species is generally considered to be indicative under optimal nutrient supply and growth conditions. General guidelines of optimal nutrient content in crops can be used for understanding the probable deficiency of nutrients (Table 14).

Whole plant analysis is conducted in order to determine the total nutrient uptake (which is usually carried out on the shoot). For plant analysis to be meaningful as a diagnostic tool, the collection of particular plant parts (tissue) at the right stage of growth for analysis is very important. Plant leaves are considered the focus of physiological activities. The concentrations of leaf nutrients appear to reflect changes in mineral nutrition. As an example, Table 15 shows the specific parts, as identified by various researchers, to be sampled from different plant species. Their concentrations are expected to reflect the true nutrient status of a growing plant (deficiency, sufficiency or excess).

The interpretation of plant analysis data is usually based on the total concentrations of nutrients in the dry matter of leaves or other suitable plant parts compared with standard values of "critical nutrient concentrations" ("critical values"). Between the nutrient concentrations of the deficiency range and those of adequate supply, there is the critical nutrient range. The critical level is that level of concentration of a nutrient in the plant that is likely to result in 90 percent of the maximum yields. The main advantage of critical values, once properly established, is their wide applicability for the same crop. Their disadvantage is that they only provide "yes or no" type of information and do not cover the entire range over which nutrient supplies need to be managed. Table 16 presents some critical values for a range of crops.

TABLE 14
General sufficiency or optimal range of nutrients in plants

Nutrients	Sufficiency or optimal range
Macronutrients	(%)
N	2.0–5.0
P	0.2–0.5
K	1.0–5.0
Ca	0.1–1.0
Mg	0.1–0.4
S	0.1–1.3
Micronutrients	(µg/g)
Zn	20–100
Fe	50–250
Mn	20–300
Cu	5–20
Mo	0.1–0.5
B	10–100

TABLE 15
Typical plant parts suggested for analysis

Crop	Part to be sampled, with age or growth stage
Wheat	Flag-leaf, before head emergence
Rice	3rd leaf from apex, at tillering
Maize	Ear-leaf before tasselling
Barley	Flag-leaf at head emergence
Pulses	Recently matured leaf at bloom initiation
Groundnut	Recently matured leaflets at maximum tillering
Soybean	3rd leaf from top, 2 months after planting
Cotton	Petiole, 4th leaf from apex, at initiation of flowering
Sugar cane	3rd leaf from top, 3–5 months after planting
Tea	3rd leaf from tip of young shoots
Potato	Most recent, fully developed leaf (half-grown)
Tomato	Leaves adjacent to inflorescence (mid-bloom)
Onion	Top non-white portion (1/3 to 1/2 grown)
Beans	Uppermost, fully developed leaves
Pea	Leaflets from most recent, fully developed leaves, at first bloom
Apple, pear	Leaves from middle of terminal shoot growth, 8–12 weeks after full bloom, 2–4 weeks after formation of terminal buds in bearing trees
Cherry	Fully expanded leaves, mid-shoot current growth in July–August
Peach	Mid-shoot leaves, fruiting or non-fruiting spurs, mid-summer leaves
Strawberry	Fully expanded matured leaf without petiole, at peak or harvest period
Banana	Petiole of 3rd open leaf from apex, 4 months after planting
Papaya	3–5-month old leaves from new flush
Pineapple	Middle third portion of white basal portion of 4th leaf from apex, at 4–6-month stage

TABLE 16
Critical nutrient concentrations for 90-percent yield for various crops

Element	Wheat & rice	Oilseed rape	Sugar cane	Alfalfa	Grass*	Citrus
N (%)	3.0	3.5	1.5	3.5	3.0	2.5
P (%)	0.25	0.3	0.2	0.25	0.4	0.15
K (%)	2.5	2.5	1.5	2.0	2.5	1.0
Mg (%)	0.15	0.2	0.12	0.25	0.2	0.2
S (%)	0.15	0.5	0.15	0.3	0.2	0.15
Mn (µg/g)	30	30	20	30	60	25
Zn (µg/g)	20	20	15	15	50	20
Cu (µg/g)	5	5	3	5	8	5
B (µg/g)	6	25	1.5	25	6	25
Mo (µg/g)	0.3	0.3	0.1	0.2	0.3	0.2

Notes:
Growth stage and plant parts used: Wheat/rice: 1–2 nodes, whole shoots; oilseed rape: pre-flowering, youngest mature leaf; sugar cane: 5–7 months, third leaf blade from top; alfalfa: pre-flowering, whole shoots; grass: pre-flowering, whole shoots; citrus: 5–7 month-old leaves from middle of non-fruiting branch.
* Not critical but optimal concentrations for cows producing 15 litres of milk per day.

Critical values determine whether immediate action such as foliar spraying is needed to correct a deficiency. Conclusions can also be drawn as to whether the amount of fertilizer applied at sowing time was sufficient or should be increased for the next crop. Where the concentration is in the toxicity range, special countermeasures are required but no application is called for. For some nutrients, e.g. Ca and Fe, the "active" (mobile) nutrient content of plants should be considered because immobilization can make the total concentrations misleading. The nutrient concentrations of green (fresh) material or of plant sap can be used as a suitable basis for interpretation in some situations.

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

Representative sampling should be done of specific plant parts at the growth stage that is most closely associated with critical values as provided by research data. Sampling criteria and procedures for individual samples are similar to those of soil testing in that the sample should be representative of the field. A predetermined, representative number of plants from a homogenous sampling unit contribute to the composition of bulk sample. The composite sample should be about 200–500 g fresh weight. Factors such as the desired precision of recommendation, the nature of the crop (seasonal or perennial) and economic considerations should be taken into account. The following procedure is suggested:

1. For analysis of seasonal crop plants, pick a few representative plants at random from each plot. Remove the shoot (aerial part) with the help of a sharp stainless steel cutter for whole shoot analysis or the desired part for analysis of specific plant parts.
2. If roots are to be included, uproot the whole plant carefully from wet soil, retaining even the fine/active roots. Dip the plant roots gently in water several times to remove adhering soil.
3. Wash with water several times.
4. Wash the samples with about 0.2 percent detergent solution to remove the waxy/greasy coating on the leaf surface.
5. Wash with 0.1M HCl followed by thorough washing with plenty of water. Give a final wash with distilled water.
6. Wash with DDW if micronutrient analysis is to be carried out.
7. Soak to dry with tissue paper.
8. Air-dry the samples on a perfectly clean surface at room temperature for at least 2–3 days in a dust-free atmosphere.
9. Put the samples in an oven, and dry at 70 °C for 48 hours.
10. Grind the samples in an electric stainless steel mill using a 0.5-mm sieve. Clean the cup and blades of the grinding mill before each sample.
11. Put the samples back in the oven, and dry again for constant weight. Store in well-stoppered plastic or glass bottles or in paper bags for analysis.

ANALYTICAL METHODS

The plant sample can be brought into solution form through digestion with acids that dissolve the solid plant parts and bring the plant nutrient in liquid form for estimation. This is called wet digestion. The plant sample can also be heated at high temperatures to destroy OM, and the ash so obtained can be dissolved in acids to bring the sample into liquid form for estimation. This method is called dry ashing.

Wet digestion

A mixture of HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 9:4:1 is used for sample digestion. It is known as tri-acid digestion. When only two acids, viz. HNO_3 and $HClO_4$ (9:4), are used, it is known as di-acid digestion.

Perchloric acid ($HClO_4$) is used primarily for increasing the efficiency of oxidation of the sample as $HClO_4$ dissociates into nascent chlorine and oxygen at high temperature, which increases the rate of oxidation or the digestion of the sample. At times, perchloric acid causes an explosion when it comes into direct contact with the plant sample. Therefore, pre-digestion of the sample with HNO_3 is considered desirable, followed by treatment with the di-acid or tri-acid mixture.

Generally, 1 g of ground plant sample is taken for analysis. It is placed in a 100-ml volumetric flask, and 10 ml of acid mixture is added and the contents are mixed by swirling. The flask is placed on a hotplate in the fumehood and heated, starting at 80–90 °C and then the temperature is raised to about 150–200 °C. Heating continues until the production of red NO_2 fumes ceases. The contents are further heated until the volume is reduced to 3–4 ml and becomes colourless, but it should not be dried. After cooling the contents, the volume is made up with the distilled water and filtered through No. 1 filter paper. This solution is used for nutrient estimation.

Di-acid digestion is used for determination of most of the elements (P, K, Ca, Mg, S, Fe, Mn, Zn and Cu). However, tri-acid digestion is preferred for P and K estimations. It cannot be used for S estimation owing to the presence of H_2SO_4. Sulphuric acid also contains many trace elements as contaminants. Therefore, micronutrients should preferably be estimated through di-acid digestion or by using a dry-ash sample solution.

Wet digestion can also be accomplished by using H_2O_2 to destroy OM followed by digestion with H_2SO_4 to dissolve the sample. In such digestion, N estimation can be carried out as per the Kjeldahl method of total N estimation as described in Chapter 3.

Dry ashing

High-temperature oxidation destroys the OM. The plant sample is ashed at 500–600 °C by placing a suitable weight (0.5–1.0 g) of the sample in a silica crucible and heating it in a muffle furnace for 4–6 hours. The ash residue is dissolved in dilute HNO_3 or

HCl, filtered through acid-washed filter paper in a 50/100-ml volumetric flask, and the volume is made up to the mark. The estimation of K, Ca, Mg and micronutrients (including B and Mo) is carried out in the dry-ashed sample solution.

Dry ashing is a preferred method for the analysis of P, K, Ca, Mg and trace elements, especially B and Mo. It is a relatively simple method and requires very little operational attention. It does not involve the use of perchloric acid. It also avoids the use of boiling acids. However, at times, incomplete recovery of some elements may be caused by:

- Volatilization of elements such as S (also Se and halogens). To avoid loss of S, $Mg(NO_3)_2$ should be mixed with plant samples while dry ashing.
- Retention of elements such as Cu on the walls of silica crucibles. Hence, platinum crucible should be used.
- Formation of compounds that are not completely soluble in the acid used for digestion.

A blank should always be carried out to account for any contamination through the acids used in the digestion.

Nitrogen

Total N in plants is estimated by the Kjeldahl method (Chapter 3). In plants, N is present in protein form, and digestion of the sample with H_2SO_4 containing digestion mixture (10 parts potassium sulphate and 1 part copper sulphate) is required for estimation. Sample size may be 0.5–1.0 g depending on the type of crop and the plant part.

The procedure for sample digestion, distillation and estimation of N is the same as for total N estimation in soil.

Phosphorus

Estimation of total P can be carried out by any of the following methods:

- gravimetric quinolinium phosphomolybdate;
- gravimetric ammonium phosphomolybdate;
- volumetric quinolinium phosphomolybdate;
- volumetric ammonium phosphomolybdate;
- spectrophotometric vanadium phosphomolybdate.

The selection of a method depends on a number of factors, the important ones are:

- speed;
- accuracy;
- reproducibility of results;
- cost of chemicals;
- applicability in the presence of most commonly occurring/interfering cations and anions.

Some of these factors are affected by the quantity and the form of the element to be estimated. Generally, gravimetric methods (which are quite accurate) can be used when the quantity of the element in the sample is quite large.

Therefore, the gravimetric quinolinium phosphomolybdate method is widely used for P estimation in fertilizer samples. The spectrophotometric vanadium phosphomolybdate method is used for P estimation in plant samples where the content is small. Similarly, volumetric methods are also considered suitable for P estimation in plant samples.

Spectrophotometric vanadium phosphomolybdate method

The P content of the plant sample is converted to orthophosphates by digestion with an acid mixture (di-acid or tri-acid). The digested sample is used for P estimation. When orthophosphates are made to react with molybdate and vanadate, a yellow-coloured vanadomolybdophosphoric heteropoly complex is formed. The intensity of the yellow colour is directly proportional to the concentration of P present in the sample, which can be read on the spectrophotometer.

The apparatus required consists of:

- a digestion block;
- a spectrophotometer;
- some beakers/flasks.

The reagents required are:

- Ammonium molybdate – ammonium vanadate in HNO_3 (vanadomolybdate): Dissolve 22.5 g of $(NH_4)_6MO_7O_2.4H_2O$ in 400 ml of distilled water. Dissolve 1.25 g of ammonium vanadate in 300 ml of boiling distilled water. Add the vanadate solution to the molybdate solution and cool to room temperature. Add 250 ml of concentrated HNO_3 and dilute to 1 litre.
- Standard phosphate solution: Dissolve 0.2195 g of analytical-grade KH_2PO_4 and dilute to 1 litre. This solution contains 50 µg P/ml.

The procedure is:

1. Preparation of the standard curve: Put 0, 1, 2, 3, 4, 5 and 10 ml of standard solution (50 µg P/ml) in 50-ml volumetric flasks. Add 10 ml of vanadomolybdate reagent to each flask and make up the volume. The P contents in these flasks are 0, 1, 2, 3, 4, 5 and 10 µg P/ml, respectively. The standard curve is prepared by measuring these concentrations on a spectrophotometer (420 nm) and recording the corresponding absorbances.
2. Take 1 g of plant sample and digest as per the wet digestion method, and make the volume up to 100 ml.
3. Put 5 ml of digest in a 50-ml volumetric flask, and add 10 ml of vanadomolybdate reagent.
4. Make up the volume with distilled water, and shake thoroughly. Keep for 30 minutes.
5. A yellow colour develops, which is stable for days and is read at 420 nm on spectrophotometer.
6. For the observed absorbance, determine the P content from the standard curve.

The relevant calculation is:

$$\text{P content } (\mu g) \text{ in 1 g of sample} = C \times df$$

$$\text{P content (g) in 100 g sample (\% P)} = \frac{C \times df \times 100}{1\,000\,000} = \frac{C \times 1\,000 \times 100}{1\,000\,000} = \frac{C}{10}$$

where:

- C = concentration of P (µg/ml) as read from the standard curve;
- df = dilution factor, which is 100 × 10 = 1 000, as calculated below:
  - 1 g of sample made to 100 ml (100 times);
  - 5 ml of sample solution made to 50 ml (10 times).
- 1 000 000 = factor for converting µg to g.

Potassium

Potassium estimation can be done on a flame photometer, an AAS or by the volumetric sodium tetraphenyl boron method. In a soil/plant analysis laboratory, the use of an AAS is very common and a large number of elements are estimated using this equipment.

Estimation by AAS

The acid-digested or dry-ashed plant sample is used for determining K.

The apparatus required consists of:

- an AAS;
- some volumetric flasks.

The reagents required are:

- Di-acid/tri-acid digestion mixture.
- KCl (AR-grade) standard solution: Dissolve 1.908 g of pure KCl in 1 litre of distilled water. This solution contains 1 mg K/ml. Take 100 ml of this solution and dilute to 1 litre. This will give 100 µg K/ml as stock solution.
- KCl working standard solution: Put 5, 10, 15 and 20 ml of stock solution in 100-ml volumetric flasks. Make up the volume. This will give 5, 10, 15 and 20 µg K/ml, respectively.

The procedure is:

1. Set up the AAS and standardize. The relevant parameters for K estimation on an AAS are:
   - lamp current = 6 m A°;
   - wavelength = 766.5 nm;
   - linear range = 0.4–1.5 µg/ml;
   - slit width = 0.5 nm;
   - integration time = 2 seconds;
   - flame = air acetylene.
2. Preparation of the standard curve: Prepare the standard curve using 0, 5, 10, 15 and 20 µg K/ml. The curve will show a linear relationship between the concentration of K and absorbance on a specific wavelength as read from the AAS.

3. Acid-digest 1 g of plant sample and make up to 100 ml. Keep the sample for estimation in the range 5–10 mg K/kg (5–10 µg K/ml) by further diluting as appropriate.
4. Prepare a blank in the same way without adding plant digested material.
5. Take an aliquot of 5 ml for estimation and make up to 100 ml. Atomize on the calibrated AAS, on which the standard curve has also been prepared.
6. Record the absorbance against each sample.
7. From the standard curve, note the concentration of K for the particular absorbance observed for the sample.

The relevant calculation is:

$$\text{K content (µg) in 1 g of sample} = C \times df$$

$$\text{K content (g) in 100 g sample (\% K)} = \frac{C \times df \times 100}{1\,000\,000} = \frac{C \times 2\,000 \times 100}{1\,000\,000} = \frac{C}{5}$$

where:

- C = concentration of K (µg/ml) as read from the standard curve;
- df = dilution factor, which is 100 × 20 = 2 000, as calculated below:
  - 1 g of sample made to 100 ml (100 times);
  - 5 ml of sample solution made to 100 ml (20 times).
- 1 000 000 = factor for converting µg to g.

Sulphur

As dry ashing leads to volatilization loss of S present in the organic combination, and the wet oxidation based on tri-acid mixture includes H_2SO_4, these two methods cannot be used for S determination in plant samples. Therefore, di-acid (HNO_3–$HClO_4$) digestion is used. The turbidimetric method as described for soil S analysis (Chapter 3) is recommended for S analysis of plant samples. The main difference is in the extraction of S. In soils, it is the available S, while in plants, it is the total S, which is brought into solution by di-acid mixture digestion.

The apparatus required consists of:

- a spectrophotometer;
- a mechanical shaker;
- some volumetric flasks.

The reagents required are the same as given for S estimation in soil (Chapter 3), except monocalcium phosphate extracting solution.

The procedure is:

1. Preparation of the standard curve: Same as for S estimation in soil.
2. Digest 1 g of plant sample in di-acid and make the volume up to 100 ml.
3. Transfer 10 ml of the aliquot to a 100-ml volumetric flask.
4. Add 1 g of sieved $BaCl_2$ and shake for 1 minute.
5. Add 1 ml of gum acacia acetic – acid solution, make the volume up to the mark and shake for 1 minute.
6. Run a blank in an identical manner.

7. Measure the turbidity 25–30 minutes after the precipitation at 440 nm.
8. Read the S content in the sample from the standard curve against the similar absorbance as noted for the sample.

The relevant calculation is:

$$\text{S content } (\mu g) \text{ in 1 g of sample} = C \times df$$

$$\text{S content (g) in 100 g sample (\% S)} = \frac{C \times df \times 100}{1\,000\,000} = \frac{C \times 000 \times 100}{1\,000\,000} = \frac{C}{10}$$

where:

- C = concentration of S (µg/ml) as read from the standard curve;
- df = dilution factor, which is 100 × 10 = 1 000, as calculated below:
  - 1 g of sample made to 100 ml (100 times);
  - 10 ml of sample solution made to 100 ml (10 times).
- 1 000 000 = factor for converting µg to g.

Calcium

Estimation by AAS is described here. However, Ca estimation in the acid digest can also be done by the EDTA titration method (Chapter 3).

The apparatus required consists of:

- an AAS;
- some volumetric flasks;
- a fumehood;
- a hotplate;
- a muffle furnace (when dry ashing has to be done).

The reagent required is:

- Standard Ca solution: Take 0.2247 g of primary standard $CaCO_3$ and add 5 ml of deionized water. Add about 10 ml of HCl to ensure complete dissolution of $CaCO_3$. Dilute to 1 litre with deionized water. This will give Ca solution of 100 µg Ca/ml. Dilute 10 ml of this solution to 100 ml to obtain 10 µg Ca/ml.

The procedure is:

1. Take 1 g of prepared plant sample. Digest in di-acid, and make the volume up to 100 ml.
2. Dilute the sample solution to 10–20 times depending on expected content of Ca, which can be estimated from the standard curve prepared for the purpose.
3. Set up and calibrate the AAS using the relevant parameters:
   - lamp current = 10 m A^0;
   - wavelength = 422.7 nm;
   - linear range = 1–4 µg/ml;
   - slit width = 0.5 nm;
   - integration time = 2 seconds;
   - flame = nitrous oxide acetylene.

4. After setting the AAS, atomize the standard solutions of different concentrations of Ca and record the absorbance for the respective concentrations of Ca. Plot the concentration of Ca on the x-axis and the corresponding absorbance on the y-axis in order to prepare the standard curve.
5. Put 5 ml of the sample solution in a 100-ml volumetric flask and make up the volume, atomize, and observe the absorbance. Note the corresponding concentration for the absorbance recorded that represents the content of Ca in the sample solution.

The relevant calculation is:

$$\text{Ca content (µg) in 1 g of sample} = C \times df$$

$$\text{Ca content (g) in 100 sample (\% Ca)} = \frac{C \times df \times 100}{1\,000\,000} = \frac{C \times 2\,000 \times 100}{000\,000} = \frac{C}{5}$$

where:

- C = concentration of Ca (µg/ml) as read from the standard curve;
- df = dilution factor, which is 100 × 20 = 2 000, as calculated below:
  - 1 g of sample made to 100 ml (100 times);
  - 5 ml of sample solution made to 100 ml (20 times).
- 1 000 000 = factor for converting µg to g.

Magnesium

Estimation by AAS is described here. However, Mg estimation in the acid digest can also be done by the EDTA titration method as described for soils (Chapter 3).

The apparatus required consists of:

- an AAS;
- some volumetric flasks.

The reagent required is:

- Standard Mg solution: Dissolve 10.141 g of $MgSO_4.7H_2O$ in 250 ml of deionized water, and make the volume up to 1 litre. This will give 1 000 µg Mg/ml solution.

Under this procedure, the preparation of the standard curve, the estimation and the calculation procedure are the same as described for Ca estimation (above). The relevant parameters for estimation by AAS are:

- lamp current = 3 m $A^{0;}$
- wavelength = 285.2 nm;
- linear range = 0–0.5 µg/ml;
- slit width = 0.5 nm;
- integration time = 2 seconds;
- flame = air acetylene.

Micronutrients

The estimation of Zn, Mn, Cu, Fe, B and Mo can be done in the same manner as the estimations of K, Ca and Mg (above) using an AAS. However, for B and Mo, chemical methods are preferred, which have been described in Chapter 3. The preparation of the standard curve for each of these metal elements is different and full descriptions are given in Chapter 3. In brief, they are:

- Zn: 1 g of pure zinc dissolved in 20 ml of HCl (1:1) and diluted to 1 000 ml gives 1 mg/ml Zn stock solution. Working solutions can be obtained by diluting the stock solution from 100 to 1 000 times to obtain 10 µg to 1 µg/ml.
- Fe: 1 g of pure iron wire is dissolved in 30 ml of HCl (1:1) and diluted to 1 000 ml to obtain 1 mg/ml of standard Fe. By diluting further, a working solution of a different concentration is obtained.
- Mn and Cu: Standard Mn and Cu solutions are prepared by dissolving 1 g each of pure Mn and Cu metal in 30 ml of HCl (1:1) and making the volume up to 1 000 ml. It will give 1 mg/ml Mn and Cu solutions. Further dilutions can be done to obtain working solutions.
- B: Dissolve 8.819 g of $Na_2B_4O_7.10H_2O$ in warm water. Dilute to 1 litre to obtain 1 mg/ml B stock solution. Dilute further for working solutions and preparation of standard curve.
- Mo: Dissolve 0.15 g of MoO_3 in 100 ml of 0.1M NaOH. Dilute to 1 litre to obtain 1 000 µg/ml or 1 mg/ml of Mo stock solution. Dilute further for working solutions and preparation of standard curve.

Table 17 lists the parameters of different hollow cathode lamps and the fuel to be used for estimation of different elements.

The estimation on an AAS is carried out first by making the standard curve for each element and then estimating the plant sample digested in acid.

The quantity of the element in the sample is calculated by multiplying the concentration observed from the standard curve by the dilution factor. This quantity is present in 1 g of sample taken for estimation. By further multiplying this value by 100, the percentage content is determined.

TABLE 17
Parameters for micronutrient estimation by AAS

Element	Wavelength	Flame	Approximate working range of standard
	(nm)		*(µg/ml)*
Zn	213.8	Air acetylene	0.4–1.5
Fe	248.3	Air acetylene	2.0–9.0
Mn	279.5	Air acetylene	1.0–3.6
Cu	324.7	Air acetylene	1.0–5.0
B	249.7	Nitrous oxide acetylene	1–4
Mo	313.3	Nitrous oxide acetylene	1–4

The relevant example calculation, which is valid for all micronutrients, is:

Micronutrient content (µg) in 1 g of sample = $C \times df$

$$\text{Micronutrient content (g) in 100 g sample (\%)} = \frac{C \times df \times 100}{1\,000\,000} = \frac{C \times 1\,000 \times 100}{000\,000} = \frac{C}{10}$$

where:

- C = concentration of micronutrient (µg/ml) as read from the standard curve;
- df = dilution factor, which is 100 × 10 = 1 000, as calculated below:
  - 1 g of sample made to 100 ml (100 times);
  - 10 ml of sample solution made to 100 ml (10 times).
- 1 000 000 = factor for converting µg to g.