COURSE GUIDE

EMT 409
SOIL ANALYSIS

COURSE TEAM
DR ABBAS IDOWU INNOCENT (COURSE WRITER)

NATIONAL OPEN UNIVERSITY OF NIGERIA

MAIN COURSE

CONTENTS **PAGE**

MODULE 1

UNIT 1 TYPES OF LABORATORY CHEMICALS

CONTENTS

1.0 INTRODUCTION

Laboratory Chemicals are reagents that are used in carrying out analysis in a standard laboratory and they are of different types and according to Ahmad (2003), they include general solvents, hydrocarbons and chlorinated solvents, acids, bases, inorganic chemicals, organic chemicals, high risk chemicals etc.

2.0 OBJECTIVES

By the end of this unit, you should be able to understand:

- the meaning of laboratory chemicals
- the different categories of laboratory chemicals
- laboratory hazards and safety precautions to be taken.

3.0 MAIN CONTENT

3.1 Laboratory Chemicals

Laboratory chemicals are reagents that are used in carrying out analysis in a standard laboratory. These chemicals are useful in carrying out analysis such as water holding capacity, cation exchange capacity, electrical conductivity, pH, soil texture, soil moisture content, lime requirement, gypsum requirement, micro and macro nutrients analysis in soil, organic matter content etc. These chemicals are of different types and American Chemical Society (2018) lists some to include oxidizing and reducing agents, peroxides, cryogenics while according to Ahmad (2003), they include, general solvents, hydrocarbons and chlorinated solvents, acids, bases, inorganic chemicals, organic chemicals, high risk chemicals etc.

3.1.1 Peroxides

These are a group of chemicals that have an oxygen-to-oxygen bond (R–O–O–R) and care must be taken when handling inorganic or organic peroxides, since they tend to be unstable and can, depending on the compound, decompose violently (American Chemical Society, 2018). A common example is hydrogen peroxide.

3.1.2 Cryogenics

These are chemicals stored at very low temperatures and they should be handled with special cryogenic gloves (American Chemical Society, 2018). Examples include dry ice (solid CO_2) and liquid nitrogen

3.1.3 Oxidizing and reducing agents

An oxidizing agent is a substance that causes oxidation or the loss of electrons from an atom, compound, or molecule while a reducing agent is a substance that causes reduction, or the gain of electrons (American Chemical Society, 2018). Oxidation and reduction always occur together. Oxidizing agents include chlorates, chromates, dichromates, hypochlorites, nitrates, nitric acid, nitrites, perchlorates, permanganates, peroxides while reducing agents include alkali metals, alkaline earth metals, hydrogen gas, carbon monoxide.

3.1.4 General solvents

A solvent is a substance in which a solute dissolve. This includes Methanol, Ethanol, 2-Propanol (IPA), Acetone, Butanol, Butyl acetate, Ethyl acetate, Ethylene glycol, etc.

3.1.5 Hydrocarbon and chlorinated solvents

A hydrocarbon is a compound containing hydrogen and carbon while chlorinated solvents are solvents that have chlorine as part of their constituents. Examples of hydrocarbon and chlorinated solvents are Toluene, Xylene, Hexane, Cyclohexane, Trichloroethylene, Dichloromethane, Chloroform, Carbon tetrachloride etc.

3.1.6 Acids

An acid is a substance which when dissolved in water releases hydrogen ions. Examples of acids are Sulphuric acid, Nitric acid, Hydrochloric acid, Acetic acid, Phosphoric acid, Oxalic acid, etc.

3.1.7 Bases

A base is a substance which when dissolved in water releases hydroxide ions. Bases include Sodium hydroxide, Potassium hydroxide, Ammonium hydroxide, Tetramethyl Ammonium hydroxide (photo resist developer) etc.

3.1.8 Inorganic and organic chemicals

Inorganic chemicals include Copper sulphate, Nickel chloride, Calcium phosphate, Erbium chloride, Ytterbium chloride, Aluminium chloride, Titanium isopropoxide, Zirconium propoxide, Silver nitrate, Potassium nitrate etc. Organic chemicals, on the other hand, include Acetic anhydride, Urea, Pyrocatechol, Butoxyethanol, Cyclopentane, Dimethylbutane, Photoresist, N, N-Dimethylformamide, 1-Methyl-2-Pyrrolidinone, etc.

3.1.9 High risk chemicals

High risk chemicals, as the name suggests are chemicals that pose high risks and must be handled with extra caution. Examples are Cyanides, Mercury compounds, Lead compounds, Arsenic compounds, Cadmium compounds, Ethylenediamine, Hydrofluoric acid, etc.

3.2 Laboratory Hazards and Safety Precautions

Due to their corrosive nature, acids and bases can irritate or even burn the eyes, irritate the skin, and cause respiratory distress (American Chemical Society, 2018). The risk is higher when they are concentrated, but even when diluted they can be hazardous. Protective equipment including chemical goggles, aprons and gloves are essential.

Hydrocarbon and volatile organic compounds are combustible or flammable and can irritate the skin. Used in a confined space, they can cause asphyxiation. They should always be used in a well-ventilated area or hood, and away from any open flames.

Mercury is a serious chronic health hazard. Although it is not readily absorbed through the skin, its greatest health hazard is due to inhalation of its vapors, usually as a result of a spill. Mercury compounds (e.g., alkyl mercury) are extremely toxic and must be handled with extreme care.

Oxidation and reduction always occur together. Oxidation-reduction reactions tend to release heat, so oxidizers and reducing agents can cause other materials to combust more readily. Mixing oxidizing agents (nitric acid) with organic materials in waste bottles has resulted in many explosions. Always store oxidizing and reducing agents away from each other, and from flammable materials.

Other safety measures according to Ahmad (2003) are listed below:

i. Always use extracted wet benches for chemical work.
ii. Always wear safety glasses or goggles at all times in the laboratory.
iii. Always wear laboratory coat/apron in the laboratory.
iv. Appropriate gloves should be worn as needed.
v. Appropriate shoes should be worn in the laboratory.
vi. Wear breathing mask as and when appropriate.
vii. Only trained personnel may use breathing apparatus.
viii. Wash hands before leaving the laboratory.
ix. Never mouth suck anything in a pipette in the laboratory.
x. No food or drink is allowed in laboratories or areas where chemicals are used or stored.
xi. No food should be stored in a laboratory refrigerator.
xii. Never eat or drink from the laboratory glassware.
xiii. Keep exposed skin covered in the laboratory.

4.0 CONCLUSION

Laboratory chemicals are useful for many purposes and they are also dangerous. Chemicals can cause severe burns and organ damage. Therefore, read the instructions on any chemical before using it and apply extra caution while using it knowing that your safety in the laboratory is not only determined by your own actions but also by the actions of those around you.

5.0 SUMMARY

In this unit, we have learnt that:

- there are various types of laboratory chemicals
- there are laboratory hazards
- safety precautions can be taken to avoid laboratory hazards.

6.0 TUTOR-MARKED ASSIGNMENT

1. Is it proper to add acid to water or water to acid while working in the laboratory?
2. List other categories of laboratory chemicals with named examples that are not mentioned in the list above.

7.0 REFERENCES/FURTHER READING

Ahmad, M. (2003). Laboratory and chemical safety guide. Pp. 1-23.

American Chemical Society (2018). Common laboratory hazards

UNIT 2 ANALYTICAL INSTRUMENTS AND PRINCIPLES OF OPERATION

CONTENTS

1.0 INTRODUCTION

Analytical instruments are of various types and each one has a specific role or roles that it performs. Analytical instruments that will be considered in this section include the colorimeter, flame analyzer, atomic absorption spectrophotometer and pH meter.

2.0 OBJECTIVES

By the end of this unit, you should be able to understand:

- the function of each instrument;
- the principle of operation of each instrument and
- the suitability of each instrument for a particular operation.

3.0 MAIN CONTENT

3.1 Colorimeter

A colorimeter is a device that measures the absorbance of particular wavelengths of light by a specific solution.

Figure 1: A digital colorimeter

This device is most commonly used to determine the concentration of a known solute in a given solution by the application of the Beer-Lambert's law.

Beer's Law: According to Beer's law when monochromatic light passes through the colored solution, the amount of light transmitted decreases exponentially with increase in concentration of the colored substance.
Lambert's Law: According to Lambert's law the amounts of light transmitted decreases exponentially with increase in thickness of the colored solution.

Beer-Lambert's 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 for a wide range of applications across the chemical and biological fields including, but not limited to, the analysis of blood, water, nutrients in soil and foodstuffs, determining the concentration of a solution, determining the rates of reaction, determining the growth of bacterial cultures and laboratory quality control (www.azo.com)

A Colorimeter is comprised of the following parts:
1. Light source
2. Filter (the device that selects the desired wavelength)

3. Cuvette chamber (the transmitted light passes through compartment wherein the solution containing the colored solution are kept in cuvette, made of glass or disposable plastic)
4. Detector (this is a photosensitive element that converts light into electrical signals)
5. Galvanometer (measures electrical signal quantitatively).

The colorimeter must be handled carefully and kept clean and dry.

3.1.1 Principle of operation

IEC (2006) explained how to operate a digital colorimeter as enumerated below:

Using cuvettes:

i. Press ON/OFF button to power the instrument.
ii. Place distilled water into one clean cuvette and store in a receptacle provided.
iii. Prepare at least two solution samples of **known** concentration and store in bottles for future use. Mark the bottles with the known concentration.
iv. Pour a small sample of each of these known concentration solution in cuvettes, mark them and store them in the receptacles provided.
v. Prepare one or more samples of **unknown** concentrations in cuvettes and store them in the receptacles provided.

If the correct light source colour to use is not known, proceed as follows:

i. Press UNITS/CAL button to select 'Transmission' in percent.
ii. Fit the square adaptor into the well, select the first wavelength (blue) and insert the square cuvette with distilled water with the smooth sides in the light path. Press and hold the 'Calibrate' button for one second then release. Meter will calibrate to 100% transmission for that particular illumination wavelength.
iii. Remove the distilled water cuvette and replace with any sample of the solution. Note the transmission reading in % for that wavelength.
iv. Using the distilled water cuvette and the same sample, repeat the above for each of the 4x wavelengths (colours) provided and choose the wavelength that provided the LOWEST reading (lowest % transmission or highest absorption). This is the light source you must use for concentration measurements of this colour of sample solution.

Now that the correct light source to use is now known, proceed as follows:

i. Select this preferred light source, **select Absorbance** and re-insert the distilled water sample. Press the CAL button for one second to calibrate to zero absorption.
ii. Using 'X' axis as 0-max concentration units and 'Y' axis as 0-2.3 Absorbance, plot firstly the distilled water reading (zero absorbance and zero concentration) and then the two known concentration sample readings, then join the three points to make a straight line graph relating absorption to concentration. This relationship between absorption and concentration is linear according to Beer's Law over a limited range ofconcentrations.
iii. Remove the known coloured reference sample and replace with unknown sample. Note the absorbance reading. Plot this reading on the graph line to find the corresponding concentration of the solution.

3.2 Flame Analyzer

It is an instrument that is meant for determining sodium and potassium; additional filters are included for measuring other elements such as calcium, barium and lithium.

Figure 2: A Flame Analyzer

A simple flame photometer consists of the following basic components:

a) The burner: a flame that can be maintained in a constant form and at a constant temperature.
b) Nebuliser and mixing chamber: a means of transporting a homogeneous solution into the flame at a steady rate.
c) Simple colour filters (interference type): a means of isolating light of the wavelength to be measured from that of extraneous emissions.
d) Photo-detector: a means of measuring the intensity of radiation emitted by the flame.

The flame analyzer must be handled carefully, kept clean and operated by a trained personnel.

3.2.1 Principle of operation

Flame photometry relies 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 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.

Table 1: Emission wavelength and flame colour of some elements

Element	Emission Wavelength (nm)	Flame Colour
Sodium (Na)	589	Yellow
Potassium (K)	766	Violet
Barium (Ba)	554	Lime Green
Calcium (Ca)	622*	Orange
Lithium (Li)	670	Red

*Note: Calcium is measured by using the calcium hydroxide band emission at 622nm as calcium main atomic emission occurs at 423nm.

Over certain ranges of concentration, the intensity of the emission is directly proportional to the number of atoms returning to the ground state. This is in turn proportional to the absolute quantity of the species volatized in the flame, i.e. light emitted is proportional to sample concentration.

It can be seen that if the light emitted by the element at the characteristic wavelength is isolated by an optical filter and the intensity of that light measured by a photo-detector, then an electrical signal can be obtained proportional to sample concentration. Such an electrical signal can be processed and the readout obtained in an analogue or digital form.

3.3 Atomic Absorption Spectrophotometer

Atomic absorption spectrophotometer (AAS) is an analytical instrument that measures the concentrations of elements (Royal Society of Chemistry, no date). There are two types of this instrument: the single beam and the double beam. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram ($\mu g\ dm^{-3}$) 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 units have four basic parts: interchangeable lamps that emit light at element-specific wavelengths, a sample aspirator, a flame or furnace apparatus for volatilizing the sample or hydride generation apparatus and a photon detector. This instrument must be kept clean and in a safe room within the laboratory to prevent theft. It must be handled by trained personnel who will be able to teach others on how to operate it efficiently.

Figure 3: An Atomic Absorption Spectrophotometer.

Atomic absorption spectrometry has many uses in different areas.

i. **Clinical analysis:** Far analyzing metals in biological fluids such as blood and urine.
ii. **Environmental analysis:** Monitoring our environment – *e.g.* finding out the levels of various elements in rivers, seawater, drinking water, air, petrol and drinks such as wine, beer and fruit drinks.
iii. **Pharmaceuticals:** In some pharmaceutical manufacturing processes, minute quantities of a catalyst used in the process (usually a metal) are sometimes present in the final product. By using AAS, the amount of catalyst present can be determined.
iv. **Industry:** Many raw materials are examined and AAS is widely used to check that the major elements are present and that toxic impurities are lower than specified – *e.g.* in concrete, where calcium is a major constituent, the lead level should be low because it is toxic.
v. **Mining:** By using AAS, the amount of metals such as gold in rocks can be determined to see whether it is worth mining the rocks to extract the gold.

3.3.1 Principle of operation

According to Chasteen (2000), the main parts of the Atomic Absorption Spectrophotometer system are a hollow cathode lamp, nebulizer, air/acetylene flame, and optical system.

The hollow cathode lamp (HCL) uses a cathode made of the element of interest with a low internal pressure of an inert gas. A low electrical current (~ 10 mA) is imposed in such a way that the metal is excited and emits a few spectral lines characteristic of that element (for instance, Cu 324.7 nm and a couple of other lines; Se 196 nm and other lines, etc.). The light is emitted directionally through the lamp's window, a window made of a glass transparent in the UV and visible wavelengths.

The nebulizer chamber thoroughly mixes acetylene (the fuel) and oxidant (air or nitrous oxide), and by doing so, creates a negative pressure at the end of the small diameter, plastic nebulizer tube. This negative pressure acts to suck ("uptake") liquid sample up the tube and into the nebulizer chamber, a process called aspiration. A small glass impact bead and/or a fixed impeller inside the chamber creates a heterogeneous mixture of gases (fuel + oxidant) and suspended aerosol (finely dispersed sample). This mixture flows immediately into the burner head where it burns as a smooth, laminar flame evenly distributed along a narrow slot in the well-machined metal burner head. Liquid sample not flowing into the flame collects on the bottom of the nebulizer chamber and flows by gravity through a waste tube to a glass waste container (remember, this is still highly acidic). For some elements that form refractory oxides (molecules hard to break down in the flame) nitrous oxide (N_2O) needs to be used instead of air (78% N_2 + 21% O_2) for the oxidant. In that case, a slightly different burner head with a shorter burner slot length is used.

Tuned to a specific wavelength and with a specified slit width chosen, the monochromator isolates the hollow cathode lamp's analytical line. Since the basis for the Atomic Absorption Spectroscopy process is atomic ABSORPTION, the monochromator seeks to only allow the light not absorbed by the analyte atoms in the flame to reach the photomultiplier tube (PMT). That is, before an analyte is aspirated, a measured signal is generated by the photomultiplier tube as light from the HCL passes through the flame. When analyte atoms are present in the flame while the sample is aspirated, some of that light is absorbed by those atoms (remember it is not the ionic but elemental form that absorbs). This causes a decrease in PMT signal that is proportional to the amount of analyte. This last is true inside the linear range for that element using that slit and that analytical line. The signal is therefore a decrease in measured light: atomic absorption spectroscopy.

3.4 pH Meter

This is an instrument used for measuring the acidity or alkalinity of an aqueous solution. It is composed of a reference electrode, a glass electrode, and a voltmeter. The basic principle of a pH meter is to measure hydrogen ions concentration. Acids dissolve in water to form positively charged hydrogen ions (H+). The greater this concentration of hydrogen ions, the stronger the acid is. Similarly, bases or alkali dissolve in water forming negatively charged ions (OH-). The stronger a base is, the higher the concentration of negatively charged hydrogen ions there are. A pH value of 7 indicates a neutral solution. Pure water should have a pH value of 7. pH values less than 7 indicate an acidic solution while pH values greater than 7 indicate an alkaline solution. A solution with a pH value of 1 is highly acidic while a solution with a pH value of 14 is highly alkaline. This instrument must be kept clean and dry when not in use.

3.4.1 Principle of operation

According to Anthoni (2005), when one metal is brought in contact with another, a voltage difference occurs due to their differences in electron mobility. When a metal is brought in contact with a solution of salts or acids, a similar electric potential is caused, which has led to the invention of batteries. Similarly, an electric potential develops when one liquid is brought in contact with another one, but a membrane is needed to keep such liquids apart.

A pH meter measures essentially the electro-chemical potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside. Because the thin glass bulb allows mainly the agile and small hydrogen ions to interact with the glass, the glass electrode measures the electro-chemical potential of hydrogen ions or the potential of hydrogen. To complete the electrical circuit, also a reference electrode is needed. Note that the instrument does not measure a current but only an electrical voltage, yet a small leakage of ions from the reference electrode is needed, forming a conducting bridge to the glass electrode. A pH meter must thus not be used in moving liquids of low conductivity (thus measuring inside small containers is preferable). The pH meter measures the electrical potential (follow the drawing clock-wise from the meter) between the mercuric chloride of the reference electrode and its potassium chloride liquid, the unknown liquid, the solution inside the glass electrode, and the potential between that solution and the silver electrode. But only the potential between the unknown liquid and the solution inside the glass electrode change from sample to sample. So all other potentials can be calibrated out of the equation.

4.0 CONCLUSION

Analytical instruments are of paramount use in laboratory analysis. Analytical instruments such as colorimeter, atomic absorption spectrophotometer, flame analyzer and pH meter considered in this section have different uses in many field of endeavours.

5.0 SUMMARY

In this unit, we have learnt that:

- each instrument operates differently
- each instrument has its own different components
- each instrument must be maintained in order to enhance its lifespan.

6.0 TUTOR-MARKED ASSIGNMENT

1. Explain in full detail how to operate a digital colorimeter.
2. Itemize the use of atomic absorption spectrophotometer in different fields.

7.0 REFERENCES/FURTHER READING

Anthoni, J.F. (2005). pH Meter Princinples.

Chasteen, T.G. (2000). Atomic Absorption Spectroscopy.

Industrial Equipment and Control Property Limited (2006). Colorimeter – digital (Instruction Sheet). Pp. 1-10.

Royal Society of Chemistry (no date). Atomic Absorption Spectrometry. The Education Department, The Royal Society of Chemistry, Burlington House, Piccadilly, London W1J 0BA

UNIT 3 SOIL SAMPLING AND SAMPLE PREPARATION

CONTENTS

1.0 INTRODUCTION

Soil is an essential factor in crop and animal production. Soil types include sand, clay, loam and silt. The nature of soil determines the type of crop that can be grown on it. Soils that support the growth of arable crops such as maize, rice etc may not support the growth of cash crops such as cocoa and rubber. Soil has the ability to absorb water and nutrients and release same to support the growth of crops.

2.0 OBJECTIVES

By the end of this unit, you should be able to understand:

- the importance of soil sampling
- the procedure for carrying out soil sampling
- how to prepare soil samples without contamination.

3.0 MAIN CONTENT

3.1 Soil Sampling and Sample Preparation

The sampling of soil intended for laboratory analysis is done by taking several small quantities from a given area. The samples are submitted to a laboratory center to identify the type and quality of the soil, and to

determine the right amount of nutrients that may be needed for the favorable growth of desirable plant and animal organisms. Soil sampling is also carried out for the purpose of other analyses such as soil texture, water holding capacity, cation exchange capacity, organic matter content etc.

3.1.1 Sampling materials

Depending on the purpose and precision required, the following tools according to Food and Agriculture Organization (FAO) (2008) may be needed for taking soil samples:

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

3.1.2 Selection of a sampling unit

A visual survey of the field should precede the actual sampling (FAO, 2008). 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.

3.1.3 Sampling

Prepare a map of the area to be covered in a survey showing different sampling unit boundaries (FAO, 2008). Enter a plan of the number of samples and manner of composite sampling on the map, designating different fields by letters (A, B, C, etc.). Traverse each area separately. Cut a slice of the plough layer at intervals of 15–20 steps or according to the area to be covered. Generally, depending on the size of the field, 10–20 spots must be taken for one composite sample.

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

samples with the help of an auger from the plough depth and collect them in the bucket.

Pour the soil from the bucket onto a piece of clean paper or cloth, and mix it thoroughly. Spread the soil evenly and divide it into quarters. Reject two opposite quarters and mix the rest of the soil again. Repeat the process until left with about 0.5 kg of the soil. Collect it and put in a clean cloth bag. Mark each bag clearly in order to identify the sample.
The bag used for sampling must always be clean and free from any contamination. If the same bag is to be used a second time, turn it inside out and remove the soil particles. Write the details of the sample on the information sheet. Put a copy of this information sheet in the bag. Tie the mouth of the bag carefully.

3.1.4 Dispatch of soil samples to the laboratory

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

Compare the number and details on the bag with the dispatch list. The serial numbers of different places should be distinguished by putting the identification mark specific for each centre.

Pack the samples properly. Wooden boxes are most suitable for long transport.

Sample bags should be packed only in clean bags never used for fertilizer or detergent packing.

3.1.5 Handling in the laboratory

As soon as the samples arrive at the soil testing laboratory, they should be checked against the accompanying information list. If the laboratory personnel have collected the samples themselves, then adequate field notes should have been kept. All unidentifiable samples should be discarded. Information regarding samples should be recorded in a register, and each sample should be given a laboratory number, in addition to the sample number, to help to distinguish it where more than one source of samples is involved.

3.1.6 Drying of samples

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

3.1.7 Post-drying care

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

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

If the soil is to be analyzed for trace elements, containers made of copper, zinc and brass must be avoided during grinding and handling. Sieves of different sizes can be obtained in stainless steel. Aluminium or plastic sieves are useful alternative for general purposes.
After the sample has passed through the sieve, it must be mixed again thoroughly.

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

3.1.8 Precautions during sampling

When sampling a soil, bear in mind the following:

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

3.2 Sampling salt-affected soils

Salt-affected soils may be sampled in two ways (FAO, 2008). Surface samples should be taken in the same way as for soil fertility analysis. These samples are used to determine the gypsum requirement of the soil. For reclamation purpose, it is necessary to know also the characteristics of lower soil depth. Therefore, such soils are sampled down to a depth of 1 m. The samples may be removed from one to two spots per 0.4 ha where the soil is uniformly salt-affected. Where patches are conspicuous, then all large patches should be sampled separately. Soil is sampled separately far soil depths (about 0.5 kg from each depth) of 0–15, 15–30, 30–60 and 60–100 cm. If a stony layer is encountered during sampling, such a layer should be sampled separately and its depth noted. This is very important and must not be ignored.

Soil samples can be removed by a spade, or if the auger is used, then care should be taken to note the depth of “concretion” (stones) or other impermeable layer (hardpan). If the soil shows evidence of profile development or distinct stratification, samples should be taken by

horizon. If a pit is dug and horizons are absent, then mark the vertical side of the pit at depths of 15, 30, 60 and 100 cm from the surface and collect about 0.5 kg soil from each layer, cutting uniform slices of soil separately. In addition to the above sampling, one surface soil sample should be taken as in the case of normal soil sampling for fertilizer recommendation.

Pack the samples and label the bags in the same way as for normal soil sampling, giving additional information about the depth of the sample.

The sheet accompanying the sample must include information on:

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

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

4.0 CONCLUSION

Soil sampling is very essential as it helps to determine soil fertility which shows the type of crops that can be grown on such soils and it also helps to reduce cost and avoid resource wastages. Sample preparation must be done following laid rules and regulations in order to avoid contamination.

5.0 SUMMARY

In this unit, we have learnt that:

- soil sampling plays an important role in precision agriculture;
- salt-contaminated soil can also be sampled and
- precautions must be taken during soil sampling and sample preparation.

6.0 TUTOR-MARKED ASSIGNMENT

1. Explain why information sheet is very important in soil sampling and sample preparation.
2. Why is the practice of passing only a portion of the ground sample through the sieve and discarding the remainder erroneous?

7.0 REFERENCE/FURTHER READING

Food and Agriculture Organization (2008). Guide to laboratory establishment for plant nutrient analysis. FAO, Rome, Italy.

UNIT 4 MACRONUTRIENT ANALYSIS IN SOIL

CONTENTS

1.0 INTRODUCTION

Macronutrients are essential nutrients that play vital role in crop growth. Their deficiencies will result in poor crop performance leading to delay in growth, maturity, flowering, fruiting etc. Three macronutrients mainly Nitrogen(N), Phosphorus (P) and Potassium (K) and their determinations in soil will be considered in this section.

2.0 OBJECTIVES

By the end of this unit, you should be able to understand:

- nitrogen determination in soil;
- phosphorus determination in soil and
- potassium analysis in soil.

3.0 MAIN CONTENT

3.1 Nitrogen Determination in Soil

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

bumping, while liquid paraffin checks frothing during heating (as recommended in total N estimation by the Kjeldahl method).

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

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

The reagents required are:

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

The procedure is:

i. Weigh 20 g of soil sample in an 800-ml Kjeldahl flask.
ii. Moisten the soil with about 10 ml of distilled water, wash down the soil, if any, adhering to the neck of the flask.
iii. Add 100 ml of 0.32 percent $KMnO_4$ solution.
iv. Add a few glass beads or broken pieces of glass rod.
v. Add 2–3 ml of paraffin liquid, avoiding contact with the upper part of the neck of the flask.
vi. Measure 20 ml of 2 percent boric acid containing mixed indicator in a 250-ml conical flask and place it under the receiver tube. Dip the receiver tube in the boric acid.
vii. Run tap-water through the condenser.
viii. Add 100 ml of 2.5 percent NaOH solution, and immediately attach to the rubber stopper fitted in the alkali trap.
ix. Switch the heaters on and continue distillation until about 100 ml of distillate is collected.

x. First, remove the conical flask containing distillate and then switch off the heaters to avoid back suction.
xi. Titrate the distillate against 0.02M H_2SO_4 in a burette until a pink colour starts to appear.
xii. Run a blank without soil.
xiii. Carefully remove the Kjeldahl flask after cooling and drain the contents in the sink.

The calculation for estimating mineralizable N is:
i. Volume of acid used to neutralize ammonia in the sample = $A - B$ ml
ii. N content in the test sample = $(A - B) \times 0.56$ mg
iii. Percent N = $(A - B) \times 0.56 \times 5$
where:
iv. A = volume of 0.02M H_2SO_4 used in titration against ammonia absorbed in boric acid;
v. B = volume of 0.02M sulphuric acid used in blank titration.
vi. 1 000 ml of 1M H_2SO_4 = 28 g N; thus, 1 ml of 0.02M sulphuric acid = 0.56 mg N. The weight of the soil sample is 20 g, thus, the factor for converting into percent N = 100/20 = 5.

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

3.2 Phosphorus Determination in Soil

The two methods most commonly used for determining the available Phosphorus (P) in soils are: Bray's Method No. 1 for acid soils; and Olsen's method for neutral and alkali soils (FAO, 2008). 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.

3.2.1 Bray's Method No. 1

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

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

The reagents required are:

- Bray's Extractant No. 1 (0.03M NH_4F in 0.025M HCl): Dissolve 2.22 g of NH_4F in 200 ml of distilled water, filter, and add to the filtrate 1.8 litres of water containing 4 ml of concentrated HCl, make the volume up to 2 litres with distilled water.
- Molybdate reagent: Dissolve 1.50 g of $(NH_4)_2MoO_4$ in 300 ml of distilled water. Add the solution to 350 ml of 10M HCl solution gradually with stirring. Dilute to 1 litre with distilled water.
- Stannous chloride solution (stock solution): Dissolve 10 g of $SnCl_2.2H_2O$ in 25 ml of concentrated HCl. Add a piece of pure metallic tin, and store the solution in a glass stoppered bottle.
- Stannous chloride solution (working solution): Dilute 1 ml of the stock solution of stannous chloride to 66.0 ml with distilled water just before use.
- Prepare fresh dilute solution every working day.

The procedure is:

- Preparation of the standard curve: Dissolve 0.2195 g of pure dry KH_2PO_4 in 1 litre of distilled water. This solution contains 50 µg P/ml. Preserve this as a stock standard solution of phosphate. Take 10 ml of this solution and dilute it to 0.5 litres with distilled water. This solution contains 1 µg P/ml (0.001 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 $SnCl_2$ solution, shake again and dilute to the 25-ml mark. After 10 minutes, read the blue colour of the solution on the spectrophotometer at a wavelength of 660 nm. Plot the absorbance reading against "µg P" and connect the points.
- 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.

- Development of colour: Take 5 ml of the filtered soil extract with a bulb pipette in a 25-ml measuring flask; deliver 5 ml of the molybdate reagent with an automatic pipette, dilute to about 20 ml with distilled water, shake and add 1 ml of the dilute $SnCl_2$ solution with a bulb pipette. Fill to the 25-ml mark and shake thoroughly. Read the blue colour after 10 minutes on the spectrophotometer at 660 nm after setting the instrument to zero with the blank prepared similarly but without the soil.

The calculation is: P(kg/ha) = A/1000000 x 50/5 x 2000000/5 = 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.

3.2.2 Olsen's method

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

The reagents required are:

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

The procedure is:

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

3.3 Potassium Analysis in Soil

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

The apparatus required consists of:

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

The reagents required are:

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

The procedure is:

- Preparation of the standard curve: Set up the flame photometer by atomizing 0 and 20 µg K/ml solutions alternatively to readings of 0 and 100. Atomize intermediate working standard solutions and record the readings. Plot these readings against the respective K contents and connect the points with a straight line to obtain a standard curve.
- 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.
- Determine the potash in the filtrate with the flame photometer.

The calculation is:
K (kg/ha) = A/1000000 x 25 x 2000000/5
where:
A = content of K (µg) in the sample, as read from the standard curve;
volume of the extract = 25 ml;
weight of the soil taken = 5 g;
weight of 1 ha of soil down to a plough depth of 22 cm is taken as 2 million kg.

4.0 CONCLUSION

From the explanation above, N, P and K analyses in soil are crucial as they indicate whether there will be any need for fertilizer application or not. It is also an established fact that cares should be taken during determinations of these macronutrients in soil in order to avoid error.

5.0 SUMMARY

In this unit, we have learnt that:

- bray's Method No. 1 and Olsen's methods are the two common methods for P analysis in soil
- in the case of soils, mineralizable Nitrogen (N) is estimated as an index of available N content and not the total N content
- potassium present in the soil is extracted with neutral ammonium acetate of 1 molarity. This is considered as plant-available K in the soils.

6.0 TUTOR-MARKED ASSIGNMENT

1. Using Bray's method No. 1, itemize stage by stage procedure for Phosphorus analysis in soil.
2. Why is it that hot Kjeldahl flasks should neither be washed immediately with cold water nor allowed to cool for long during Nitrogen determination in soil?

7.0 REFERENCES/FURTHER READING

Bray, R.H. and Kurtz. L.T. (1945). Determination of total, organic and available forms of phosphorus in soils. *Soil Sci.*, 59: 30–45.

Food and Agriculture Organization (2008). Guide to laboratory establishment for plant nutrient analysis. FAO, Rome, Italy.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U.S. Dep. Agric. 939.

Subbiah, B.V. and Asija, G.L. (1956). A rapid procedure for the determination of available nitrogen in soils. *Curr. Sci.*, 25: 259–260.

Toth, S.J. & Prince, A.L. 1949. Estimation of cation exchange capacity and exchangeable Ca, K and Na contents of soils by flame photometric techniques. *Soil Sci.*, 67: 439–445.

MODULE 2

Unit 1 Features and Functions of a Standard Soil Testing Laboratory
Unit 2 Special Techniques and Precautions in Micronutrient Analysis
Unit 3 Evaluation of Analytical Data

UNIT 1 FEATURES AND FUNCTIONS OF A STANDARD SOIL TESTING LABORATORY

CONTENTS

1.0 INTRODUCTION

Soil is the major source of nutrients for crops. Soil also provides support for plant growth in various ways. Knowledge about soil health and its maintenance is critical to sustaining crop productivity. Essential plant nutrients such as N, P, K, Ca, Mg and S are called macronutrients, while Fe, Zn, Cu, Mo, Mn, B and Cl are called micronutrients. It is necessary to assess the capacity of a soil to supply nutrients in order to supply the remaining amounts of needed plant nutrients (total crop requirement - soil supply).

2.0 OBJECTIVES

By the end of this unit, you should be able to understand:

- the features of a standard soil testing laboratory;
- the functions of a standard soil test and
- laboratory safety measures.

3.0 MAIN CONTENT

3.1 Soil Testing Laboratories

Standard soil testing laboratories are considered nerve centres for nutrient management and crop production systems (FAO, 2008). A standard soil testing laboratory building should have suitable separate rooms for different purposes and for performing different operations as described below

Room 1: Reception, sample receipt, and dispatch of reports.
Room 2: Sample storage and preparation room
Room 3: Nitrogen digestion/distillation room (with fumehood for digestion).
Room 4: Instrument room to house:

- atomic absorption spectrophotometer (AAS);
- flame photometer;
- spectrophotometer;
- pH meter, conductivity meter;
- ovens;
- centrifuge;
- balances;
- water still.

Room 5: Chemical analysis room:

- to prepare reagents and chemicals, and to carry out their standardization;
- to carry out extraction of soil and nutrient samples with appropriate chemicals/reagents;
- to carry out titration, colour development, precipitation, filtration, etc.;
- all other types of chemical work.

Room 6: Storage room for chemicals and spare equipment.
Room 7: Office room with computers for data processing and record keeping.

The air temperature of the laboratory and work rooms should be maintained constant at 20–25 °C. Humidity should be kept at about 50

percent. Temperature and humidity often affect soil and fertilizer samples. Temperature also affects some chemical operations. Hence, maintaining the temperature and humidity as specified is critical.

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

Care is required in order to ensure that acids and hazardous chemicals are stored in separate and safe racks. An inventory of all the equipment, chemicals, glassware and miscellaneous items in a laboratory should be maintained.

3.2 Functions of a Standard Soil Testing Laboratory

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

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

3.3 Laboratory Safety Measures

According to FAO (2008), special care is required while operating equipment, handling chemicals and in waste disposal.

3.3.1 Equipment

Electrical cables, plugs and tubing need proper checking in order to avoid accidents.

Various types of gas cylinders needed in the laboratory, such as acetylene, nitrous oxide and liquefied petroleum gas, must be kept under watch and properly sealed/capped, and they must be stored in ventilated cupboards.

3.3.2 Chemical reagents

Hazardous chemicals should be stored in plastic bottles. While working with chemicals, such as perchloric acid, a fumehood must be used. Chemicals must be labeled properly, indicating their hazardous nature. Bottles with inflammable substances need to be stored in stainless-steel containers.

3.3.3 Waste disposal

Each country has special rules and methods for the disposal of hazardous waste. Cyanides, chromates, arsenic (As), selenium (Se), cobalt (Co) and molybdate are commonly used but hazardous chemicals should never be disposed of in the laboratory sink but collected in a metal container for proper disposal.

3.3.4 General rules and requirements in a soil testing laboratory

General safety rules and requirements for personnel/students working in a laboratory according to FAO (2008) are:

1. Learn safety rules and the use of first-aid kits. Keep the first-aid kit handy in a conspicuous place in the laboratory.
2. Personal safety aids, such as laboratory coats, protective gloves, safety glasses, face shields and proper footwear, should be used.
3. Observe normal laboratory safety practice in connecting equipment to the power supply, in handling chemicals and in preparing solutions of reagents.
4. All electrical work must be done by qualified personnel.
5. Maintain an instrument manual and logbook for each item of equipment in order to avoid mishandling, accidents and damage to equipment.
6. Keep work tables/spaces clean. Clean up spillage immediately.
7. Wash hands after handling toxic/hazardous chemicals.
8. Never suck the chemicals by mouth but use automatic pipetting devices.

9. Use forceps/tongs to remove containers from hotplates/ovens/furnaces.
10. Do not use laboratory glassware for eating/drinking.
11. Never open a centrifuge cover until the machine has stopped.
12. Add acid to water and not water to acid when diluting the acid.
13. Always put labels on bottles, vessels and wash-bottles containing reagents, solutions, samples and water.
14. Handle perchloric acid and hazardous chemicals in fumehoods.
15. With the wet oxidation method of sample digestion, destroy organic matter (OM) first with nitric acid.
16. Read the labels on the bottles before opening them

4.0 CONCLUSION

Standard soil testing laboratories are very useful in soil science as they play a pivotal role in many analytical estimations such as soil structure analysis, cation exchange capacity, water holding capacity, organic matter content etc.

5.0 SUMMARY

In this unit, we have learnt that:

- an inventory of all the equipment, chemicals, glassware and miscellaneous items in a laboratory should be maintained
- a standard soil testing laboratory should have many rooms for different purposes
- proper air circulation in the laboratory is vital in order to prevent hazardous and toxic fumes and gases from remaining in the laboratory for long so as to prevent health risks.

6.0 TUTOR-MARKED ASSIGNMENT

1. Of what relevance are separate rooms within a standard soil testing laboratory.
2. List other functions of a standard soil testing laboratory that are not mentioned above.

7.0 REFERENCE/FURTHER READING

Food and Agriculture Organization (2008). Guide to laboratory establishment for plant nutrient analysis. FAO, Rome, Italy.

UNIT 2 SPECIAL TECHNIQUES AND PRECAUTIONS IN MICRONUTRIENT ANALYSIS

CONTENTS

1.0 INTRODUCTION

For the estimation of micronutrients in soils, it is the plant-available form that is critical and not the total content (FAO, 2008). 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 Zinc (Zn), Copper (Cu), Iron (Fe), Manganese (Mn), Boron (B) and Molybdenum (Mo), and the same are dealt with here.

Micronutrients are present in different forms in the soil. Among the most deficient ones is Zn, which is present as the divalent cation Zn^{2+}. Maize, citrus, legumes, cotton and rice are especially sensitive to Zn deficiency. Iron is present mostly in sparingly soluble ferric oxide forms, which occur as coatings of aggregate or as separate constituents of the clay fraction. Soil redox potential and pH affect Fe availability. The Fe form that is predominantly taken up by plants is $Fe^{2+.}$ The uptake of Fe is inhibited by phosphate levels caused by the formation of insoluble iron phosphate. Chemically, Mn behaves in soil in the same way as Fe. Soil Mn originates primarily from the decomposition of ferromagnesian rocks. It is taken up by the plants as Mn^{2+} ions although it exists in many oxidation states. Manganese and phosphate are mutually antagonistic. Copper, like zinc, exists in soils mainly as divalent ions, Cu^{2+}. It is usually adsorbed by clay minerals or associated with organic matter although they have little or no effect on its availability to crops. High phosphate fertilization can induce

Cu deficiency. Molybdenum usually occurs as MoO_3, MoO_5 and MoO_2. These oxides are transformed slowly to soluble molybdates (MoO_4), which is the form taken up by plants. Boron deficiency occurs mostly in the light-textured acid soils when they are leached heavily through irrigation or heavy rainfall.

There are different extractants for assessing plant-available nutrient (element) content in soils. The elements so extracted can be estimated quantitatively through chemical methods or instrumental techniques. Table 1 below lists commonly used extractants for different elements.

Table 2: Commonly used extractants for micronutrients

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

Source: FAO, 2008.

2.0 OBJECTIVES

By the end of this unit, you should be able to understand:

- whether a soil can provide enough micronutrients for optimal crop production
- the preparation of standard curve for some micronutrients
- the role of Atomic Absorption Spectrophotometer in micronutrient estimation.

3.0 MAIN CONTENT

3.1 Micronutrient Extraction

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

The estimation of elements in the extract is done with the help of an AAS.

Table 2 details the critical limits for DTPA-extractable micronutrient elements as proposed by Lindsay and Norvell (1978).

Table 3: Critical limits for DTPA-extractable micronutrients

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

Source: Lindsay and Norvell, 1978.

Diethylenetriamine pentaacetic acid is an important and widely used chelating agent that combines with free metal ions in the solution to form soluble complexes of elements. To avoid excessive dissolution of $CaCO_3$, which may release occluded micronutrients that are not available to crops in calcareous soils and may give erroneous results, the extractant is buffered in slightly alkaline pH. Triethanolamine (TEA) is used as buffer because it burns cleanly during atomization of extractant solution while estimating on an AAS. The DTPA has a capacity to complex each of the micronutrient cations as 10 times of its atomic weight. The capacity ranges from 550 to 650 mg/kg depending on the micronutrient cations.

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

1. Add 1.967 g of DTPA and 13.3 ml of TEA in 400 ml of distilled water in a 500-ml flask.
2. Put 1.47 g of $CaCl_2.2H_2O$ in a separate 1000-ml flask. Add 500 ml of distilled water and shake to dissolve.

3. Add DTPA+TEA mixture to the $CaCl_2$ solution and make the volume up to 1 litre. Adjust the pH to 7.3 by using 1M HCl before making the volume.

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

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

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

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

Atomic absorption spectroscopy can be applied successfully for estimating Zn, Cu, Fe and Mn. For specific estimation with an AAS, hollow cathode lamps specific to specific elements are used. Table 3 lists the relevant parameters.

Table 4: Parameters for estimation of micronutrients using an AAS

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

Source: FAO, 2008.

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 1000 µg/ml or 1 mg/ml (1000 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 4 details the quantities of chemical required to make 1 litre of standard solution of 100 µg/ml for different elements.

Table 5: Specifications for preparing micronutrient standard solutions

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

Mn	100	Manganese sulphate ($MnSO_4.H_2O$)	0.3075

Source: FAO, 2008.

In the case of Zn, Cu and Fe, 1000 µg/ml (1000 ppm) standard solutions are preferably prepared by dissolving 1.0g of pure metal wire and making the volume up to 1 litre as per the method described under each element. The solution is diluted to obtain the required concentration. In the case of Mn, $MnSO_4.H_2O$ is preferred.

3.1.1 Preparation of standard curve for zinc

The reagents required are:

- Standard Zn solution: Weigh 1.0 g of pure zinc metal in a beaker. Add 20 ml of HCl (1:1). Keep for a few hours, allowing the metal to dissolve completely. Transfer the solution to a 1-litre volumetric flask. Make up the volume with glass-distilled water. This is 1000 µg/ml Zn solution. For preparing the standard curve, refer to the 1000 µg/ml solution as solution A. Dilute 1 ml of standard A to 100 ml in order to obtain a 10 µg/ml solution, to be designated standard B.
- Glass-distilled or demineralized acidified water of pH 2.5 ± 0.5: Dilute 1 ml of 10 percent sulphuric acid to 1 litre with glass-distilled or mineralized water and adjust the pH to 2.5 with a pH meter using 10 percent H_2SO_4 or NaOH. This solution is called acidified water.
- Working Zn standard solutions: Pipette 1, 2, 4, 6, 8 and 10 ml of standard B solution in 50-ml numbered volumetric flasks and make the volume up with DTPA solution to obtain 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 µg/ml zinc. Stopper the flasks and shake them well. Fresh standards should be prepared every time when a fresh lot of acidified water is prepared.

The procedure is:

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

Special points to note are:

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.

3.1.2 Preparation of standard curve for copper

The reagents required are:

- Standard Cu solution: Weigh 1 g of pure copper wire on a clean watch glass and transfer it to a 1-litre flask. Add 30 ml of HNO_3 (1:1) and make up to the mark by glass-distilled water. Stopper the flask and shake the solution well. This is 1000 µg/ml Cu solution and it should be stored in a clean bottle for further use. Dilute 1 ml of 1000 µg/ml solution of Cu to 100 ml to obtain 10 µg/ml of standard Cu solution.
- Glass-distilled or de-mineralized acidified water of pH 2.5 ± 0.5: Same as that for Zn (above).
- 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 forth night.

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.

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

ml of 100 µg/ml solution and dilute each to 100 ml to obtain 2, 3, 8, 12 and 16 µg/ml of Fe solution.

The procedure is:

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

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 ($MnSO_4.H_2O$) 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 1000 µg/ml Mn. A secondary dilution of 5 ml to 100 ml with acidified water gives a 50 µg/ml solution.
- Glass-distilled or demineralized acidified water of pH 2.5 ± 0.2: Same as that for Zn (above).
- Working Mn standard solutions: The standard curve is prepared by taking lower concentrations of Mn in the range of 0–10 µg/ml. Take 1, 2, 4, 6 and 8 ml of 50 µg/ml solution, and make the volume up with DTPA solution to 50 ml to obtain 1, 2, 4, 6 and 8 µg/ml working standards.

The procedure is:

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

3.1.5 Available boron estimation

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

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

The extraction procedure for the methods presented below is:

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:

i. Lamp current: 20 m A°;
ii. Wavelength: 249.7 nm;
iii. Linear range: 1–10 µg/ml;
iv. Slit width: 0.2 nm;
v. Integration time: 2.0 seconds;
vi. 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:

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

The procedure is:

1. Atomize the working standards on an AAS using acetylene nitrous oxide as fuel instead of air acetylene fuel (as used for other micronutrients) at a wavelength of 249.7 nm.
2. Prepare a standard curve of known concentration of B by plotting the absorbance values on the y-axis against their respective B concentration on the x-axis. Measure the absorbance of the soil sample extract and determine the B content in the soil from the standard curve.

The relevant calculation is:

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

where:

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

Estimation by colorimetric method

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

The apparatus required consists of:

- An analytical balance;
- A flask or beaker;
- A volumetric flask;
- Some funnels;
- Some No. 42 filter paper;
- A spectrophotometer.

The reagents required are:

i. Azomethine-H: Dissolve 0.45 g of azomethine-H and 1.0 g of L-ascorbic acid in about 100 ml of deionized or double-distilled water. If the solution is not clear, it should be heated gently in a water-bath or under a hot water tap at about 30 °C until it dissolves. Every week, a fresh solution should be prepared and kept in a refrigerator.
ii. Buffer solution: Dissolve 250 g of ammonium acetate in 500 ml of de-ionized or double-distilled water, and adjust the pH to about 5.5 by adding slowly about 100 ml of glacial acetic acid, stirring continuously.
iii. EDTA solution (0.025 M): Dissolve 9.3 g of EDTA in de-ionized or double-distilled water, and make the volume up to 1 litre.
iv. Standard stock solution: Dissolve 0.8819 g of $Na_2B_4O_7.10H_2O$ (AR-grade) in a small volume of de-ionized water, and make the volume up to 1000 ml to obtain a stock solution of 100 µg B/ml.
v. 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 de-ionized or double-distilled water, and measure absorbance at 420 nm. This will give reading for standard solution with B concentration of 0, 0.05, 0.10, 0.20, 0.40 and 0.80 µg B/ml.

The relevant calculation is:
Content of B in the soil (µg/g or mg/kg) = C × dilution factor (10)
where:
C (µg/ml) = concentration of B as read from the standard curve against the absorbance reading of the soil solution on the spectrophotometer;
dilution factor = 10, which is calculated as follows:

- Weight of the soil taken = 25 g;
- Volume of extractant (water) added = 50 ml;
- First dilution = 2 times;
- Volume of the filtrate taken = 5 ml;
- Final volume of filtrate after colour development = 25 ml;
- Second dilution = 5 times;
- Total dilution = 2 × 5 = 10 times.

Important points to note are:

i. 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.
ii. The amount of charcoal added may vary with the organic matter 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.

3.1.6 Available molybdenum estimation

Molybdenum is a rare element in soils, and it is present only in very small amounts in igneous and sedimentary rocks (FAO, 2008). The major inorganic source of Mo is molybdenite (MoS_2). The total Mo content in soils is perhaps the lowest of all the micronutrient elements.

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

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

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

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

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

- Lamp current: 7 m A°;
- Wavelength: 313.3 nm;
- Linear range: 1–4 µg/ml;
- Slit width: 0.2 nm;
- Integration time: 2.0 seconds;
- Flame: acetylene nitrous oxide.

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

to ensure that the concentration of the element in solutions relates perfectly to the absorbance.

The apparatus required consists of:

- A centrifuge and some 50-ml centrifuge tubes;
- An automatic shaker;
- An AAS.

The reagents required are:

i. Ammonium acetate solution (NH_4OAc) 1.0M: Dissolve 77.09 g of ammonium acetate in 1 litre of distilled water, and adjust the pH to 7.0.
ii. Glass-distilled acidified water of pH 2.5: Same as that for Zn estimation (above).
iii. 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.
iv. 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 2000 rpm for 5 minutes or until the supernatant is clear.
4. Decant the solution into a 100-ml volumetric flask.
5. Repeat steps 2–4.
6. Make up the volume to 100 ml with ammonium acetate.
7. Atomize the working standards on an AAS at a wavelength of 313.5 nm. Prepare a standard curve of known concentration of Mo by plotting the absorbance values on the y-axis against their respective Mo concentration on the x-axis.
8. Measure the absorbance of the soil sample extract and determine the Mo content in the soil from the standard curve.

The relevant calculation is:
Content of Mo in the sample (mg/kg) = C µg/ml × 20 (dilution factor).
where:

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

Estimation by colorimetric method

The apparatus required consists of:

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

The reagents required are:

- 50 percent potassium iodide solution: Dissolve 50 g in 100 ml of double-distilled water (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 (analytical reagent)-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 organic matter and oxalates. Keep overnight.

4. Digest the contents with 5 ml of HNO_3–$HClO_4$ mixture (4:1), followed by 10 ml of 4M H_2SO_4 and then with H_2O_2, each time bringing to dryness.
5. Add 10 ml of 0.1M HCl and filter. Wash the filter paper, first with 10 ml of 0.1M HCl and then with 10 ml of DDW, until the volume of the filtrate is 100 ml.
6. Run a blank side by side (without soil).
7. Put 50 ml of the filtrate in 250-ml separatory funnels, add 0.25 ml of ferrous ammonium sulphate solution and 20 ml of DDW, and shake vigorously.
8. Add excess of potassium iodide solution and clear the liberated iodine by adding ascorbic acid drop by drop while shaking vigorously.
9. Add 1 ml of tartaric acid and 2 ml of thiourea solution, and shake vigorously.
10. Add 5 drops of dithiol solution, and allow the mixture to stand for 30 minutes.
11. Add 10 ml of iso-amyl acetate, and separate out the contents (green colour) in colorimeter tubes/cuvettes.
12. Read the colour intensity at 680 nm (red filter).
13. Preparation of standard curve: Measure 0, 2, 5, 10, 15 and 20 ml of the working standard Mo solution containing 1 mg/litre Mo in a series of 250-ml separatory funnels. Proceed for colour development as described above for sample aliquots. Read the colour intensity and prepare the standard curve by plotting Mo concentration against readings.

The relevant calculation is.
Content of Mo in the soil (µg/g or mg/kg) = C µg/ml × dilution factor (0.5)
where:
C (µg/ml) = concentration of Mo as read from the standard curve against the absorbance reading of the soil solution on the spectrophotometer;
dilution factor = 0.5, which is calculated as follows:

- Weight of the soil taken = 25 g;
- Volume of extract = 250 ml;
- First dilution = 10 times;
- Volume of the filtrate taken = 200 ml;
- Filtrate digested (concentrated) to 100 ml;
- Volume of concentrated filtrate taken = 50 ml;
- Second dilution = 0.25 times;
- Volume of solvent (iso-amyl acetate) used for extraction = 10 ml;
- Third dilution (50 ml extracted by 10 ml) = 0.2 times;
- Total dilution = 10 × 0.25 × 0.2 = 0.5 times.

4.0 CONCLUSION

Estimation of micronutrients involves different procedures which must be carefully handled. These procedures involve estimation by atomic absorption spectrophotometer and colorimeter and the use of reagents.

5.0 SUMMARY

In this unit, we have learnt that:

- there are parameters for estimation of micronutrients using an AAS
- micronutrients are present in different forms in the soil
- colorimeter also plays an important role in micronutrient estimation.

6.0 TUTOR-MARKED ASSIGNMENT

1. Compare and contrast micronutrient estimation by colorimeter and an AAS.
2. Why is excess amount of charcoal not advisable during Boron estimation?

7.0 REFERENCES/FURTHER READING

Berger, K.C. & Truog, E. 1939. Boron determination in soils and plants. *Ind. Eng. Chem. Anal. Ed.*, 11: 540–545.

Lindsay, W.L. and Norvell, W.A. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.*, 42: 421–448.

Food and Agriculture Organization (2008). Guide to laboratory establishment for plant nutrient analysis. FAO, Rome, Italy.

UNIT 3 EVALUATION OF ANALYTICAL DATA

CONTENTS

1.0 INTRODUCTION

This section provides information on the evaluation and interpretation of data. Statistical methods or approaches for evaluating analytical data are described in this section. Direction for scientifically acceptable treatment and interpretation of data is also mentioned. Many descriptive statistics, such as the mean and standard deviation, are in common use (www.uspnf.com).

Other statistical tools, such as outlier tests, can be performed using several different, scientifically valid approaches, and examples of these tools and their applications are also included.

2.0 OBJECTIVES

By the end of this unit, you should be able to understand:

- measures of central tendency
- determination of sample size
- comparison of Analytical methods.

3.0 MAIN CONTENT

3.1 Measurement Principles and Variation

All measurements are, at best, estimates of the actual (“true” or “accepted”) value for they contain random variability (also referred to as random error) and may also contain systematic variation (bias). Thus, the

measured value differs from the actual value because of variability inherent in the measurement. If an array of measurements consists of individual results that are representative of the whole, statistical methods can be used to estimate informative properties of the entirety, and statistical tests are available to investigate whether it is likely that these properties comply with given requirements. The resulting statistical analyses should address the variability associated with the measurement process as well as that of the entity being measured. Statistical measures used to assess the direction and magnitude of these errors include the mean, standard deviation, and expressions derived there from, such as the coefficient of variation (CV, also called the relative standard deviation, RSD). The estimated variability can be used to calculate confidence intervals for the mean, or measures of variability, and tolerance intervals capturing a specified proportion of the individual measurements.

The use of statistical measures must be tempered with good judgment, especially with regard to representative sampling. Most of the statistical measures and tests cited in this chapter rely on the assumptions that the distribution of the entire population is represented by a normal distribution and that the analyzed sample is a representative subset of this population.

The normal (or Gaussian) distribution is bell-shaped and symmetric about its center and has certain characteristics that are required for these tests to be valid. If the assumption of a normal distribution for the population is not warranted, then normality can often be achieved (at least approximately) through an appropriate transformation of the measurement values. For example, there exist variables that have distributions with longer right tails than left. Such distributions can often be made approximately normal through a log transformation. An alternative approach would be to use “distribution-free” or “nonparametric” statistical procedures that do not require that the shape of the population be that of a normal distribution. When the objective is to construct a confidence interval for the mean or for the difference between two means, for example, then the normality assumption is not as important because of the central limit theorem. However, one must verify normality of data to construct valid confidence intervals for standard deviations and ratios of standard deviations, perform some outlier tests, and construct valid statistical tolerance limits. In the latter case, normality is a critical assumption. Simple graphical methods, such as dot plots, histograms, and normal probability plots, are useful aids for investigating this assumption.

A single analytical measurement may be useful in quality assessment if the sample is from a whole that has been prepared using a well-validated, documented process and if the analytical errors are well known. The

obtained analytical result may be qualified by including an estimate of the associated errors. There may be instances when one might consider the use of averaging because the variability associated with an average value is always reduced as compared to the variability in the individual measurements. The choice of whether to use individual measurements or averages will depend upon the use of the measure and its variability.

Variability is associated with the dispersion of observations around the center of a distribution. The most commonly used statistic to measure the center is the sample mean

$$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n} = \frac{x_1 + x_2 + \ldots + x_n}{n}$$

For instance, the mean of the following set of values, 3,7,5 is (3+7+5)/3 which is 5.

The median is the middle value when we arrange our data from the smallest to the largest value. When the data set is made up of an odd number of entries, the median is the middle value What is the median for the values in parenthesis (7,3,1,9,5)? The first thing is to arrange the values in ascending order, 1,3,5,7,9. The median, therefore is 5.

For an even number of entries, the median is the average of the $n/2$ and the $(n/2) + 1$ values, where n is the size of the data set.

Method variability can be estimated in various ways. The most common and useful assessment of a method's variability is the determination of the standard deviation based on repeated independent measurements of a sample. The sample standard deviation, s, is calculated by the formula:

$$s = \sqrt{\sum_{i=1}^{n} (x_i - \overline{x})^2 / (n-1)}$$

in which x_i is the individual measurement in a set of n measurements; and x is the mean of all the measurements. The square of the standard deviation is the VARIANCE.

Variance = s^2

The relative standard deviation (RSD) is then calculated as:

$$RSD = \frac{S}{x}$$

% RSD is calculated by multiplying the above formula by 100.

If the data requires log transformation to achieve normality (e.g., for biological assays), then alternative methods are available.

A control sample is defined as a homogeneous and stable sample that is tested at specific intervals sufficient to monitor the performance of the method for which it was established. Test data from a control sample can be used to monitor the method variability or be used as part of system suitability requirements. The control sample should be essentially the same as the test sample and should be treated similarly whenever possible. A control chart can be constructed and used to monitor the method performance on a continuing basis.

A precision study should be conducted to provide a better estimate of method variability. The precision study may be designed to determine intermediate precision (which includes the components of both "between run" and "within-run" variability) and repeatability ("within-run" variability). The intermediate precision studies should allow for changes in the experimental conditions that might be expected, such as different analysts, different preparations of reagents, different days, and different instruments. To perform a precision study, the test is repeated several times. Each run must be completely independent of the others to provide accurate estimates of the various components of variability. In addition, within each run, replicates are made in order to estimate repeatability.

A confidence interval for the mean may be considered in the interpretation of data. Such intervals are calculated from several data points using the sample mean (x) and sample standard deviation (s) according to the formula:

$$\left(\bar{x} - t_{\alpha/2,n-1} \frac{s}{\sqrt{n}}, \bar{x} + t_{\alpha/2,n-1} \frac{s}{\sqrt{n}} \right),$$

in which $t_{\alpha/2,n-1}$ is a statistical number dependent upon the sample size (n), the number of degrees of freedom ($n - 1$), and the desired confidence level ($1 - \alpha$). Its values are obtained from published tables of the Student t-distribution. The confidence interval provides an estimate of the range within which the "true" population mean (μ) falls, and it also evaluates the reliability of the sample mean as an estimate of the true mean. If the same experimental set-up were to be replicated over and over and a 95% (for example) confidence interval for the true mean is calculated each time, then 95% of such intervals would be expected to contain the true mean, μ. One cannot say with certainty whether or not the confidence interval derived from a specific set of data actually collected contains μ. However, assuming the data represent mutually independent measurements randomly generated from a normally distributed population the procedure used to construct the confidence interval guarantees that 95% of such confidence intervals contain μ. Note that it is important to define the population appropriately so that all relevant sources of variation are captured.

3.2 Outlying Results

Occasionally, observed analytical results are very different from those expected. Aberrant, anomalous, contaminated, discordant, spurious, suspicious or wild observations; and flyers, rogues, and mavericks are properly called outlying results. Like all laboratory results, these outliers must be documented, interpreted, and managed. Such results may be accurate measurements of the entity being measured, but are very different from what is expected. Alternatively, due to an error in the analytical system, the results may not be typical, even though the entity being measured is typical. When an outlying result is obtained, systematic laboratory and process investigations of the result are conducted to determine if an assignable cause for the result can be established. Factors to be considered when investigating an outlying result include but are not limited to human error, instrumentation error, calculation error, and product or component deficiency. If an assignable cause that is not related to a product or component deficiency can be identified, then retesting may be performed on the same sample, if possible, or on a new sample. The

precision and accuracy of the method, the reference standard, process trends, and the specification limits should all be examined. Data may be invalidated, based on this documented investigation, and eliminated from subsequent calculations.
If no documentable, assignable cause for the outlying laboratory result is found, the result may be tested, as part of the overall investigation, to determine whether it is an outlier.

However, careful consideration is warranted when using these tests. Two types of errors may occur with outlier tests: (a) labeling observations as outliers when they really are not; and (b) failing to identify outliers when they truly exist. Any judgment about the acceptability of data in which outliers are observed requires careful interpretation.

“Outlier labeling” is informal recognition of suspicious laboratory values that should be further investigated with more formal methods. The selection of the correct outlier identification technique often depends on the initial recognition of the number and location of the values.

Outlier labeling is most often done visually with graphical techniques. “Outlier identification” is the use of statistical significance tests to confirm that the values are inconsistent with the known or assumed statistical model. Several tests exist for detecting outliers and these include Extreme Studentized Deviate (ESD) Test, Dixon's Test, and Hampel's Rule.

3.3 Comparison of Analytical Methods

It is often necessary to compare two methods to determine if their average results or their variabilities differ by an amount that is deemed important. The goal of a method comparison experiment is to generate adequate data to evaluate the equivalency of the two methods over a range of concentrations. Some of the considerations to be made when performing such comparisons are discussed in this section.

3.3.1 Precision

Precision is the degree of agreement among individual test results when the analytical method is applied repeatedly to a homogeneous sample. For an alternative method to be considered to have “comparable” precision to that of a current method, its precision must not be worse than that of the current method by an amount deemed important. A decrease in precision (or increase in variability) can lead to an increase in the number of results expected to fail required specifications. On the other hand, an alternative method providing improved precision is acceptable.

One way of comparing the precision of two methods is by estimating the variance for each method (the sample variance, $s2$, is the square of the sample standard deviation) and calculating a one-sided upper confidence interval for the ratio of (true) variances, where the ratio is defined as the variance of the alternative method to that of the current method. The one-sided upper confidence limit should be compared to an upper limit deemed acceptable, *a priori*, by the analytical laboratory. If the one-sided upper confidence limit is less than this upper acceptable limit, then the precision of the alternative method is considered acceptable in the sense that the use of the alternative method will not lead to an important loss in precision.

Note that if the one-sided upper confidence limit is less than one, then the alternative method has been shown to have improved precision relative to the current method.

The confidence interval method just described is preferred to applying the two-sample *F*-test to test the statistical significance of the ratio of variances. To perform the two-sample *F*-test, the calculated ratio of sample variances would be compared to a critical value based on tabulated values of the *F* distribution for the desired level of confidence and the number of degrees of freedom for each variance. Tables providing *F*-values are available in most standard statistical textbooks. If the calculated ratio exceeds this critical value, a statistically significant difference in precision is said to exist between the two methods. However, if the calculated ratio is less than the critical value, this does not prove that the methods have the same or equivalent level of precision; but rather that there was not enough evidence to prove that a statistically significant difference did, in fact, exist.

3.3.1 Accuracy

Comparison of the accuracy of methods provides information useful in determining if the new method is equivalent, on the average, to the current method. A simple method for making this comparison is by calculating a confidence interval for the difference in true means, where the difference is estimated by the sample mean of the alternative method minus that of the current method.

The confidence interval should be compared to a lower and upper range deemed acceptable, *a priori*, by the laboratory. If the confidence interval falls entirely within this acceptable range, then the two methods can be considered equivalent, in the sense that the average difference between them is not of practical concern. The lower and upper limits of the confidence interval only show how large the true difference between the two methods may be, not whether this difference is considered tolerable.

Such an assessment can only be made within the appropriate scientific context.

The confidence interval method just described is preferred to the practice of applying a *t*-test to test the statistical significance of the difference in averages. One way to perform the *t*-test is to calculate the confidence interval and to examine whether or not it contains the value zero. The two methods have a statistically significant difference in averages if the confidence interval excludes zero. A statistically significant difference may not be large enough to have practical importance to the laboratory because it may have arisen as a result of highly precise data or a larger sample size. On the other hand, it is possible that no statistically significant difference is found, which happens when the confidence interval includes zero, and yet an important practical difference cannot be ruled out. This might occur, for example, if the data are highly variable or the sample size is too small. Thus, while the outcome of the *t*-test indicates whether or not a statistically significant difference has been observed, it is not informative with regard to the presence or absence of a difference of practical importance.

3.4 Determination of Sample Size

Sample size determination is based on the comparison of the accuracy and precision of the two methods and is similar to that for testing hypotheses about average differences in the former case and variance ratios in the latter case, but the meaning of some of the input is different. The first component to be specified is δ, the largest acceptable difference between the two methods that, if achieved, still leads to the conclusion of equivalence. That is, if the two methods differ by no more than δ, they are considered acceptably similar. The comparison can be two-sided as just expressed, considering a difference of δ in either direction, as would be used when comparing means. Alternatively, it can be one-sided as in the case of comparing variances where a decrease in variability is acceptable and equivalency is concluded if the ratio of the variances (new/current, as a proportion) is not more than $1.0 + \delta$. A researcher will need to state δ based on knowledge of the current method and/or its use, or it may be calculated. One consideration, when there are specifications to satisfy, is that the new method should not differ by so much from the current method as to risk generating out-of-specification results. One then chooses δ to have a low likelihood of this happening by, for example, comparing the distribution of data for the current method to the specification limits. This could be done graphically or by using a tolerance interval.

In general, the choice for δ must depend on the scientific requirements of the laboratory.

The next two components relate to the probability of error. The data could lead to a conclusion of similarity when the methods are unacceptably different (as defined by δ). This is called a false positive or Type I error. The error could also be in the other direction; that is, the methods could be similar, but the data do not permit that conclusion. This is a false negative or Type II error. With statistical methods, it is not possible to completely eliminate the possibility of either error. However, by choosing the sample size appropriately, the probability of each of these errors can be made acceptably small. The acceptable maximum probability of a Type I error is commonly denoted as α and is commonly taken as 5%, but may be chosen differently. The desired maximum probability of a Type II error is commonly denoted by β. Often β, is specified indirectly by choosing a desired level of $1 - \beta$, which is called the "power" of the test. In the context of equivalency testing, power is the probability of correctly concluding that two methods are equivalent. Power is commonly taken to be 80% or 90% (corresponding to a β of 20% or 10%), though other values may be chosen. The protocol for the experiment should specify δ, α, and power. The sample size will depend on all of these components.

4.0 CONCLUSION

Analytical data can be evaluated using statistical methods and such methods include the use of mean, median, standard deviation, relative standard deviation, percent relative standard deviation, variance etc.

5.0 SUMMARY

In this unit, we have learnt that:

- the use of statistical measures must be tempered with good judgment, especially with regard to representative sampling
- there may be instances when one might consider the use of averaging because the variability associated with an average value is always reduced as compared to the variability in the individual measurements
- all measurements are, at best, estimates of the actual ("true" or "accepted") value for they contain random variability.

6.0 TUTOR-MARKED ASSIGNMENT

1. The following masses were recorded for 12 different quarters in a particular country (all given in grams):

5.683	5.549	5.548	5.552
5.620	5.536	5.539	5.684
5.551	5.552	5.554	5.632

Report the mean, median, range, standard deviation and variance for this data (Hint: Range is the difference between the largest value and the smallest value).

2. Differentiate between Type I error and Type II error.

7.0 REFERENCE/FURTHER READING

www.uspnf.com