

# **SOS 515 (SOIL AND PLANT ANALYSIS)**

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## **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 know 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,  $\text{CaCO}_3$  and texture in drier areas the presence of Na and gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) is also of concern.

## **Types of Sampling**

- 1) *Simple random sampling*
- 2) *Systematic sampling*
- 3) *Stratified sampling*

## **Phases of Soil Testing**

- 1) **Sample collection:** This should be such that it reliably reflects the average status of a field for the parameter considered.
- 2) **Extraction or digestion and nutrient determination:** The reagent used and the procedures adopted should quantify all or a portion of the element in the soil which is related to availability to the plant i.e. it should be correlated with plant growth.
- 3) **Interpreting the analytical results:** The units of measurement should reliably indicate if a nutrient is deficient, adequate, or in excess.
- 4) **Fertilizer recommendation:** This is based upon the soil test calibrated for field conditions, and considers other factors such as yield target, crop nutrient requirement, management of the crop, soil type, and method of fertilizer application.

## **PROCEDURES**

### **1) Soil Sampling**

Soil sample should be composed of several subsamples representing a seemingly uniform 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°C for 24/72 hours.

- 3) Mechanical grinding to produce a material suitable for analysis,
- 4) Grinding of a dry sample.
- 5) Final drying at 65°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.

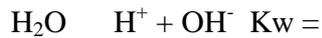
**Wet ashing:** can be accompanied by use of nitric, sulphuric or perchloric acid in different combinations

**Dry ashing:** this is done in a muffle furnace at temp of 600°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<sup>+</sup>. P means – log.

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



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

At 25°C  $K_w = 10^{-14}$  (moles litre<sup>-1</sup>)

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

In pure water the concentration of (H<sup>+</sup>) and (OH<sup>-</sup>) are equal

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

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

$$\therefore (\text{H}^+) = 10^{-7}, \quad (\text{OH}^-) = 10^{-7}$$

$$\therefore \text{pH} = 7 \quad \text{of pure water}$$

$$\text{pOH} = 7 \quad \text{of pure water}$$

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<sub>3</sub>.

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;  $\text{NH}_4^+$  producing and  $\text{NH}_4^+$  containing fertilizer like urea and  $(\text{NH}_4)_2\text{SO}_4$  (3) Acid rain (4) Decomposition of organic matter, here  $\text{CO}_2$  evolved react with soil water to form  $\text{H}_2\text{CO}_3$  (5) Hydrolysis of aluminum.



### **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  $\text{CaCl}_2$ ) 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 :  $\text{H}_2\text{O}$  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 fatigue.)

#### **Potentiometric Method**

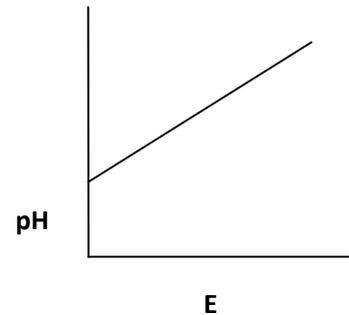
This is an instrumental method and involves measurement of potential. It is based on the principle that if we use pH sensitive electrode (selective or specific electrode), the potential generated is proportional to the  $H^+$  concentration. i.e.  $E = K(H^+)$

It is based on the Nerst equation.

$$E = E^0 \pm 0.059/n \log [H^+] \quad \text{ie } E^0 \pm 0.059/n = K, \text{ holds only at } 25^\circ\text{C}.$$

pH is also known to be equal to  $(E - K) / 0.059 @ 25^\circ\text{C}$

hence the temperature should be adjusted to  $25^\circ\text{C}$ .



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

#### 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<sub>2</sub> evolved during decomposition. This is achieved by destroying the CO<sub>3</sub> with conc. H<sub>2</sub>SO<sub>4</sub>. It only works in a very close analytical train.
- 2) Determination from the total Nitrogen values. It is assumed that 5% of SOM is N. (100/5 x value), this particular method is based on an assumption which may not hold at all time. It is known that N content of SOM could vary from as low as 3% to as high as 8%.
- 3) **Weight loss:** - This is achieved by destroying the SOM and estimated by difference in weight loss before and after the destruction. SOM is destroyed by (1) chemical method by the addition of H<sub>2</sub>O<sub>2</sub> or (2) By ignition in a furnace. Weight loss method is not a very accurate method because it may not get all the OM destroyed; it is however used when there is no other method.
- 4) **Estimation of the oxidizable carbon:-** This is the most popular method and most accurate. There are several techniques under this, but the most popular is the Walkley and Black (1939) method.

#### **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<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and conc.

H<sub>2</sub>SO<sub>4</sub> then you back titrate with ferrous solution because the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and H<sub>2</sub>SO<sub>4</sub> 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  
 $12/4 = 3/1000 = 0.003g$

$$\therefore \% \text{ org C} = \frac{\text{Titre value of blank (A)} - \text{Titre value of sample (B)} \times \text{Normality of titrant} \times 100}{\text{X } 0.003 \times 1.33}$$

Weight of soil taken

$$\therefore \text{Org. matter} = \text{Total Org. C} \times 1.724$$

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

### 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  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , 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  $\text{NO}_3^-$  for many reasons.

- 1) The possibility of leached  $\text{NO}_3^-$  polluting the underground water i.e.  $\text{NO}_3^-$  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 determining total Nitrogen.

- 1) **Dumass (1831)**:- This is a dry oxidation procedure.
- 2) **Kjeldahl method**:- This is the widely used method for determining total nitrogen and there are many form of this method viz macro, micro, semi-micro systems. The Kjeldahl method is made u of two steps viz: digestion step and distillation step.

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  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ . There are some modification viz:

- 1) **Salicylic (e.g. aspirin) acid modification**:- In this modification the sample is pre-treated with salicylic acid dissolved in conc.  $\text{H}_2\text{SO}_4$ , the  $\text{NO}_3^-$  with the salicylic acid form nitro

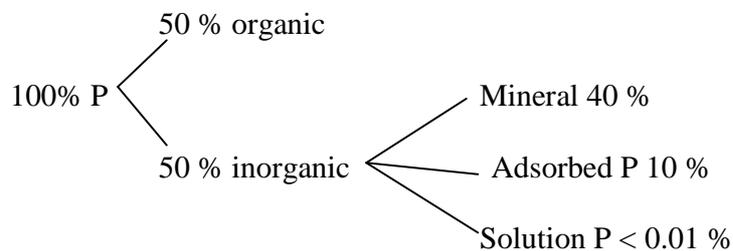
compound, the nitro compound in acid medium will be converted to amino compound and the sample is then treated normally by adding all the reagent required for digestion in ordinary Kjeldahl system.

## Determination of Phosphorus

### Chemistry of P in the Soil

Plant takes their P in form of  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$ . 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:

- 1) Bray 1            0.03M NH<sub>4</sub>F in 0.025N HCl
- 2) Bray 2            0.03M NH<sub>4</sub>F in 0.1N HCl
- 3) Olsen            0.5M NaHCO<sub>3</sub>, pH 8.5
- 4) Hunter            0.05M NaHCO<sub>3</sub> in 0.01M EDTA
- 5) Mehlich<sup>1</sup>        0.05N HCl + 0.025N H<sub>2</sub>SO<sub>4</sub>
- 6) Egner            0.02N Ca-lactate + 0.02N HCl
- 7) Ambic I          0.25M NH<sub>4</sub>HCO<sub>3</sub> + 0.01M (NH<sub>4</sub>)<sub>2</sub> EDTA + 0.01M NH<sub>4</sub>F + superfloc
- 8) Citric acid        1% citric acid
- 9)                    0.01M CaCl<sub>2</sub> solution

### **Bray 1 Extractant**

0.03M NH<sub>4</sub>F in 0.025N HCl, here the F<sup>-</sup> ion complexes Al and Fe forming AlF and FeF (AlPO<sub>3</sub> and FePO<sub>3</sub> are P forms in the soil). Since Al and Fe is removed from AlPO<sub>3</sub> and FePO<sub>3</sub> then the P is left available for determination. NH<sub>4</sub>F also chelates Al and Fe in solution.

### **Bray 2 Extractant (0.03M NH<sub>4</sub>F in 0.1N HCl)**

This is also based on the same principle as Bray 1 however, because of the stronger strength of the acid in Bray 2, it is also able to dissolve some mineral P (rock phosphate, Apatite).

### **Olsen (0.5M NaHCO<sub>3</sub> at pH 8.5)**

At high pH, P is held by Ca as Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>



If  $\text{Ca}^{2+}$  is removed, more  $\text{Ca}_3(\text{PO}_4)_2$  will be dissociated to counteract the effect of the removal (Le-Chatelier's principle) hence Ca is removed by  $\text{NaHCO}_3$  even, Ca has a strong affinity for  $\text{CO}_3^{2-}$  to form  $\text{CaCO}_3$ , hence more  $\text{Ca}_3(\text{PO}_4)_2$  dissolves.

If we continue to remove Ca by precipitating it as  $\text{CO}_3^{2-}$ , the reaction goes to the right, more and more P will be released into solution. In addition  $\text{NaHCO}_3$  in solution will also have  $\text{NaOH}$ , the  $\text{NaOH}$  will react with Fe in the  $\text{FePO}_3$  to form  $\text{Fe}(\text{OH})_3$ , this will also release more P into solution.

### 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 molybdate and  $\text{NH}_4\text{MoO}_4$ . 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 by 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.  $\text{NH}_4^+$  (ammonium) ion is often used. The salt widely used is  $\text{NH}_4\text{OAc}$ , by adding this  $\text{NH}_4^+$  is furnished and all other cations will have been displaced. The cations will then be determined and summed up to give the total

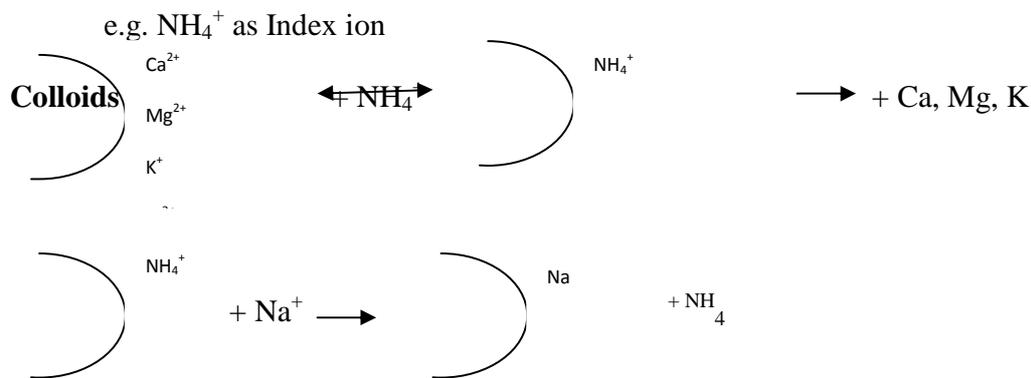


**Colloids** +  $\text{NH}_4^+$       **Colloids** +  $\text{Ca}^{2+}, \text{Mg}^{2+}, \text{K}^+$

colloid

usually the  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  is determined using atomic absorption spectrophotometer (AAS) while  $\text{Na}^+$  and  $\text{K}^+$  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



With soil and  $\text{NH}_4$ , shake for 1 hour filter, the filtrate has cations, residue (solid) has  $\text{NH}_4^+$  return the residue to the beaker, then look for a displacing ion (monovalent cation) usually  $\text{Na}^+$  in form of acetate. Hence  $\text{NH}_4^+$  in solution is equivalent to all the cations.

### **Determination of Available Sulphur**

The best extractant for S is  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , it must contain about 500 ppm  $\text{PO}_4^{3-}$ . 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 turbidometric method, here  $\text{BaCl}_2$  is added to the extract.

$\text{BaCl}_2 + \text{SO}_4^{2-} \longrightarrow \text{BaSO}_4 + 2\text{Cl}^-$ ,  $\text{BaSO}_4$  is formed, this is a turbid suspension, the turbidity 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  $\text{BaCl}_2$  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 several 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 lamp.

## **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.

## ➤ . Plant Analysis

- 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.

- 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 sample 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.

❖ . General Purposes of plant Analysis

- To diagnose or confirm Diagnoses of Visible Symptoms  
Plant analysis is required in order to confirm tentative identification of plant nutrient deficiency symptoms, though tissue tests should be used with some restraint, they have proved to be an effective tool in convincing farmers of nutrient shortages.
- To identify hidden trouble  
Certain nutrient deficiencies may not show specific symptoms other than reduced yield and lack of vigor. Plant analysis can identify such deficiencies and lead to measures to be taken for their correction
- Determining the total elemental uptake by a crop  
Plant nutrients applied to the soil for nutrient deficiency correction would be monitored to know whether they actually entered the plant, if no yield response is obtained, it may be concluded that the applied nutrients were not lacking whereas they may not have been absorbed. Reasons for non absorbance of the nutrients could be detected by the researcher, this may be due to reasons like soil reactions, unfavorable nutrient placement or whether nutrient was absorbed but was ineffective in promoting growth.
- To indicate interactions or antagonisms among nutrients.  
At times, the addition of certain nutrients reduces the amount of other nutrients in the plant. Additional data from plant analyses will accelerate the development of solutions to practical problems in the application of micronutrients and secondary nutrients.
- As an aid to understanding internal functioning of plants.  
Analysis of plants either whole or parts periodically through the season under varied environmental conditions show great differences among crops. Plant analyses are useful in showing the mobility of elements within the plant and the zones of concentration.
- To suggest additional tests to identify a particular trouble.  
Plant analyses at times point to the need for soil tests to identify the specific cause of trouble in a plant

➤ Sampling and Analyzing tissue samples

The validity and usefulness of any plant analysis hinge upon an intelligent and realistic approach to the problem of obtaining a reliable sample. If the sample collected does not properly represent the commodity where it was obtained, all the careful and costly work put into the analysis that follow will be wasted because results will not be valid

- ❖ Factors to be considered before sample collection:
  - Nutrient element heterogeneity
 

Location: The composition of nutrient element in a particular plant varies with month, hour on the same day, soil type and among different parts of the plant. A specific plant part at a specific location must be selected when sampling. Mature leaves just below the growing tips of main branches and stems should be sampled, this is because physiologically young tissues undergo rapid changes in nutrient composition and therefore should not be sampled.
  - Statistical Considerations
 

After the particular part of the plant to be sampled have been determined, enough sample to adequately represent the sampled population should be taken. Large variations occur in nutrient concentration from plant to plant than within single plant parts, therefore, if sampling material is carefully selected, a small sample will adequately represent the nutrient content of a single plant. Intensive sampling procedure is however needed when many plants are involved in order to represent the nutrient content of the plants in question.
  - Sampling techniques
 

Leaves are generally preferred when sampling, these should be mature leaves just below the growing tips on the main branches and stems of plants. The number of of plants to sample depend on such factors as: the general condition of the plants, soil homogeneity, and the purpose for which the samples are to be taken. As many plants as possible should be sampled and it is best to take samples during a particular time of the day under calm climatic conditions. It is recommended that samples should be taken just prior to or at the time plants begin their reproductive stage.
  - What not to sample
 

Plants which are covered with soil, dust, damaged by insects, mechanically injured or diseased

Dead plant tissues

Moisture or temperature stressed plants
- ❖ Transportation
 

Plant samples should be transported under refrigerated conditions if there is going to be a considerable time lag after sampling and transport to the laboratory and if they could not be decontaminated immediately, they should be kept under refrigerated condition, this is

to allow for easy and efficient cleaning. Plant samples should not be transported to the laboratory in sealed packages, they should be opened to the atmosphere.

➤ **Sample Preparation**

After sampling, plants are subjected to four different preparatory steps before actual chemical analysis:

- **Cleaning the material to remove surface contamination or Decontamination:** Plant samples collected may be covered with a thin film of dust which may be difficult to be removed by mechanical wiping or brushing, the presence of dust can affect the concentrations of Fe and Mg in the sampled plant if not removed, therefore, dust must be removed before the leaf material is dried. Dust can be removed by washing plant tissues in 0.1-0.3 % phosphorus free detergent solutions followed by rinsing in pure water. The washing procedure should be done as quickly as possible so as to prevent the loss of nutrients like K, Ca and Na through leaching.

- **Drying**  
Plant tissue samples should be dried as rapidly as possible after washing, delay in drying them may result in:

Considerable loss in dry weight

Breaking down of protein to simpler nitrogenous compounds

Samples should be dried at 65°C in a dust – free forced-draft electrical oven to a constant weight. This temperature satisfies the two separate requirements that must be met when drying plant material for analysis, they are:

A sufficiently high temperature to destroy the enzymes responsible for decomposition processes

Optimum temperature for moisture removal without appreciable thermal decomposition

Plant samples are therefore not recommended to be air dried because the temperature when air dried will not satisfy the above requirements

- **Grinding**  
Plant samples are to go through the process of grinding after drying, this will allow for greater ease of manipulation and ensure greater uniformity in composition

When dealing with a large number of samples, hand grinding may be too laborious and time wasting, therefore, mechanical grinding may be preferred. Mills should be carefully selected particularly when analyzing for micronutrients, mills which minimize the possibility of contamination should be used.

Equipments having grinding surfaces of either steel, stainless steel or agate are

recommended. Examples of grinding equipments are: hammer mill, wiley mill, agate or glass mortar with pestle.

- Storage

Dried ground plant material should not be stored in the shelf for more than two months to avoid becoming moldy. Samples to be kept for longer periods should be stored in a sterilized sealed bottle in a refrigerator at

-5°C.

- Laboratory Analysis

Most methods developed for the analysis involve ashing the plant tissue to

destroy the organic components leaving the various elements for analysis. Some

scientists have proposed the use of extraction procedures which utilize dried,

green tissue. There are two methods of ashing plant samples:

- Wet ashing: Plant tissue is decomposed by digesting the plant sample in strong acid solutions such as  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$  and  $\text{HClO}_4$ . (Special precautions against explosions must be taken when  $\text{HClO}_4$  is used). Wet digestion procedure is to be preferred for analysis of trace elements and elements which could be lost by volatilization in the dry ashing procedure (N, Cl, S). Since boron can be lost by steam distillation as  $\text{H}_3\text{BO}_3$  and can be volatilized during wet ashing, the wet digestion procedure is not applicable for analysis of this element. There is the danger of loss of P by volatilization as  $\text{H}_3\text{PO}_4$  during  $\text{HClO}_4$  digestion if the temperature exceeds 230°C.
- Dry ashing: Dry ashing consists of heating the sample to a temperature sufficiently high to burn off the carbon. This is normally carried out in a muffle furnace at temperatures not exceeding 500°C. Ashing may require two or more hours depending on the type of tissue. For highly carbonaceous tissues a longer ashing period may be necessary. The use of high walled crucibles are preferred over open flat vessels. Boron can be determined only by dry ashing since this element is volatilized during wet ashing. Heating of sample should not be too rapid during dry ashing, if heating is too rapid, the sample might burst into flames and excessively high temperatures will be reached. High temperatures are to be avoided because of the following reasons:
  - Elements like Na, K, P, Cl, S and B might be lost as volatile compounds. No loss of P is expected below 600°C.
  - Salts present in the ash might fuse and form a fused mass surrounding particles of incompletely burned tissue.
  - Complex silicates might form as siliceous residues in the ashed material. Such residues frequently contain significant amounts of trace elements and possibly some Na and K.
- ❖ Method of analysis
  - The following are the commonly used methods for the analysis of plant tissue ash:
    - Colorimetric
    - Flame emission

- Atomic absorption spectroscopy
  - Mass spectrometry
  - Neutron activation analysis
  - X-ray fluorescence
  - AutoAnalyzer technique
- ❖ Total Nitrogen in plant tissue is determined by Micro-Kjeldahl method
  - ❖ Phosphorus in plant tissue is determined by Vanado-Molybdate method
  - ❖ Potassium is determined by dry ashing and read on flame photometer.

➤ Plant Analysis as an aid in fertilizing crops

Plant analysis can serve as an efficient guide in crop fertilization. Plant analysis is a study of the relationship of the nutrient content of the plant to its growth. Concentrations of mineral nutrients in specific plant parts are used as guides to indicate how well plants are supplied with these nutrients at a certain time of sampling. Such reference concentrations provide a tool to assist the agronomist in evaluating nutrient disorders and at improving fertilizer practices. A plant's nutrient status could be more accurately described using concentration ranges. The procedure commonly used is that of comparing a plant's concentration of elements with its sufficiency or critical range. The concentration of each element found during analysis is reported as "less than" "greater than" or "within the range".

Nutrient Sufficiency Ranges of Corn at specific plant parts and time of Sampling

Element	Jones (1969) Ear Leaf at Silk	Neubert, et al., (1969) Ear Leaf at Silk	Lockman (1969) 30-45 days after emergence
N %	2.76 – 350.0	2.60 – 400.0	3.5 – 5.0
P	0.25 - 0.40	0.25 – 0.50	0.4 – 0.8
K	1.71 – 2.50	1.70 – 3.00	3.9 – 5.0

Ca	0.21 – 1.00	0.31 – 0.50	0.3 – 0.8
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Sufficiency Ranges for Soybean Leaves Small and Ohlrogge, 1973

Element	Sufficiency Range
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N %	4.26 – 5.50
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P	0.26 – 0.50
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K	1.71 – 2.50
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Ca	0.36 – 2.00
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Mg	0.26 – 1.00
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Fe	51.0 – 350.0
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Cu	10.0 – 30.0
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Zn	21.0 – 50.0
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- Operation and Management of a soil testing and plant analysis laboratory

There are three types of soil and plant analysis namely:

- Laboratories operated by a university or educated institution
  - Objective: To provide a service in the field of soil and plant analysis with the objective of acquiring data to support or confirm the research or to acquire information that would be useful in designing educational programs for students and other persons concerned with soil fertility and plant nutrition.
- Industrial Laboratories: These are laboratories operated by a company, usually a fertilizer company.
  - Objective: Promoting the use or sale of the product manufactured or distributed by the company owning or operating the laboratory
- Commercial Laboratory
  - To operate in such a manner as to return a profit for the investment required to provide the service

The common purpose of all the above laboratories is to provide a service to the agricultural laboratory.

The successful operation of a commercial laboratory requires careful study, planning and organization. The overall organization includes the people, facilities for housing the laboratory and the instrumentation.

### PERSONNEL

- Class I Technicians: People who are primarily responsible for the receiving and preparation of soil and plant samples for analysis. They also involved in recording of test results.
- Class II Technicians: These are people trained to perform routine soil analyses such as pH, phosphorus, potassium e.t.c. A knowledge of chemistry is useful but not mandatory for this set of people. It is however much more efficient to have this class of technicians become specialists in a particular phase of the soil testing operation as this will improve their proficiency.
- Class III Technicians: This category includes people who have a working knowledge of chemistry and are responsible for the operation of more complex instruments such as the atomic absorption spectrophotometer. Because of the variety of tests that are performed and the dilutions and other calculations that are necessary, a formal education in chemistry with a good mathematics background is important.
- Class IV Technicians: This category includes persons performing Kjeldahl nitrogen tests, sulfur analysis and other wet chemical tests. Those to be employed to this position should have a good knowledge of chemistry this is because wet chemistry and techniques such as titration require a good working knowledge of chemistry. The difference between class III and IV technicians lies in the fact that people who prefer

- to work with atomic absorption, for example, usually do not like to work with the wet chemistry tests that are performed by class IV technicians.
- Class V Technicians: These are trained in the operation of instrumentations such as the direct-reading emission spectrograph for plant tissue analysis and gas chromatography. Formal education in chemistry and related sciences is a must for people in this category. This people may or may not operate the instruments but should direct or supervise instrument operation.
  - Supervisory Personnel: The primary purpose of this supervisor is to make certain that that all of the technicians are utilized efficiently and that samples are flowing through the laboratory smoothly and in proper sequence. The person occupying this post should be trained in the management of people.
  - Technical Director: This person will work closely with the supervisor, must have a working knowledge of chemistry i. e. should know the technique of actually performing various analysis. He/She should be responsible for the quality control programme.
  - Finance Officer: Financial records are important to the success of the laboratory, and bookkeeping is a necessary part of the laboratory operation. It is important that accurate accounting methods be employed. Accounts receivable should be monitored continuously to assure adequate cash flow.
  - The Agronomist: This performs a vital role. It is their responsibility to read the reports and discuss it personally with the customer. The competence and ability of the agronomist in many cases will determine the success or failure of the operation regardless of how correct/rapid the analyses may be and whether the laboratory is using the most sophisticated instrumentation to process results. His ability to interpret and make recommendations is of utmost importance.

## ➤ FACILITIES

**SIZE OF THE BULIDING:** This will depend on the volume of business and instrumentation involved. The building should be large enough to accommodate all the instrumentation and allow adequate working space for technicians, clerical personnel, storage and receiving of chemicals and samples.

### ❖ Sections of the building

- Receiving dock and Storage: This is utilized for receiving of all chemical supplies as well as soil and plant samples
- Soil sterilization area: Devoted to heat-treating soil samples once they have been tested and before they are discarded. This is mandatory under Federal regulations for laboratories receiving soil samples from quarantine areas.
- Soil Preparation room: Soil samples are placed in trays for movement into the laboratory testing area. Soil samples are stored in this area until they have been tested and reports sent out
- Plant Preparation room: This is where plant and feed materials are kept. There should be drying and storing facilities for the plant material as well as space

for grinding equipment. Adequate exhaust system is essential in both the soil preparation and plant tissue analysis preparation rooms. Soil and plant preparation rooms should be separated so as to avoid any contamination of plant tissue material by soil.

- Air conditioning and heating systems: These should be adequate in the laboratory to maintain a uniform temperature as much as possible from day to day and from month to month. Unusual variations in temperature within the laboratory can upset the calibration of some equipment. There should also be electrostatic air cleaners so as to remove dust and foreign contaminants from the air being circulated throughout the laboratory.
- Employees' lounge: There should be enough space for employees where they can have a private area for coffee breaks and launch. Employees are not to eat in the laboratory to avoid contaminations. Smoking is absolutely prohibited in any of the laboratory sections
- Executive Conference room: This is where discussions with customers, management discussions and meetings with employees take place.
- Business office or Reception: This is where customers would be greeted as they enter the building and they may be directed to the proper area. Visitors should not be allowed in any of the laboratory sections without prior approval with exception to the business, storage and receiving areas and executive conference rooms. Windows should be installed on the inner walls along the main hallway so that visitors touring the lab may view the operations without interfering with the employees or introducing any contamination into the various areas.

➤ **INSTRUMENTATION:**

- Racks; For storage and drying soil and plant samples
- Grinders: For grinding soil and plant samples
- Soil sterilizer: For soil sterilization
- pH meters: For pH determination of soil and water samples
- Spectrophotometer: For phosphorus determination
- Flame photometer: For potassium and sodium determinations
- Atomic absorption spectrophotometer: For the determination of trace elements, calcium and magnesium
- Kjeldahl equipment: For total and ammonium nitrogen determination
- Furnaces: For ashing of plant samples
- Weighing balance: This should be accurate to 0.001 g.
- Fume cupboards
- Incinerator: A place where all containers from which soil and plant samples are received
- Fire extinguisher: In case of fire incident.

➤ **CHEMICALS:**

High grade chemicals and standards should be purchased and kept at the recommended temperature.

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