COURSE CODE:	*SOS 515*
COURSE TITLE:	*Soil and Plant Analysis*
NUMBER OF UNITS:	*3 Units*
COURSE DURATION:	*Three hours per week*

COURSE DETAILS:

Course Coordinator:	**Dr. Jamiu Oladipupo Azeez** B. Agric., M. Agric., PhD
Email:	azeezjo@unaab.edu.ng
Office Location:	Room 151, COLPLANT
Other Lecturers:	Dr (Mrs.) F. A. Olowokere

COURSE CONTENT:

Collection and preparation of soil and plant samples. The principles of soil testing. Dissolution for total elemental analysis. Testing for available nutrients in the soil. Testing for pH and lime requirement. Interpretation of analytical results. Principles and practices in plant analysis. Sampling and analyzing tissue samples. Plant analysis as aid in fertilizing crops. Operation and management of a soil testing and plant analysis laboratory.

Practical: Sampling techniques. Handling of soil and plant samples. Analysis of soil and plant samples for total elements and for available nutrients.

COURSE REQUIREMENTS:

This is a compulsory course for all students in the College of Plant Science and Crop Production (COLPLANT). In view of this, students are expected to participate in all course activities and have a minimum of 75 % attendance to be able to write the final examination.

READING LIST:

1. Brady, N. C. and R. R. Weil. *The Nature and Properties of Soils*. 12th ed. Prentice-Hall, New Jersey: Prentice-Hall Incorporated, 1999.
2. Carter, M. R. (ed.). *Soil Sampling and Methods of Analysis*. Lewis, Florida: CRC Press Incorporated, 2000.

3. Landon, J. R. (ed.). *Booker Tropical Soil Manual (A handbook for soil survey and agricultural land evaluation in the tropics and subtropics).* Longman, New York: Longman Scientific & Technical, 1991.
4. Anderson, J. M. and J. S. I. Ingram. *Tropical Soil Biology and Fertility (A handbook of Methods).* 2nd ed.CAB, Oxon: CAB International, 1993.
5. Tan, K. H. Soil Sampling, Preparation, and Analysis. Marcel Dekker, Incorporated, New York. 1996.
6. Walsh, L. M. and J. D. Beaton. *Soil Testing and Plant Analysis.* Soil Science Society of America, Inc Madison, Wisconsin USA. 1973.
7. Agbede O. O. *Understanding Soil and Plant Nutrition*. Salman Press & Co Nig Ltd. Keffi Nassarawa State. 2009

LECTURE NOTES

COLLECTION AND PREPARATION OF SOIL AND PLANT SAMPLES

Soil and plant analysis is a diagnostic instrument for soil fertility and basis for fertilizer recommendation; to known where and where not fertilizer is to be applied. Obtaining accurate and precise values has always being the basis of soil analysis.

From agronomic view, the aims of soil and plant analysis are:

1) To satisfy the demand for soil classification data.
2) To generate information for management and improvement of the soil.
3) To determine the ecological effect of some agricultural production and environmental pollution.
4) To evaluate soil fertility in order to recommend fertilizer.

It is important to have a clear idea about the purpose of any soil analysis as this will help determine sampling technique, sample preparation methods, elements or fractions to be determined and the analytical techniques to be employed.

General Principles of Soil and Plant Sampling

It is necessary to procure a test sample that will be representative of the soil or plant under investigation and to prepare the test sample for analysis. This is because sampling errors are commonly greater than analytical errors. Analytical value can serve as an accurate description of the soil or plant if the followings are true:

1) The gross sample accurately represents the whole soil/plant from which it was taken.
2) No changes occur in the gross and subsamples prior to analysis.
3) The subsamples analysed represents the gross sample accurately.
4) The analysis determines a true value of the soil/plant characteristics under investigation.

A soil or field may be assessed for its capability of providing a crop with essentials nutrients in several ways:

1) Field plot fertilizer trials
2) Greenhouse pot experiments
3) Crop deficiency symptoms
4) Plant analysis
5) Rapid tissue or sap analysis
6) Biological tests such as growing microorganisms
7) Soil testing prior to cropping

All the approaches can be used in research, the latter one is most amenable and popular and one upon which recommendations for farmers can be based. On the other hand, plant analysis is a postmortem approach and one that should be interpreted in the light of soil test results.

Most soil tests primarily focuses on elements in most demand by crops which are supplied by fertilizers: N, P and K, others are Ca, Mg and S. In drier areas micronutrients such as Fe, Zn, Mn, Cu and B are often measured. As nutrient behavior in soils is governed by soil properties and environmental conditions, measurement of such properties is often required.

These include pH, salinity, organic matter, $CaCO_3$ and texture in drier areas the presence of Na and gypsum ($CaSO_4.2H_2O$) is also of concern.

Types of Sampling

1) ***Simple random sampling***

2) ***Systematic sampling***

3) ***Stratified sampling***

Phases of Soil Testing

1) Sample collection

2) Extraction or digestion and nutrient determination

3) Interpreting the analytical results

4) Fertilizer recommendation

PROCEDURES

1) Soil Sampling

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

(A) Composite sampling

(B) Time of Sampling

(C) Depth of Sampling

(D) Sampling Tools

2) Field Processing

3) Laboratory Processing

LABORATORY FACTORS OF IMPORTANCE TO SOIL EXTRACTION

These are factors that have significant impact on the test results. They include means of shaking, rate of reciprocation, type of extraction vessel, extraction time and laboratory temperature.

1) Extraction vessel shape

2) Shaking vs stirring

3) Shaking rates

4) Extraction time

5) Laboratory temperature

PLANT SAMPLING FOR ANALYSIS

From the nutritional standpoint, plant analysis is based on the principle that the concentration of a nutrient within the plant is an integral value of all the factors that have interacted to affect it. Plant analysis involves the determination of nutrient concentration in diagnostic plant part(s) sampled at recommended growth stage(s) of the crop. In a way plant analysis complements soil analysis. There are reliable sampling criteria and procedures for most of the world's commercial crops.

Laboratory Processing

Some steps are followed for processing the sampled plant tissues:

1) *Cleaning plant tissue to remove dust, pesticide and fertilizer residues*

2) Immediate drying in an oven to stop enzymatic activity, usually at 65^{o}C for 24/72 hours.

3) Mechanical grinding to produce a material suitable for analysis,

4) Grinding of a dry sample.

5) Final during at 65^{o}C of ground tissue to obtain a constant weight upon which to base the analysis.

6) Storing in appropriate container.

DISSOLUTION FOR TOTAL ELEMENTAL ANALYSIS

It is important to have a clear idea about the purpose of any soil analysis as this will help determine sampling technique, sample preparation methods, elements or fractions to be determined and the analytical techniques to be employed. There are several types of soil analysis viz:

1) Elemental analysis

2) Fractional analysis

3) Total elemental analysis (TEA)

TEA determine the quantity of an element present in the soil without reference to the quality (available form or polluted form). TEA is achieved by either wet or dry ashing.

***Wet ashing*:** can be accompanied by use of nitric, sulphuric or perchloric acid in different combinations

***Dry ashing*:** this is done in a murfle furnace at temp of 600^{o}C but with high temperature

Testing for Soil pH and Soil Acidity and Lime Requirement

pH measures relative acidity and alkalinity whereas soil acidity means the total amount of acid present in the soil. Quantitatively we use the pH scale in order to remove unwieldy figures e.g. 0.056M H^+. P means – log.

The pH scale could be derived from the ionization of water.

H_2O $H^+ + OH^-$ Kw =

activity of pure solid, liquid or gas in solution is 1.

At 25^{o}C Kw = 10^{-14} (moles litre^{-1})

$\therefore (H^+)(OH^-) = -14$

In pure water the concentration of (H^+) and (OH^-) are equal

$(H^+)(OH^-) = 10^{-14}$

$x \quad x = x^2 = 10^{-14}$ $\therefore x = 10^{-14/2} = 10^{-7}$

$\therefore (H+) = -7, \quad (OH^-) = -7$

$\therefore$ pH = 7 of pure water

POH = 7 of pure water

pH scale runs between 0 and 14 and that pH 7 is neutral.

Application of pH to Soil

Most mineral soil in the humid region has pH range between 3.5 to 7, while those of arid region have a range between 6.8 – 8.8. pH above 9 are found in alkali Na saturated soil and pH below 3.5 are found in acid organic soil (peat).

pH is one of the most enlightening attributes of the soil, whether the soil pH is high or low will depend on the solubility of certain compounds in the soil. pH of around 4 signifies the presence of free acids in the soil (usually from oxidation of sulphides), pH of 5.5 and below indicates the likely presence of $CaCO_3$.

Measurement of pH means the H concentration in solution and its called the active acidity, the potential/reserve acidity is that left within the microcell. Cations in exchange site is in constant equilibrium with that in solution.

pH measures the active acidity while potential acidity is determined by titration using a base.

Causes of soil acidity: (1) Leaching loss of bases (2) Application of fertilizer especially N fertilizer; NH_4^+ producing and NH_4^+ containing fertilizer like urea and $(NH_4)_2SO_4$ (3) Acid rain (4) Decomposition of organic matter, here CO_2 evolved react with soil water to form H_2CO_3 (5) Hydrolysis of aluminum.

$\therefore\ Al^{3+} + 3H_2O \qquad Al(OH)_3 + 3H^+$

Importance of Soil pH in Crop Production

Determination of pH

There are 2 basic methods of determining the soil pH viz (1) colorimetric and (2) potentiometric method

In either method, the sample has to be prepared. The soil sample is weighed, then decision on the type of slurry to prepare (water slurry (distilled water)) or salt solution (KCl or $CaCl_2$) 0.01m conc. of the salts are used. Decision on the ratio of water to soil or salt solution to soil, usually 1:1 or 2:1 (salt or water: soil). It is recommended that slurry should be shaken and read immediately because if allowed to settle, the potential difference as a result of the junction is avoided when settling is not allowed the actual reading is gotten.

Colorimetric Method of pH Measurement

This entails the formation of colour with soil : H_2O or salt solution mixture. The colour formation is made possible by the addition of a universal indicator (indicator with large pH range), the colour is then matched with colour charts of known pH. (Demerit – slower, less precise colour blindness and eye fatique.)

Potentiometric Method

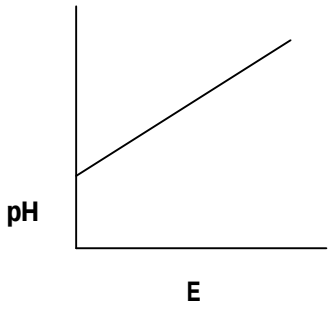
This is an instrumental method and involves measurement of potential. It is based on the principle that if we use pH sensitive electrode (selective or specific electrode), the potential generated is proportional to the H^+ concentration. i.e. $E = K(H^+)$

It is based on the Nerst equation.

$E = E^0 \pm 0.059/n \log [H+]$ ie $E^0 \pm 0.059/n = K$, holds only at 25^oC.

pH is also known to be equal to $(E - K) / 0.059$ @ 25^0C

hence the temperature should be adjusted to 25^oC.



The pH is directly related to E. To establish this straight line, a minimum of two or more points is required. To establish this straight line, you have to calibrate the pH meter with standard buffers. There are 3 standard buffers pH 4, 7 and 9. The choice of buffer is a function of the experience, if acid soil use pH 4, 6 or 7 if alkaline use 6 or 7 and 9. If no knowledge of soil pH use 4 and 9.

Factors Affecting pH Measurement

1) Suspension effect
2) Dilution effect
3) Sodium effect

Lime Requirement

This is the amount of lime required to neutralize the acidity of the soil to a desired pH. There are several methods of determining lime requirement, out of which five are very common: (1) Field plot techniques (2) Titration with a base (soil/base titration) (3) Incubation studies (4) Use of buffer (5) Green house techniques

1) Field plot techniques/green house
2) Titration with a base
3) Incubation studies
4) Use of buffer

Soil Organic Matter

Determination of SOM

SOM is the plant and animal remains or debris at all stages of decomposition. Decomposed parts are called humus.

1) Measurement of CO_2 evolved during decomposition.
2) Determination from the total Nitrogen values.
3) Weight loss
4) Estimation of the oxidizable carbon

Walkley and Black Procedure

This is a chromic acid oxidation procedure; it involves the oxidation of the SOM by chromic acid. In practice the chromic acid is generated insitu by the reaction between $K_2Cr_2O_7$ and conc. H_2SO_4 then you back titrate with ferrous solution because the $K_2Cr_2O_7$ and H_2SO_4 is added in excess. By this we determine the oxidizable organic carbon, however not all the Organic Carbon is oxidizable, but we know that about.

1) 75 % of the organic carbon in organic matter is oxidizable hence to convert org. carbon = 100 / 75 = 1.33
2) Only about 58% of total organic matter is organic carbon. So to convert org. carbon to org. matter = 100/58 = 1.724
3) Milli-equivalent weight of carbon in (g) = 0.003

   12/4 = 3/1000 = 0.003g

$$\therefore \textbf{ \% org C} = \frac{(A) - (B) \textbf{ X Normality of titrant X } 100 \textbf{ X } 0.003 \textbf{ X } 1.33}{\text{Weight of soil taken}}$$

Where: Titre value of blank (A)

Titre value of sample (B)

$\therefore$ **Org. matter** = Total Org. C **X** 1.724

$$\textbf{Org. matter} = \frac{(A) - (B) \text{ X N X } 100 \textbf{ X } 0.003 \textbf{ X } 1.33}{\text{Weight of sample}}$$

Testing for Available Nutrients

Available nutrient is that portion of soil nutrient, whose variations (increase or decrease) are reflected in the growth/yield of the crop. The major nutrients of interest in this course are nitrogen, P, K, Ca, Mg, Na, Mn, Fe, etc.

Soil Nitrogen

This is perhaps the most needed nutrient element in most soils. About 90% of total N in the soil is in organic combination. In most soil, N content ranges as low as 0.01% to as high as 0.5%. Total N content of Nigerian soil is around 0.02 – 0.2% and the critical level is 0.15%.

Methods of Determining N Levels in Soil

Plant take N as NO_3^- and NH_4^+, hence both are important in plant uptake. There is however, the interconversion of both in the soil to different forms. In recent time, attention is focused on NO_3^- for many reasons.

1) The possibility of leached NO_3^- polluting the underground water i.e. NO_3^- going below root zone of plants.
2) From point of view of crop need.

However, so far in Nigeria, total nitrogen is used mainly as the index of N availability to crops.

Total Nitrogen Determination

There are 2 classical methods of determing total Nitrogen.

1) ***Dumass (1831)*:-** This is a dry oxidation procedure.
2) ***Kjeldahl method*:-** The two step Kjeldahl system does not take into consideration the following compounds N-O compounds and the N-N compounds therefore, the two way system has to be modified in order to include N-O compounds as NO_3^-, NO_2^-. One of the modification is the salicylic (e.g. aspirin) acid modification.

Determination of Phosphorus

Chemistry of P in the Soil

Plant takes their P in form of HPO_4^{2-} and $H_2PO_4^-$. Unfortunately the soluble form of P in the soil at any particular time is very small that it will not satisfy the crop yield.

Labile P is the pool of P that replenishes soil P immediately the soluble P is depleted. Therefore available P = labile P + solution P. Labile P varies from soil to soil, hence the extractant varies too from soil to soil.

Criteria for Selecting Extractant for P

1) The extractant should rapidly dissolve or desorb P and it should be time independent after 30 minutes.
2) It should maintain O.M. and soil clays in a flocculated form (no dispersion of OM or soil minerals).
3) It should not precipitate after dissolution.
4) It should not contain excess salts, buffers, or ions that will interfere with the analytical determination.
5) It should be easy to prepare, store or disposed of.

In practice some of the commonly used extractant include: Bray 1, Bray 2, Olsen, Hunter, Mehlich[1], Egner, Ambic I, Citric acid, 0.01M $CaCl_2$

Determination of Extracted P

There are several methods of doing this, but the most common is the molybdate method. The classical molybdate method involves the use of certain reagents like Na vernadate and NH_4MoO_{10}. When these reagents react with P in solution, yellow phosphomolybdate is formed and the intensity of the yellow colour is determined colorimetrically. However, the yellow colour is not very sensitive and there is a limit to its detection, hence to enhance the sensitivity of the colour, it is reduced to blue colour buy the addition of stannous chloride (tin chloride). Another common method is the use of antimony potassium tartrate and ascorbic acid solution to generate a blue colour, whose intensity is a function of the P concentration.

Exchangeable Cations

Two principal methods used in determining total CEC are:

1) ***Summation method*:-** All the cations are displaced by a saturated solution of the displacing ion, usually a monovalent ion. NH_4^+ (ammonium) ion is often used. The salt widely used id NH_4OA_c, by adding this NH_4^+ is furnished and all other cations

will have been displaced. The cations will then be determined and summed up to give the total CEC.

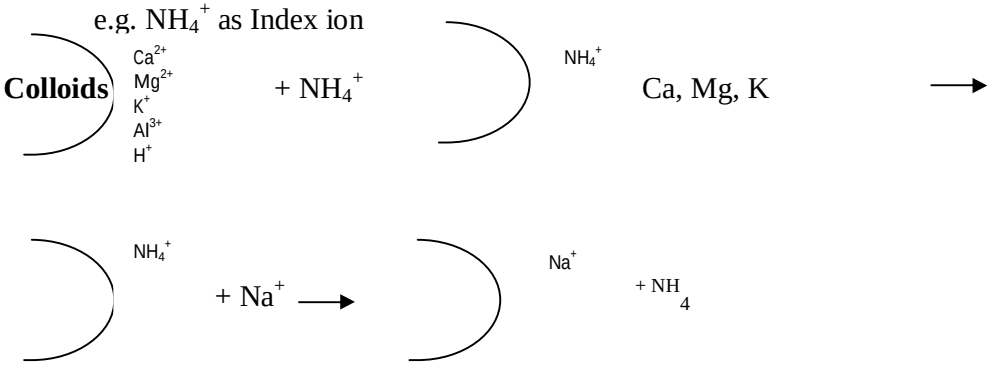
Colloids $+ NH_4^+$ **Colloids** $+ Ca^{2+}, Mg^{2+}, K^+$

colloid

usually the Ca^{2+} and Mg^{2+} is determined using atomic absorption spectrophotometer (AAS) while Na^+ and K^+ are determined using flame photometer, H and Al by AAS and by NaOH titration.

2) ***Displacement method*:** here we figure out (i) Displacing ion (ii) Index ion

e.g. NH_4^+ as Index ion



With soil and NH_4, shake for 1 hour filter, the filtrate has cations, residue (solid) has NH_4^+ return the residue to the beaker, then look for a displacing ion (monovalent cation) usually Na^+ in form of acetate. Hence NH_4^+ in solution is equivalent to all the cations.

Determination of Available Sulphur

The best extractant for S is $Ca(H_2PO_4)_2$, it must contain about 500 ppm PO_4^{3-}. Phosphorus is more specifically fixed whereas S is not specifically fixed i.e. the adsorption energy is higher in P than in S (P is more tightly held than S). Therefore P can easily displace S on the adsorption site.

Extract and determine S by colorimetry, gravimetry but most common is turbidemetric method, here $BaCl_2$ is added to the extract.

$BaCl_2 + SO_4^{2-} \longrightarrow BaSO_4 + 2Cl^-$, $BaSO_4$ is formed, this is a turbid suspension, the rabidity of the solution is determined, hence to make it stay, a stabilizer is added e.g. Gelatin/Cum acacia, the resulting solution is determined by use of a spectrophotometer at 420nm wavelength.

- To remove any colour (to ascertain that only turbidity is measured and not colour), this is achieved by adding a decolorizer e.g. activated charcoal; this is added to the filtrate and then refilter before adding $BaCl_2$ and measuring.
- Turbidimeter functions even in the presence of colour because it records reflection and refraction.

Micro-Nutrients

They are Cu, Zn, Co, Mo, B, Fe, Mn. They are essential to crop growth but needed in small amount as far as fertilizer need is concerned, however they have equal importance as the macro elements. Micronutrient analysis is not common in most analysis because of severl reasons as:

1) Since their presence is in trace levels, hence the instrument used for the analysis must be highly sensitive; this is not only very costly but also not available in most laboratories.
2) Since they are present in trace amount, containers used for them may contaminate the sample to the extent that the error level could be very high (e.g. 90%) and therefore it requires well-trained personnel to handle micronutrient analysis.

Extraction

by EDTA + HCl, DTA + HCl, Acid etc. for boron we can use hot-water and immediately they are extracted, we can use AAS to determine them, depending on the availability of lamp as every element has its own lam.

PLANT ANALYSIS

a. **Definition**

Plant analysis can be defined as the quantitative determination of the concentration of an element or extractable fraction of an element in a sample from a particular part or portion of a crop.

b**. Principles and Practices**

Plant analysis is used as an index of available nutrient element supply. Plant growth or yield are compared with the elemental concentrations contained in the dry matter of the entire plant or plant structures such as leaves, petioles, fruit or grain sampled at different times during their development. Plant analysis gives the overall picture of the nutrient levels within the plant at the time the nutrient was taken. The use of plant analysis is based on the principle that the nutrient level present is as a result of all factors affecting the growth of the plant.

2. **Some uses of Plant Analysis**

i. It is used to determine if an element is essential for plant growth, development and

maturation.

ii. It is used to verify the element associated with a phenotypic or nutrient deficiency or toxicity symptom

iii Establishment of optimum concentrations or critical values for elements associated with optimum or maximum economic yields

iv. Determining the total elemental uptake by a crop which could be used to estimate the nutrient element requirement per unit of production

v. Determining the availability and recovery of an applied element in fertilizer in crop response experiments

3. **Sampling and Analyzing tissue samples**

A. Factors to be considered before sample collection:

i. Nutrient element heterogeneity

ii. Statistical considerations

B. Sampling Techniques

Factors on which the number of plants to sample are dependent:

i. General condition of the plants

ii. Soil homogeneity

iii. Purpose for sampling

C. Sample Preparation

Plant samples are to be subjected to the following preparatory steps before the actual chemical analysis:

1. Storage and transport of the fresh material prior to cleaning and drying
2. Cleaning the material to remove surface contamination or Decontamination
3. Drying to stop enzymatic reactions and prepare the material for grinding
4. Mechanical grinding to reduce the material to a fineness suitable for analysis
5. Storage of the tissue powder prior to analysis

D **Plant Analysis**

Most of the elements contained in plant tissue are present as constituents of the plant tissue rather than as water soluble inorganic anions or cations. Consequently, organic matter of plant tissue must be destroyed before the mineral elements can be determined.

i. Methods of organic matter destruction:

a. Wet Ashing- Decomposition of plant tissue by digesting in strong acid solutions

b. Dry Ashing- Heating plant samples to a temperature sufficiently high to burn off the carbon

ii. Methods of determining elements in plant samples:

- Total Nitrogen
  Method: Micro-Kjeldahl
- Phosphorus
  Method: Vanado-Molybdate
- Potassium
  Method: Flame emission
- Calcium, magnesium, manganese, Zinc and copper determination
  Method: Atomic absorption

4. Plant Analysis as an aid in fertilizing crops

This is based on the concept of critical nutrient concentration.

Definition : Critical level is defined for a given form of nutrient and plant part as that concentration above which sufficiency occurs and below which deficiency occurs.

There are established critical or sufficiency ranges for specific crops and elements, when nutrient concentrations are below the established sufficiency range, additional nutrients would be required.

- Students would be provided with established critical or sufficiency ranges of some common crops

5. **Operation and management of a soil testing and plant analysis laboratory**

5.1 Types of laboratories:

- University or educational Institute laboratory

Objectives: Data acquisition to support or confirm research or to acquire information useful in designing educational programs for students and other persons concerned with soil fertility and plant nutrition

- Industrial laboratory

Objectives: Promoting the use or sale of the product manufactured or distributed by the company owning or operating the laboratory

- Commercial laboratory

Objectives: To operate in a manner as to return a profit for the investment required to provide the service

5.2 Facilities:

- Receiving dock: Used for receiving chemical supplies, soil and plant samples
- Soil grinding or crushing room: Where soils are prepared for analysis
- Soil sterilization area: Used for heat treating soils
- Plant and feed samples preparation room: Where plant samples are prepared for analysis
- Vibration free benches: On which analytical balances and delicate instruments would be placed
- Equipment room: Where laboratory equipments would be kept
- A lockable room or cabinet: For safe storage of chemicals
- A well defined area for disposing of laboratory wastes, washing and drying glassware

5.3 Safety: Ready access should be provided to protective and first aid supplies

5.4 Electricity Supply: There should be reliable electricity supply since most analytical procedures involves the use of equipment powered by electricity

5.5 Water Supply and quality: Water is the wellspring of laboratory performance hence the laboratory should be supplied with regular and clean water

5.6 Management: This includes technicians, supervisory personnel, technical director and the manager

5.7 Record keeping: Data must be recorded in specific laboratory record books. Records which contain primary data should not leave the laboratory.