COURSE GUIDE

SLM 309
SOIL ANALYTICAL TECHNIQUES

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INTRODUCTION

All scientists generally depend on analysis as a major means of solving scientific problems. This is to enable them proffer solutions to numerous questions on the content of the materials with which they work. The methods by which these analyses are carried out are referred to as analytical techniques. The types of analytical techniques may vary from field to field. This variation stems primarily from differences in the nature of materials with which people in each field works.

Soil science is branch of applied sciences that makes use of the principles of physics, chemistry, biology and mathematics in the study of soil. This is because the soil consists of matter (living and non-living) interacting together physically, chemically and biologically to provide ecosystem services at all levels of consideration. For a soil scientist to be able therefore to answer questions on the constituents of soil, he needs to carry out one form of analysis or the other. Methods of analysis in soil science consist of chemical, biological and physical techniques.

WHAT YOU WILL LEARN IN THIS COURSE

The course consists of modules in units and a course guide. This course guide tells you briefly what the course is about, what course materials you will be using and how you can work with these materials. In addition, it advocates some general guidelines for the amount of time you are likely to spend on each unit of the course in order to complete it successfully. It gives you guidance in respect of your Tutor-Marked Assignment in the assignment file. There will be regular tutorial classes that are related to the course. It is advisable for you to attend these tutorial sessions. The course will prepare you for the challenges you will meet in the field of soil pedology and classification.

COURSE AIMS

The aim of the course is not complex. The course aims to provide you with an understanding of soil survey and land evaluation; it also aims to provide you with solutions to problems with soil classification in the field.

COURSE OBJECTIVES

To achieve the aims set out, the course has a set of objectives. Each unit has specific objectives which are included at the beginning of the unit.

You should read these objectives before you study the unit. You may wish to refer to them during your study to check on your progress. You should always look at the unit objectives after completion of each unit. By doing so, you would have followed the instructions in the unit. Below are the comprehensive objectives of the course as a whole. By meeting these objectives, you should have achieved the aims of the course as a whole. In addition to the aims above, this course sets to achieve some objectives.

Thus, after going through the course, you will be able to:

- explain precision, accuracy and operational variation in analytical techniques
- identify different types of laboratory chemicals
- discuss major analytical instruments and their principles of operations
- examine how to carry out plant and soil sampling and sample preparation
- carry out nitrogen and phosphorus determinations in soil and plants
- carry out potassium analysis in soil, plants and fertilisers
- evaluate analytical data
- explain special techniques and precautions in carrying out micro-nutrient analysis
- discuss features and functions of a standard soil-testing laboratory.

WORKING THROUGH THE COURSE

To complete this course, you are required to read each study units, read the textbook and other materials which may be provided by the National Open University of Nigeria. Each unit contains self-assessment exercises and at certain points in the course you would be required to submit assignment for assessment purpose. At the end of the course there is a final examination. The course should take you a total of 17 weeks to complete. Below you will find listed all the components of the course, what you have to do and how should allocate your time to each unit in order to complete the course on time and successfully. The details that you spend a lot of time to read. I would advise that you avail yourself the opportunity of attending the tutorial sessions where you have the opportunity of comparing your knowledge with that of other people.

THE COURSE MATERIALS

The main components of the course are:

1. The Course Guide
2. Study Units
3. References/Further Reading
4. Assignments
5. Presentation Schedule

STUDY UNITS

The study units in this course are as follows:

Module 1 The Basics of Analytical Laboratory

Unit 1 Features and Functions of a Standard Laboratory
Unit 2 Safety Measures in the Laboratory
Unit 3 Quality Assurance Control
Unit 4 Precision and Accuracy
Unit 5 Laboratory Chemicals

Module 2 Soil and Plant Analysis

Unit 1 Soil Sampling and Sample Preparation
Unit 2 Plant Sampling and Sample Preparation
Unit 3 Nitrogen Determination in Soil and Plant
Unit 4 Phosphorus Determination in Soil and Plant
Unit 5 Potassium Determination in Soils, Plants and Fertilisers

Module 3 Analytical Instruments and Principles of Operation

Unit 1 Principles and Operations of Colorimeter
Unit 2 Principles and Operations of Flame Photometer
Unit 3 Principles and Operation of Atomic Absorption Spectrophotometer (AAS)
Unit 4 Principles and Operation of A pH Meter

Module 4 Techniques and Precautions in Micronutrients Analysis, Features and Functions of a standard Soil-Testing Laboratory

Unit 1 Analytical Techniques for Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn)
Unit 2 Analytical Techniques for available Boron (B) and Molybdenum (Mo)

PRESENTATION SCHEDULE

The presentation schedule included in the course material gives you the important dates for the completion of tutor-marking assignments and attending tutorials. You are required to submit all your assignments by due date. You should guide against falling behind in your work.

ASSESSMENT

There are two types of assessments in the course. First is the tutor-marked assignments; and the second is a written examination.

In attempting the assignments, you are expected to apply the information, knowledge and techniques gathered during the course. The assignments must be submitted to your course tutor for formal Assessment in accordance with the deadlines stated in the Presentation Schedule and the Assignments File. The works you submit to your course tutor for assessment constitute 30 % of the total course mark.

At the end of the course, you will need to sit for a final written examination of two hours' duration. This examination will constitute 70% of your total course mark.

TUTOR-MARKED ASSIGNMENT

There are three Tutor-Marked Assignments (TMAs) to be submitted in this course. The TMAs constitute 30% of the total score. You are encouraged to work all the questions thoroughly.

Assignment questions for the units in this course are contained in the Assignment File. You will be able to complete your assignments from the information and materials contained in your set books, reading and study units. However, it is desirable that you demonstrate that you have read and researched more widely than the required minimum. You should use other references to have a broad viewpoint of the subject and also to give you a deeper understanding of the subject.

When you have completed each assignment, send it, together with a TMA form, to your tutor. Make sure that each assignment reaches your tutor on or before the deadline given in the Presentation File. If for any reason, you cannot complete your work on time, contact your tutor before the assignment is due to discuss the possibility of an extension. Extensions will not be granted after the due date unless there are exceptional circumstance.

FINAL EXAMINATION AND GRADING

The final examination will be of two-hour duration and have a value of 70% of the total course grade. The examination will consist of questions which reflect the types of self-assessment practice exercises and tutor-marked problems you have previously encountered. All areas of the course will be assessed.

Revise the entire course material using the time between finishing the last unit in the module and that of sitting for the final examination. You might find it useful to review your self-assessment exercises, tutor-marked assignments and comments on them before the examination. The final examination covers information from all parts of the course.

HOW TO GET THE MOST FROM THIS COURSE

In distance learning, the study units replace the university lecturer. This is one of the great advantages of distance learning; you can read and work through specially designed study materials at your own pace and at a time and place that suit you best.

Think of it as reading the lecture instead of listening to a lecturer. In the same way that a lecturer might set you some reading to do, the study units tell you when to read your books or other material, and when to embark on discussion with your colleagues. Just as a lecturer might give you an in-class exercise, your study units provides exercises for you to do at appropriate points.

Each of the study units follows a common format. The first item is an introduction to the subject matter of the unit and how a particular unit is integrated with the other units and the course as a whole. Next is a set of learning objectives. These objectives let you know what you should be able to do by the time you have completed the unit.

You should use these objectives to guide your study. When you have finished the unit you must re-check whether you have achieved the

objectives. If you make a habit of doing this, you will significantly improve your chances of passing the course and getting the best grade.
The main body of the unit guides you through the required reading from other sources. This will usually be either from your set books or from a readings section.

Self-assessments are interspersed throughout the units, and answers are given at the end of the units. Working through these tests will help you achieve the objectives of the unit and prepare you for the assignments and the examination. You should do each self-assessment exercises as you come to it in the study unit. Also, ensure to master some major historical dates and events during the course of studying the material.

If you run into any trouble, consult your tutor. Remember that your tutor's job is to help you. When you need help, don't hesitate to call and ask your tutor to provide it. The following is a practical strategy for working through the course:

1. Read this Course Guide thoroughly.
2. Organise a study schedule. Refer to the `Course overview' for more details. Note the time you are expected to spend on each unit and how the assignments relate to the units. Important information, e.g. details of your tutorials, and the date of the first day of the semester is available from study centre. You need to gather together all this information in one place, such as your diary or a wall calendar. Whatever method you choose to use, you should decide on and write in your own dates for working breach unit.
3. Once you have created your own study schedule, do everything you can to stick to it. The major reason that students fail is that they get behind with their course work. If you get into difficulties with your schedule, let your tutor know before it is too late for help.
4. Turn to Unit 1 and read the introduction and the objectives for the unit.
5. Assemble the study materials. Information about what you need for a unit is given in the `Overview' at the beginning of each unit. You will also need both the study unit you are working on and one of your set books on your desk at the same time.
6. Work through the unit. The content of the unit itself has been arranged to provide a sequence for you to follow. As you work through the unit you will be instructed to read sections from your set books or other articles. Use the unit to guide your reading.
7. Up-to-date course information will be continuously delivered to you at the study centre.

8. Work before the relevant due date (about 4 weeks before due dates), get the Assignment File for the next required assignment. Keep in mind that you will learn a lot by doing the assignments carefully. They have been designed to help you meet the objectives of the course and, therefore, will help you pass the exam. Submit all assignments no later than the due date.
9. Review the objectives for each study unit to confirm that you have achieved them. If you feel unsure about any of the objectives, review the study material or consult your tutor.
10. When you are confident that you have achieved a unit's objectives, you can then start on the next unit. Proceed unit by unit through the course and try to space your study so that you keep yourself on schedule.
11. When you have submitted an assignment to your tutor for marking do not wait for its return `before starting on the next units. Keep to your schedule. When the assignment is returned, pay particular attention to your tutor's comments, both on the tutor-marked assignment form and also written on the assignment. Consult your tutor as soon as possible if you have any questions or problems.
12. After completing the last unit, review the course and prepare yourself for the final examination. Check that you have achieved the unit objectives (listed at the beginning of each unit) and the course objectives (listed in this Course Guide).

TUTORS AND TUTORIALS

There are some hours of tutorials (two-hour session) provided in support of this course. You will be notified of the dates, times and location of these tutorials. Together with the name and phone number of your tutor, as soon as you are allocated a tutorial group.

Your tutor will evaluate and comment on your assignments, keep a close watch on your progress and on any difficulties you might encounter during the course. You must mail your tutor-marked assignments to your tutor well before the due date (at least two working days are required). They will be marked by your tutor and returned to you as soon as possible.
Do not hesitate to contact your tutor by telephone, e-mail, or discussion board if you need help. The following might be circumstances in which you would find help necessary. Contact your tutor if you:

- do not understand any part of the study units or the assigned readings
- have difficulty with the self-assessment exercises

- have a question or problem with an assignment, with your tutor's comments on an assignment or with the grading of an assignment.

You should try your best to attend the tutorials. This is the only chance to have face to face contact with your tutor and to ask questions which are answered instantly. You can raise any problem encountered in the course of your study. To gain the maximum benefit from course tutorials, prepare a question list before attending them. You will learn a lot from participating in discussions actively.

SUMMARY

On successful completion of the course, you would have developed sufficient critical thinking skills with the material necessary for efficient and effective discussion on issues related to soil survey and land evaluation; you will also be able to proffer solutions to problems with soil classification in the field.

We wish you success in the course and hope that you will find it exciting.

MAIN COURSE

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MODULE 1 THE BASICS OF ANALYTICAL LABORATORY

Unit 1 Features and Functions of a Standard Laboratory
Unit 2 Safety Measures in the Laboratory
Unit 3 Quality Assurance Control
Unit 4 Precision and Accuracy
Unit 5 Laboratory Chemicals

UNIT 1 FEATURES AND FUNCTIONS OF A STANDARD SOIL LABORATORY

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1.0 Introduction
2.0 Objectives
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        3.1.1 Standard Laboratory Building
        3.1.2 Choosing a Laboratory for Analytical work
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

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 fertiliser samples. Temperature also affects some chemical operations. Hence, maintaining the temperature and humidity as specified is critical.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the features and functions of a standard laboratory
- choose a good laboratory for analytical works.

3.0 MAIN CONTENT

3.1 Features and Functions of a Standard Laboratory

Among other things, proper air circulation is important as this will 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 (FAO, 2008 has suggested a suitable format).

3.1.1 Standard Laboratory Building

A safe laboratory building should have suitable separate rooms for different purposes and for performing different operations as described below (figure 20):

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

e. Room 5. Chemical analysis room (separate for soil/plant and fertilisers):
   - 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.

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

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

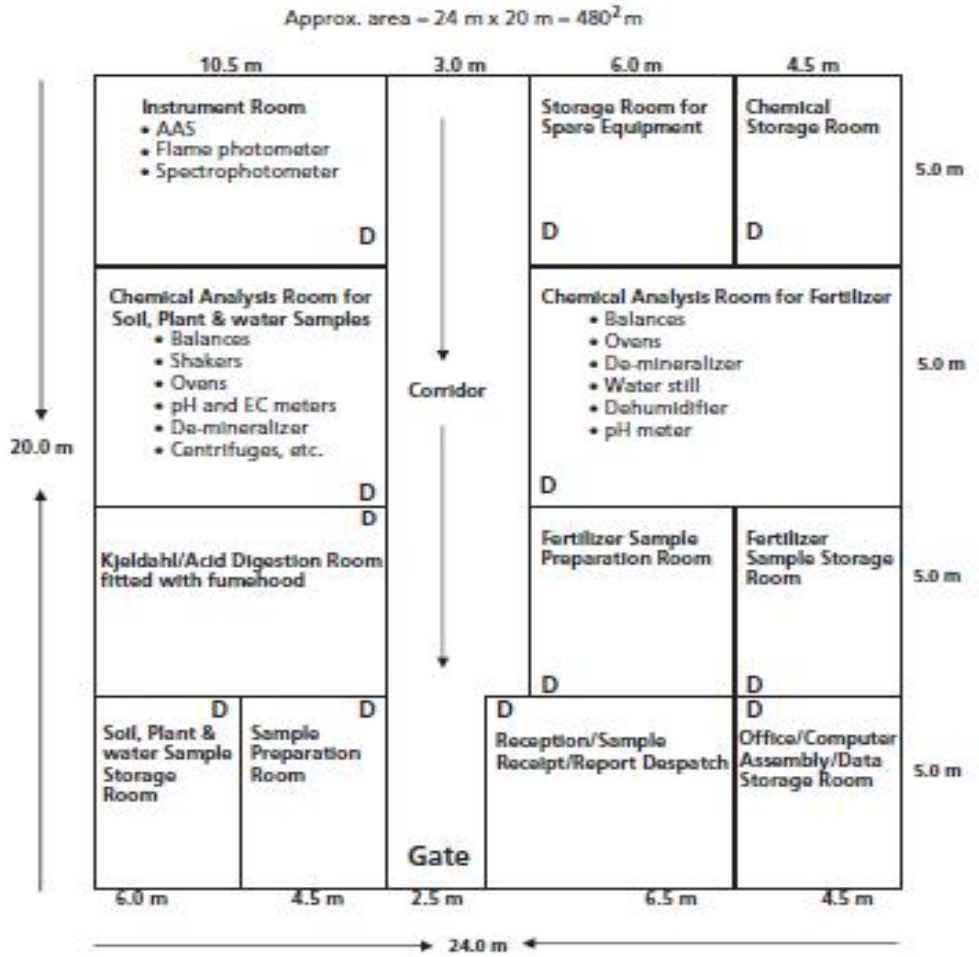

Fig 1.1: Laboratory Floor Plan for Soil, Plant and Fertiliser Analysis

3.1.2 Choosing a Laboratory for Analytical work

Selecting a laboratory that can supply fast and accurate results may be time-consuming; however, the time spent in choosing a good laboratory can quickly pay for itself in the form of accurate fertilizer recommendations, desired crop responses, and better economic returns. A high degree of variability has been observed in fertilizer recommendations among laboratories. Specifically, some studies have shown that when the same soils were sent to different laboratories requesting a fertilizer recommendation for a specific crop and yield goal, significantly different recommendations have been provided *(*Jacobsen *et al.*, 2002*)*. Fertilizer recommendations can differ because of differences in test results and/or the approach used to develop a recommendation.

In general, laboratories' methods are consistent and provide reliable results for Nitrate-N (NO_3-N), and K, whereas test results for soil organic matter are least reliable and test results for P are intermediate (Miller, 2013). However, researchers have found substantial differences in soil analytical results that may affect fertiliser recommendations. In a study of 10 laboratories used by northern Great Plains consultants and producers, Nitrate-N concentrations reported for the same soil ranged from 3 to 95 pounds N per acre *(*Jacobsen *et al.*, 2002*)*. This difference would alter fertilizer recommendations substantially. Phosphorus test results varied from 7 to 42 ppm, and averaged 28 ppm, in one of the four soils tested in this study. Phosphorus fertilizer would likely be recommended for a soil with a 7 ppm test result, yet would likely be wasted on this soil if the actual value was similar to the average soil test P value of 28 ppm (well above the critical level). Selecting a laboratory belonging to a proficiency testing program which should greatly enhance accuracy.

Some of the differences in results may be due to different analytical methods. For example, laboratories either use Bray, Olsen or Mehlich P tests, all of which use different extractants. In Montana's alkaline soils, P is typically tested using Olsen P, also known as bicarbonate-P. Olsen P is fairly robust and works below pH 7, while Bray does not generally work well above pH 7. Bray and Mehlich test results do not convert readily to Olsen P. Because P fertilizer guidelines for Montana crops are based on Olsen P, ask the soil testing lab to only use this test. Unfortunately, some laboratories do not report which test they use. Therefore, it is critical that the user of soil analytical results be aware of the actual analytical procedure since different tests may produce different results.

Fertiliser recommendations are based on a sufficiency, build, or maintenance approach. The sufficiency approach only suggests fertilization when there are insufficient amounts of nutrients in the soil for the present crop. With the sufficiency approach, "critical" values are used to determine whether a field should be fertilised. The critical value is the nutrient test result above which only minimal yield responses are observed when fertilizer is added. A maintenance approach replaces nutrients removed by crop harvest without substantially changing the soil test level, whereas a build approach increases the soil test level over time. The build approach is generally only practiced at low soil test levels, and maintenance is generally only done at higher soil test levels.
The resulting economic differences between different philosophies and different laboratories can be large. For example, in a multi-year study of fertilizer recommendations from six different laboratories, fertiliser recommendations from one lab were double those of another lab, yet were no significant differences in yields (Follet*et al.*, 1984). In another study, the greatest economic return was four times higher than the least economic return based on six different labs' fertilizer recommendations (Davis *et al.*, 1999), demonstrating the importance of accurate fertilizer recommendations.

Growers can assist laboratories in making accurate recommendations by selecting realistic yield goals. These must reflect the soil type and area climate. A recommendation that is satisfactory on calcareous soil in a semi-arid area may be very different than one for a soil containing the same nutrient level but from an area with different soil type and climate.

3.1.3 Assessing Laboratory Accuracy

Any quality assurance/ quality control (QA/ QC) data supplied by a laboratory should have both a "true" value and a measured value for each analysis (pH, Nitrate-N, P, etc.) for a certified soil, sometimes referred to as an "external standard." The true values reported are typically the average results from many reputable laboratories for that standard, producing a "mean" value. The Agriculture Laboratory Proficiency Program reports the mean, while the North American Proficiency Testing Program reports the "median" value, which is the concentration that has the same number of labs reporting higher values as lower values, and is often close to the mean. By subtracting the difference between the true and the laboratory measured concentration and dividing this difference by the true value, you can obtain a relative estimate of "error" (accuracy), that is, how close the laboratory's test comes to the true value. You can compare the errors reported by laboratories that you are considering using, and choose a laboratory that has a low error (high accuracy) for the test(s) that you are most interested in (e.g., Nitrate-N, P).

Laboratories should also have high "precision," which is a measure of how consistent the results are for the same sample. Some laboratories may report the "standard deviation," which is a measure of how precise the results are (low standard deviation implies high precision).

4.0 CONCLUSION

In general, laboratories' methods are consistent and may provide reliable results for Nitrate-N (NO_3-N), and K, whereas test results for soil organic matter are least reliable and test results for P are intermediate. However, researchers have found substantial differences in soil analytical results that may affect fertilizer recommendations.

5.0 SUMMARY

Quality assurance/ quality control (QA/ QC) data supplied by a laboratory should have both a "true" value and a measured value for each analysis (pH, Nitrate-N, P, etc.) for a certified soil, sometimes referred to as an "external standard." The true values reported are typically the average results from many reputable laboratories for that standard, producing a "mean" value.

6.0 TUTOR-MARKED ASSIGNMENT

1. What are the major climatic factors to be considered while building a standard laboratory and why?
2. Name different rooms in a standard laboratory and their main contents.
3. Outline major instruments installed in the instrument room of a standard laboratory.
4. Discuss why it is very necessary to be careful while choosing a laboratory for analysis.
5. Discuss why accuracy is very important in soil analytical technique.

7.0 REFERENCES/FURTHER READING

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UNIT 2 SAFETY MEASURES IN THE LABORATORY

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        3.2.1 Different Categories Where Safety is Needed
        3.2.2 Handling Contaminations
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

Safety is an important consideration in any soil/plant laboratories even though it is frequently overlocked. To avoid injuries that may lead to deformation or even death, all safety precaution in the Laboratory must be strictly adhered to. Therefore, safety is of interest to every employee who works in the labs as well as any organizations which operate laboratories. All staff, not minding his/her grade, technical skills or employment status should be properly educated on the subject of safety when laboratories are concerned especially at commencement of work. From time to time, reminders of such regulations should be done to encourage staff familiarity with such regulations.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the safety precautions of a soil science laboratory
- identify safety precautions in the laboratory to avoid any casualty.

3.0 MAIN CONTENT

3.1 Important Facts to Know

Always double-check the name of the reagent to be used and the name of the reagent you are using. Reagent bottles should remain stoppered, except when you are actually pouring solutions out of them. Always replace the stopper or lid of stock solutions or stains. Be sure to put them on the container they came from. Take only as much as you need and never return leftover solutions to a reagent bottle. Discard leftovers in the proper container. Also note the following:

i. Do not use your thumb as a stopper. Swirl gently or put a piece of parafilm over the opening to mix solutions.
ii. Label all test tubes and other containers with contents.
iii. Do not pour reagents and chemicals down the sink. Dispose of these only in designated containers.
iv. Put plant remains in the designated containers. Do not discard solids or plant materials down the sinks. They will clog up the sink. Use specified containers for such wastes
v. Be sure to use caution when using razor blades to prepare lab materials. Put the used blades into the designated container from which you obtained them. Do not leave them loose on the desks or counters. Never put razor blades in the wastepaper basket.
vi. Your instructor will review with you the location and, where applicable, use of the safety equipment in the laboratory including: emergency phone, first aid kit, fire extinguisher, eyewash

3.2 Laboratory Safety Measures

Laboratory is not a place of playing or carrying out any careless activity. Any one entering laboratory should be very careful and be conscious of what he/she has gone there to do. However, rules pertaining to safety can be extensive, different categories of concerns have been noted (Rashid, *et al.*, 2007).

3.2.1 Different Categories Where Safety is Needed

1. General attitude
2. Instrument operation
3. Accidents
4. Chemicals
5. Furnace, Ovens and Hot plates

6. Handling gasses
7. Maintenance
8. Eating and drinking
9. Protective Equipment
10. Waste disposals

General safety rules in different categories to ensure safety

1. General Attitude

i. Develop a positive attitude towards laboratory safety
ii. Observe normal laboratory safety practices
iii. Maintain a safe and clean work environment
iv. Avoid working alone

2. Instrument operation

i. Follow safety precautions provided by the manufacturer when operating instruments
ii. Monitor instruments while they are in operation
iii. Do not open centrifuge cover until machine has completely stopped
iv. Atomic Absorption Spectrophotometer (AAS) must be vented to the atmosphere, ensure that the drain trap is filled with water before igniting the burner

3. Accidents

i. Learn what to do in case of emergencies (fire, spill of chemicals, etc.), firefighting equipment must be readily accessible in the event of fire.
ii. Learn emergency first aid. First aid supplies are a necessity and laboratory staff should be well trained in their use.
iii. Immediately seek medical attention if affected by chemicals, use first aid until medical aid is available
iv. Access to eye-wash fountains and safety showers must not be locked. Fountain and showers should be regularly checked for proper operation

4. Chemicals

i. Add acid to water and not water to acid when diluting the acid.
ii. Always put labels on bottles, vessels and wash-bottles containing reagents, solutions, samples and water.
iii. Handle perchloric acid and hazardous chemicals in fumehoods.

iv. With the wet oxidation method of sample digestion, destroy organic matter (OM) first with nitric acid.
v. Read the labels on the bottles before opening them.
vi. Wash hands after handling toxic/hazardous chemicals.
vii. Never suck the chemicals by mouth but use automatic pipetting devices.

5. Maintenance

i. All electrical, plumbing and instrument maintenance work should be done by qualified personnel
ii. Fume hoods should be checked routinely
iii. As most equipment operate on low wattage, use of Uninterrupted power supply (UPS) is necessary to provide stable power and allows the completion of any batch measurement in the event of power outage

6. Eating and drinking

i. Do not eat, drink or smoke in the laboratory. This is essential both for reasons of health and to reduce contamination
ii. Do not use laboratory glassware for eating/drinking.
iii. Do not store food in the laboratory

7. Protective equipment

i. Use personal safety equipment as follow:

- **Body protection**: Use laboratory coat and chemical resistant apron
- **Hand protection**: use gloves particularly when handling concentrated acids, bases and other hazardous chemicals
- **Dust mask:** usually needed when grinding soil
- **Eye protection:** Use safety glasses with side shields. Persons wearing contact lens should always wear safety glasses in the laboratory. Make sure the people you work with know you are using contact lenses and it should not be worn around corrosives.
- **Full face shield**: wear face shields over safety glasses in experiments involving corrosive chemicals
- **Foot protection:** proper foot wear should be used; sandals should be worn in the laboratories.

8. Waste disposal

i. Liquid waste should be poured carefully down the sink with sufficient water to dilute and flush it away.

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

3.2.2 Handling Contaminations

One of the most insidious enemies in any laboratory is contamination and therefore its sources must be identified and eliminated.

Some common sources of contaminations are as follow:

1. External dusts blown from the surrounding environment
2. Internal dusts resulting from cleaning operations
3. Cross- contaminations derived from handling many samples at the same time (e.g. handling plant and soil samples together)
4. Failure to store volatile reagents well away from the samples
5. Washing materials especially soap powder
6. Smoking in the laboratory

4.0 CONCLUSION

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. 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. 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.

5.0 SUMMARY

You have learnt that safety precautions in the laboratory include but not limited to the following: Food and drink must not be allowed in lab unless food or drinks are provided as a part of the lab requirement for the intended analysis. Even though lab tables and counters are wiped down before each lab set up, as a result of some laboratory exercises, chemical residues may be present on the tables. For labs exercises involving food or drinks, lab assistants and instructors will follow procedures that allow safe consumption. Smoking is totally not

acceptable in all University buildings. Shoes must be worn in lab. If you have very long hair, please tie it back in the laboratory.

Handle chemicals, reagents, and stains carefully and follow all warnings. All bottles and containers are labeled as to contents and potential hazards. If, for example, a label says avoid contact with substance and fumes, do so. For potentially hazardous chemicals, information on the hazards, proper handling, and clean-up is provided on Material Safety Data Sheets (MSDS). These are available in the lab. It is highly recommended that you spend the first few minutes of the lab consulting the MSDS. Read all precautions in the laboratory manual and on labels and follow directions exactly.

6.0 TUTOR-MARKED ASSIGNMENT

1. Why is safety a very important consideration in a soil science laboratory?
2. Mention 10 different categories where safety is needed in a soil science laboratory
3. Give at least five safety precautions in five of the categories mentioned above.
4. What are the sources of contaminations in the laboratory and how can they be handled?

7.0 REFERENCE/FURTHER READING

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UNIT 3 QUALITY ASSURANCE CONTROL

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        3.2.4 Validation of Analysis Procedures
    3.3 Quality Control of Analytical Procedures
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1.0 INTRODUCTION

In order to achieve uniformity of expression and good understanding, this study material adopts the definitions of the terms quality, quality assurance and quality control as defined by the International Standardisation Organisation (ISO) and also those compiled in FAO (2008). 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. 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. 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.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the meaning of quality assurance control
- define quality, quality assurance and quality control.

3.0 MAIN CONTENT

3.1 Laboratory Quality Assurance/Control

i. Quality

A product can be said to possess good quality if it meets the predetermined parameters. 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. Analytical laboratory therefore is considered to be of good quality 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.

ii. Quality assurance

Quality assurance according to ISO 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.

iii. 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:

a. Check on the performance of the instruments;
b. Calibration or standardization of instruments and chemicals;
c. Adoption of sample check system as a batch control within the laboratory;
d. External check: inter-laboratory 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).

3.2 Quality Control of Analytical Procedures

3.2.1 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.

3.2.2 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.

3.2.3 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 recognise the material as such.

3.2.4 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.

1. **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 practiced 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.

The following steps have to be implemented to achieve Internal quality control

a. Use a blank and a control (standard) sample of known composition along with the samples under analysis.
b. 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 than5, 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:

i. The sample is homogenous.
ii. The sample material is stable.
iii. The material has uniform and correct particle size as sieved through a standard sieve.
iv. 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.

2. 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 inter-laboratory 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.

3.3 Inter-Laboratory Sample and Data Exchange Programme

Where an error is suspected in the procedure and the uncertainty cannot be resolved readily, it is advisable to have the sample analysed in another laboratory of the same system/organisation. 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 inter-laboratory 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 organisations 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:

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

Developed by Wageningen Evaluating Programme for Analytical Laboratories (WEPAL) of Wageningen Agricultural University, the Netherlands.

Other programmes run by Wageningen Agricultural University are:

3. International Sediment Exchange for Tests on Organic Contaminants (SETOC);
4. 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 fertiliser 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.

4.0 CONCLUSION

You have learnt that analytical laboratory is considered to be of good quality 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. This will enable an acceptable judgement on the product quality. You have also studied quality assurance according to ISO, which 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. An 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 is an important part of quality assurance, and it is 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.

5.0 SUMMARY

You have learnt that 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. When this must have been achieved, analysis on an unknown sample can start. Apart from independent standards, certified reference samples can also be used as “standard”. Such samples are gotten 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.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define the following according to International Standardisation Organisation definitions:
   i. Quality
   ii. Quality Assurance
   iii. Quality Control

2. State four steps that must be followed in quality control system, that will ensure that the results delivered are acceptable and verifiable.

3. Mention and briefly explain 4 Quality controls of analytical procedures.

4. Write short notes on the following;
   i. Validation of own procedure
   ii. Validation of standard procedure

5. State four Inter-laboratory sample and data exchange programme you studied and the body that developed them.

7.0 REFERENCES/FURTHER READING

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UNIT 4 PRECISION AND ACCURACY

CONTENTS

2.0 Introduction
2.0 Objectives
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3.1.1 Precision/Repeatability/Reproducibility
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1.0 INTRODUCTION

The issues of accuracy and precision are vital considerations for the analyst and can be determining factors in any method selection (Jones 2001). Accuracy is the ability to obtain the "true" value and is dependent to a large degree on the availability and use of reliable standards. Precision, on the other hand, is a measure of the degree of variability of an obtained result determined by repeated analyses of the same sample through all the steps from sample preparation to the final obtained result. Rayment *et al.*, (2000) looked at the percent coefficients of variation for a number of commonly performed soil analysis procedures, found that the pH determination had the lowest (<5%), that determinations for Cl, organic C, Nitrate-N, DTPA Zn and Mn, and exchangeable Ca, K, and Mg ranged from 10 to 20%, that exchangeable Na was between 20 and 30%, and that Olsen P and Bray P were greater than30%. They also looked at intra-laboratory RSDs (%), finding that the greatest precision was obtained for the determination of pH (RSD of 1.6%), that determinations for ammoniun-N, Bray P1, and organic carbon had RSDs of6.2, 6.3, and 8.3%, respectively, and that Nitrate-N (Cd reduction) and Olsen P had RSDs of 14.5 and 11.8%, respectively. All these evaluations would suggest that there may be an inherent variance in a particular method and/or that the methodology associated with these assay procedures should be more carefully examined to determine the source of variance.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the meaning of accuracy and precision in soil analytical procedures
- explain what to do to ensure accuracy and precision in soil analytical technic.

3.0 MAIN CONTENT

3.1 Precision and Accuracy

Precision is defined as the agreement without conflict or 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.

3.1.1 Precision/Repeatability/Reproducibility

Precision is a term that describes an instrument's degree of freedom from random errors. If a large number of readings are taken of the same quantity by a high precision instrument, then the spread of readings will be very small. Precision is often, though incorrectly, confused with accuracy. High precision does not imply anything about measurement accuracy. A high precision instrument may have a low accuracy. Low accuracy measurements from a high precision instrument are normally caused by a bias in the measurements, which is removable by recalibration. The terms repeatability and reproducibility mean approximately the same but are applied in different contexts as given below.

Repeatability describes the closeness of output readings when the same input is applied repetitively over a short period of time, with the same measurement conditions, same instrument and observer, same location and same conditions of use maintained throughout.

Reproducibility describes the closeness of output readings for the same input when there are changes in the method of measurement, observer,

measuring instrument, location, conditions of use and time of measurement. Both terms thus describe the spread of output readings for the same input. This spread is referred to as repeatability if the measurement conditions are constant and as reproducibility if the measurement conditions vary. The degree of repeatability or reproducibility in measurements from an instrument is an alternative way of expressing its precision.

Figure 1 below shows the results of tests on three industrial robots that were programmed to place components at a particular point on a table. The target point was at the centre of the concentric circles shown, and the red x-marks represent the points where each robot actually deposited components at each attempt. Both the accuracy and precision of Robot 1 are shown to be low in this trial. Robot 2 consistently puts the component down at approximately the same place but this is the wrong point. Therefore, it has high precision but low accuracy. Finally, Robot 3 has both high precision and high accuracy, because it consistently places the component at the correct target position.

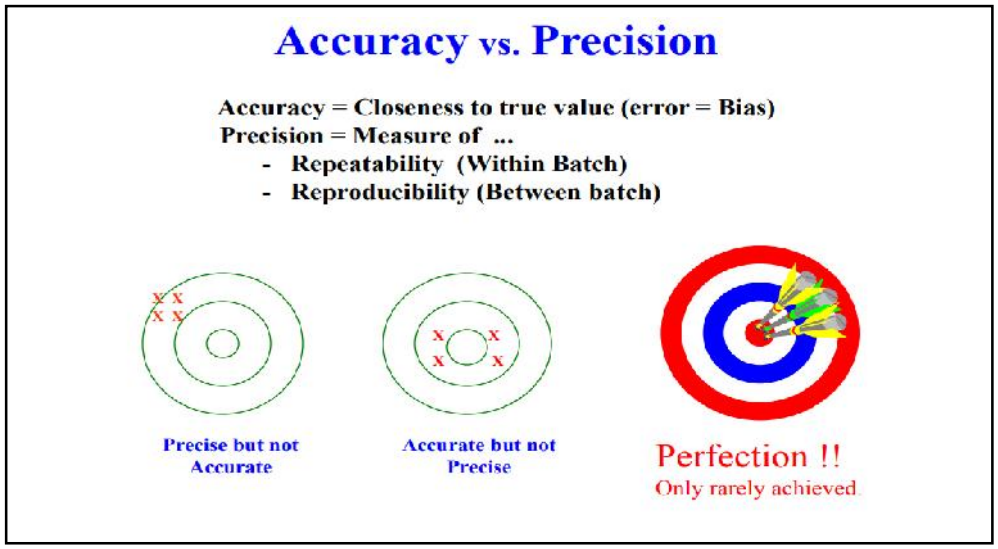

Fig. 1.2: Test of Accuracy and Precision

3.2 Errors

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.

Part of improving chemical analysis is to be able to identify the place where errors can occur and be able to evaluate their magnitude. There is possibility of making mistakes at any step of the analysis. These possible mistakes are called error. One way to find out if you have made mistake is to repeat the whole procedure for several replicate samples or replicates. The more the replicates the higher the level of certainty of the results.

3.2.1 Sources of Errors

Errors could be classified based on the sources as:

1. Personal error: These are errors that emanate from the person carrying out the analysis (the analyst). It could be due to lack of experience, dirty or unorganized work habit, lack of attention to details, or personal defect that may pose hindrance to accurate judgement (e.g. colour blindness).

2. Method errors: These are errors that emanate due to use of wrong method of analysis. Some methods may not be able to eliminate certain interferences in the course of analysis, thus leading to error.

3. Instrumental Errors: These are errors resulting from improper functioning of the instrument used for the measurement. It could be due to improper calibration or low sensitivity of instrument.

Errors could also be classified based on the whether or not the magnitude can be ascertained and correction made or not. Errors originating from fixed case are referred to as determinate or

1. *Systematic error*; while those originating from arbitrary or indeterminate processes are **Random errors**.

Measuring Errors

When several runs of replicates are made on a sample, it is not likely that the results would be exactly the same. We can make use of the Arithmetic mean (average) with deviation from mean (absolute deviation, or relative deviation).

3.3 Detection Limits

In the analysis for elements, particularly trace elements in soils, plants and fertilisers 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.

4.0 CONCLUSION

Accuracy is the ability to obtain the "true" value and is dependent to a large degree on the availability and use of reliable standards. Precision, on the other hand, is a measure of the degree of variability of an obtained result determined by repeated analyses of the same sample through all the steps from sample preparation to the final obtained result.

5.0 SUMMARY

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.

6.0 TUTOR-MARKED ASSIGNMENT

1. i. Define Precision and accuracy.
   ii. With the aid of diagram, show relationship between precision and accuracy.
2. What is the relationship between precision, repeatability and reproducibility?
3. i. What do you understand by error in an analytical process?
   ii. State four sources of error.
4. Explain fully what you know about detection limits.

7.0 REFERENCES/FURTHER READING

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UNIT 5 LABORATORY CHEMICALS

CONTENTS

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1.0 INTRODUCTION

After soil samples are received at a laboratory, a number of tests can be performed. These tests require different types of laboratory chemicals which are combined at different levels depending on the parameters being analysed for. This is called soil testing or soil analysis. A general understanding of soil testing will help you know how the results can be interpreted and to appreciate the accuracy of analytical results. Soils supply most of the mineral nutrition for higher plants through the plant's root system. The root system extracts nutrients from the soil over a long period of time; two to three months for most annual crops, years for perennial crops.

In contrast, a soil test determines the soil's nutrient supplying capacity by mixing soil for only a few minutes with a strong extracting solution (often an acid or a combination of acids). The soil reacts with the extracting solution, releasing some of the nutrients. The solution is filtered and assayed for the concentration of each nutrient. The nutrient concentration is then related to field calibration research that indicates the yield level reached with varying soil nutrient concentrations. This method works very well for some nutrients, but is less accurate for others, for example those nutrients supplied largely from organic matter (OM) decomposition such as nitrogen and sulfur. This is primarily due to the difficulty of estimating or predicting the rate at which OM will decompose and release these nutrients in plant-available forms.

Individual analyses included in a 'standard' or 'routine' soil test varies from laboratory to laboratory, but generally include soil pH, and available phosphorus (P) and potassium (K). They sometimes also include available calcium (Ca) and magnesium (Mg), salinity, and often

include an analysis of OM content and soil texture. Most laboratories offer nitrogen (N), sulfur (S), and micronutrient analyses for additional cost. The methods used to test soils vary depending on chemical properties of the soil. For example, tests used for measuring soil P are quite different in the acidic soils common in the southeastern U.S. than those used in the alkaline soils of the southwest. Analysis of southwestern soils with methods tailored for acidic soils will provide erroneous results. Therefore, it is important to be aware of the methods used by test labs, and to select methods that are regionally appropriate. Local laboratories will generally use methods appropriate for your soils and your laboratory should provide you with test method information.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss key chemicals used for laboratory analysis
- explain key soil properties that can be analyzed using analytical chemicals
- examine what standardisation means.

3.0 MAIN CONTENT

3.1 Reagents and Standards

Reagents: Reagents are chemicals used in carrying out soil or plant analysis in the laboratory. Reagents should be of **reagent** or **analytical** grade. The storage requirements for many of the reagents are frequently specified to ensure reliable performance. Commercially prepared reagents are sometimes available, particularly extraction reagents and standards; however, users are advised to test the quality of these reagents and standards before use.

Standards: For many, the use of commercially prepared standards, whose reliability is high, is convenient and saves time both in the preparation and verification testing required for user-prepared standards. Therefore, whenever possible, the use of commercially prepared standards is highly recommended. However, the source and labeling of standards are important considerations, ensuring freedom from analytes in a standard that may be included in a multi-element assay, as well as ensuring that the characteristics of the matrix (mix of cations and anions) and the acid content, whether nitric (HNO_3) or hydrochloric (HCl), or both, will not affect or interfere with the analytical procedure being used.

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

1. 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.

2. Normality

The normality of a solution is defined as 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. 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.

3.2 Types of Laboratory Chemicals

List of Reagents, Standards, pH Buffers, Acids, and Indicators, and Preparation of Standard Acids, Bases, and Buffers

A. Reagents

i. Aluminum chloride ($AlCl_3 \cdot 6H_2O$)
ii. Aluminum sulfate [$Al_2(SO4)_3$]
iii. Ammonium acetate ($NH_4C_2H_3O_2$)
iv. Ammonium chloride (NH4Cl)

v. Ammonium hydroxide (NH4OH)
vi. Ammonium iron sulfate [(NH4)2Fe(SO4)2·6H2O]
vii. Ammonium molybdate [(NH4)6Mo7O24·4H2O]
viii. Ammonium nitrate (NH4NO3)
ix. Ammonium sulfate [(NH4)2SO4]
x. Antimony metal (Sb)
xi. Calcium acetate [Ca(C2H3O2)2]
xii. Calcium carbonate (caco3)
xiii. Calcium chloride (cacl2·2H2O)
xiv. Calcium hydroxide [Ca(OH)2]
xv. Calcium nitrate [Ca(NO3)2·H2O]
xvi. Calcium oxide (CaO)
xvii. Calcium sulfate (CaSO4·5H2O)
xviii. CALGON (sodium heximetaphosphate)
xix. Citric acid ($C_6H_8O_7$)
xx. Copper sulfate (Cuso4·5H2O)
xxi. Potassium chloride (KCl)
xxii. Potassium dichromate (K2Cr2O7)
xxiii. Potassium dihydrogen phosphate (KH2PO4)
xxiv. Potassium hydroxide (KOH)
xxv. Potassium nitrate (KNO3)
xxvi. Potassium permanganate (kmno4)
xxvii. Potassium sulfate (K2SO4)

B. Reagents for Preparation of Standards

i. Ammonium dihydrogen phosphate (NH4H2PO4)
ii. Barium chloride ($BaCl_2$)
iii. Barium carbonate ($BaCO_3$)
iv. Barium nitrate [$Ba(NO3)_2$]
v. Boric acid (H_3BO_3)
vi. Calcium carbonate ($CaCO_3$)
vii. Calcium nitrate [$Ca(NO_3)_2$]
viii. Chromium (Cr) metal
ix. Chromium chloride ($CrCl_3·6H_2O$)
x. Cobalt (Co) metal
xi. Cobalt chloride (cocl2)
xii. Copper (Cu) metal
xiii. Copper oxide (CuO)
xiv. Humicacid
xv. Iron (Fe) wire
xvi. Iron oxide (Fe_2O_3)
xvii. Lead (Pb) metal
xviii. Lead nitrate [Pb(NO3)2]
xix. Lead oxide (PbO)
xx. Magnesium chloride (MgCl2)

C. Reagents for Preparation of pH Buffers

i. Boric acid (H3BO3)
ii. Citric acid (C6H8O7)
iii. Disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$)
iv. Monopotassium phosphate (KH_2PO_4)
v. Potassium chloride (Kcl)
vi. Potassium phthalate (KHC8H4O4)
vii. Sodium hydroxide (NaOH)

D. Concentrated Acids

i. *Glacial* acetic acid (CH_3COOH)
ii. Hydrochloric acid (Hcl)
iii. Hydrofluoric acid (HF)
iv. Nitric acid (HNO_3)
v. Perchloric acid ($HClO_4$)
vi. Phosphoric acid (H_3PO_4)
vii. Sulfuric acid (H_2SO_4)

E. Indicators

i. Bromocresol green
ii. Bromocresol purple
iii. Bromothymol blue
iv. Erichrome Black T
v. Malachite green oxalate
vi. Methyl red
vii. Methylene blue
viii. Phenolphathalein
ix. *n*-phenylanthranilic acid

4.0 CONCLUSION

You have learnt that soil reacts with the extracting solution during soil or plant analysis, releasing some of the nutrients. The solution is usually filtered and assayed for the concentration of each nutrient. The nutrient concentration is then related to field calibration research that indicates the yield level reached with varying soil nutrient concentrations. This method works very well for some nutrients, but is less accurate for others, for example those nutrients supplied largely from organic matter (OM) decomposition such as nitrogen and sulfur. This is primarily due to the difficulty of estimating or predicting the rate at which OM will decompose and release these nutrients in plant-available forms.

5.0 SUMMARY

You have studied that major chemicals used in the laboratory include the following; Reagents, Standards, pH Buffers, Acids, and Indicators, and Preparation of Standard Acids, Bases, and Buffers.

6.0 TUTOR-MARKED ASSIGNMENT

1. Mention at least seven soil properties analysed for in the soil science laboratory.
2. Explain briefly what you understand by the following:
   i. Reagents
   ii. Standards
   iii. Molarity
   iv. Normality
3. List at least seven analytical chemicals in the following categories:
   i. Reagents for the preparation of standards
   ii. Reagents for the preparation of pH buffers
   iii. Concentrated acids
   iv. Indicators

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MODULE 2 SOIL AND PLANT ANALYSIS

Unit 1 Soil Sampling and Sample Preparation
Unit 2 Plant Sampling and Sample Preparation
Unit 3 Nitrogen Determination in Soil and Plant
Unit 4 Phosphorus Determination in Soil and Plant
Unit 5 Potassium Determination in Soils, Plants and Fertilisers

UNIT 1 SOIL SAMPLING AND SAMPLE PREPARATION

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3.0 Main Content
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3.1.2 Soil Sampling Methods
3.1.3 Sampling Techniques
3.1.4 General Soil Sampling Guidelines
3.1.5 Soil Sampling Procedure
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3.1.7 Steps Adopted in Preparing Samples for Laboratory Analysis
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1.0 INTRODUCTION

Soil sampling is a method that can be used for exploration of soil resources that lack obvious surface manifestations. Soils that are above or adjacent to a “hidden” system will have a unique chemistry that can be indicative of such system at depth and a zone of relatively high permeability. 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.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the meaning of soil sampling and sample preparation
- explain the meaning of crop sampling and sample preparation
- examine the basic procedures, techniques and equipment's used in soil and crop sampling and sample preparations.

3.0 MAIN CONTENT

3.1 Soil Sampling and Sample Preparation

3.1.1 Soil Sampling

The major objectives of soil sampling have been to determine the average nutrient status of a field and to provide some measure of nutrient variability in a field. Soil sampling for precision agriculture is the trending dimension of agricultural researches and has these same objectives with some modifications. Instead of a field, producers are interested in areas within fields. They also are interested in relating trends in soil fertilizer levels to other field properties that are predictable or easily measured. Knowledge of factors influencing soil nutrient levels including soil type, topography, cropping history, manure application, fertilizer application and leveling for irrigation will help the producer determine the most effective sampling approach. The basic principles of soil sampling still apply to precision sampling. An adequate number of samples should be collected to accurately characterise nutrient levels. The samples should be collected to the proper depth for non-mobile and mobile nutrients. Samples should be handled and stored to minimize contamination and degradation.

3.1.2 Soil Sampling Methods

Properly collecting soil samples is the most important step in any nutrient/soil amendment management program. When considering soil sampling methods, the following should be borne in mind:

i. Soil sampling should reflect tillage,
ii. past fertiliser/soil amendment placement,
iii. cropping patterns (and corresponding irrigation requirements),
iv. soil type (including drainage and slope characteristics) and perhaps
v. old field boundaries (such as old feedlots, windrows, altered stream beds, etc.).

Trends toward reduced and/or zero tillage and technology for variable rate fertilisation (VRF) have especially demanded that soil samples be taken more comprehensively and intensively for more accurate fertiliser and soil amendment application. This section will discuss the many methods used for taking an accurate soil sample using various methods and under several different types of tillage situations.

The most commonly used method for soil sampling would be based on soil types. Fields are split into sampling areas that contain similar soils. Hillsides are kept separate from bottoms since the soil types will vary. Soil survey maps, if applicable, can help organise the soil types throughout the sampling area. Samples will not necessarily need to be collected for every soil type; however, similar soils should be kept together. Sampling maps can be kept to note the locations of the cores for subsequent sampling. The sampling area will be dependent on the soils and topography. Generally, an area of 40 acres is considered the maximum size. Smaller sampling areas may be needed if the soils are quite variable or a production problem is apparent.

Once the sampling area is determined, a sufficient number of cores should be taken to acquire a representative sample. This is generally 10 to 20 cores. The depth of sample for surface soils would be 0 to 6 inches or as deep as the primary tillage. Deeper samples to 24 or 36 inches can be taken for residual nitrate-nitrogen. These deep samples would be kept separate from the surface samples and noted accordingly on the bag and submittal form.

3.1.3 Sampling Techniques

Soil variability is a major concern when trying to obtain a representative soil sample. The strategy used to sample a field can address this challenge. Information collected during a site assessment can assist in

choosing an appropriate strategy for a particular field. Some of the sampling techniques that can be adopted include:

- Random composite sampling
- Directed random composite sampling
- Benchmark sampling
- Landscape-directed benchmark sampling
- Grid sampling
- Transect Sampling

1. Random Composite Sampling

Random composite sampling involves taking samples in a random pattern across a field, while avoiding unusual or problem soil areas. This strategy is most appropriate for fields less than 30 ha (80 ac), that have been uniformly cropped in the recent past and have little natural variation. This is the most common method of sampling presently used in most research works in Nigeria. For random sampling, collect cores from 15 to 20 sites and separate each core by depth to obtain representative bulk samples for each depth.

2. Directed Random Sampling

Directed or managed random sampling is a modified version of a random sampling strategy. This pattern is suited to fields or areas where it is difficult to identify a single dominant area that would represent most of the field. The study field is therefore sub-divided into management zones based on unique characteristics. For instance, if there are noticeable differences in yield throughout a field, management zones might be comprised of below average, average and above-average yielding areas. Take15 to 20 cores randomly from each management zone. A single field may require several bulk samples depending on the number of management zones. This technique might also be appropriate for areas with more than one soil type, fields with hummocky (rolling)landforms, and fields under strip-crop management.

3. Benchmark Sampling

Benchmark sampling involves selecting a small (30 m by30 m) representative site on a field. This site is used as a guide for fertilizing that entire field. Select probe sampling sites in a grid pattern within the benchmark area and prepare a composite sample for each soil depth. Sampling from the same small area each year reduces sampling variability and better reflects changes in soil nutrient level from year to year. Benchmark sampling sites should be marked with a GPS or by other means. When first using this sampling technique, it can be difficult

to select a benchmark site that best represents a field. Therefore, in the first year, it may be necessary to sample and analyse a number of potential benchmark sites. Initially, the costs for laboratory analysis will be higher but the most representative benchmarking site will be identified. If a single site does not adequately represent a field, it may be necessary to maintain multiple benchmark areas (i.e., directed benchmark sampling, see next section).

Features that helps in selecting a benchmark site

i. Look for features such as soil colour and landscape to identify where different soil types occur.
ii. Select a site that has characteristics similar to most of the field or the dominant soil type.
iii. Observe crop development patterns to assist in identifying different soil conditions. At the beginning of the growing season differences in crop establishment and vigor are more apparent, making a representative location easier to identify.
iv. Potential benchmark sites can also be selected based on yield,
v. Aerial photos or topographic maps, this gaining popularity particularly with increased use of GPS.
vi. GPS coordinates help to identify and locate the benchmark site for sampling each year.

4. Directed Benchmark Sampling

Directed benchmark sampling is a variation on the benchmark technique. It involves establishing multiple benchmark areas and management zones, based on topography or other characteristics. This strategy can be used when major areas within fields have distinct and well-defined features related to moisture (e.g., texture, slope). Management zones can be identified using soil surveys, detailed elevation mapping, aerial black and white photographs, yield maps or remote sensed images.

Directed benchmark sampling is only warranted if distinct areas are managed individually. For example, a soil analysis from a benchmark site in a low area suggests that it might respond to higher rates of N compared to a benchmark site on an upland area. Even without variable rate application capabilities, N application could be increased by other means to optimize yield in low areas.

5. Grid Sampling

Grid sampling is the most intense and expensive sampling strategy. It uses a systematic method to reveal fertility patterns and assumes there is

no topographic reason for fertility patterns to vary within a field. For grid sampling, a field is divided into small areas or blocks. A sample location within each block (e.g. the center point) is sampled 3 to 10 times. Sampling frequency may range from one sample from each 60 m ×60 m (0.5 ac) area of the field to one sample from each2 ha (5 ac) of the field. In general, the smaller the sampling unit, the greater the accuracy.

6. Transect Sampling

A transect is a path along which one counts and records occurrences of the species of study. It requires an observer to move along a fixed path and to count occurrences along the path and, at the same time (in some procedures), obtain the distance of the object from the path. This results in an estimate of the area covered and an estimate of the way in which detectability increases from probability 0 (far from the path) towards 1 (near the path). Using the raw count and this probability function, one can arrive at an estimate of the actual density of objects.

In soil sampling especially along a slope or a toposequence, a transect is usually made from the crest of the slope to its bottom and sampling carried out along the transect line.

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.

3.1.4 General Soil Sampling Guidelines

i. For any soil sampling Technique: Take 15 to 20 cores for each representative bulk sample. This number of samples is based on statistical precision.
ii. Each core will be segmented into lengths that represent depths of 0 to 15 cm, 15 to 30 cm and 30 to 60 cm.
iii. Separate the segmented cores by depth into clean, labeled plastic pails. Thoroughly mix the content of each pail, crushing any lumps in the process.

iv. Avoid using metal pails to collect samples because they can alter the results of micronutrient tests.
v. Take a single sub-sample (0.5 kg) for each sampling depth and submit for analysis.
vi. For hilly fields with knolls, slopes, or depressions, take samples from crest or top slope, mid-slope and foot slope positions to get a representative sample each part of the field.
vii. Avoid sampling obvious areas of unusual variability such as: saline areas, eroded soils, old manure piles, burn piles, haystacks, corrals, fence rows, old farmsteads or any other unusual areas.
viii. Soils within 15 m (50ft) of field borders or shelterbelts and within 50 m (150ft) of built-up-roads should be avoided or sampled separately.
ix. Always sample prior to manure or fertiliser applications.

3.1.5 Soil Sampling Procedure

i. Prepare a map of the area to be covered in a survey showing different sampling unit boundaries.

ii. 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.).
iii. 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.
iv. Generally, depending on the size of the field, 10–20 spots must be taken for one composite sample.
v. Scrape away surface litter to obtain a uniformly thick slice of soil from the surface to the plough depth from each spot.
vi. 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.
vii. In this way, collect samples from all the spots marked for one sampling unit.
viii. In the case of hard soil, take samples with the help of an auger from the plough depth and collect them in the bucket.
ix. 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.
x. Repeat the process until left with about0.5 kg of the soil. Collect it and put in a clean cloth bag. Mark each bag clearly in order to identify the sample.
xi. The bag used for sampling must always be clean and free from any contamination.

3.1.6 Methods for Sub-Sampling Soils

1. Mixing

The bulk soil sample should be thoroughly homogenized by mixing with a spatula, stirring rod, or other implement. As much of the sample as possible should be loosened and mixed together. No segregation of the sample by aggregate size should be apparent after mixing. Dip into the center of the mixed sample to obtain a subsample.

2. Weighing

Subsamples should be weighed into a tared vessel with a minimum precision of +/- 1%(e.g., 5.0 +/- 0.05 g). If a separate container is used to weigh and transfer subsamples to extraction vessels, it should be brushed out between samples to avoid cross-contamination.

3. Scooping

Dip into the center of the homogenized bulk sample with a standard soil scoop, filling it heaping full without pushing against the side of the soil container. Holding the scoop firmly, tap the handle three times with a spatula, two or three inches from the sample. Hold the spatula perpendicular to the top of the scoop and strike off the excess soil (Peck, 1988). If weight basis results are needed, empty the scoop contents into a tared weighing vessel and record the weight.

4. Depth of Soil Samples

Remove organic debris, rocks and trash from the surface of the soil sampling areas before the sample is collected the soil sample to plough layer. Select the sampling spot using any of the sampling techniques discussed earlier. Collect all the samples in a bucker. Depth of sampling is desired according to the purpose.

1. Soil fertility (15-25 cm)
2. Salinity and alkalinity (1m)
3. Establishment of gardens (2m)
4. Soil survey profile of (1-1.5m) depth

Keep record of the areas sampled and simple sketch map for reference.

Precautions

When sampling a soil, bear in mind the following:

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

vi. The information sheet should be filled in clearly with a copying pencil.

3.1.7 Steps adopted in preparing Samples for Laboratory Analysis

After complete collection of soil samples from field the organic residues like tree leaves, twigs, dung etc. and gravels, stones and other unwanted material should be kept out and sample should be prepared for laboratory analysis by adopting following steps.

1. Drying
2. Grinding
3. Sieving
4. Mixing
5. Partitioning
6. Weighing
7. Labelling
8. Storing

The soil samples should be air dried first and then grinding should be followed. A wooden mortar and pestle should be used for grinding to avoid contamination in the soil sample. After grinding soil samples should be sieved with 2mm mesh sieve and all the samples are then mixed thoroughly by spreading over cloth or paper. From this bulk soil

sample one representative soil sample should be collected following quartering method of portioning. About 250 to 500 g of soil sample is sufficient which should be stored in dry and clean poly bags, screw cap jars or card board boxes with proper labeling.

3.1.8 Sampling Tools and Sample Preparation

A sampling tool should be:

- uncontaminated
- approximately uniform in cross section to the desired depth
- provide reproducible sampling units

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

1. blades: trowel, spade, shovel, spoon, knife, cutlass
2. tubes: open-sided and plain cylinders
3. augers: wood-bit, post-hole, sheathed auger
4. a clean bucket or a tray or a clean cloth – for mixing the soil and subsampling;
5. cloth bags of a specific size;
6. a copying pencil for markings, and tags for tying cloth bags;
7. soil sample information sheet.

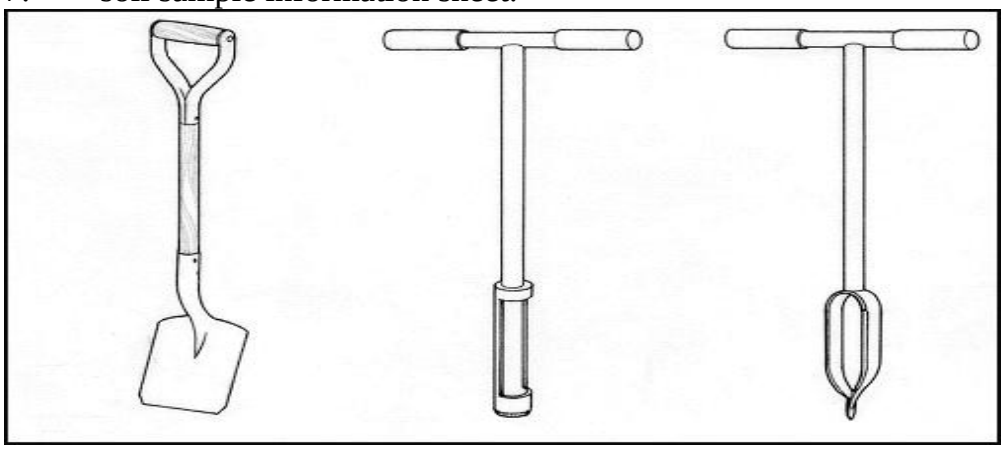

Fig. 2.1: Blade, Tube, And Auger (Left to Right)

For comparison over periods of time, take soil samples at approximately the same time of the year (e.g. before planting). When making comparisons, consider other factors like weather conditions, crops, treatments, seasonal fluctuations.

Select sampling depth according to the purpose of sampling. For soil fertility evaluations in annual crops, sample at a depth of 0 to 15 or 0 to 20 cm. For perennial crops (e.g. trees), take deeper samples since tree roots often grow deep into the soil.

Soil samples usually need preparation before laboratory analysis:

- **Air-drying.** Crush large soil clods to facilitate drying. Do not dry at high temperature. During air-drying, avoid contamination (i.e. from dust, gases, rain, etc.). Air-drying usually takes one week.
- **Crushing.** Crush the sample in a mortar using a rubber or porcelain-capped pestle (Figure 6). The mortar is usually made from porcelain.

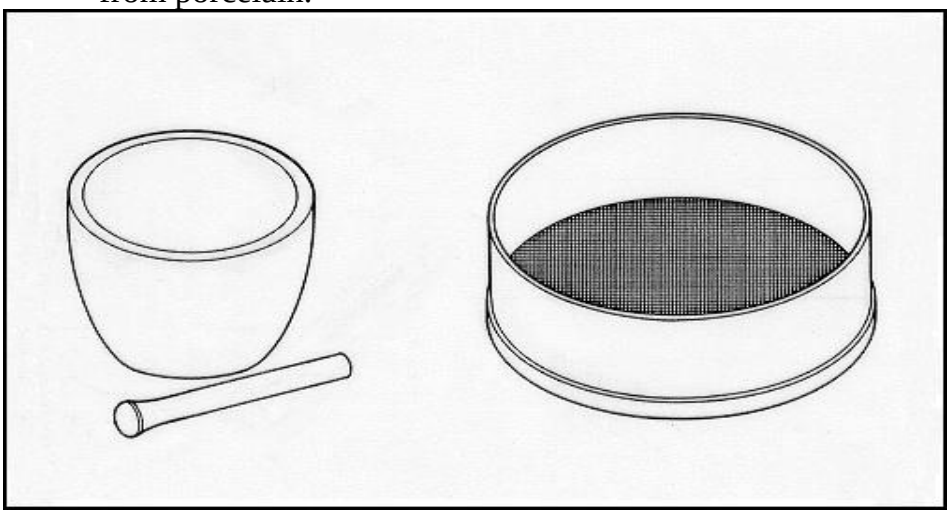

Fig. 2.2: Mortar and Pestle, Sieve

- **Pulverising:** Not all samples require pulverizing. The subsampling error is a function of the ratio between the average weight of the largest particles and the weight of the subsample. If the subsample being analysed is small (i.e. for total nitrogen and organic carbon analysis), the sample has to be pulverised to a fine powder (less than 0.5 mm).
- **Sieving:** Sieve the soil through a 2 mm sieve made of brass, stainless steel, or plastic. Use plastic sieves when micronutrients are to be analyzed.
- **Mixing and storage:** Mix samples thoroughly, then store in clean closed containers (i.e. polyethylene bags or bottles). Label the containers.

4.0 CONCLUSION

Soil Sampling should be carried out in such a manner that can maximise their use as a soil fertility index based on comparison between sampling events. Consistency, in the areas of season, location (aided by GPS techniques), crop rotation, soil type and sampling depth must be maintained as this will help in proper soil test interpretation. Inconsistencies in any of these areas of soil sampling collection will lessen the interpretation value of soil test changes that occurred since the last soil sample was taken. Along with consistency, soil samples should

reflect past soil and fertilizer/amendment management of a given field, taking into account tillage, crop rotation, fertilizer/ amendment placement and also soil characteristics (texture, slope and drainage). Following these guidelines will allow soil tests to be used more effectively for nutrient management and crop diagnostics.

5.0 SUMMARY

You have learnt that the major objectives of soil sampling have been to determine the average nutrient status of a field and to provide some measure of nutrient variability in a field. Through soil sampling and analysis, soil scientists are able to relate trends in soil fertilizer levels to other field properties that are predictable or easily measured. Knowledge of factors influencing soil nutrient levels including soil type, topography, cropping history, manure application, fertiliser application and leveling for irrigation will help the producer determine the most effective sampling approach or technique.

6.0 TUTOR-MARKED ASSIGNMENT

1. What do you understand by soil sampling?
2. What are factors to consider in choosing sampling method?
3. Mention and discuss the sampling techniques you studied.
4. Outline the guidelines for soil sampling.
5. State and explain sampling tools and how they are used for sampling soils.
6. How do we carry out soil preparations before analysis?

7.0 REFERENCES/FURTHER READING

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UNIT 2 PLANT SAMPLING AND SAMPLE PREPARATION

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1.0 INTRODUCTION

Plant and soil testing enable 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). Sample collection is critical for plant analysis as plant nutrient composition varies with age, the portion of the plant sampled, and many other factors. Mistakes or carelessness in selecting, collecting, handling, preparing, or shipping plant tissue for analysis can result in unreliable data, which may lead to incorrect interpretations and recommendations. Standards, against which the sample is evaluated, have been selected to represent the plant part and time of sampling that best define the relationship between nutrient composition and plant growth. Deviation from the prescribed protocol severely limits this interpretations capability. It is, therefore, critical to follow a standard sampling procedure. However, when plant analysis is being used to confirm a suspected nutrient deficiency, the samples should be taken as early in the season as possible so that the deficiency can be corrected and minimise the potential yield loss. Plants showing abnormalities usually continue to accumulate nutrients even if growth is impaired by some limiting factor.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the meaning of crop sampling and sample preparation
- carry out crop sampling and sample preparation.

3.0 MAIN CONTENT

3.1 Crop Sampling and Sample Preparation

3.1.1 Crop Sampling

Crop sampling is crucial for crop tissue analysis to enable researchers in the field of crop science carry out sustainable research works. This will lead to adequate crop improvements, genetic enhancement and modifications as well as improved productivity. Crop sampling has to be done with utmost carefulness as any mistakes or carelessness in selecting, collecting, handling, preparing, or shipping plant tissue for analysis can result in unreliable data, which may lead to incorrect interpretations and recommendations. Samples should not be taken from plants that obviously have been stressed from causes other than nutrients. Do not take samples from plants that are dead or insect damaged; Are mechanically or chemically injured; Have been stressed by too much or too little moisture (i.e., flooding or drought); Have been stressed by abnormally high or abnormally low temperature. Sample normal and abnormal areas when a nutrient deficiency is suspected (even without visual symptoms), or there is a need to compare different areas in a field, it is recommended that similar plant parts be collected separately from both the affected plants and adjacent normal plants that are at the same stage of growth. In this way, a better evaluation can be made between the nutritional status of healthy and abnormal plants of the same variety grown under the same conditions.

Precautions during crop sampling

The following guidelines have to be followed during sampling as serious error may occur which may lead to grave consequences. Such precautions are as follow:

i. Samples should not be taken from plants that obviously have been stressed from causes other than nutrients.
ii. Do not take samples from plants that are dead or insect damaged
iii. Are mechanically or chemically injured
iv. Have been stressed by too much or too little moisture (i.e., flooding or drought);
v. Have been stressed by abnormally high or abnormally low temperature.

vi. Sample normal and abnormal areas when a nutrient deficiency is suspected (even without visual symptoms), or there is a need to compare different areas in a field, it is recommended that similar plant parts be collected separately from both the affected plants and adjacent normal plants that are at the same stage of growth.

3.1.2 Plant Tissue Sample Preparation

After a plant sample has been collected, it should be prepared for shipment or delivery to the laboratory. Roots or foreign material attached to the sample should be removed and discarded. Plant tissue must then be dusted off to remove soil particles. **Do not wash** tissue since soluble nutrients will be leached out of the sample. If tissue is to be mailed, the sample should be air-dried above a heating vent or in the sun for one to two days to avoid mold formation during shipment. Place the plant sample in a paper bag in a large paper envelope for shipment. Do not pack the sample tightly into the mailing container or put samples in plastic or polyethylene bags as this will also promote mold development. Plant samples that are delivered to the laboratory do not need to be air-dried if they are delivered within one day after sampling. Samples to be delivered directly to the laboratory at a later date may be kept frozen or air-dried until they are delivered. Include Soil Sample Soil test results for pH, organic matter, phosphorus, and potassium (routine test) can be useful for correlating with plant analysis results to pinpoint a nutrient problem. A composite soil sample, consisting of five or more cores, taken to a depth of 6-7 inches, should be taken from the same area where the plant sample was collected. For row crops, avoid the fertilizer band by sampling from the middle of the row. Put the sample into a soil sample bag or other waterproof container and label the soil sample with the same field and sample number as that assigned to the tissue sample. Package corresponding plant and soil samples together, but make certain soil sample bags do not open in transit as spilled soil will contaminate plants. No additional fee is charged for routine soil analysis when submitting along with a plant sample. Special soil test requests for Ca, Mg, S, B, Mn, or Zn are assessed an extra fee.

Precautions during plant tissue sample preparation

i. Roots or foreign material attached to the sample should be removed and discarded.
ii. Plant tissue must then be dusted off to remove soil particles.
iii. Do not wash tissue since soluble nutrients will be leached out of the sample.
iv. If tissue is to be mailed, the sample should be air-dried above a heating vent or in the sun for one to two days to avoid mold formation during shipment.

v. Place the plant sample in a paper bag in a large paper envelope for shipment.
vi. Do not pack the sample tightly into the mailing container or put samples in plastic or polyethylene bags as this will also promote mold development.
vii. Plant samples that are delivered to the laboratory do not need to be air-dried if they are delivered within one day after sampling.
viii. Samples to be delivered directly to the laboratory at a later date may be kept frozen or air-dried until they are delivered.
ix. Include Soil Sample Soil test results for pH, organic matter, phosphorus, and potassium (routine test) can be useful for correlating with plant analysis results to pinpoint a nutrient problem.
x. A composite soil sample, consisting of five or more cores, taken to a depth of six to seven inches, should be taken from the same area where the plant sample was collected.

3.1.3 What to Do with Samples

A “Plant Analysis Information Sheet” should be filled out for any samples submitted. Use a separate information sheet for each sample. Plant samples, corresponding soil samples, and accompanying information sheets can be obtained and turned in at the County Extension Office especially in the United states. Samples may also be sent or delivered to the laboratory directly. The University of Wisconsin laboratory that conducts the plant analysis program is the Soil and Plant Analysis Laboratory at Madison. Some, but not all, private laboratories also analyse plant tissues; therefore, you should check with your laboratory on the specific services they provide before submitting the samples.

3.1.4 What the Analysis Report Will Include

The report will show the concentration of N, P, K, Ca, Mg, S, Zn, Mn, B, Cu, Fe, Al, and Na in the plant sample. If a soil was submitted with the plant sample, soil analyses for pH, organic matter, P, K, and any special soil test results will also be reported. In addition, the analytical levels of nutrients in the plant and soil will be interpreted to reflect nutrient deficiencies, toxicities, or imbalances by the sufficiency range approach, and if calibration data are available, the nutrient ratio method. When warranted, fertiliser recommendations will be made based on the analytical results. Most commonly grown field vegetables and fruit crops will receive these interpretations and recommendations. For those plant materials where calibration data are not available, these analytical results will be provided without interpretation.

3.1.5 Procedure for Plant Preparation for Analysis

The following procedure is suggested:

i. For analysis of seasonal crop plants such as maize, cowpea, or any kind of vegetables, 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.
ii. 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.
iii. Wash with water several times.
iv. Wash the samples with about 0.2 percent detergent solution to remove the waxy/greasy coating on the leaf surface.
v. Wash with 0.1M HCl followed by thorough washing with plenty of water. Give a final wash with distilled water.
vi. Wash with DDW if micronutrient analysis is to be carried out.
vii. Soak to dry with tissue paper.
viii. Air-dry the samples on a perfectly clean surface at room temperature for at least 2–3 days in a dust-free atmosphere.
ix. Put the samples in an oven, and dry at 70 °C for 48 hours.
x. 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.
xi. 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.

3.1.6 Parts of Crops Analysed

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 1 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 2 presents some critical values for a range of crops.

Table 1: General sufficiency or optimal range of nutrients in plants

Nutrients	Sufficiency or optimal level
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

Source: FAO, 2008

Table 2: Crop Part to be sampled, with age or growth stage

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 tasseling
Barley Flag	leaf at head emergence
Pulse	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	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)
Onions	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	Papaya 3–5-month old leaves from new flush
Pineapple	Middle third portion of white basal portion of 4^{th} leaf from apex, at 4–6-month stage

Source: FAO, 2008

4.0 CONCLUSION

You have learnt that samples should not be taken from plants that obviously have been stressed from causes other than nutrients. Do not take samples from plants that are dead or insect damaged; Are mechanically or chemically injured; Have been stressed by too much or too little moisture (i.e., flooding or drought); Have been stressed by abnormally high or abnormally low temperature. Sample normal and abnormal areas when a nutrient deficiency is suspected.

5.0 SUMMARY

The analytical levels of nutrients in the plant and soil will be interpreted to reflect nutrient deficiencies, toxicities, or imbalances by the sufficiency range approach, and if calibration data are available, the nutrient ratio method. When warranted, fertilizer recommendations will be made based on the analytical results. Most commonly grown field vegetables and fruit crops will receive these interpretations and recommendations. For those plant materials where calibration data are not available, these analytical results will be provided without interpretation.

6.0 TUTOR-MARKED ASSIGNMENT

1. What is plant sampling and why is it crucial in plant analysis?
2. What are the precautions to take while carrying out crop sampling?
3. State 10 precautions during plant tissue sample preparation.
4. Mention at least 10 parameters to be measured during plant analysis.
5. Discuss what to do with samples after collection.

6. Outline the procedure for plant preparation for analysis.

7.0 REFERENCES/FURTHER READING

FAO (2008). "Guides to Laboratory Establishment for Plant Nutrient Analysis." *Fertiliser and plant nutrition bulletin* Vol. 19. Rome.

http://www.soils.wisc.edu/extension/pubs/pa_sampling.pdf

UNIT 3 NITROGEN DETERMINATION IN SOIL AND PLANT

CONTENTS

1.0 Introduction
2.0 Objectives
3.0 Main Content
    3.1 Nitrogen Determination in Soil
    3.2 Nitrogen Determination in Plant
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

Total Nitrogen 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.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss how to determine total nitrogen (TN) in a soil sample
- explain how to determine nitrogen content of a plant sample.

4 MAIN CONTENT

3.1 Nitrogen Determination in Soils

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 NH3 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 volatilisation 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 (CuSO4.H2O) (AR-grade).
- Potassium sulphate or anhydrous sodium sulphate (AR-grade).
- 35-percent sodium hydroxide solution: Dissolve 350 g of solid NaOH inwater and dilute to 1 litre.
- 0.1M NaOH: Prepare 0.1M NaOH by dissolving 4.0 g of NaOH in waterand make the volume up to 1 litre. Standardize against 0.1N potassiumhydrogen phthalate or standard H2SO4.
- 0.1M HCl or 0.05M H2SO4: Prepare approximately the standard acidsolution and standardize against 0.1M sodium carbonate.
- Methyl red indicator.
- Salicylic acid for reducing NO3 to NH4, if present in the sample.
- Devarda's alloy for reducing NO3 to NH4, 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 K2SO4 and 30 ml of H2SO4.
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 H2SO4) 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:

$$\text{Percent N} = \frac{1.401\ [\ (V1\ M1 - V2M2) - (V3M1 - V4M2)]}{W} \times df$$

where:

- $V1$ – millilitres of standard acid put in receiving flask for samples;
- $V2$ – millilitres of standard NaOH used in titration;
- $V3$ – millilitres of standard acid put in receiving flask for blank;
- $V4$ – millilitres of standard NaOH used in titrating blank;
- $M1$ – molarity of standard acid;
- $M2$ – 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 H2SO4 corresponds to 1.401 g of N.

The following precautions should be observed:

- The material should not solidify after digestion.
- No NH4 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.

3.2 Nitrogen Determination in Plant

Total N in plants is estimated by the Kjeldahl method (as discussed above for N determination in soils). In plants, Nis present in protein form, and digestion of the sample with H2SO4 containing digestion mixture (10 parts potassium sulphate and 1part 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.

4.0 CONCLUSION

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.

5.0 SUMMARY

You have learnt that Total N in soil and plants is estimated by the Kjeldahl. In plants, N is present in protein form, and digestion of the sample with H2SO4 containing digestion mixture (10 parts potassium sulphate and one-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.

6.0 TUTOR-MARKED ASSIGNMENT

1. State commonly used catalysts to accelerate the digestion process in N determination in soil and plants.
2. Mention four apparatus used in N determinations.
3. Outline the reagents used in N determination.
4. Give the procedure used in N determination using Kjeldahl method.
5. State the equation used to calculate the percentage N content of soils.

7.0 REFERENCES/FURTHER READING

FAO (2008). "Guides to Laboratory Establishment for plant nutrient analysis." *Fertiliser and Plant Nutrition Bulletin* Vol. 19. Rome.

UNIT 4 PHOSPHORUS DETERMINATION IN SOIL AND PLANT

CONTENTS

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2.0 Objectives
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    3.1 Phosphorus Determination in Soil
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5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

Phosphorus Determination in Plant and Soil

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. Estimation of total P in plant materials 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.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- determine available P in soil using Bray's Method No. 1 for acid soils; and Olsen's method for neutral and alkali soils
- determine total P in plant material using spectrophotometric vanadium phosphomolybdate.

3.0 MAIN CONTENT

3.1 Available P Determination in Soils

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

Table 3: Wavelenght and corresponding colour ranges

Wavelenght	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: FAO, 2008

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 NH4F in 0.025M HCL): Dissolve 2.22 g ofNH4F in 200 ml of distilled water, filter, and add to the filtrate 1.8 litres of water containing 4 ml of concentrated HCl, make the volume up to 2 litres with distilled water.
- Molybdate reagent: Dissolve 1.50 g of (NH4)2MoO4 in 300 ml of distilled water. Add the solution to 350 ml of 10M HCl solution gradually with stirring. Dilute to one litre with distilled water.
- Stannous chloride solution (stock solution): Dissolve 10 g of SnCl2 2H2O in25 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 KH2PO4 in one 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 mgP/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 SnCl2solution, 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 of660 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 SnCl2 solution with a bulb pipette. Fill to the 25-mlmark 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:

$$P(kg/ha) = \frac{A}{1000\,000} \times \frac{50}{5} \times \frac{2000\,000}{5} = 4A$$

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 14 shows a standard curve prepared by the (FAO, 2008) for estimation of available P by Bray's Method No. 1 while establishing a soil testing laboratory.

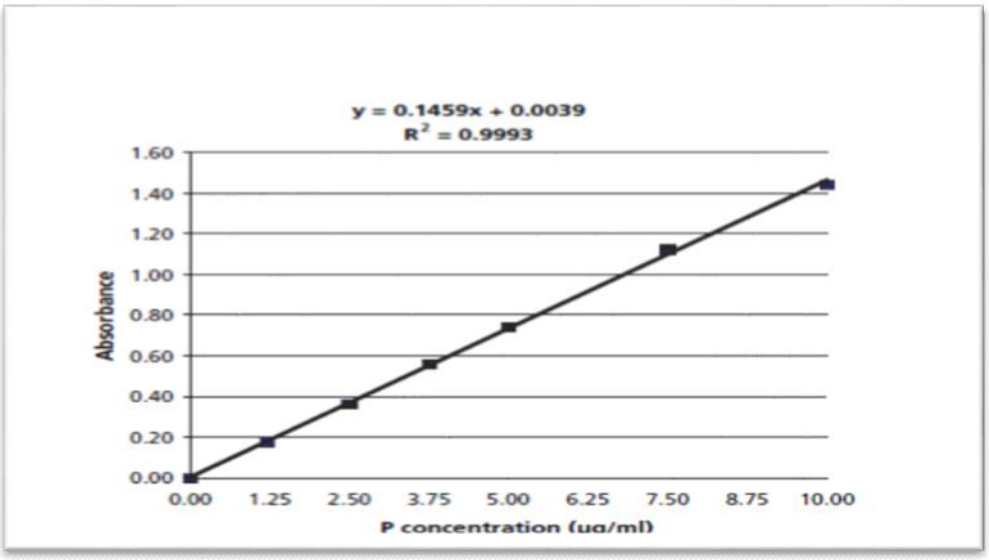

Fig. 2.3: Standard curve for P on Spectrophotometer (FAO, 2008)

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 one litre ofdistilled 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 for30 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.

3.2 Phosphorus Determination in Plants

Estimation of total P in plants 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 fertiliser 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 Pestimation in plant samples.

3.2.1 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 Pestimation. When orthophosphates are made to react with molybdate and vanadate, a yellow-coloured vanadomolybdophosphoricheteropoly 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 HNO3 (vanadomolybdate): Dissolve 22.5 g of (NH4)6MO7O2.4H2O in 400 ml of distilled water. Dissolve1.25 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 HNO3 and dilute to 1 litre.
- Standard phosphate solution: Dissolve 0.2195 g of analytical-grade KH2PO4and dilute to one 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.

2. The standard curve is prepared by measuring these concentrations on a spectrophotometer (420 nm) and recording the corresponding absorbances.

3. Take 1 g of plant sample and digest as per the wet digestion method, and make the volume up to 100 ml.

4. Put 5 ml of digest in a 50-ml volumetric flask, and add 10 ml of vanadomolybdate reagent.

5. Make up the volume with distilled water, and shake thoroughly. Keep for30 minutes.

6. A yellow colour develops, which is stable for days and is read at 420 nm on spectrophotometer.

7. For the observed absorbance, determine the P content from the standard curve.

The relevant calculation is:

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

$$\text{P content (g) in 100 g sample (\% P)} \quad \frac{C \times df \times 100}{1000\,000} = \frac{C \times 1000 \times 100}{1000\,000} \quad \frac{C}{10}$$

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.

5 CONCLUSION

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. Estimation of total P in plant materials 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.

5.0 SUMMARY

You have learnt that Bray's Method No. 1 for acid soils; and Olsen's method for neutral and alkali soils are used for P determination in soils while estimation of P in plant materials can be done by gravimetric quinolinium phosphomolybdate; gravimetric ammonium

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

6.0 TUTOR-MARKED ASSIGNMENT

1. State the two major methods used for determining P content of soils. What factors determine the one to use?
2. State five methods used for P determination in plants. What factors determine the one to use?
3. Mention the apparatus and reagents used for P determination in soils using Bray No 1 and Olsen's methods.
4. What are the procedures to use while using Bray No1 and Olsen's Method for P determination in soils?
5. Outline the apparatus, reagents and procedure used in Spectrophotometric vanadium phosphomolybdate method of P determination in plants.

7.0 REFERENCES/FURTHER READING

Bray, R.H. & Kurtz. L.T. (1945). "Determination of Total, Organic and Available Forms of Phosphorus in Soils." *Soil Sci.*, 59: 30–45.

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UNIT 5 POTASSIUM DETERMINATION IN SOILS, PLANTS AND FERTILISERS

CONTENTS

1.0 Introduction
2.0 Objectives
3.0 Main Content
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7.0 References/Further Reading

1.0 INTRODUCTION

Potassium present in the soil is first 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. Potassium estimation in plants 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. Analysis of fertilizers for K is very necessary as it is manufactured to supply most nutrients that plants need for survival. The sample received for analysis is recorded in the laboratory with adequate details, and a laboratory code number is assigned in order to identify the sample and to keep its identity confidential. About half of the sample is ground, sieved through a 1 mm sieve, and stored in a sample bottle for analysis. The remaining half is kept unground for particle size estimation. The samples are stored in an airtight glass bottle or taken for analysis in a moisture-free room (fitted with a dehumidifier) as most fertilizers are hygroscopic in nature.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- determine contents of soil, plants and fertilisers.

3.0 MAIN CONTENT

3.1 Available Potassium(K) Determination in Soil

Potassium present in the soil is extracted with neutral ammonium acetate of1 molarity. This is considered as plant-available K in the soils. It is estimated with the help of a flame photometer (FAO, 2008).

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 ammoniumacetate (NH4C2H3O2) in one litre of water. Check the pH with bromothymolblue or with a pH meter. If not neutral, add either ammonium hydroxide oracetic acid as per the need in order to neutralise it to pH 7.0.
- Standard potassium solution: Dissolve 1.908 g of pure KCl in one litre of distilled water. This solution contains 1 mg K/ml. Take 100 ml of this solution and dilute to one litre with ammonium acetate solution. This gives0.1 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 molarammonium acetate solution. These solutions contain 0, 5, 10, 15 and 20 µg K/ml, respectively.

The procedure is:

2.0 Preparation of the standard curve: Set up the flame photometer by atomizing0 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.

3.0 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.

4.0 Determine the potash in the filtrate with the flame photometer. The calculation is:

$$\text{K (kg/ha)} = x \times 25 \times \frac{A}{1000\,000} \times \frac{2000\,000}{5}$$

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 as2 million kg.

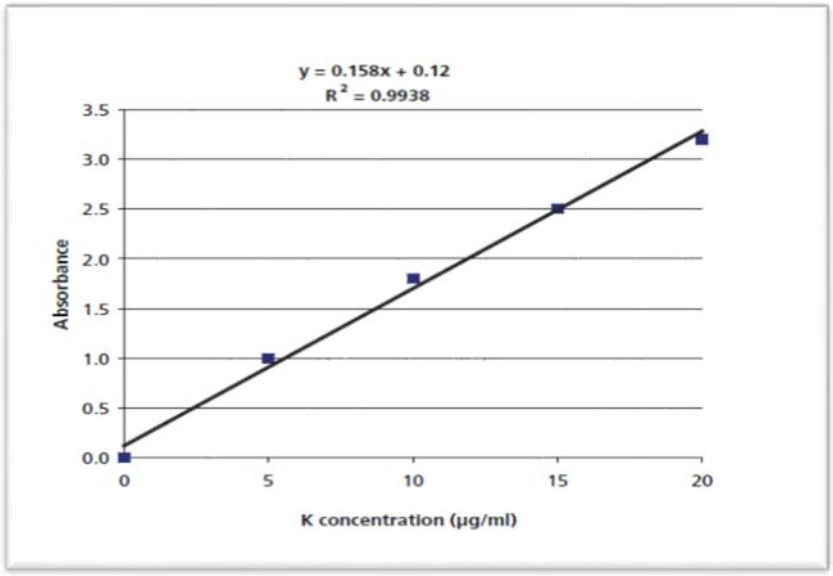

Fig. 2.4: Standard curve for K on Flame Photometer

Figure 15 above shows an example of a standard curve for estimating K using the flame
photometer method.

3.2 Potassium Determination in Plant

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

3.2.1 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 one litre of distilled water. This solution contains 1 mg K/ml. Take 100 ml of this solution and dilute to one 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 in100-ml volumetric flasks. Make up the volume. This will give 5, 10, 15 and20 µg K/ml, respectively.

The procedure is:

1. Set up the AAS and standardise. 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. Atomise 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:

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

K content (g) in 100 g sample (%K) = $\frac{C \times df \times 100}{1000\ 000}$ $\frac{C \times 2000 \times 100}{1000\ 000}$ $\frac{C}{5}$

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.

3.3 Potassium Determination in fertilisers

3.3.1 Sample Collection and Preparation

The collection of a representative sample of a fertiliser is an important step in fertiliser analysis and quality control. The method of sample collection depends on the type and source of fertiliser. Fertilisers are manufactured commodities. They are stored in silos at the manufacturing site for different periods. They are transported using various means (e.g. ship, rail and road) and stored again at various locations including port godowns, warehouses and dealers' shops. Fertilisers are stored/moved both in bulk and as bagged. Therefore, the sample collection methods can be classified broadly as concerning:

- collection from bulk stock in godowns;
- collection from ship hatches or while the ship is being loaded/unloaded;
- collection from bagged stock in godowns of various types;
- sample collection from damaged stock.

Scale of sampling

The number of samples to be collected from a given stock depends on the quantity available in the stock. In a ship, each hold/hatch is treated separately from the others, and samples are collected accordingly. In practice, one representative sample is taken from 100 tonnes of material. A similar scale of sampling is followed for factory silos where bulk fertilizers are stored.

In the case of bagged material, stored in smaller quantities (e.g. at dealers' godowns), generally, 1 sample (minimum) is drawn from 10

bags; 2 samples from 100 bags, 6–7 samples from 1 000 bags; and 10 samples from 2 000 bags. There is no fixed number of samples that can be defined to represent a given quantity. However, it is necessary to ensure that the sample is truly representative of the lot it designates.

In the case of bulk material being loaded into or unloaded from ships, samples are taken from the conveyor belt at certain intervals of time. The material so collected is stored in a container, and from such quantity collected, three representative samples each weighing about 400–500 g are taken. Samples from the bulk material are collected with the help of sampling cups made of corrosion-free metal. Bagged fertilizers are sampled with the help of a sampling probe/tube. This is often a slotted double tube with a solid cone tip made of stainless steel or brass, about 60–65 cm long, about 1.5 cm in diameter, and with a slot width of about 1.2–1.3 cm. The sampling probe should be inserted diagonally from one corner to another, keeping the slit down and rotated while withdrawing. The samples collected from the bags or bulk representing one lot is composited and, through the quartering method of leaving aside the portions of two opposite quarters successively, three identical samples of about 450–500 g each are retained for analysis. These samples are labelled with details showing:

- the name of the fertiliser
- the source
- the date when collected
- the signature, name and details of authority of the person who collected the sample.

Unlike soil, plant and water samples, fertiliser samples are generally collected intriplicate. Of the three samples, one goes to the designated laboratory for analysis; another goes to the owner of the fertiliser (e.g. ship’s captain, godown manager, or dealer), and the third sample is kept for further reference by the sample collector. Such a procedure is necessary because in the event of deficiencies in the quality parameters, there has to be provision to analyse the reference sample before holding the party concerned responsible for the poor quality of the fertiliser manufactured/imported/sold to the farmers.

3.3.2 Potassium Determination in Fertilisers

In all potassic fertilisers, K is generally present in water-soluble form. Therefore, itis estimated directly in fertiliser solution either gravimetrically, volumetrically or flame photometrically. In manures and organic fertilisers, wet digestion with acid is required prior to determination of K in order to bring the element into solution form, as

described for plant digestion in the previous section. The methods used for K determination in fertilisers and manures are:

- gravimetric perchloric acid method
- gravimetric chloroplatinate method
- gravimetric and volumetric cobaltinitrite method
- gravimetric and volumetric sodium tetraphenyl boron (STPB) method.

The AOAC-based STPB volumetric method is commonly used in laboratories because of its accuracy and simplicity.

STPB method

Potassium from the fertiliser sample is first extracted with water or ammoniumoxalate. The K in extracted solution is precipitated with an excess of STPBas potassium tetraphenyl boron. The excess of STPB is back-titrated with benzalkonium chloride (BAC) or quaternary ammonium chloride using Clayton yellow as indicator:

$Na[B(C_6H_5)_4]+K$ ~~$K[B(C_6H_5)_4]$~~ $+ Na^+$.

Interference of $NH4^+$ takes place during K precipitation. It is avoided by complexing NH4+ with formaldehyde under slightly alkaline conditions before precipitation of K. The chlorides and sulphates do not interfere in the titration.

The apparatus required consists of:

- some volumetric flasks and beakers
- a burette / semi-microburette
- some filter papers.

The reagents required are:

- Sodium hydroxide solution (20 percent): Dissolve 20 g of NaOH in 100 ml of distilled water.
- Formaldehyde (HCHO) solution (37 percent).
- STPB solution (about 1.2 percent): Dissolve 12 g of STPB in about 800 ml of water. Add 20–25 g of Al(OH)3, stir for five minutes, and filter through No.42 filter paper (or equivalent) into a one litre volumetric flask. Rinse the beaker sparingly with water and add to the filtrate. Collect the entire filtrate, add two ml of 20 percent NaOH solution, dilute to volume (one litre) with water, and mix. Let it stand for 48 hours, and then standardize (as described below). Adjust (by using K salt of known composition

for prior standardisation by trial and error) so that 1 ml of STPB = 1 percent K2O. Store at room temperature.

- BAC or quaternary ammonium chloride solution (about 0.625 percent): Dilute 50 ml of 12.8 percent BAC to one litre with water, mix and standardise (as described below). If a different concentration is used, adjust the volume accordingly (BAC of 0.625 percent strength is required so the dilution can be done according to the concentration available).
- Clayton yellow (0.04 percent) indicator: Dissolve 40 mg of Clayton yellow powder in 100 ml of water.
- Ammonium oxalate solution [(NH4)2 C2O4] (four percent): Dissolve 40 g of ammonium oxalate in one litre of distilled water.

The procedures for standardizing the solutions are:

- BAC solution: Put 1 ml of STPB solution in a 250 ml Erlenmeyer flask; add 20–25 ml of water, one ml of 20 percent NaOH, 2.2 ml of HCHO, 1.5ml of four percent ammonium oxalate, and 6–8 drops of Clayton yellow indicator. Titrate to pink end point with BAC solution, using a 10 ml semi microburette. Adjust by increasing or decreasing the strength of the BAC solution so that 2 ml = 1 ml of STPB solution (keeping 1 ml STPB = 1 percentK2O).

- STPB solution: Dissolve 2.5 g of KH_2PO_4 in about 150 ml of water in a 250ml volumetric flask, add 50 ml of 4 percent ammonium oxalate solution, dilute to volume with water, and mix. Transfer 15 ml of aliquot (51.92 mg ofK2O or 43.10 mg of K) to a 100 ml volumetric flask, add 2 ml of 20 percent NaOH, 5 ml of HCHO and 43 ml of STPB solution. Dilute to volume (100ml) with water, and mix thoroughly. Let it stand for 5–10 minutes, and then pass through dry No. 42 filter paper. Transfer 50 ml of aliquot of filtrate to a 250 ml Erlenmeyer flask, add 6–8 drops of Clayton yellow indicator, and titrate excess STPB with BAC solution to pink end point. Calculate factor (f)by: f = percent K2O/ml of STPB solution

$$= \frac{34.61}{43 - \text{ml of BAC used for standardization}}$$

where, 34.61 = % K2O present in standard KH2PO4.

The procedure is:

1. K extraction/preparation of sample solution: Dissolve a known weight (2.5g) of straight K fertilizer (MOP, SOP, potassium magnesium sulphate) in 200ml of distilled water, and make the volume up to 250 ml for estimation. For NPK complex fertilizers or NPK fertilizer mixtures, dissolve the sample in125 ml of water, add 50 ml of 4 percent ammonium oxalate solution, and boil for 30 minutes; after cooling, filter through dry No. 12 filter paper, and make the volume up to 250 ml for further estimation.

2. Transfer 15 ml of aliquot of sample solution to a 100 ml volumetric flask and add 2 ml of 20 percent NaOH and five ml of HCHO.

3. Add 1 ml of standard STPB solution for each 1 percent of K2O expected in the sample plus an additional 8 ml in excess in order to ensure complete precipitation.

4. Dilute to volume (100 ml) with water, mix thoroughly, let it stand for 5–10minutes, and pass it through No. 12 filter paper (or equivalent).

5. Transfer 50 ml of filtrate to a 250 ml Erlenmeyer flask, add 6–8 drops of Clayton yellow indicator, and titrate excess STPB with standard BAC solution to pink end point.

The relevant calculation is:
% K_2O = (ml STPB used - ml BAC used) x *f*

where, *f* = % K_2O/ml of STPB solution. This factor applies to all fertilisers where 2.5 g of sample is diluted to 250 ml, and 15 ml of aliquot is taken for analysis. To express the results as K rather than K_2O, substitute 28.73 for 34.61 in calculating the value of *f*.

4.0 CONCLUSION

Potassium present in the soil is extracted with neutral ammonium acetate of 1 molarity. This is considered as plant-available K in the soils. Potassium from the fertilizer sample is first extracted with water or ammonium oxalate. The K in extracted solution is precipitated with an excess of STPB as potassium tetraphenyl boron. Potassium estimation can be done on a flame photometer, an Atomic Absorption Spectrophotometer (AAS) or by the volumetric sodium tetraphenyl boron method.

5.0 SUMMARY

You have learnt that 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. Potassium estimation in plants can be done on a flame photometer, an AAS or by the volumetric sodium tetraphenyl boron method. In all potassic fertilisers, K is generally present in water-soluble form. Therefore, it is estimated directly in fertilizer solution either gravimetrically, volumetrically or flame photometrically.

6.0 TUTOR-MARKED ASSIGNMENT

1. What is the reagent used to extract K present in the soil?
2. What are the names of the equipment used to test for K in in Soils and Plants?
3. State the methods used for K determination in fertilisers and manures.
4. How can sampling be carried out in fertilisers.
5. Outline the apparatus used in the laboratory to determine K in soils, plant and fertilisers.
6. Discus the procedures used to determine K in soils, plants and fertilisers.

7.0 REFERENCE/FURTHER READING

FAO (2008). “Guides to Laboratory Establishment for Plant Nutrient Analysis.” *Fertiliser and Plant Nutrition Bulletin* Vol. 19. Rome.

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MODULE 3 ANALYTICAL INSTRUMENTS AND PRINCIPLES OF OPERATION

Unit 1 Principles and Operations of Colorimeter
Unit 2 Principles and Operations of Flame Photometer
Unit 3 Principles and Operation of Atomic Absorption Spectrophotometer (AAS)
Unit 4 Principles and Operation of A pH Meter

UNIT 1 PRINCIPLES AND OPERATIONS OF COLORIMETER

CONTENTS

1.0 Introduction
2.0 Objectives
3.0 Main Content
3.1 Colorimeter
3.1.1 Principles of Colorimeter
3.1.2 Using the Instrument
3.1.3 Uses of Colorimeters
3.1.4 Parts of a Colorimeter
3.1.5 Standard Operation Procedure
3.1.6 Pre-Caution Step
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

A colorimeter is a light-sensitive instrument which measures the transmittance and absorbance of light passing through a liquid sample. The device measures the intensity or concentration of the color that develops when you introduce a specific reagent into a solution. The colorimeters are highly sensitive devices that can measure the concentration and intensity of a particular color that is used in a product. There are mainly two different types of colorimeters that are used in industries that are colour densitometers and color photometers. The color densitometers measure the colour density of primary colors in a color combination in a test sample. The color photometers are used for measuring the reflectance of a color as well as the transmission.

One type of colorimeter can find the concentration of a substance in solution, based on the intensity of color of the solution. If you are testing

a colorless solution, you add a reagent that reacts with the substance, producing a color. Most types colorimeter have a wide range of applications, which may include; laboratory research, environmental analysis of water quality, analysis of soil components, monitoring of hemoglobin content in blood and analysis of chemicals used in various industrial settings. As has been stated earlier, there are two types of colorimeters — color densitometers, which measure the density of primary colors, and color photometers, which measure the color reflection and transmission.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- examine what colorimeter and colorimetry is all about
- identify the main types of colorimeter
- measure the absorbance of particular wavelengths of light by a specific solution.
- determine the concentration of a known solute in a given solution by the application of the Beer-Lambert law which state that the concentration of a solute is proportional to the absorbance.
- discuss the theory and the principles of the colorimeter.
- identify the standard operation procedure to operate the colorimeter correctly.

3.0 MAIN CONTENT

3.1 Colorimeter

Colorimeter has been defined earlier as a light-sensitive device used for measuring the transmittance and absorbance of light passing through a liquid sample. Colorimetry is the scientific color measurement which is used to express color in numerical terms and to measure the color differences between the specimens. The specimens can be paints, textiles, plastics, food and other products that may reflect or transmit colour. Colorimeter is an instrument for psychophysical analysis by measuring the amount of light passing through a liquid. This instrument provides measurements that correlate with human eye-brain perception. Besides, the colorimeter is basically like a spectrophotometer but less complex as the spectrophotometer allows selection of any wavelength of light. Colorimeter measures the colour through three wide-band filters which corresponding to the spectral sensitivity curves.

A light source creates a beam of light that shines through a sample. The colorimeter then measures the amount of light transmitted or absorbed electronically and provides colorimetric data as tri-stimulus values

(XYZ, L, a, b). The design of the tristimulus colorimetry is about duplicate the response of the human eyes. A light source, three glass filters with transmittance spectra that duplicate the X, Y and Z curves and a photocell are required. This helps to get the reading of XYZ represents the colour of the sample. Drawback of XYZ system is not visually uniform, that means one unit of colour measurement in one area of the solid was visually different from the same unit in another area. Normally, the values of tristimulus are used to determine the direction and amount of any color difference if a color match is accurate.

The colorimeter provided in this lab is Colour Flex colorimeter from the Hunter Lab. Colour Flex is a self-contained colour measurement spectrophotometer which had been introduced in this lab. It can be used in production or in the laboratory for inspecting raw materials and evaluating the final product. Apart from this, the Colour Flex is ideal for measuring powders, granules, pastes, liquids and opaque as it has its port-up or port-forward measurement orientations. The Colour Flex require glass sample cup to hold the sample for measure and has a hole to insert the glass sample cup according to its size. Specialized versions of the Colour Flex are available for the citrus industry and the tomato industry. These systems include specialises calibration standards and measurement scales appropriate for the industry.

Colorimeter vs Spectrophotometer

As stated above, colorimeter has similarity with spectrophotometer although the spectrophotometer is much more complex in their operations. Like colorimeters, spectrophotometers are used to measure the colour absorbing properties of a substance. The key difference between the two is that the spectrophotometer measures the transmittance and reflectance as a function of wavelength, whereas the colorimeter measures the absorbance of specific colours. Spectrophotometers measure the transmittance and reflectance for all colours of light and show how they vary as the colour is changed. Colorimeters operate only in the visible portion of the electromagnetic spectrum whereas spectrophotometers work with infrared as well as visible light. Spectrophotometers will produce valid results for Beer’s law and can effectively be used as colorimeters but are much higher in cost and complexity.

Fig. 3.1: Different Colours

Colorimetry is the scientific field of determining the concentration of a coloured compound in a solution. A colorimeter, also known as a filter photometer, is an analytical machine that acts as the tool quantify a solutions concentration by measuring the absorbance of a specific wavelength of light.

3.1.1 Principles of Colorimeter

The colorimeter uses the Beer-Lambert law or principles to detect the absorbance of the wavelength. This law states that the light absorption when passes through a medium are directly proportional to the concentration of the medium. When a colorimeter is used, there is a ray of light with a certain wavelength is directed towards a solution. Before reaching the solution the ray of light passes through a series of different lenses. These lenses are used for navigation of the colored light in the colorimeter. The colorimeter analyses the reflected light and compares with a predetermined standard. Then a microprocessor installed in the device is used for calculation of the absorbance of the light by the solution. If the absorption of the solution is higher than there will be more-light absorbed by the solution and if the concentration of the solution is low, then more lights will be transmitted through the solution.
Beer-Lamberts law is commonly written as: $A = \varepsilon cl$

Where, A is the absorbance, ε (epsilon) is the molar absorptivity, c is the concentration of the solution and l is the length that the light passes through (also known as the mean free path). Aside from this, if there is a continual changing of the solution, i.e. it is a reaction, then % of transmittance against time is generally used.

Colorimeters are used to detect colour and determine the solutions concentration, i.e. when a wavelength is passed through a sample, some of the light is absorbed and some passes through. It is the wavelengths

of light that pass through that are detected. By knowing which wavelengths have passed through, the detector can also work out which coloured wavelengths were absorbed. If the solution to be tested is colourless, a common procedure is to introduce a reagent that reacts with the solution to produce a coloured solution. The results are compared against known standards. To measure concentrations, the amount of light absorbed is dependent upon the amount of solute (also known as the analyte as it is the species being measured) in the solution- a higher concentration of dissolved solute means that more light will be absorbed, and vice versa, hence, the concentration can be backed out from the absorption of specific wavelengths.

3.1.2 Using the Instrument

With a conventional colorimeter, you will need to calibrate the instrument (using the solvent alone) and use it to determine the absorbance values of several standard solutions containing a solute at known concentrations. (If the solute produces a colorless solution, add a reagent that reacts with the solute and generates a color.) Choose the light filter or LED that gives the highest absorbance values. Plot the data to obtain a graph of absorbance versus concentration. Then use the instrument to find the absorbance of the test solution, and use the graph to find the concentration of the solute in the test solution. Modern digital colorimeters may directly show the concentration of the solute, eliminating the need for most of the above steps.

3.1.3 Uses of Colorimeters

Besides being valuable for basic research in chemistry laboratories, colorimeters have many practical applications which include the following:

i. They are used to test for water quality, by screening for chemicals such as chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc and hydrazine.
ii. They are also used to determine the concentrations of plant nutrients (such as phosphorus, nitrate and ammonia) in the soil or hemoglobin in the blood.
iii. To identify substandard and counterfeit drugs.

3.1.4 Parts of a Colorimeter

A colorimeter has three main parts: a light source, a cuvette that holds the sample solution and a photocell that detects the light transmitted through the solution. To produce coloured light, the instrument may be equipped with either colored filters or specific LEDs. The light

transmitted by the solution in the cuvette is detected by a photocell, producing a digital or analog signal that can be measured. Some colorimeters are portable and useful for on-site tests, while others are larger, bench-top instruments useful for laboratory testing.

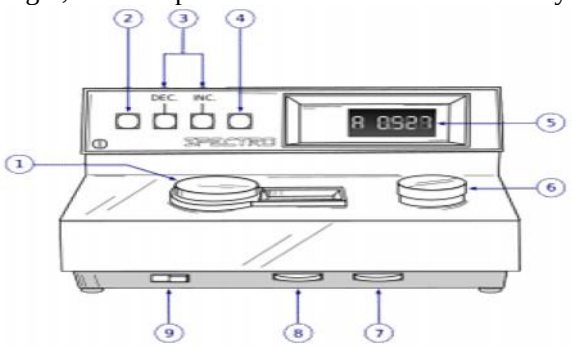

Fig. 3.2: A Typical Colorimeter

Parts of a typical colorimeter drawn above

1. Wavelength selection
2. Printer button
3. Concentration factor adjustment
4. UV mode selector (Deuterium lamp)
5. Readout
6. Sample compartment
7. Zero control (100% T)
8. Sensitivity switch
9. ON/OFF switch

The essential parts of a colorimeter are:

- a **light source** (often an ordinary low-voltage filament lamp);
- an adjustable aperture
- a set of colored filters
- a cuvette to hold the working solution
- a detector (usually a photoresistor) to measure the transmitted light
- a meter to display the output from the detector.
- a voltage regulator, to protect the instrument from fluctuations in mains voltage;
- a second light path, cuvette and detector. This enables comparison between the working solution and a "blank", consisting of pure solvent, to improve accuracy.

There are many commercialized colorimeters as well as open source versions with construction documentation for education and for research.

Filters: Changeable [Filter (optics)|optics filters] are used in the colorimeter to select the wavelength which the solute absorbs the most, in order to maximize accuracy. The usual wavelength range is from 400 to 700 [nanometer] (nm). If it is necessary to operate in the [ultraviolet] range, then some modifications to the colorimeter are needed. In modern colorimeters the filament lamp and filters may be replaced by several (light-emitting diode) of different colours. The Measurement of Colour.

Cuvettes: In a manual colorimeter the cuvettes are inserted and removed by hand. An automated colorimeter (as used in an auto analyser) is fitted with a flow-cell through which solution flows continuously.

Output: The output from a colorimeter may be displayed by an analogue or digital meter and may be shown as transmittance (a linear scale from 0-100%) or as absorbance (a logarithmic scale from zero to infinity). The useful range of the absorbance scale is from 0-2 but it is desirable to keep within the range 0-1 because, above 1, the results become unreliable due to scattering of light.

In addition, the output may be sent to a chart recorder, data logger, or computer.

3.1.5 Standard Operation Procedure

ColorFlex Colorimeter

i. The ColorFlex is placed on a flat and stable surface where near an electrical outlet.
ii. The system is turned on by pressing the Red (lightning bolt) key and is allowed to warm up for at least two hours before use.
iii. Before measuring sample, the instrument must be calibrated. Steps to standardize the colorimeter are carried out as below:
iv. The Down Arrow key is pressed until the menu is reached and then the standardise is selected by pressing the read key.
v. As instructed, the sample pot should be covered with the black glass first. The black glass is covered at the sample port with the shiny side toward the port and the arrow on the glass should be pointed towards the scientist.
vi. The “thunderstorm” button is pressed.
vii. The sample pot then is covered with the white tile. Same with the black tile, the sample port is covered with the shiny side toward the port and the arrow on the glass should be pointed towards the scientist.

viii. The "thunderstorm" button is pressed and the values are showed on the periphery of the tile.
ix. A message which indicates the instrument is ready to read will be displayed when the standardisation of the instrument is completed. The value of L*, a* and b* should be 50.87, -25.11 and 14.98 respectively.
x. The sample cup must be cleaned before put the sample into it. Make sure that the sample must be at least fully covered the bottom surface of the cup.
xi. After closed the cover, the cup is put onto the sensor to measure the sample and take the reading. The L, a, b value will be show on the screen after the "thunderstorm" button is pressed and wait for a moment.
xii. The readings are recorded.
xiii. Steps 5-7 are repeated for different samples.

3.1.6 Pre-Caution Step

1. Make sure that the setting of the colorimeter is set as default setup before conduct the experiment to obtain accuracy data.
2. The standard plates must be cleaned and make sure that it is free from dust and fingerprint.
3. After doing the calibration, put the black glass and the white tile back to the box to avoid scratching.
4. The volume, size and weight of the sample must be standardized (constant). The amount of the samples must at least cover fully the bottom surface of the sample cup.
5. The sample must be covered with the non-transparent black-coloured cover (light trap) when the readings are being taken.

This helps to avoid the light sensitive colorimeter from the disturbance of other light sources.

4.0 CONCLUSION

You have learnt that a colorimeter is composed of many parts. The principles are based around light, a light source is required and usually takes the form of a filament lamp. Other components include an adjustable aperture to let the light through, coloured filters to filter specific wavelengths of light, a cuvette to hold the solution (commonly made of quartz), a photodetector to measure the transmitted light and a meter to quantify the values into a readable output. The coloured filters are chosen to select the wavelength in which the dissolved solute will absorb the most. For most experiments the common wavelength range is between 400 and 700 nm, but when some analytes absorb in the ultraviolet range (less than 400 nm) then modification of the colorimeter

is generally required. This normally takes the form of removing the filament lamp and replacing it with light-emitting diode(s) of a specific colour. The output can be either analogue or digital in nature and, depending of the principle used, will give either an absorbance (0-infinity logarithmic output) or a %transmittance (0-100%) readout. The ideal output for an absorbance measurement is between 0 and 2, but it is desirable to have a reading between 0 and 1, as above 1 the results can become unreliable due to the scattering of light. The readout is usually in the form of a spectrum.

5.0 SUMMARY

You have studied that most calorimeters will require calibration, which is the solvent alone and not the measurable contents with the solvent-i.e. a standard or 'blank' solution. The calibration allows the absorbance of the solvent to be measured, also known across many instruments as the background noise. Once measured, the solvent absorption values are removed from any future readings, allowing the absorbance (or %transmittance) to be calculated (and plotted on a spectrum) for the desired analyte(s) without noise interference. There are a wide variety of colorimeters out there, where some colorimeters are large machines and generally used for a wide-range of laboratory analyses, but some colorimeters are now hand-held and can be used for on-site analyses such as the determination of *in-situ* water and soil samples. In the case of handheld colorimeters, a numerical readout is the common procedure as opposed to a spectrum found on the larger laboratory machines.

6.0 TUTOR-MARKED ASSIGNMENT

1) Define Colorimeter and Colorimetry, what are the two main types of colorimeter and their uses.
2) State Beer-Lambert's law, how does colorimeter use this principle in its operation.
3) Describe the function of colorimeter, what is the similarity of colorimeter to spectrophotometer.
4) With the aid of a diagram, show a typical colorimeter and label it appropriately.
5) Mention five parts of a colorimeter and their functions.
6) State the standard operation procedures of a colorimeter, list five precautions to take while using a colorimeter.

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UNIT 2 PRINCIPLES AND OPERATIONS OF FLAME PHOTOMETER

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1.0 Introduction
2.0 Objectives
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1.0 INTRODUCTION

In 1980s the following men; Bowling Barnes, David Richardson, John Berry and Robert Hood produced an instrument to measure the low concentrations of sodium and potassium in a solution. The instrument was named Flame photometer. The principle of flame photometer is based on the measurement of the emitted light intensity when a metal is introduced into the flame. The wavelength of the colour gives information about the element and the colour of the flame gives information about the amount of the element present in the sample.

Flame photometry is one of the branches of atomic absorption spectroscopy. It is also known as flame emission spectroscopy. Currently, it has become a necessary tool in the field of analytical chemistry. Flame photometer can be used to determine the concentration of certain metal ions like sodium, potassium, lithium, calcium and cesium etc. In flame photometer spectra the metal ions are used in the form of atoms. The International Union of Pure and Applied Chemistry (IUPAC) Committee on Spectroscopic Nomenclature has named this technique as flame atomic emission spectrometry (FAES).

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- explain the principle and operations of flame photometer
- discuss the working procedures of flame photometer
- differentiate parts of flame photometer and their functions
- state the advantages and disadvantages of use of flame photometer.

3.0 MAIN CONTENT

3.1 Flame Photometer

Flame photometry depends upon the fact that the compounds of the alkali and alkaline earth metals can be thermally dissociated in a flame and that some of the atoms produced will be further excited to a higher energy level. When these atoms return to the ground state they emit radiation which lies mainly in the visible region of the spectrum. Each element will emit radiation at a wavelength specific for that element. The compounds of the alkali and alkaline earth metals dissociate into atoms when introduced into the flame. Some of these atoms further get excited to even higher levels. But these atoms are not stable at higher levels. Hence, these atoms emit radiations when returning back to the ground state. These radiations generally lie in the visible region of the spectrum. Each of the alkali and alkaline earth metals has a specific wavelength. The table below gives details of the measurable atomic flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the colour produced.

Flame photometry is a process wherein the emission of radiation by neutral atoms is measured. The neutral atoms are obtained by introduction of the sample into flame. Hence the name flame photometry. Since radiation is emitted, it is also called as flame emission spectroscopy.

3.1.3 Flame Photometer Working Principle

1. When a solution of metallic salt is sprayed as fine droplets into a flame. Due to the heat of the flame, the droplets dry leaving a fine residue of salt. This fine residue converts into neutral atoms.
2. Due to the thermal energy of the flame, the atoms get excited and after that return to ground state. In this process of return to ground state, excited atoms emit radiation of specific wavelength.

This wavelength of radiation emitted is specific for every element.

3. This specificity of the wavelength of light emitted makes it a qualitative aspect. While the intensity of radiation depends on the concentration of element. This makes it a quantitative aspect.
4. The process seems to be simple and applicable to all elements. But in practice, only a few elements of Group IA and group IIA (like Li, Na, k & Ca, Mg) are only analyzed. The radiation emitted in the process is of a specific wavelength. Like for Sodium (Na) 589nm yellow radiation, Potassium 766nm violet radiation.

Table 3.1: Wavelengths and flame colors of some alkali and alkaline earth metals

Element	Emitted wavelength	Flame color
Sodium (Na)	589 nm	Yellow
Potassium (K)	766 nm	Violet
Barium (Ba)	554 nm	Lime green
Calcium (Ca)	622 nm	Orange
Lithium (Li)	670 nm	Red

Fig. 3.4: Jenway Flame Photometer

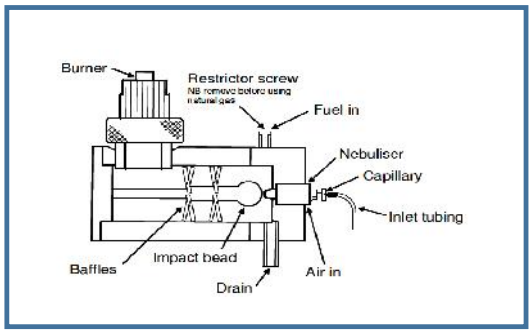

Fig. 3.5: Sample System

3.1.2 Components of Flame Photometer

A simple flame photometer consists of the following basic components:

1. **Source of flame:** A Burner in the flame photometer is the source of flame. It can be maintained in at a constant temperature. The temperature of the flame is one of the critical factors in flame photometry.

2. **Nebuliser:** Nebuliser is used to send homogeneous solution into the flame at a balanced rate.

3. **Optical system:** The optical system consists of convex mirror and convex lens. The convex mirror transmits the light emitted from the atoms. Convex mirror also helps to focus the emissions to the lens. The lens helps to focus the light on a point or slit.

4. **Simple colour filters:** The reflections from the mirror pass through the slit and reach the filters. Filters will isolate the wavelength to be measured from that of irrelevant emissions.

5. **Photo-detector:** The intensity of radiation emitted by the flame is measured by photo detector. Here the emitted radiation is converted to an electrical signal with the help of photo detector. These electrical signals are directly proportional to the intensity of light.

3.1.3 Working Procedure

i. Both the standard stock solution and sample solution are prepared in fresh distilled water.
ii. The flame of the photometer is calibrated by adjusting the air and gas. Then the flame is allowed to stabilize for about five minutes.
iii. Now the instrument is switched on and the lids of the filter chamber are opened to insert appropriate colour filters.
iv. The readings of the galvanometer are adjusted to zero by spraying distilled water into the flame.
v. The sensitivity is adjusted by spraying the most concentrated standard working solution into the flame. Now the full scale deflection of the galvanometer is recorded.
vi. Again distilled water is sprayed into the flame to attain constant readings of galvanometer. Then the galvanometer is readjusted to zero.
vii. Now each of the standard working solutions is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
viii. Finally, sample solution is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
ix. Calculate the mean of the galvanometer reading.
x. Plot the graph of concentration against the galvanometer reading to find out the concentration of the element in the sample.

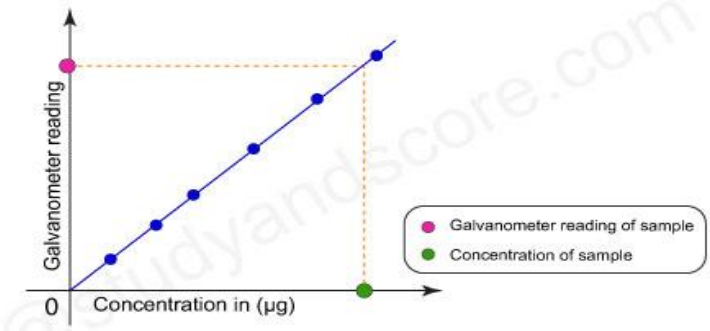

Fig.3.6: Flame Photometer Graph

- The solvent is first aspirated to obtain fine solid particles.
- These molecules in the solid particles are moved towards the flame to produce gaseous atoms and ions.
- These ions absorb the energy from the flame get excited to high energy levels from the ground state.
- But as these ions are unstable, they return back to ground state. While returning they emit characteristic radiation.
- The intensity of emitted light is proportional to the concentration of the element.

The oxidants in flame photometer are mainly air, oxygen or nitrous oxide. The temperature of the flame depends on the ratio of fuel and oxidant.

3.1.4 Process of Operation of Flame Photometer

The processes occurring during flame photometer analysis are summarised below:

- **Desolvation:** Desolvation has to do with drying a sample in a solution. The metal particles in the solvent are dehydrated by the flame and thus solvent is evaporated.
- **Vaporisation:** The metal particles in the sample are also dehydrated. This also will lead to the evaporation of the solvent.
- **Atomisation:** Atomisation is the separation of all atoms in a chemical substance. The metal ions in the sample are reduced to metal atoms by the flame.
- **Excitation:** The electrostatic force of attraction between the electrons and nucleus of the atom helps them to absorb a particular amount of energy. The atoms then jump to the higher energy state when excited.
- **Emission:** Since the higher energy state is unstable the atoms jump back to the ground state or low energy state to gain stability. This jumping of atoms emits radiation with characteristic wavelength. The radiation is measured by the photo detector.

3.1.5 Applications of Flame Photometer

1. Flame photometer can be applied both for quantitative and qualitative analysis of elements. The radiations emitted by the flame photometer are characteristic to

particular metal. Hence with the help of Flame photometer we can detect the presence of any specific element in the given sample.

2. The presence of some group II elements is critical for soil health. We can determine the presence of various alkali and alkaline earth metals in soil sample by conducting flame test and then the soil can be supplied with specific fertiliser.
3. The concentrations of Na+ and K+ ions are very important in the human body for conducting various metabolic functions. Their concentrations can be determined by diluting and aspirating blood serum sample into the flame.

4. Soft drinks, fruit juices and alcoholic beverages can also be analysed by using flame photometry to determine the concentrations of various metals and elements.

3.1.6 Advantages of Flame Photometer

1. The method of analysis is very simple and economical.
2. It is quick, convenient, selective and sensitive analysis.
3. It is both and qualitative and quantitative in nature.
4. Even very low concentrations (parts per million/ppm to parts per billion/ppb range) of metals in the sample can be determined.
5. This method compensates for any unexpected interfering material present in the sample solution.
6. This method can be used to estimate elements which are rarely analysed.

3.1.7 Disadvantages of Flame Photometer

In spite of many advantages, this analysis technique has quite a few disadvantages:

i. The accurate concentration of the metal ion in the solution cannot be measured.
ii. It cannot directly detect and determine the presence of inert gases.
iii. Though this technique measures the total metal content present in the sample, it does not provide the information about the molecular structure of the metal present in the sample.
iv. Only liquid samples may be used. Also sample preparation becomes lengthy in some cases.

v. Flame photometry cannot be used for the direct determination of each and every metal atom. A number of metal atoms cannot be analysed by this method. The elements such as carbon, hydrogen and halides cannot be detected due to their non-radiating nature.

3.1.8 Operating Precautions

i. The fuel gases used in the flame photometers are inflammable and therefore can be very hazardous. Cylinders of fuel gas should always be stored and used in line with the supplier's recommendation.

ii. It may be possible that some quantity of fuel will escape from the instrument during the ignition sequence. The amount of fuel is harmless if it is small although may smell slightly. If the smell of fuel gas persists the instrument should be immediately shut down and the source of the leakage determined by using a soap solution on the hose joints.
iii. Don't allow the instrument running unattended while the flame is alight. The top of the instrument chimney unit becomes very hot when running and may lead to severe burns when you mistakenly touch it.
iv. The exhaust gases from the flame are very hot and the area approximately one metre above the chimney must be avoided. Never attempt to look down the chimney whilst the flame is running. Always use the inspection window.
v. The instrument uses potentially hazardous electrical supplies. Never remove covers from the instrument without first ensuring that it has been isolated completely from the AC mains supply.
vi. If the instrument is used in a pathology laboratory, all samples should be handled with the caution normally accorded to those known to contain pathogenic organisms. Care must also be taken when carrying out maintenance on instruments that have been used in these environments. A bactericidal agent should be used when cleaning parts during routine maintenance.

4.0 CONCLUSION

You have learnt that flame photometry depends upon the fact that the compounds of the alkali and alkaline earth metals can be thermally dissociated in a flame and that some of the atoms produced will be further excited to a higher energy level. Flame photometer can be applied both for quantitative and qualitative analysis of elements. The radiations emitted by the flame photometer are characteristic to particular metal. Hence through the help of

Flame photometer we can detect the presence of any specific element in the given sample. The presence of some group II elements is critical for soil health. We can determine the presence of various alkali and alkaline earth metals in soil sample by conducting flame test and then the soil can be supplied with specific fertiliser.

5.0 SUMMARY

You have learnt the working procedures of the flame photometer which include calibration of the flame of the photometer by adjusting the air and gas. Then the flame will be allowed to stabilize for about five minutes. Now the instrument is switched on and the lids of the filter chamber are opened to insert appropriate colour filters. The readings of the galvanometer are also adjusted to zero by spraying distilled water into the flame. The solvent is first aspirated to obtain fine solid particles. These molecules in the solid particles are moved towards the flame to produce gaseous atoms and ions. These ions absorb the energy from the flame get excited to high energy levels from the ground state. But as these ions are unstable, they return back to ground state. While returning they emit characteristic radiation. The intensity of emitted light is proportional to the concentration of the element.

6.0 TUTOR-MARKED ASSIGNMENT

1. What do you understand as the flame photometer? State its working principles.
2. State five processes of operation of the flame photometer and give four applications of the instrument.
3. What are the advantages and the disadvantages of use of flame photometer? Give at least five each.
4. Carefully outline the working procedures of the flame photometer.
5. Carefully state six working precautions of the instrument when using it for operation.

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UNIT 3 PRINCIPLES AND OPERATION OF ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

CONTENTS

1.0 Introduction
2.0 Objectives
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1.0 INTRODUCTION

Trace element analysis is very important in many fields which encompass ecological science, food analysis, forensic science, material science, soil and plant science as well as in environmental studies. In order to carry out the analysis, many techniques varying in degree of sensitivity and convenience have been developed. These include atomic absorption spectrometry, inductively coupled plasma-atomic emission spectrometry, X-ray fluorescence, neutron activation analysis, mass spectrometry and proton induced X-ray emission. Atomic absorption spectrometry will be discussed in this unit. It is worthy to note that atomic absorption spectrometer and atomic absorption spectrophotometer are always used interchangeably and both are always abbreviated as AAS, a few slight differences have been noted; a spectrometer tells you which wavelengths of light is absorbed and which wavelengths of light is reflected. A spectrophotometer measures the relative intensity of the light absorbed or reflected at a particular wavelength of light. Spectrometer is a term that applied to instruments that operate over a very wide range of wavelengths, from gamma rays and x-rays into the far infrared, however if the instrument is designed to measure the spectrum in absolute units rather than relative units, then it is typically called spectrophotometer. In this unit, we will use the two interchangeably.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the principles and operations of AAS

- explain the different components of the AAS and functions.

3.0 MAIN CONTENT

3.1 Atomic Absorption Spectrophotometer (AAS)

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (μgdm–3) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another, higher, energy level. Atomic absorption spectrometry has many uses in different areas of chemistry.

Fig. 3.7: Flame Atomic Absorption Spectroscopy Instrument (Wikipedia)

3.1.1 How it Works

Atoms of different elements absorb characteristic wavelengths of light. Analysing a sample to see if it contains a particular element means using light from that element. For example, with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample. In AAS, the sample is atomised – i.e. converted into ground state free atoms in the vapour state – and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapour, the more radiation is absorbed. The amount of light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample. Consequently, an atomic absorption spectrometer needs the

following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed.

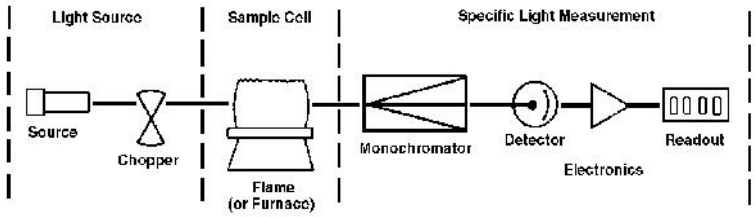

Fig. 3.8: Basic Atomic Absorption Spectrometer

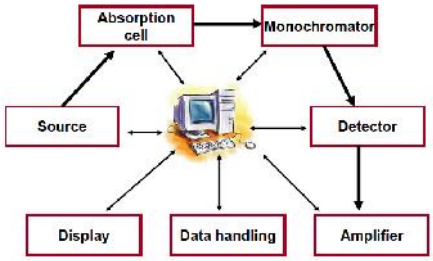

Fig.3.9: Schematic Diagram of Atomic Absorption Spectrometer

3.1.2 Principles of operation of AAS

The technique makes use of the atomic absorption spectrum of a sample in order to assess the concentration of specific analytes within it. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

3.1.3 The Basic Components

Every absorption spectrometer must have components which fulfill the three basic requirements. There must be:

1. **Light source:** A light source which emits the sharp atomic lines of the element to be determined is needed. The most widely known and recognized source is the hollow cathode lamp. These lamps are designed to emit the atomic spectrum of a particular element, and specific lamps are selected for use depending on the element to be determined.
2. **Sample cell:** An atomic vapor must be generated in the light beam from the source. This is generally accomplished by introducing the sample into a burner system or electrically heated furnace aligned in the optical path of the spectrophotometer.
3. **Means of specific light measurement:** The selection of a specific source and a particular wavelength in that source is what allows the determination of a selected element to be made in the presence of others. The wavelength of light which is isolated by the monochromator is directed onto the detector, which serves as the ‘‘eye’’ of the instrument.

1. Light Sources

An atom absorbs light at discrete wavelengths. In order to measure this narrow light absorption with maximum sensitivity, it is necessary to use a line source, which emits the specific wavelengths which can be absorbed by the atom. Narrow line sources not only provide high sensitivity, but also make atomic absorption a very specific analytical technique with few spectral interferences. The two most common line sources used in atomic absorption are the ‘‘hollow cathode lamp’’ and the ‘‘electrodeless discharge lamp.’’

i. The Hollow Cathode Lamp

The hollow cathode lamp is an excellent, bright line source for most of the elements determinable by atomic absorption.

ii. The Electrodeless Discharge Lamp

The atomic absorption determination of these elements can often be dramatically improved with the use of brighter, more stable sources such as the ‘‘electrodeless discharge lamp’’.

2. Optical Considerations Photometers

The portion of an atomic absorption spectrometer's optical system which conveys the light from the source to the monochromator is referred to as the *photometer*. Three types of photometers are typically used in atomic absorption instruments: single-beam, double-beam and what might be called compensated single-beam or pseudo double-beam.

i. Single-Beam Photometers

The instrument represents a fully functional ''single-beam'' atomic absorption spectrometer. It is called ''single-beam'' because all measurements are based on the varying intensity of a single beam of light in a single optical path.

ii. Double-Beam Photometers

An alternate photometer configuration, known as ''double-beam'' uses additional optics to divide the light from the lamp into a ''sample beam'' (directed through the sample cell) and a ''reference beam'' (directed around the sample cell).

iii. Alternative Photometer Designs

Many alternative system designs which provide advantages that are similar to those of double beam optical systems and the light throughput characteristic of single-beam systems.

3. The Atomic Absorption Atomiser (The Sample Cell)

i. Pre-Mix Burner System

The sample cell, or atomiser, of the spectrometer must produce the ground state atoms necessary for atomic absorption to occur. This involves the application of thermal energy to break the bonds that hold atoms together as molecules.

ii. Impact Devices

The sample aerosol is composed of variously sized droplets as it is sprayed into the mixing chamber. Upon entering the flame, the water in these droplets is vaporised. The remaining solid material must likewise be vaporised, and chemical bonds must be broken to create free ground state atoms.

iii. Nebulisers, Burner Heads and Mounting Systems

- Several important factors enter into the nebulizer portion of the burner system. In order to provide efficient nebulization for all types of sample solution, the nebulizer should be adjustable. Stainless steel has been the most common material used for construction of the nebuliser because of its durability and affordability.
- Burner heads typically are constructed of stainless steel or titanium. All-titanium heads are preferred as they provide extreme resistance to heat and corrosion.
- A ‘‘quick change’’ atomizer mount is an important item to facilitate convenient changeover from one device to another without the use of tools.

4. Electronics

i. Precision in Atomic Absorption Measurements

Observed precision will improve with the period of time over which each sample is read. Where analyte concentrations are not approaching detection limits, integration times of one to three seconds will usually provide acceptable precision. When approaching instrument detection limits where repeatability is poor, precision can be improved by using even longer integration times, up to 10 seconds. In most instances; however, there is little advantage to using integration times longer than 10 seconds.

ii. Calibration of the Spectrometer

- Most modern atomic absorption instruments include microcomputer-based electronics.
- The microcomputer provides atomic absorption instruments with advanced calculation capabilities, including the ability to calibrate and compute concentrations from absorbance data conveniently and accurately, even for nonlinear calibration curves.
- In the linear region, data on as little as one standard and a blank may be sufficient for defining the relationship between concentration and absorbance.
- However, additional standards are usually used to verify calibration accuracy. Where the relationship becomes nonlinear, however, more standards are required.

- The accuracy of a calibration computed for a nonlinear relationship depends on the number of standards and the equations used for calibration.
- For the equation format which optimally fits atomic absorption data, it has been experimentally shown that accurate calibration can be achieved with a minimum of three standards plus a blank, even in cases of severe curvature.
- Figure 8 illustrates the accuracy of microcomputer-calculated results for cobalt with single standard ‘‘linear’’ and three-standard ‘‘nonlinear’’ calibrations. After the instrument was calibrated using the specified procedure, a series of standards were analysed.
- Figure 8 shows plots of the actual concentrations for those standards versus the measured values for both calibration procedures.
- The results obtained with ‘‘linear’’ calibration are accurate only where the absorbance: concentration relationship is linear, up to about 5 mg/mL.

The results obtained with three-standard ‘‘nonlinear’’ calibration are still accurate at 30mg/mL, significantly extending the useful working range. For versatility, current instruments allow fitting of more than three standards to these same basic equations.

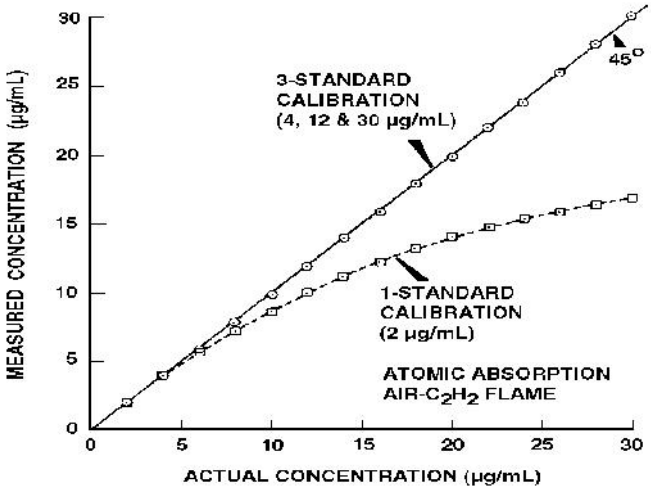

Fig. 3.10: Cobalt Calibration Accuracy

5. Automation of Atomic Absorption

i. Automated Instruments and Sample Changers

One of the greatest contributions to the efficiency of the analytical laboratory is the automated atomic absorption spectrometer. Automatic samplers were the first step in freeing the analyst from the monotonous task of manually introducing each and every sample. However, the real advancement in analysis automation came in the late 1970's, when automated multi-element atomic absorption was introduced. In addition to automatic sample introduction, these instruments offer automatic setup of instrument parameters to preprogrammed values.

ii. Automated Sample Preparation

The introduction of commercial systems based on techniques such as flow injection have directly addressed the need for automated sample preparation capabilities. Flow injection techniques can be used to automate relatively simple procedures such as dilution or reagent addition. They can also be used to automate complex chemical pretreatments, including analyte pre-concentration and cold vapor mercury and hydride generation procedures.

iii. The Stand-alone Computer and Atomic Absorption

Stand-alone and personal computers have extended the automation and data handling capabilities of atomic absorption even further. These computers can interface directly to instrument output ports to receive, manipulate, and store data and print reports in user selectable formats. Also, data files stored in personal computers can be read into supplemental software supplied with the system or third-party software such as word processor, spreadsheet and database programs for open-ended customization of data treatment and reporting.

4.0 CONCLUSION

You have learnt that atoms of different elements absorb characteristic wavelengths of light. Analysing a sample to see if it contains a particular element means using light from that element. For e.g. with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample. In AAS, the sample is atomised – i.e. converted into ground state free atoms in the vapour state – and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapour, the more radiation is absorbed. The amount of light absorbed is proportional to the number of lead atoms. A calibration curve is

constructed by running several samples of known lead concentration under the same conditions as the unknown.

5.0 SUMMARY

You have been taught that the three main requirements that the components of every AAS must fulfill include light source, sample cell and means of specific light measurement. A light source which emits the sharp atomic lines of the element to be determined. Sample cell ensures an atomic vapor must be generated in the light beam from the source. This is generally accomplished by introducing the sample into a burner system or electrically heated furnace aligned in the optical path of the spectrophotometer. Means of specific light measurement: The selection of a specific source and a particular wavelength in that source is what allows the determination of a selected element to be made in the presence of others.

6.0 TUTOR-MARKED ASSIGNMENT

1. Briefly explain the AAS instrument and mention five other instruments in that category.
2. Explain how the AAS instrument works and state its principles of operation.
3. Mention and briefly explain the three basic components of the AAS.
4. What do you understand as the Photometer? State three types of photometers you studied.
5. Write short notes on the following components of a sample cell or atomic absorption atomiser.
   i. Pre-mix burner system
   ii. Impact devices
   iii. Nebulisers, burner heads and mounting systems
6. Give a detailed explanation on how calibration of the spectrometer can be carried out.
7. What is the difference and similarity of colorimeter to AAS?

7.0 REFERENCES/FURTHER READING

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UNIT 4 PRINCIPLES AND OPERATION OF A PH METER

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1.0 INTRODUCTION

A **pH meter** is an instrument used to measure acidity or alkalinity of a solution - also known as pH. pH is the unit of measure that describes the degree of acidity or alkalinity. It is measured on a scale of 0 to 14. pH is however, negative logarithm to base 10 of hydrogen ion concentrations of a substance say soil. A rough indication of pH can be obtained using pH papers or indicators, which change color as the pH level varies. These indicators have limitations on their accuracy, and can be difficult to interpret correctly in colored or murky samples. If it turns pink, it's acid I think—you probably learned that useful phrase once upon a time, along with the second half of the same rhyme: "and if it turns blue, it's an alkali true." Measuring acids and alkalis (bases) with litmus paper is something pretty much every one learns how to do in the secondary school. It's relatively easy to compare your little strip of wet paper with the colors on a chart and figure out how acidic or alkaline something, is on what's called the **pH scale**. But sometimes that's too crude a measurement and can lead to unexpected errors especially when complex materials are involved. If you keep tropical fish, for example, or you're a gardener with specimens like soil of a certain acidity or alkalinity, getting things wrong with the litmus risks killing off your prized pets or your plants. That's why many people invest in a meter that

can measure pH directly. We will soon have a critical look at what pH meters are and how they work.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the basic principles and operations of a pH meter
- explain how pH meter can be calibrated.

3.0 MAIN CONTENT

3.1 pH Meter

A **pH meter** is an instrument used to measure acidity or alkalinity of a solution - also known as pH. pH is the unit of measure that describes the degree of acidity or alkalinity. It is measured on a scale of 0 to 14.
The quantitative information provided by the pH value expresses the degree of the activity of an acid or base in terms of hydrogen ion activity. The pH value of a substance is directly related to the ratio of the hydrogen ion [H+] and the hydroxyl ion [OH-] concentrations. If the H+ concentration is greater than OH-, the material is acidic; i.e., the pH value is less than 7. If the OH- concentration is greater than H+, the material is basic, with a pH value greater than 7. If equal amounts of H+ and OH- ions are present, the material is neutral, with a pH of 7. Acids and bases have free hydrogen and hydroxyl ions, respectively. The relationship between hydrogen ions and hydroxyl ions in a given solution is constant for a given set of conditions, either one can be determined by knowing the other.

3.1.1 What Does pH Mean?

The pH (always written little p, big H) of a substance is an indication of how many hydrogen ions it forms in a certain volume of water. There's no absolute agreement on what "pH" actually stands for, but most people define it as something like "power of hydrogen" or "potential of hydrogen." Now this is where it gets confusing for those of you who don't like math. The proper definition of pH is that it's *minus the logarithm of the hydrogen ion activity in a solution* (or, if you prefer, the logarithm of the reciprocal of the hydrogen ion activity in a solution). Gulp. What does that mean?

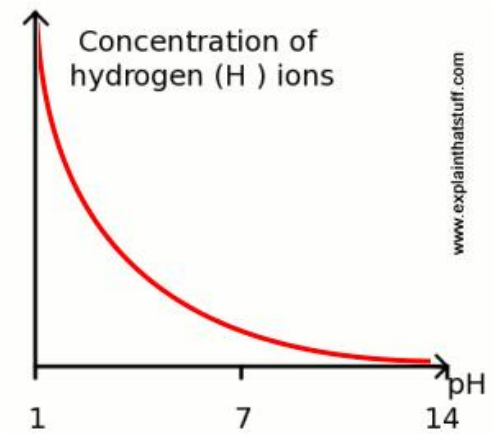

Fig. 3.11: Curve of Concentration of H ions

It's simpler than it sounds. Let's unpick it a bit at a time. Suppose you have some liquid sloshing about in your aquarium and you want to know if it's safe for those fishes you intend to keep. You get your pH meter and stick it into the "water" (which in reality is a mixture of water with other things dissolved in it). If the water is very acidic, there will be lots of active hydrogen ions and hardly any hydroxide ions. If the water is very alkaline, the opposite will be true. Now if you have a thimble-full of the water and it has a pH of 1 (it's unbelievably, instantly, fish-killingly acidic), there will be one million times (10 to the power of 6, written 10^6) more hydrogen ions than there would be if the water were neutral (neither acidic nor alkaline), with a pH of 7. That's because a pH of 1 means 10^1 (which is just 10), and a pH of 7 means 10^7(10 million), so dividing the two gives us 10^6 (one million). There will be 10 million million (10^{13}) more hydrogen ions than if the water were extremely alkaline, with a pH of 14. Maybe you can start to see now where those mysterious pH numbers come from?

However, if we decide to invent a scale of acidity and start it off at very acidic and call that 1. Then something neutral will have far fewer (one millionth or 10^{-6} times as many hydrogen ions) and something alkaline will have fewer still (that's one 10 trillionth, or one 10 million millionth, or 10^{-13} times as many). Dealing with all these millions and billions and trillions is confusing and may be difficult to handle, so we just take a logarithm of the number of hydrogen ions and refer to the power of ten we get in each case. In other words, the pH means simply looking at the (probably gigantic) number of hydrogen ions, taking the power of 10, and removing the minus sign. That gives us a pH of 1 for extremely acidic, pH 7 for neutral, and pH 14 for extremely alkaline. "Extremely alkaline" is another way of saying **incredibly weakly** acidic.

3.1.1.1 Definition of pH

pH is the negative logarithm to base ten of hydrogen ion concentration of soil or any substance considered.
$pH = -\log_{10}[H^+]$

How to calculate pH and $[H^+]$

The equilibrium equation yields the following formula for pH:
$pH = -\log_{10}[H^+]$
$[H^+] = 10^{-pH}$

In other words, pH is the negative log of the molar hydrogen ion concentration. Or, the molar hydrogen ion concentration equals 10 to the power of the negative pH value. It's easy to do this calculation on any scientific calculator because it will have a "log" button. (This is not the same as the "ln" button, which refers to the natural logarithm!)

Example:

Calculate the pH for a specific $[H^+]$. Calculate pH given $[H^+] = 1.4 \times 10^{-5}$ M
$pH = -\log_{10}[H^+]$
$pH = -\log_{10}(1.4 \times 10^{-5})$
$pH = 4.85$

Example:

Calculate $[H^+]$ from a known pH. Find $[H^+]$ if pH = 8.5
$[H^+] = 10^{-Ph}$
$[H^+] = 10^{-8.5}$
$[H^+] = 3.2 \times 10^{-9}$ M

Example:

Find the pH if the H^+ concentration is 0.0001 moles per liter.
$pH = -\log[H^+]$

Here it helps to rewrite the concentration as 1.0×10^{-4} M, because if you understand how logarithms work, this makes the formula:

$pH = -(-4) = 4$
Or, you could simply use a calculator and take:
$pH = -\log(0.0001) = 4$

Usually you aren't given the hydrogen ion concentration in a problem, but have to find it from a chemical reaction or acid concentration. Whether this is easy or not depends on whether you're dealing with a strong acid or a weak acid. Most problems asking for pH are for strong acids because they completely dissociate into their ions in water. Weak acids, on the other hand, only partially dissociate, so at equilibrium a solution contains both the weak acid and the ions into which it dissociates.

Example:

Find the pH of a 0.03 M solution of hydrochloric acid, HCl.

Hydrochloric acid is a strong acid that dissociates according to a 1:1 molar ratio into hydrogen cations and chloride anions. So, the concentration of hydrogen ions is exactly the same as the concentration of the acid solution.

[H^+ = 0.03 M
pH = - log (0.03)
pH = 1.5
pH and pOH

You can easily use the pH value to calculate pOH, if you recall:

pH + pOH = 14

This is particularly useful if you're asked to find the pH of a base, since you'll usually solve for pOH rather than pH.

3.1.2 Principle of pH Measurement

The pH of a substance indicates how many hydrogen ions (H+) it forms in a certain measured volume of water. A pH meter shows the value as to how acidity or alkalinity a liquid is. The basic principle of the pH meter is measuring the density of hydrogen ions. Positively charged hydrogen ions (H+) is formed when acids dissolve in water. A stronger acid is measured by the greater dense accumulation of hydrogen ions. Bases or alkaline substances dissolve in water forming negatively charged hydrogen ions (OH-). A high concentration of negatively charged hydrogen ions forms a stronger base. A number of hydrogen ions present in a solution dissolved in some amount of water determine the pH. pH value of solutions ranges from 1 to 14. Solutions with pH value 1 is highly acidic with solutions with pH value 14 are highly basic. A pH meter is used to determine the pH of different soil and plant materials.

A pH meter is made of a pH probe which passes the electrical signals to the meter and the meter shows the pH value of the solution. The glass probe contains two electrodes, a reference electrode and a sensor electrode which are in the form glass tubes. One contains saturated potassium chloride solution and other contains pH 7 buffer. The sensor electrode bulb interpolates permeable or porous glass membrane with metal salts and silica coatings. A silver wire coated with silver chloride is submerged in a pH buffer in the bulb. Another silver wire with silver chloride coating is immersed in the saturated potassium chloride solution.

3.1.2.1 Making Accurate pH Measurements

For accurate measurements, pH meters have to be properly calibrated by dipping it into buffers which are test solution of known pH and adjusting the meter accordingly. You can alternatively correct the measurement yourself by calibrating the pH meter and making measurements at broadly the same measurements.

3.1.3 Operations of pH Meter

When the probe is immersed in a liquid solution for measurement, hydrogen (H+) ions accumulate around the bulb replacing the metal ions from the bulb. An exchange of ions occurs which generates an electric flow that is captured by the silver wire. pH meter computes the voltage of the electric flow by changing it into pH value by comparing the generated voltage with the reference electrode. A high voltage is realized when there is a higher accumulation or density of hydrogen ions due to increased acidity of the solution. Reading in a meter decreases due to increased voltage. Likewise, hydrogen ions decrease when there is an increase in alkalinity. In other words, an increase in hydroxyl ions concentration decreases the voltage and increases the pH value in the meter.

All-inclusive, working principle of the pH meter and pH sensor depends upon the exchange of ions from sample solution to pH 7 buffer (inner solution) of glass electrode through the glass membrane. Continuous use of the glass integument decreases the porosity of the glass thus decreases the performance and efficiency of the probe.

3.1.4 Care for a pH Meter

Caring for a pH meter depends on the types of electrode in use. However, the following have to be borne in mind while using a pH meter:

i. Study the manufacturer's recommendations.
ii. When used frequently, it is better to keep the electrode moist, since moisturising a dry electrode takes a long time, accompanied by signal drift. However, modern pH meters do not mind their electrodes drying out provided they have been rinsed thoroughly in tap water or potassium chloride.
iii. When on expedition, measuring sea water, the pH meter can be left moist with sea water. However, for prolonged periods, it is recommended to moist it with a solution of potassium chloride at pH=4 or in the pH=4.01 acidic calibration buffer.
iv. pH meters do not like to be left in distilled water.
v. Note that a pH probe kept moist in an acidic solution, can influence results when not rinsed before inserting it into the test vial.
vi. Remember that a liquid of pH=4 has 10,000 more hydrogen ions than a liquid of pH=8. Thus a single drop of pH=4 in a vial measuring 400 drops of pH=8 really upsets measurements!
vii. Remember also that the calibration solutions consist of chemical buffers that 'try' to keep pH levels constant, so contamination of your test vial with a buffer is really serious.

3.1.5 Challenges of pH Measurement Applications

The list below illustrates the types of problems that you can expect when measuring pH and how to handle them.

i. Instrumentation is frequently the source of disturbance for pH systems, through repeatability error, measurement noise, or valve hysteresis.
ii. In-line pH loops will oscillate, regardless of controller modes and tuning, if set points are on the steep parts of the titration curves.
iii. pH electrode submersion assemblies with un-encapsulated terminations below the liquid surface will eventually have wet terminations.
iv. Reagent control valves that are not close-coupled to the injection point on in-line systems will cause reagent delivery delays large enough to describe the tools of your trade n words that may seem foreign.
v. You need either a flow meter or a seer to diagnose reagent delivery problems.
vi. Flow feedforward signals should be multiplied by pH controller outputs and employed to operate reagent valves directly or to establish reagent flow control set points.
vii. Transportation delays to pH electrodes in analyser houses will exceed mixing deadlines - such that increasing comfort in

checking the electrodes is offset by decreasing comfort in checking trend recordings.

viii. Injection electrodes should be preferred to sample holder assemblies whenever possible to reduce maintenance problems and improve response times - but not all injection electrodes are created equal.

ix. Large tanks are fine if you don't have to control them; use the volume upstream to reduce reagent consumption or downstream to reduce control error. If you can't make-up your mind where to use one, put it downstream.

x. Install one or three but never two electrodes for a pH measurement.

3.1.6 The pH of Known Substances

The values for pH make more sense when compared with that of known substances. Note that the pH scale is logarithmic and that each next value contains times less hydrogen ions.
A Ph=0 contains the most, and is highly acidic.

0 5% Sulphuric acid, H_2SO_4, battery acid.
1 0.1 N HCl, hydrochloric acid (1.1)
2 Lemon juice. Vinegar (2.4-3.4)
3 wine (3.5-3.7)
4 Orange juice. Apple juice (3.8). Beer. Tomatoes.
5 Cottage cheese. Black coffee. Rain water 5.6.
6 Milk. Fish (6.7-7). chicken (6.4-6.6).
7 Neutral: equal numbers of hydrogen and hydroxyl ions. Blood (7.3-7.4). Distilled water without CO_2, after boiling.
8 Sea water (8.1). Egg white.
9 Borax. baking soda.
10 Milk of magnesia, Magnesium hydroxide $Mg(OH)_2$.
11 Household ammonia
12 Photographic developer, household bleach
13 Oven cleaner
14 Sodium lye NaOH, 1 mol/litre.

3.2 Soil pH Meter

Measure soil pH and temperature in the field or laboratory with the pH-HH-SOIL meter. The pH of the soil can either be measured directly or a

slurry can be prepared. The pH-HH-SOIL meter effectively measures the pH of the soil pore water.

Fig. 3.11: pH-HH-SOIL Meter

Features of the pH-HH-SOIL Meter

- Specialized soil pH electrode
- Multi-level LCD display
- User friendly operation using only 2 buttons
- On-screen tutorial messages
- Automatic Temperature Compensation
- Automatic one or two-point calibration
- BEPS (Battery Error Prevention System) alerts the user in the event that low battery power could adversely affect readings
- Battery % displayed on startup
- Compact, heavy-duty, and waterproof

How pH influences soil and its soil properties

Soil can be acid, neutral or alkaline, according to its pH value. Most plants prefer a pH range from 5.5 to 7.5; but some species prefer more acid or alkaline soils. Nevertheless, every plant requires a particular range of pH, for optimum growth.

pH strongly influences the availability of nutrients and the presence of microorganisms and plants in the soil. For example, fungi prefer acidic conditions whereas most bacteria, especially those supplying nutrients to the plants, have a preference for moderately acidic or slightly alkaline soils. In fact, in strongly acidic conditions, nitrogen fixing and the mineralization of vegetable residual is reduced. Plants absorb the nutrients dissolved in the soil water and the nutrient solubility depends largely on the pH value. Hence, the availability of elements is different at different pH levels.

Each plant needs elements in different quantities and this is the reason why each plant requires a particular range of pH to optimize its growth. For example, iron, copper and manganese are not soluble in an alkaline environment. This means that plants needing these elements should theoretically be in an acidic type of soil. Nitrogen, phosphorus, potassium and sulfur, on the other hand, are readily available in a pH range close to neutrality. Furthermore, abnormal pH values, increase the concentration of toxic elements for plants. For example, in acid conditions, there can be an excess of aluminum ions in such quantities that the plant cannot tolerate. Negative effects on chemical and physical structure are also present when pH values are too far from neutral conditions (break up of aggregates, a less permeable and more compact soil).

The pH-HH-SOIL meter features a large multi-level LCD screen which displays both pH and temperature readings simultaneously with 0.01 pH resolution and an accuracy of ±0.05 pH. The LCD screen also has indicators for calibration status and stability, as well as on-screen tutorial messages. The pH-HH-SOIL has many advanced features that are found in more expensive portable instrumentation including automatic calibration, buffer recognition, and temperature compensation. The battery percent level is displayed at start up alerting the user to the remaining battery power that is available. The supplied pH electrode is a glass body, amplified pH electrode with a built-in temperature sensor designed specifically for measuring the pH in soils.

4.0 CONCLUSION

You have learnt that a **pH meter** is an instrument used to measure acidity or alkalinity of a solution - also known as pH. pH is the unit of measure that describes the degree of acidity or alkalinity. It is measured on a scale of 0 to 14. A rough indication of pH can be obtained using pH papers or indicators, which change color as the pH level varies. These indicators have limitations on their accuracy, and can be difficult to interpret correctly in colored or murky samples. pH meter is the easiest way of testing for the pH of any substance in solution as it measures the H+ and OH- concentrations of the substance. If the H+ concentration is greater than OH-, the substance is acidic; i.e., the pH value is less than 7. If the OH- concentration is greater than H+, the substance is basic, with a pH value greater than 7. If equal amounts of H+ and OH- ions are present, the substance is neutral, with a pH of 7.

5.0 SUMMARY

You have therefore learnt what a pH meter is and how it is used to measure the pH of any substance(s). pH meter will produce an accurate result when it is calibrated before use, that is inserting the electrode in a buffer solution (usually a solution with known pH). Adequate precautions have to be taken to ensure accuracy while using a pH meter.

6.0 TUTOR-MARKED ASSIGNMENT

1. What is soil pH meter?
2. How can you care for a pH meter?
3. Define soil pH and state its formula.
4. State the principle of pH measurement.
5. What are the challenges of pH measurement applications?
6. Briefly discuss the influence of pH on soil properties.

7.0 REFERENCES/FURTHER READING

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MODULE 4 TECHNIQUES AND PRECAUTIO MICRONUTRIENTS ANALYSIS

Unit 1 Analytical Techniques for Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn)
Unit 2 Analytical Techniques for available Boron (B) and Molybdenum (Mo)

UNIT 1 ANALYTICAL TECHNIQUES FOR ZINC (ZN), COPPER (CU), IRON (FE) AND MANGANESE (MN)

CONTENTS

1.0 Introduction
2.0 Objectives
3.0 Main Content
3.1 Techniques of Analysing for Zn, Cu, Fe and Mn
3.1.1 Preparation of Standard Curve for Zinc
3.1.2 Preparation of Standard Curve for Copper
3.1.3 Preparation of Standard Curve for Iron
3.1.4 Preparation of Standard Curve for Manganese
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

Micronutrients are the nutrient elements that are needed by crop in a small quantity. However, 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 and Mn and the same are hereby discussed.

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 mostly taken up by plants is Fe2+. The uptake of Fe is affected 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 Mn2+ ions although it exists in many oxidation states. Manganese and phosphate are mutually antagonistic. Copper, as zinc, exists in soils mainly as divalent ions, Cu2+. 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 fertilisation can induce Cu deficiency.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss some basic micronutrients needed for optimal crop productivity
- identify the basic analytical techniques for determining Zn, Cu, Fe and Mn content of soils.

3.0 MAIN CONTENT

3.1 Techniques of Analysing for Micronutrients (Zn, Cu, Fe and Mn)

Ethylenediaminetetraacetic acid (EDTA) with ammonium acetate is commonly used for the extraction of many elements. Diethylenetriaminepentaacetic 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. 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 4 shows commonly used extractants for micronutrients (FAO, 2008). The estimation of elements in the extract is done with the help of an AAS.

Table 5 details the critical limits for DTPA-extractable micronutrient elements as proposed by Lindsay and Norvell (1978). Diethylenetriaminepentaacetic acid is an important and widely used chelating agent that combines with free metal ions in the solution to form soluble complexes of elements.

Table 4.1: Commonly used extractants for micronutrients

Elements	Extractants
Zinc	EDTA + ammonium acetate, EDTA + ammonium carbonate, DTPA + CaCl2, HCl, HNO3 and dithiozone + ammonium acetate
Copper	EDTA, EDTA + ammonium acetate, ammonium bicarbonate + DTPA, HCl and HNO3
Iron	EDTA, DTPA, EDTA + ammonium acetate, HCl and HNO3
Manganese	Hydroquinone, ammonium phosphate, DTPA and EDTA + ammonium acetate
Boron	Hot water and mannitol + CaCl2
Molybdenum	Ammonium oxalate, ammonium acetate, ammonium fluoride and water

Source: FAO (2008)

Table 4.2: Critical limits for DTPA-extractable micronutrients

Available	Micronutrients			
(µg/g soil)	Zn	Cu	Fe	Mn
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

Source: Lindsay and Norvell (1978)

To prepare DTPA 0.005M, 0.01M CaCl2.2H2O 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 a500-ml flask.
2. Put 1.47 g of CaCl2.2H2O in a separate 1 000-ml flask. Add 500 ml of distilled water and shake to dissolve.
3. Add DTPA+TEA mixture to the CaCl2 solution and make the volume up to1 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(AAS).

Volumetric methods such as EDTA and KMnO4 titrations are used for estimating Zn and Mn, and Fe, respectively. Copper can be estimated by titration with Na2S2O3.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.

Spectrometric methods include the following for Zn, Fe, Mn and Cu respectively

- 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 atomised. A hollow cathode lamp (which precedes the atomiser) 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 6 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 7 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, MnSO4.H2O is preferred.

Table 4.3: 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/liter)	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 4.4: Specifications for preparing micronutrient standard solution

Element	Concentration of stock solution (µg/ml)	Salt to be used	Quantity of salt required (g/liter)
Zn	100	Zinc sulphate (ZnSO4.7H2O)	0.4398
Cu	100	Copper sulphate (CuSO4.5H2O)	0.3928
Fe	100	Ferrous sulphate (FeSO4.7H2O) or Ferrous	0.4964

		ammoniumsulphate	
Mn	100	Manganese sulphate (MnSO4.H2O)	0.3075

3.1.1 Preparation of Standard Curve for Zinc

The reagents required are:

i. 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.

ii. Glass-distilled or demineralised acidified water of pH 2.5±0.5: Dilute 1 ml of 10 percent sulphuric acid to 1 litre with glass-distilled or mineralised water and adjust the pH to 2.5 with a pH meter using 10 percent H2SO4 or NaOH. This solution is called acidified water.

iii. 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:

i. Flaming the solutions: Atomize the standards on an AAS at a wavelength of213.8 nm (Zn line of the instrument).

ii. 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.

Precautions

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

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

As an example, Figure 16 shows a standard curve prepared by the FAO, 2008 for Zn estimation while establishing a soil testing laboratory.

3.1.2 Preparation of Standard Curve for Copper

The reagents required are:

i. 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 HNO3 (1:1) and make up to the mark by glass-distilled water. Stopper the flask and shake the solution well. This is1 000 µg/ml Cu.

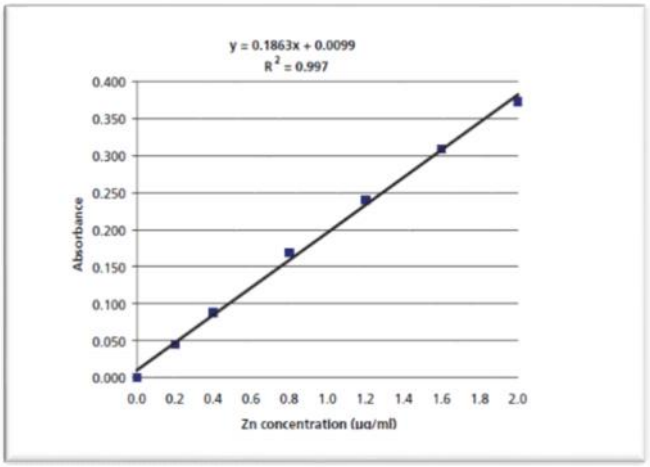

Fig. 4.1: Standard curve of Zn on an AAS

ii. 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.

iii. Glass-distilled or demineralised acidified water of pH 2.5±0.5: Same as that for Zn above

iv. 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 17 shows a standard curve prepared by the authors for Cu estimation while establishing a soil testing laboratory.

3.1.3 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.

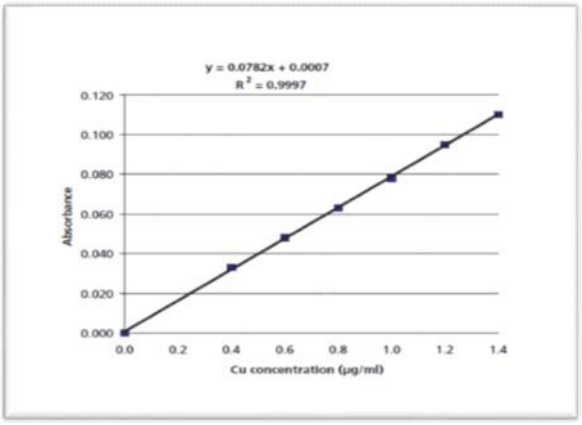

Fig. 4.2: Standard curve of Cu on an AAS

Transfer it to a 1 litre 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 demineralised acidified water of pH 2.5 $\pm$ 0.5: Same as that for Zn (above).

Working Fe standard solutions: Pipette 10 ml of Fe stock solution in a100-ml volumetric flask, and dilute to volume with DTPA solution.

This is100 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 18 shows a standard curve prepared by FAO, 2008 for Fe estimation while establishing a soil testing laboratory.

3.1.4 Preparation of Standard Curve for Manganese

The reagents required are:

Standard Mn solution: Weigh 3.0751 g of AR-grade manganese sulphate (MnSO4.H2O) on a clean watch glass and transfer it to a 1-litre flask through 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.

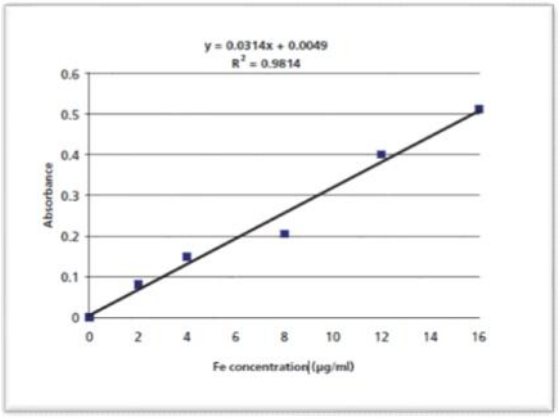

Fig. 4.3: Standard Curve of Fe on an AAS

- Glass-distilled or demineralized acidified water of pH 2.5 $\pm$ 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 and8 ml of 50 µg/ml solution, and make the

volume up with DTPA solution to50 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 19 shows a standard curve prepared by FAO, 2008 for Mn estimation while establishing a soil testing laboratory.

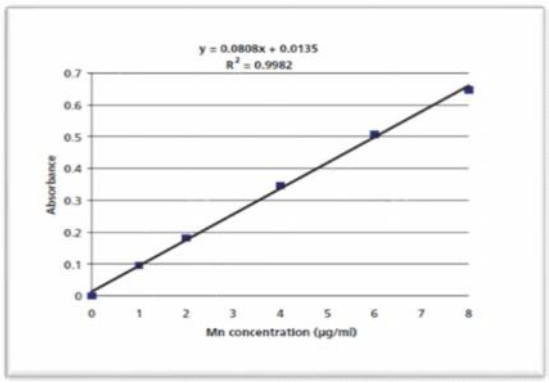

Fig. 4.4: Standard curve of Mn on an AAS

3.2 Procedure for Extraction of Micronutrient 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.

3.3 Estimation of Micronutrients 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.

4.0 CONCLUSION

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 mostly taken up by plants is Fe2+. The uptake of Fe is affected 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.

5.0 SUMMARY

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.

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.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define micronutrients and mention four (4) micronutrients you studied in this unit.
2. Mention five crops that are sensitive to Zn deficiency in soils.
3. In a tabular form state five micronutrients and their extractants.
4. State three major reagents used in preparation of standard curve for Zn.
5. What is the procedure for preparation of standard curve for Zn?
6. Outline the precautions to be taken while preparing standard Zn curve.

7.0 REFERENCES/FURTHER READING

FAO (2008). “Guides to Laboratory Establishment for Plant Nutrient Analysis.” *Fertiliser and Plant Nutrition Bulletin* Vol. 19. Rome.

Lindsay, W.L. & Norvell, W.A. (1978). “Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper.’ *Soil Sci. Soc. Am. J.,* 42: 421–448.

UNIT 2 ANALYTICAL TECHNIQUES FOR AVAILABLE BORON (B) AND MOLYBDENUM (MO)

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1.0 Introduction
2.0 Objectives
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        3.2.2 Estimation by Colorimetric Method
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5.0 Summary
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1.0 INTRODUCTION

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 analyzed 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. Boron deficiency occurs mostly in the light-textured acid soils when they are leached heavily through irrigation or heavy rainfall.

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. Molybdenum usually occurs as MoO3, MoO5 and MoO2. These oxides are transformed slowly to soluble molybdates (MoO4), which is the form taken up by plants.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- discuss the basic analytical techniques for determining available B and Mo content of soils.

3.0 MAIN CONTENT

3.1 Techniques for Analysing Available B

3.1.1 The Extraction Procedure for B

i. Put 25 g of soil in a quartz flask or beaker.
ii. Add about 50 ml of double-distilled water (DDW) and about 0.5 g of activated charcoal.
iii. 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 Na2B4O7.10H2O 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. Atomise 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).

3.1.2 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 deionised 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 deionised 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 Na2B4O7.10H2O (AR-grade) in a small volume of deionised 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.

Precautions

- 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.

3.2 Techniques for Analysing Available Mo

3.2.1 Extraction of Available Mo

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 (NH4OAc) 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 MoO3 (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. Atomise 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.

1. 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).

3.2.2 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 HNO3–HClO4 mixture (4:1), followed by 10 ml of 4M H2SO4 and then with H2O2, 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.

4.0 CONCLUSION

Boron deficiency occurs mostly in the light-textured acid soils when they are leached heavily through irrigation or heavy rainfall. The total Mo content in soils is perhaps the lowest of all the micronutrient elements. In the soil solution, Mo exists mainly as HMoO4 ion under acidic condition, and as $MoO4^{2-}$ 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.

5.0 SUMMARY

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. Boron deficiency occurs mostly in the light-textured acid soils when they are leached heavily through irrigation or heavy rainfall. 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.

6.0 TUTOR-MARKED ASSIGNMENT

1. Why is calorimetric method preferred over AAS in B analysis in soil?
2. Outline the extraction procedure for B.
3. What are the specifications/relevant parameters for estimation of B on an AAS?
4. Discus B estimation by colorimetric method under the following headings:
   a. Apparatus
   b. Reagents
   c. Procedure
   d. Precautions
5. Discus the estimation of Mo by colorimetric method under the following headings:
   a. Apparatus
   b. Reagents
   c. Procedure
   d. Precautions

7.0 REFERENCES/FURTHER READING

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