# PRACTICAL MANUAL

# Manures, Fertilizers and Soil Fertility Management

## APS 316 3(2+1)

Dr. Bharat Lal

Dr. Susheel Kumar Singh

2020

College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi-284003, Uttar Pradesh

**Syllabus: Manures, Fertilizers and Soil Fertility Management APS 316 3(2+1)**

Introduction of analytical instruments and their principles, calibration and applications, Colorimetry and flame photometry. Estimation of soil organic carbon, Estimation of alkaline hydrolysable N in soils. Estimation of soil extractable P in soils. Estimation of exchangeable K, Ca and Mg in soils. Estimation of soil extractable S in soils, Estimation of DTPA extractable Zn in soils. Estimation of N in plants. Estimation of P in plants. Estimation of K in plants. Estimation of S in plants.

**Name of Students** ........................................................................................

**Roll No.** ...................................................................................................

**Batch** .......................................................................................................

**Session** ....................................................................................................

**Semester** ..................................................................................................

**Course Name :** .........................................................................................

**Course No. :** .............................................................................................

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Date: Course Teacher

# CONTENTS

Experiment No. 1

## Objective: To identify different analytical instruments

**Weighing balance**: ……………………………………………………..……

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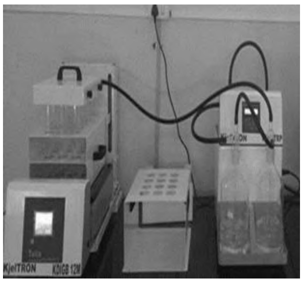

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**Atomic absorption spectrophotometer (AAS):** ……………………………

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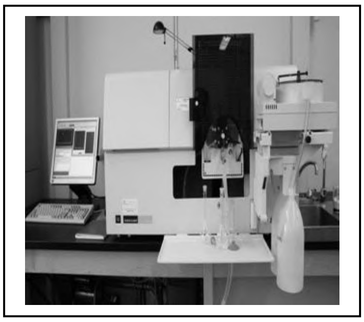

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**X-ray diffractometer:** ……………………………………………………………

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**Uses:**……………………………………………………………………………………

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## Experiment No. 2

## Objective: To study about the working principle of pH meter.

The pH Measurement specifies the degree of relative acidity or alkalinity of an aqueous solution at a given temperature. Theoretically, pH is derived from the word “Pondus Hydrogenii” which means “Potential Hydrogen” or power of hydrogen ion, pH is represented in the form of an equation as the negative logarithm of the hydrogen ion concentration.

**Components of pH meter:**……………………………………………………………………………………

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**Draw the well labeled diagram of pH meter**

**Working principle of pH meter:**……………………………………………………………………………

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**Calibration of pH meter**……………………………………………………………………………..

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## Experiment No. 3

## Objective: To study about the working principle of conductivity meter

The conductivity meter measures the soluble salt concentration for soil and water samples which helps to determine the salinity hazard.

**Working principle of conductivity meter:**

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**Draw the diagram of conductivity meter**

**Calibration of EC meter:**……………………………………………………………………………………

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## Experiment No. 4

## Objective: To study about the working principle of flame-photometer

During 1980s Bowling Barnes, David Richardson, John Berry and Robert Hood developed an instrument to measure the low concentrations of sodium and potassium in a solution. They named this instrument as Flame photometer. Flame photometer can be used to determine the concentration of certain metal ions like sodium, potassium, lithium, calcium and cesium etc.

**Working principle:** ……………………..……………………………………………………………………

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**Parts of the flame-photometer:**

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**Simple color filter:**…………………………………………………………………………………………

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**Photo-detector:**……………………………………………………………………………………...

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**Draw the flame-photometer with their components**

**Processes of flame-photometer:**…………………………………………………………………

**Desolvation:**………………………………………………………………………………………….

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**Vaporization:**……………………………………………………………………………………....

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**Atomization:**…………………………………………………………………………………………

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**Sketch the process of flame-photometer**

**Working procedure of flame-photometer:**……………………………………………………………………

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**Applications of flame-photometer:**……………………………………………………………………………….

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## Experiment No. 5

## Objective: To study about the working principle of spectrophotometer

A spectrophotometer is an instrument used to measure absorbance at various wavelengths. It can be operated in UV (Ultraviolet) region, visible spectrum as well as IR (Infrared) region of the electromagnetic spectrum.

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**Draw the internal diagram of single and double beam spectrophotometer**

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**Sample holder:** ………………………………………………………………………………………………

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**Beam splitter:** ………………………………………………………………………………………………

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**Measuring device: :**……………………………………………………………………………………………

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## Experiment No. 6

## Objective: To study about the working principle of atomic absorption spectrophotometer (AAS)

The Atomic absorption (AA) spectrometer is used to analyze metals (Fe, Mn, Zn, Cu, Ni, Pb and Cr etc.) at very low concentrations, typically in the parts per million (ppm) or parts per billion (ppb) ranges.

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**Monochromator**:…………………………………………………………………………………………

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**Detector:**……………………………………………………………………………………………………

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**Calibration curve:**…………………………………………………………………………………...

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**Draw the various components of AAS**

**Applications of AAS:** ………………………………………………………………………………

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## Experiment No. 7

## Objective: To estimate the organic carbon content in soil sample

Walkley-Black rapid titration method (wet combustion) is extremely useful. Soil organic carbon is oxidized to $CO_2$ in presence of an excess of oxidizing agent such as a mixture of potassium dichromate and sulphuric acid. It is actually the chromic acid formed by the action of potassium dichromate and sulphuric acid which oxidizes the carbon. The excess of dichromate is determined by blank titration with ferrous ammonium sulphate (redox-titration).

**Reagents required:** ……………………………………………………………………………………………

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**Procedure for organic carbon determination in soil:** ……………………………………………………

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**Chemical Reactions:** ………………………………………………………………………………………

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**Calculations:**

$$2K_2Cr_2O_7 + 8H_2SO_4 = 2K_2SO_4 + Cr_2(SO_4)_3 + 8H_2O + 6O$$

$$3C + 6O = 3CO_2$$

$$2K\ Cr\ O \xrightarrow{\text{evolve}} 6O \xrightarrow{\text{oxidise}} 3C$$

$$(2 \times 294)\text{ g } K_2Cr_2O_7 \equiv (3 \times 12)\text{ g C}$$

Hence; $49\text{ g K Cr O} \equiv \frac{3 \times 12}{2 \times 294} \times 49 = 3\text{ g C}$

Now 49 g $K_2Cr_2O_7$ dissolved in 1 litre gives 1(N) – solution

i.e. 1000 ml 1(N) $K_2Cr_2O_7 \equiv 3$ g C

1 ml 1(N) K2Cr2O7 ≡ 0.003 g C

Alternatively,

**Organic Carbon (%) =** $\frac{\mathbf{10\ (B-T)}}{\mathbf{B}} \times \mathbf{0.003} \times \frac{\mathbf{100}}{\mathbf{w}}$

where,

B = Volume (ml) of ferrous ammonium sulphate solution required for blank titration.

T = Volume (ml) of ferrous ammonium sulphate (ml) needed for sample titration

W = weight of soil sample in g

More precisely, Organic carbon value considering recovery factor of 0.77 can be calculated using the formula.

$$= \frac{10\ (B-T)}{B} \times 0.003 \times \frac{100}{w} \times \frac{1}{0.77} \times \frac{(100+m)}{100}$$

Where, m = air dry moisture

% organic matter = % organic carbon x 1.724

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**Interpretations:**

| **Low Organic carbon** | **Medium Organic carbon** | **High Organic carbon** |
|---|---|---|
| < 0.50 % | 0.50 – 0.75 % | > 0.75 % |

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## Experiment No. 8

## Objective: To determine the mineralizable nitrogen in soil

In the alkaline permanganate method (Subbiah and Asija, 1956) nitrogen is released by the alkaline permanganate solution and estimated by the usual ammonia distillation procedure, the distillate being absorbed in standard acid and excess acid back titrated with standard alkali using methyl red indicator.

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**Procedure for nitrogen estimation in soil:** ………………………………………………………………

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**Calculations:**

$$\text{Available nitrogen in soil (Kg/ha)} = \frac{(S-B)\times 0.00028}{20} \times 10^{6} \times 2.24$$

$$= (S-B) \times 31.36$$

Where, S is the titre value for soil sample

B is titre value for blank sample

The factor 0.00028 is arrived at by

$2NH_4OH + H_2SO_4 = (NH_4)_2SO_4 + 2H_2O$

Or 98 g of $H_2SO_4$ (or 1L of 2N $H_2SO_4$) ≡ 28g N

Or 1 ml of 0.02N $H_2SO_4$ ≡ 0.00028 g N

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**Interpretations**

| **Low Nitrogen** | **Medium Nitrogen** | **High Nitrogen** |
|---|---|---|
| < 280 Kg/ha | 280 – 560 Kg/ha | > 560 Kg/ha |

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## Experiment No. 9

## Objective: To determine the available phosphorus in soils by Olsen method (1954)

An alkaline (pH 8.5) bicarbonate solution can repress the concentration of calcium ions by precipitation as calcium carbonate and of aluminium and ferric ions as hydroxides. Thus phosphate ions concentrations are increased and available phosphate can be extracted from soil by shaking with alkaline $NaHCO_3$ and filtering. The 0.5 (M) sodium bicarbonate adjusted to pH 8.5 actually controls the ionic activity of calcium, through the solubility product of calcium carbonate, during the extraction of calcareous soils.

**Reagents required:** ……………………………………………………………………………………………

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**Working procedure for P estimation in soil sample**

**Phosphorus extraction from soil:** ……………………………………………………………………………

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**Color development and phosphorus detection:** ………………………………………………………………

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**Chemical reactions during phosphorus estimation**

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**Calculations:**

$$\text{Available P (Kg/ha)} = \frac{Q \times V \times 2.24 \times 10^6}{A \times S \times 10^6} = \frac{Q \times V \times 2.24}{A \times S}$$

$$= Q \times 8.96$$

Where, Q = quantity P (Sample reading), V = volume of olsen’s reagent used

A = Volume of aliquot used for color development, S = weight of sample (g)

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**Interpretations**

| **Low Nitrogen** | **Medium Nitrogen** | **High Nitrogen** |
|---|---|---|
| < 12.5 Kg/ha | 12.5 - 25 Kg/ha | > 25 Kg/ha |

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**What are the precautions should kept in mind during P analysis**

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## Experiment No. 10

## Objective: To determine the available phosphorus in soils by Bray's P-1 (Bray and Kurtz, 1945)

Under acidic soil conditions ($pH < 6.5$), Bray's P-1 (or Bray No.1) is used for the phosphorus determination, which involves soil extraction with a solution consisting of 0.03N NH4F and 0.025N HCl is widely followed. The fluoride complexes Al and Fe in soil, thus releasing some bound P besides the easily acid-soluble P (largely Ca-P). This extractant is suitable for soils containing less than 2% calcite or dolomite because in calcareous soils, carbonates quickly neutralize the acid, resulting in less extraction of P.

**Reagents required:** …………………………………………………………………………………………………

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**Working procedure for P estimation in soil sample:** ………….……………………………………………

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**Calculations:**

$$\text{Available P (Kg/ha)} = \frac{Q \times V \times 2.24 \times 10^6}{A \times S \times 10^6} = \frac{Q \times V \times 2.24}{A \times S}$$

$$= Q \times 4.48$$

Where, Q = quantity P (Sample reading), V = volume of olsen's reagent used

A = Volume of aliquot used for color development, S = weight of sample (g)

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**Interpretations**

| **Low Nitrogen** | **Medium Nitrogen** | **High Nitrogen** |
|---|---|---|
| < 34 Kg/ha | 34 - 68 Kg/ha | > 68 Kg/ha |

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## Experiment No. 11

## Objective: To estimate the available potassium in soil by neutral normal ammonium acetate method (Hanway and Heidel, 1952)

The readily exchangeable plus water soluble $K^+$ is determined in the neutral normal ammonium acetate extract of the soil. The $NH_4^+$ ion provide a sharp and quick separation from the exchange sites while it determine by using flame-photometer.

**Reagents required:** …………………………………………………………………………………………

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**Procedure for potassium determination in soil:** ……………………………………………………………

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### Calculations

$$\text{Available K (Kg /ha)} = R - B \times \frac{25}{5} \times 2.24 = R \times 11.2$$

Where,

R = ppm of K in the extract, obtained from the standard curve (soil sample reading).
B = reading of blank sample (without soil)

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### Interpretations

| Low Potassium | Medium Potassium | High Potassium |
|---|---|---|
| < 135 Kg/ha | 135 - 335 Kg/ha | > 335 Kg/ha |

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### Experiment No. 12

## Objective: To determine the extractable calcium and magnesium in soil- Versenate (EDTA) method

The Ca in solution is titrated with 0.01N EDTA using first the indicator ammonium purpurate (murexide) which at the pH of 12.0 change color from red to purple at the end point of titration. Ca + Mg in solution can be titrated with 0.01N EDTA using Erichrome Black T as indicator at pH 10.0 in the presence of Ammonium chloride-ammonium hydroxide buffer. At the end point color changes from wine red to blue or green. The Mg content is determined by the difference.

**Reagents required**

**For Ca determination:** ……………………………………………………………………………………………

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**For Ca + Mg determination:** ………..………………………………………………………………………………

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**Procedure**

**Ca determination:** ………………………………………………………………………………………………

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**Ca + Mg determination:** ………..………………………………………………………………………………

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**Mg determination:** ………..…………………………………………………………………………………

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**Calculations:**

$$\text{Ca (me/L)} = \frac{\text{EDTA Used (ml)X Normality of EDTA}}{5} \text{ X } 1000$$

Ca (ppm) = Ca (me/L) X equivalent wt. of Ca (20)

$$\text{Ca+ Mg (me/L)} = \frac{\text{EDTA Used (ml)X Normality of EDTA}}{5} \text{ X } 1000$$

Ca+Mg (ppm) = Ca +Mg(me/L) X equivalent wt. of Ca+Mg (32)
Mg (me/L) = Ca+ Mg (me/L) – Ca (me/L)
Mg (ppm) = Ca+ Mg (ppm) – Ca (ppm)

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## Experiment No. 13

## Objective: To determine the available sulphur in soil

When the soil solution is shaken with $CaCl_2$ (0.15 %), the chloride ions displace the adsorbed sulphate during extraction. The filtrate is analyzed for sulphur by turbidimetry method as outlined by Chesin and Yien (1950), in which turbidity produced due to the precipitation of $SO_4^{-2}$ as $BaSO_4$ is measured on a spectrophotometer at a wavelength of 420 nm or corresponding to blue filter.

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**Preparation of standard solution for S estimation in soil:** ………………………………………………

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**Working procedure for S estimation in soil:** ……………………………………………………………

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**Calculations:**

Available S in soil (mg/Kg) = $\mathrm{S} - \mathrm{B} \times \frac{50}{10} \times \frac{1}{10}$

Where, S = Soil sample reading
B = Blank sample reading

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**Interpretations:**

| **Low Sulphur** | **Medium Sulphur** | **High Sulphur** |
|---|---|---|
| < 11.25 mg/Kg | 11.25 – 17.50 mg/Kg | > 17.50 mg/Kg |

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## Experiment No. 14

## Objective: To determine the available zinc in soil by DTPA-$CaCl_2$ method

Lindsay and Norvill (1978) developed a method using DTPA (Diethylene triamine Penta Acetic Acid). This method has been adopted in all the laboratories engaged in the analyses of available Zn, Cu, Mn and Fe. Chelating agents offer great promise for assessing readily available micronutrient cations in soil. These agents combine with free metal ions in solution to from soluble complexes. DTPA is an efficient complexing agent for all the essential micronutrient cations, viz. Cu, Fe, Mn and Zn. With the advent of hollow cathode lamps along with precision detecting device all the estimation are now possible in a single extraction.

**Reagents required:** ……………………………………………………………………………………………

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**Prepare the extracting solution for Zn determination in soil:** ……………………………………………

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**Procedure for Zn determination in soil sample:** ……………………………………………………………

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**Calculation**

$$\text{Micronutrients content in soil (mg/Kg or ppm)} = R \times 2$$

Where, R is the reading

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**Interpretations:**

| **Micronutrients** | **Deficient** | **Sufficient** | **High level** |
|---|---|---|---|
| Available Zn (mg/Kg) | <0.6 | 0.6- 1.2 | > 1.2 |

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## Experiment No. 15

## Objective: To determine the total nitrogen in plant sample

For determination of total N in plant, organic N in plant sample is converted to NH4-N by digestion with concentrated H2SO4 containing substances (K2SO4 or Na2SO4) that promote this conversion. The NH4-N the digest is determined from the amount of NH3 liberated by distillation of the digest with an alkali (NaOH). Ammonia absorbed in the boric acid containing mixed indicator is determined with a standard acid.

**Reagents required:** ……………………………………………………………………………………………

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**Reactions**

**Sample digestion:** ………...……………………………………………………………………………

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**Distillation:** ……………………………………………………………………………………………

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**Titration:** ………………………………………………………………………………………………

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**Calculations**:

Amount (g) of N in the sample (S) = (ml of acid used for sample - ml of acid used for blank) x normality of acid x 14 x $10^{-3}$.

N in sample (%) = (S x 100) / Sample weight (g)

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## Experiment No. 16

## Objective: To determine the total P in plant sample

The digested plant sample will be used for the phosphorus determination in plant samples. Ammonium molybdate and ammonium metavanadate helps to develops color and the color intensity is measured by the spectrophotometer at 470 nm wavelengths.

### A) Digestion of plant sample:

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**Wet digestion (Di-acid or tri-acid mixture)**

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**Procedure for plant digestion**: ……………………………………………..………………………………..

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### B) Phosphorus analysis from digested sample

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1. **Procedure for p estimation:** ……………………………………………………………

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**Calculations**

P in plant material (ppm or as mg kg$^{-1}$) = $\text{Reading from standard curve X}\frac{50}{5}\text{X}\frac{100}{\text{Wt of sample(g)}}$

P in plant material (%) = P in plant material (ppm) x 10$^{-4}$

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## Experiment No. 17

## Objective: To estimate the total K in plant sample

Plant sample digested by di-acid mixture and sample introduced into the flame, K atoms further get excited to even higher levels when get energy. But these atoms are not stable at higher levels. Hence, these atoms emit radiations when returning back to the ground state. These radiations generally lie in the visible region of the spectrum and emitted radiation is proportional to the ion concentration.

**Reagents required:** ……………………………………………………………………………………………

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**Calculations**

$$\text{K in plant material (ppm)} = \frac{\text{Flame photometer reading} \times 100 \times 25}{5 \times \text{sample wt (g)}}$$

$$\text{K in plant material (\%)} = \text{K in plant material (ppm)} \times 10^{-4}$$

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## Experiment No. 18

## Objective: To determine the total S in plant sample

In the estimation of total sulphur in plant sample, wet digest is taken from di-acid digestion method (as described for phosphorous) and then determined by barium sulphate turbidimetry method. During wet digestion of the sample, all the plant sulphur converted to sulphate form, which when treated with $BaCl_2$ is precipitated as $BaSO_4$. This provides turbidity to the solution which is proportional to the amount of sulphate present. Measurement of this turbidity provides the means for quantitative determination of sulphur. Gum acacia solution is added to help in stabilizing turbidity.

**Required Reagents:** ……………………………………………………………………………………………

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**Calculations**

$$\text{S in plant tissue (\%)} = \text{Reading from standard curve X } 10^{-4}\text{X}\left[\frac{50}{5}\text{X}\frac{100}{\text{Wt of sample(g)}}\right]$$

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***APPENDICES***

## WORKING PRINCIPLE OF pH METER

**Components:** Measuring electrode, reference electrode, temperature sensor and the sample solution being measured.

**Working Principle:** The pH Meter measures the voltage of an electro chemical cell and based on the temperature sensor determines the pH of a solution. The overall potential or the voltage is the algebraic sum of the potentials of the measuring electrode, reference electrode and the liquid Junction. The reference electrode provides a stable voltage as it has a fixed concentration of potassium chloride solution which is a neutral solution. On the contrary, the potential of the measuring electrode depends only on the pH of the solution. The potential difference (voltage) between a glass membrane of measuring electrode and a reference electrode which is dipped in the sample solution to be tested is measured. When the two electrodes are dipped in the sample solution, ion-exchange process occurs, where in some of the hydrogen ions move towards the outer surface of the measuring electrode and replaces some of the metal ions inside it. The potential difference of glass electrode and sample solutions will be recorded in the galvanometer.

**Calibration:** Calibration is done by dipping the measuring electrode into buffer solution of known pH i.e., 4.0, 7.0 and 9.2 pH, which helps in interpreting millivolt reading as pH measurement of the sample solution at the given temperature

### Applications of pH measurement

- pH measurement is very crucial parameters for soil evaluation.
- Major crops require pH 6.5 to 7.5 for their optimum growth.
- pH controls the availability of various nutrient in soil.
- It is also used in food industry especially for dairy products like cheese, curds, yogurts, etc.
- pH also identifies the salt affected soil.
- pH level monitoring is essential in water quality for irrigation.

## WORKING PRINCIPLE OF CONDUCTIVITY METER

**Principle:** Salinity in soil and water is characterized as the total content of the dissolved inorganic solute. It is conventionally determined by measuring the electrical conductivity. The electrical conductivity is a measure of the ability of a salt solution to carry electric current by the migration of ions under the influence of an electric field, as ions are the carrier of electricity. Like a metallic conductor, solutions also obey Ohm's law. Increase in temperature promotes dissociation of the salts with a consequent rise in conductivity at the rate of approximately 2% for each degree Celsius rise in temperature. The unit of specific conductance is the reciprocal of specific resistance in mhos /cm or dS/m. At infinite dilution, the ions are theoretically independent of each other and each ion has its contribution to the total conductance. Thus,

$$\lambda \propto \; = \Sigma (\lambda^{+}) + \Sigma (\lambda^{-})$$

where, $\lambda \propto$ is the total conductance,
$\lambda^{+}$ is the conductance of cations and
$\lambda^{-}$ is the conductance of anions

**Calibration of EC meter:** The cell constant, k, of a conductivity cell is determined by measuring the electrical conductance of a standard KCl solution at the same temperature according to the equation:

$$K = \frac{L}{C}$$

where, L and C are the known specific electrical conductance and the measured conductance of the standard solution (usually 0.02M KCl) in the given cell in dS /m. The specific conductance (L) of 0.02M KCl is 2.39 dS /m at 18 °C and 2.77 dS /m at 25 °C. The measured conductance C, of a test solution in dS /m multiplied by the cell constant (k) gives the specific conductance, L in dS /m of the test solution (L = kC).

### Procedure

- Switch on the instrument. Allow at least 15-20 minutes for warm up.
- Wash the electrode with distilled water and wipe dry with tissue paper.
- Use 0.02M KCl solution to calibrate the meter.
- Wash and fill the cell with the KCl solution. Adjust the meter to read the standard conductivity at that temperature or adjust the cell constant with the help of calibration knob.
- Wash the electrode with distilled water, followed by the test solution.
- Record the conductivity reading.

### Precautions

- Before using a new conductivity cell, place it in distilled water for 24 hours.
- When not in use, keep the cell in distilled water.
- Keep the cells perfectly clean for accurate readings.
- Rinse the cell with the specimen before making the measurement.

## WORKING PRINCIPLE OF FLAME-PHOTOMETER

**Principle:** The compounds of the alkali and alkaline earth metals (Group II) dissociate into atoms when introduced into the flame. Some of these atoms further get excited to even higher levels. But these atoms are not stable at higher levels. Hence, these atoms emit radiations when returning back to the ground state. These radiations generally lie in the visible region of the spectrum. Each of the alkali and alkaline earth metals has a specific wavelength.

| Elements | Emitted Wavelength | Flame color |
|---|---|---|
| Sodium | 589 nm | Yellow |
| Potassium | 766 nm | Violet |
| Barium | 554 nm | Lime green |
| Calcium | 622 nm | Orange |
| Lithium | 670 nm | Red |

$$I = K \times C^n$$

Where: I = Intensity of emitted light
C = Concentration of the element
K = Proportionality constant

Then I = K × C. In other words, the intensity of emitted light is directly related to the concentration of the sample.

The intensity of the emission is directly proportional to the number of atoms returning to the ground state. And the light emitted is in turn proportional to the concentration of the sample.

**Parts of flame photometer:** A simple flame photometer consists of the following basic components:

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

| Fuel oxidant mixture | Temperature (˚C) |
|---|---|
| Natural gas – Air | 1700 |
| Propane - Air | 1800 |
| Hydrogen - Air | 2000 |
| Hydrogen - Oxygen | 2650 |
| Acetylene – Air | 2300 |
| Acetylene - Oxygen | 3200 |
| Acetylene – Nitrous oxide | 2700 |
| Cyanogen – Oxygen | 4800 |

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

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

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

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

**Processes:**

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

**Working procedure**

- Both the standard stock solution and sample solution are prepared in fresh distilled water.
- The flame of the photometer is calibrated by adjusting the air and gas.
- Then the flame is allowed to stabilize for about 5 min.
- Now the instrument is switched on and the lids of the filter chamber are opened to insert appropriate colour filters.
- The readings of the galvanometer are adjusted to zero by spraying distilled water into the flame.
- The sensitivity is adjusted by spraying the most concentrated standard working solution into the flame.
- Now the full-scale deflection of the galvanometer is recorded.
- Again, distilled water is sprayed into the flame to attain constant readings of galvanometer.
- Then the galvanometer is readjusted to zero.
- Now each of the standard working solutions is sprayed into the flame for three times and the readings of galvanometer are recorded.

- After each spray, the apparatus must be thoroughly washed.
- Finally sample solution is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- Calculate the mean of the galvanometer reading. Plot the graph of concentration against the galvanometer reading to find out the concentration of the element in the sample.

**Applications:**

- Flame photometer can be applied both for quantitative and qualitative analysis of elements.
- The presence of some group II elements is critical for soil health. We can determine the presence of various alkali (lithium, sodium, potassium, rubidium, cesium, and francium) and alkaline earth metals (beryllium, magnesium, calcium, strontium, barium, and radium) in soil sample by conducting flame test and then the soil can be supplied with specific fertilizer.
- The concentrations of $Na^+$ and $K^+$ ions are very important in the human body for conducting various metabolic functions. Their concentrations can be determined by diluting and aspirating blood serum sample into the flame.
- Soft drinks, fruit juices and alcoholic beverages can also be analyzed by using flame photometry to determine the concentrations of various metals and elements.

**Advantages:**

- The method of analysis is very simple and economical.
- It is quick, convenient, selective and sensitive analysis.
- It is both and qualitative and quantitative in nature.
- Even very low concentrations (parts per million/ppm to parts per billion/ppb range) of metals in the sample can be determined.
- This method compensates for any unexpected interfering material present in the sample solution.

**Disadvantages:**

- The accurate concentration of the metal ion in the solution cannot be measured.
- It cannot directly detect and determine the presence of inert gases.
- Though this technique measures the total metal content present in the sample, it does not provide the information about the molecular structure of the metal present in the sample.
- Only liquid samples may be used. Also sample preparation becomes lengthy in some cases.
- Flame photometry cannot be used for the direct determination of each and every metal atom.
- The elements such as carbon, hydrogen and halides cannot be detected due to their non-radiating nature.

## WORKING PRINCIPLE OF SPECTROPHOTOMETER

**Principle:** Spectrophotometer is based on the photometric technique which states that When a beam of incident light of intensity $I_0$ passes through a solution, a part of the incident light is reflected (Ir), a part is absorbed (Ia) and rest of the light is transmitted (It)

Thus,

$$I_0 = Ir + Ia + It$$

The working principle of the Spectrophotometer is based on Beer-Lambert's law which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution.

$$A \propto cl$$

Where, A = Absorbance / Optical density of solution
c = Concentration of solution
l = Path length

**Types of Spectrophotometers**

**Single beam spectrophotometer:** Single beam spectrophotometer operates between 325 nm to 1000 nm wavelength using the single beam of light. The light travels in one direction and the test solution and blank are read in the same.

**Double beam spectrophotometer:** It operates between 185 nm to 1000 nm wavelength. It has two photocells. This instrument splits the light from the monochromator into two beams. One beam is used for reference and the other for sample reading. It eliminates the error which occurs due to fluctuations in the light output and the sensitivity of the detector.

**Parts of Spectrophotometers:** There are 7 essential parts of a spectrophotometer

- **Light source** – In spectrophotometer three different sources of light are commonly used to produce light of different wavelength. The most common source of light used in the spectrophotometer for the visible spectrum is a tungsten lamp. For Ultraviolet radiation, commonly used sources of are the hydrogen lamp and the deuterium lamp. Nernst filament or globar is the most satisfactory sources of IR (Infrared) radiation.
- **Monochromator** – To select the particular wavelength, prism or diffraction grating is used to split the light from the light source.
- **Sample holder** – Test tube or cuvettes are used to hold the colored solutions. They are made up of glass at a visible wavelength.
- **Beam splitter** – It is present only in double beam spectrophotometer. It is used to split the single beam of light coming from the light source into two beams.

- **Mirror** – It is also present only and double beam spectrophotometer. It is used to the right direction to the splitted light from the beam splitter.
- **Photodetector system** – When light falls on the detector system, an electric current is generated that reflects the galvanometer reading.
- **Measuring device** – The current from the detector is fed to the measuring device. The meter reading is directly proportional to the intensity of light.

**Working in the Spectrophotometers**

- When using a Spectrophotometer, it requires being calibrated first which is done by using the standard solutions of the known concentration of the solute that has to be determined in the test solution. For this, the standard solutions are filled in the cuvette and placed in the cuvette holder in the spectrophotometer
- There is a ray of light with a certain wavelength that is specific for the assay is directed towards the solution. Before reaching the solution the ray of light passes through a series of the diffraction grating, prism and mirrors. These mirrors are used for navigation of the light in the spectrophotometer and the prism splits the beam of light into different wavelength and the diffraction grating allows the required wavelength to pass through it and reaches the cuvette containing the standard or test solutions.
- When the monochromatic light (light of one wavelength) reaches the cuvette some of the light is reflected and some part of the light is absorbed by the solution and the remaining part is transmitted through the solution which falls on the photo-detector system. The photo-detector system measures the intensity of transmitted light and converts it into the electrical signals that are sent to the galvanometer.
- The galvanometer measures the electrical signals and displays it in the digital form
- If the absorption of the solution is higher than there will be more light absorbed by the solution and if the absorption of the solution is low then more lights will be transmitted through the solution which affects the galvanometer reading and corresponds to the concentration of the solute in the solution.
- In double beam spectrophotometers, the beam splitters are present which splits the monochromatic light into two beams one for the standard solution and the other for test solution. In this, the absorbance of standard and the test solution can be measured at the same time and any no. of test solutions can be analyzed against one standard. It gives more accurate and precise results, eliminates the errors which occur due to the fluctuations in the light output and the sensitivity of the detector.

**Applications**

- It used for the determination of the course of the reaction by measuring the rate of formation and disappearance of the light absorbing compound in the range of the visible & UV region of electromagnetic spectrum.
- It used to measure the different total and available nutrients in soil (P, S, B and Mo)
- It also determines the total nutrient analysis of different plant samples, which based on color formations.

## WORKING PRINCIPLE OF ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

**Principle:** The technique uses basically the principle that free atoms (gas) generated in an atomizer can absorb radiation at specific frequency. Atomic-absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The element concentration is determined from the amount of absorption concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration. Atomic absorption is a very common technique for detecting metals and metalloids in environmental samples.

**Components**

- **Light Source:** Hollow cathode lamps are the most common radiation source in AAS. It contains a tungsten anode and a hollow cylindrical cathode made of the element to be determined. These are sealed in a glass tube filled with an inert gas (neon or argon). Each element has its own unique lamp which must be used for that analysis.
- **Nebulizer:** Suck up liquid samples at controlled rate. It creates a fine aerosol spray for introduction into flame and mix the aerosol and fuel and oxidant thoroughly for introduction into flame.
- **Atomizer**: Atomization is separation of particles into individual molecules and breaking molecules into atoms. This is done by exposing the analyte to high temperatures in a flame or graphite furnace.
  - **Flame atomizer:** To create flame, we need to mix an oxidant gas and a fuel gas. In most of the cases air-acetylene flame or nitrous oxide acetylene flame is used. The liquid or dissolved samples are typically used with flame atomizer.
  - **Graphite tube atomizer:** Uses a graphite coated furnace to vaporize the sample. In sample, samples are deposited in a small graphite coated tube which can then be heated to vaporize and atomize the analyte. The graphite tubes are heated using a high current power supply.
- **Monochromator:** This is a very important part in an AAS. It is used to separate out all of the thousands of lines. A monochromator is used to select the specific wavelength of light which is absorbed by the sample and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others.
- **Detector:** The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube,

whose function is to convert the light signal into an electrical signal proportional to the light intensity. The signal could be displayed for read out or further fed into a data station for printout by the requested format.

- **Calibration Curve**: A calibration curve is used to determine the unknown concentration of an element in a solution. The instrument is calibrated using several solutions of known concentrations. The absorbance of each known solution is measured and then a calibration curve of concentration vs absorbance is plotted. The sample solution is fed into the instrument, and the absorbance of the element in this solution is measured. The unknown concentration of the element is then calculated from the calibration curve

**Applications:** Determination of even small amounts of metals (lead, mercury, calcium, magnesium, etc) as follows:

- Environmental studies: drinking water, ocean water, soil and plant analysis.
- Food industry.
- Pharmaceutical industry.
- Used for the micronutrient's analysis of soil and plant samples.

## ORGANIC CARBON CONTENT IN SOIL SAMPLE

**Reactions:** Oxidation of Carbon

$K_2Cr_2O_7 + 4H_2SO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3O$ (× 2)

$3C + 60 \rightarrow 3CO2$

$2K_2Cr_2O_7 + 8H_2SO_4 + 3C \rightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 + 8H_2O + 3CO_2$

Titration

$FeSO_4(NH_4)_2 SO_4 . 6H_2O \rightarrow FeSO_4 + (NH_4)_2 SO_4 + 6H_2O$ (× 2)

$2FeSO_4 + H_2SO_4 + O \rightarrow Fe_2(SO_4)_3 + H_2O$

$2FeSO_4(NH_4)_2 SO_4 . 12H_2O + H_2SO_4 + O \rightarrow 2(NH_4)_2SO_4 + Fe_2(SO_4)_3 + 13H_2O$

Action of diphenylamine indicator

$2C_6 H_5 NHC_6 H_5 \frac{[O]}{-H2O} \rightarrow 2(C_6 H_5 . NHC_6 H_4) \frac{[O]}{-H2O} \rightarrow C_6H_5N\text{-} C_6H_4C_6H_4N\text{-}C_6H_5$

Diphenylamine diphenyl benzidine (violet)

### Reagents

- Standard potassium dichromate solution 1(N). Heat, $K_2Cr_2O_7$ in an air oven for 4 hours at 105°C. Dissolve 49.04 of pure $K_2Cr_2O_7$ in distilled water and dilute to one litre.
- Ferrous ammonium sulphate (N/2). Dissolve 196 g ferrous ammonium sulphate, $FeSO_4(NH_4)_2 SO_4 . 6H_2O$ in water. Add 25 ml of concentrated $H_2SO_4$ and dilute to one litre.
- Redox indicator. Use any one of the following:
  a) Diphenylamine indicator: Dissolve 0.5 g of diphenylamine in a mixture of 100 ml conc. sulphuric acid and 20 ml distilled water and store in a colored bottle.
  b) Ferroin indicator: Dissolve 1.485 g 1,10 phenanthroline monohydrate in about 80 ml water (warm if necessary and then cool) and added 0.69 g ferrous sulphate heptahydrate. Stir to dissolve and dilute to 100 ml.
- Concentrated sulphuric acid (sp.gr.1.84), 96% concentration or more
- Orthophosphoric acid (85%).

### Procedure

- Take 1.0 g of the soil sample (weighed to the nearest milligram) into a clear and dry 500 ml conical flask.
- Add 10 ml of 1(N) $K_2Cr_2O_7$ by means of a pipette and swirl gently.
- Then add 20 ml of concentrated $H_2SO_4$ rapidly into the solution and immediately mix by swirling gently at first and then vigorously for a total of one minute.
- Keep the flask on an asbestos pad for 30 minutes.
- Add 200 ml of distilled water and add 10 ml of orthophosphoric acid also add 1ml of diphenylamine indicator. A blue violet color will appear.
- Titrate with ferrous ammonium sulphate solution till color changes from blue violet to green (If ferroin indicator is used the colour change at the endpoint is from blue to red).
- If more than 8 ml of the dichromate solution is consumed repeat the estimation with a smaller quantity (0.25 – 0.50g) of the soil sample.
- Simultaneously carry out a blank determination using all the reagents similarly but no soil sample.

## MINERALIZABLE NITROGEN IN SOIL

### Reagents

- 0.32% potassium permanganate solution–freshly prepared
- 2.5% sodium hydroxide solution–freshly prepared
- Standard sulphuric acid 0.02(N)
- Standard sodium hydroxide 0.02(N).
- Methyl red indicator (Dissolve 1 gm methyl red in 200 ml of rectified spirit)
- Liquid paraffin (extra pure)
- Glass beads

### Procedure

- Weigh accurately 20 g of the soil sample in a distillation flask.
- Add 20 ml of distilled water, 100 ml of potassium permanganate solution and 100 ml of 2.5 percent sodium hydroxide solution (the frothing during boiling is prevented by adding liquid paraffin (1 ml) and bumping by adding a few glass beads.
- Immediately after alkali addition connect to the distillation apparatus and distill the contents in Kjeldahl assembly at a steady rate.
- Pipette out 25ml of standard sulphuric acid (0.02N) in a conical flask.
- Add methyl red indicator and dip the end of delivery tube in it.
- Distil the ammonia gas from the distillation flask for about 30–40 minutes until distillation is completed and collect about 100 ml of the distillate.
- Back titrate the excess acid with standard alkali i.e., 0.02 N NaOH. (Color change at end point is usually pink to faint yellow or straw).
- Perform a blank without sample.

## AVAILABLE PHOSPHORUS IN SOILS BY OLSEN METHOD (1954)

### Reagents

- Olsen's reagent; 0.5 M Sodium bicarbonate solution (pH 8.5); Dissolve 42.0 g $NaHCO_3$ (L.R.) in double distilled water to give one litre of the solution. Adjust the pH to 8.5 with small amounts of 10% NaOH.
- Activated charcoal (free of P) or Darco G-60
- Dickman and Bray's chloromolybdic acid reagent; Weigh 15g of ammonium molybdate (AR) and dissolve in 300 ml of warm water (50°C), cool and filter if necessary. To this, add 400 ml of 10N HCl and make up the volume to one litre. Mix thoroughly and store in an amber glass stopper bottle.
- Stannous chloride solution; Stannous chloride solution; Dissolve 10g of crystalline stannous chloride by warming and store in an amber-coloured bottle, carefully avoiding contact with air. This is 40% stock solution of $SnCl_2$. Just before use prepare freshly diluted stannous chloride. Pipette 1 ml 40% $SnCl_2$ into 330 ml of double distilled water (A piece of tin metal (AR) added to the stock solution will preserve the stock solution for a long time).

### Procedure

- Accurately weigh 2.5 g of the soil sample in a 100 ml conical flask and to it add 50 ml of Olsen's reagent.
- Add 1 teaspoon of phosphorus free charcoal.
- Shake the suspension for 30 minutes on a platform type shaker.
- Filter the solution through Whatman 40 dry filter paper into clean and dry beakers. Perform a blank without soil (if the filtrate is not clear, return it to the conical flask containing the sample, add more charcoal, shake quickly and filter again).

### Color Development

- Pipette 5 ml of the soil extract into a 25 ml volumetric flask.
- To it add 5 ml of Dickman and Bray's chloromolybdic acid reagent must be added drop by drop with constant shaking till the effervescence due to $CO_2$ evaluation ceases.
- Wash the neck of the flask with double distilled water until the contents are diluted to about 22 ml. Add 0.25 ml (5 drops) of the 0.1 M stannous chloride.
- Make the volume upto the mark with double distilled water.
- Measure the intensity of the blue color spectrophotometrically at 660 nm after 5 minutes. Determine the concentration of P from the standard curve perform a blank.

**Reactions:** Exchange reaction

Exchange complex] Phosphate + $HCO_3^-$ → Exchange complex] HCO3 + Phosphate

Chemical reaction

$$Ca_3(PO_4)_2 + 6NaHCO_3 \rightarrow 3Ca(HCO_3)_2 + 2Na_3PO_4$$

$$3Ca(HCO_3)_2 \rightarrow 3CaCO_3 + 3H_2CO_3$$

$$2Na_3PO_4 + 3H_2CO_3 \rightarrow 2H_3PO_4 + 3Na_2CO_3$$

$$Ca_3(PO_4)_2 + 6NaHCO_3 \rightarrow 3CaCO_3 + 2H_3PO_4 + 3Na_2CO_3$$

Color development

$$(NH_4)_6Mo_7O_{24}.\ 4H_2O + 6HCl \rightarrow 7H_2MoO_4 + 6NH_4Cl$$

(Ammonium molybdate) (Molybdenic acid)

$$H_3PO_4 + 12H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12H_2O$$

(Phosphate) (Phosphomolybdate yellow coloured complex)

$$\text{Phosphomolybdate} + SnCl \xrightarrow{\text{reduction}} \text{Reduced phosphomolybdate}$$

(molybdenum-blue complex of approximate composition)

### Standard Curve for Phosphorus

- Primary phosphate standard; 50 ppm of phosphorus; Dry (AR) grade potassium dihydrogen phosphate ($KH_2PO_4$) in an air oven at 40° – 50°C for one hour and cool in a desiccator. Weigh accurately 0.2195 gm of $KH_2PO4$ and dissolve in about 400 ml of double distilled water in a 1 litre volumetric flask. Then add 25 ml of 7(N) $H_2SO_4$ (approx) and make up the volume to 1000 ml with double distilled water. This gives 50 ppm 'P' solution (Addition of $H_2SO_4$ preserves the solution indefinitely but should be stored in soft glass bottle rather than one of pyrex to minimize contamination with arsenic)
- Prepare 2 ppm standard (secondary/working solution) from the prepared 50 ppm by proper dilution (20 ml of 50 ppm stock diluted exactly to 500 ml for preparing 2 ppm standard).

- From this 2 ml stock prepare different concentrations of P in 25 ml volumetric flask viz. 0.2, 0.4, 0.6, 0.8, 1.0 ppm by pipetting requisite volume of 2 ml stock.
- Add 5 ml of extracting reagent (Olsen's) and the color is developed by adding 5 ml of chloromolybdic acid reagent and stannous chloride (1 ml or less).
- Make up the volume with double distilled water and take the readings after 4–5 minutes at 660 nm wavelength after properly adjusting the blank (0.00 ppm) to 100% transmittance or 0.00% absorbance. All reagents including the extraction solution and sample processing chemicals must be included in each of the standard solutions and in the blank employed for preparing the calibration curve. The standard curve is plotted by taking the spectrophotometer readings along the Y axis (ordinate) and the concentration of P (ppm) along the x-axis (abscissa).

### Precautions for P estimation

- For a satisfactory phosphorus procedure constant conditions must be maintained in the blank, standard and test solutions.
- Alkaline washing powders (which often contains) phosphates must not be used for glassware cleaning. All glass wares should be cleaned with chromic acid and thoroughly washed with double distilled water. For final clearing the glassware may be dipped in or rinsed with 6 N HCl after apparently clean, then thoroughly washed with double distilled water.
- Double distilled water must be used for all purposes in P estimation.
- The reagents and filter papers should be as free of phosphorus as possible.
- Pyrex glass apparatus, particularly new ones are to be avoided to minimize contamination with arsenic, which interferes in the analysis.
- Molybdenum blue solutions must not be kept in the volumetric flask after completion of experiment. The volumetric flasks and the spectrophotometer cuvettes must be washed immediately after use.

## AVAILABLE PHOSPHORUS IN SOILS BY BRAY'S P-1 (BRAY AND KURTZ, 1945)

### Reagents

- Bray's P-1 extractant: Dissolve 1.110 g of AR grade ammonium fluoride in one litre of 0.025N HCl.
- 1.5% Dickman and Bray's reagent: Dissolve 15 g of AR grade ammonium molybdate in 300 mL of warm water, cool and add exact 350 mL of 10N HCl. Make the volume to one litre.
- 40% $SnCl_2$ stock solution: Weigh 10 g pure stannous chloride in a 100 mL glass beaker. Add 25 mL of conc. HCl and dissolve by heating. Cool, transfer to an amber-coloured bottle and store in dark after adding a small piece of Zn metal (AR grade) to prevent oxidation. From this, prepare a dilute $SnCl_2$ solution (0.5 mL diluted to 66 mL) immediately before use.
- Standard stock solution: Weigh 0.439 g of AR grade $KH_2 PO_4$ dried in oven at 60°C for 1 hour in a one litre beaker, add about 500 mL of distilled water and dissolve. Add 25 mL of approx. 7N $H_2SO_4$ and make the volume to one litre. This is 100 mg P $L^{-1}$ solution.
- Standard working solution: Dilute a suitable volume of 100 mg $PL^{-1}$ solution 50 times to get 2 mg $PL^{-1}$ solution.

### Procedure

- Weigh 5 g of soil sample in a 150 mL conical flask.
- Add 50 mL of Bray's P-1 extractant and shake for 5 minutes.
- Filter through Whatman No.1 filter paper quickly so as to collect the filtrate within 10 minutes.
- Transfer 5 mL aliquot into a 25 mL volumetric flask.
- Add 5 mL of ammonium molybdate solution, shake a little and dilute to about 22 mL
- Add 1 mL of diluted $SnCl_2$ (0.5 mL diluted to 66 mL), mix by shaking a little, and make up the volume.
- Run a blank without soil under identical conditions.
- Measure the intensity of the blue colour developed, using 660 nm wavelength (red filter).

### Preparation of Standard Curve for P

- Take a series of 25 mL volumetric flasks.
- Pipette out 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mL of 2 mg $L^{-1}$ P solution.
- Add 5 mL of the extractant (Bray's or Olsen's).
- Add ammonium molybdate as for Bray's and proceed to develop blue colour as described earlier.
- Measure blue colour intensity and draw a standard curve by plotting concentrations of P in ug against absorbance readings.
- If a straight line is obtained, find out a factor for each reading.

## AVAILABLE POTASSIUM IN SOIL BY NEUTRAL NORMAL AMMONIUM ACETATE METHOD (HANWAY AND HEIDEL, 1952)

### Reagents

- Neutral normal ammonium acetate solution; Dilute 60 ml glacial acetic acid (99.5%) and 75 ml concentrated ammonia solution (sp. gr. 0.91, 25% $NH_3$) to one litre. Mix well, cool and adjust the pH to 7.0 with dilute acetic acid or ammonia solution.
- Potassium chloride solution: 1000 ppm stock solution; Dissolve 1.907 g of AR grade potassium chloride (dried at 60°C for 1 hr.) in distilled water and make up the volume to 1 litre.

### Procedure

- Weigh 5 g soil sample in a 25 ml conical flask.
- Add 25 ml of neutral normal ammonium acetate (pH = 7) and shake for 25 minutes.
- Filter immediately through a dry filter paper (Whatman No.1).
- Reject first few ml of the filtrate.
- Determine the potassium concentration in the extract flame-photometrically after necessary setting and calibration of the instrument.

### Standard curve for potassium

- From the mother stock solution (1000 ppm K), prepare 2, 5, 10, 15 and 20 ppm K solutions in 50 ml volumetric flask by proper dilution.
- Construct the standard curve by plotting the flamephotometer readings along Y-axis and the different concentrations (ppm) along X-axis.

## EXTRACTABLE CALCIUM AND MAGNESIUM IN SOIL- VERSENATE (EDTA) METHOD

### Required reagents:

### For Ca Determination

- 0.01N Versenate (EDTA) solution: Dissolve 2.0 g of EDTA in distilled water and make the volume in to 1liter volumetric flask.
- Sodium hydroxide (NaOH) (pH 10): 10 g of AR grade NaOH is dissolved in 90 mL of distilled water.
- Ammonium purpurate (murexide) indicator: 0.2 g of murexide is mixed with 40 g of powered potassium sulphate ($K_2SO_4$).

### For Ca + Mg Determination

- 0.01N Versenate (EDTA) solution: Dissolve 2.0 g of EDTA in distilled water and make the volume in to 1liter volumetric flask.
- Ammonium chloride-ammonium hydroxide buffer ($NH_4Cl$-$NH_4OH$) (pH 10): Dissolve 67.5 g pure Ammonium chloride in 570 mL on conc. Ammonium hydroxide and made to 1 liter and adjusted to pH 10.
- Eriochrome black T indicator (EBT): Dissolve 0.5 g of EBT (Solochrome black) and 4.5 g of hydroxylamine hydrochloride AR grade in 100 mL of 95% ethyle alcohol.

### Procedure:

### Ca Determination

- Weight 10 g of soil sample in 150 mL conical flask, then add 50 mL of 1 N ammonium acetate solution and shake for 5 minutes, Filter through Whatman no. 1 filter paper.
- Pipette out 5 mL of soil extract in a porcelain dish (8 cm dia). Add about 25 mL distilled water to it.
- Add 5 mL of NaOH solution and then 50 g of murexide indicator, stirred well and the solution is titrated with standard 0.01 N EDTA till the color change from red to purple. The volume of EDTA used is noted.

### Ca + Mg Determination

- Same as Ca determination pipette out 5 mL of soil extract in a porcelain dish (8cm dia). Add about 25 mL distilled water to it.
- Add 1 mL of $NH_4Cl$-$NH_4OH$ buffer solution and then 3-5 drop of EBT indicator, stirred well and the solution is titrated with standard 0.01 N EDTA till the color change from wine red to blue or green. The volume of EDTA used is noted.

### Mg Determination

- The Mg content of the soil is determined from the difference between the contents of Ca + Mg and Ca.

## AVAILABLE SULPHUR IN SOIL

### Reagent

- CaCl2 (0.15%). Dissolve 1.5 g of $CaCl_2$ dihydrate in distilled water and make the vol. to 1 lit.
- Stabilizing agent or conditioning agent: Dissolve 75 g NaCl in 250 ml of distilled water in a 500 ml volumetric flask and add 30 ml of concentrated HCl followed by 100 ml ethanol and 50 ml glycerol with constant stirring. Make the volume to 500 ml.
- BaCl2.2 H2O
- Standard sulphate solution: Dissolve 0.5434 g of AR grade $K_2SO_4$ in distilled water and dilute to 1 L. This is 100 ppm S solution.

**Standard preparation:** Pipette out 0, 0.5, 1.0, 1.5, 2.0, 2.5 ml of 100 ppm sulphur solution into 50 ml volumetric flask and to this add 5 ml of conditioning agent and a pinch of $BaCl_2$. Make the volume to the mark to prepare the working standard of 0, 1, 2, 3, 4 and 5 ppm respectively. After 10 minutes, the turbidity developed in the standards is measured in a spectrophotometer at a wave length of 420 nm.

### Procedure

- Take 10 g of soil into a 250 ml of conical flask.
- To this add 50 ml of $CaCl_2$ (0.15 %) solution and shake for 30 min.
- Filter through Whatman No. 1 filter paper.
- Pipette out 10 ml of extract into a 25 ml volumetric flask, add little amount of distilled water followed by 2.5 ml stabilizing agent and a pinch of $BaCl_2$.
- Shake the contents and make up the volume to the mark with distilled water.
- Measured the sulphur content in a spectrophotometer at a wave length of 420 nm.

## AVAILABLE ZINC IN SOIL BY DTPA-$CACL_2$ METHOD

### Reagents

- DTPA= 0.0005 M (formula weight 393.35)
- $CaCl_2.H_2O$= 0.01 M solution
- TEA = 0.1 M solution.

**Extracting solution:** Dissolve 13.1 ml reagent grade TEA, 1.967g DTPA (AR grade) and 1.47g of $CaCl_2$ in 100 of glass distilled water. Allow DTPA to dissolve and then dilute it to approximately 900 ml. Adjust the pH 7.3 ±0.05 with 1;1 HCl (while stirring) and dilute to 1litre (while stirring) and dilute to 1 litre.

### Procedure

- Weight 10 g of air-dried soil in a 125 ml conical flask or polypropylene storage bottle.
- Then add 20 ml of the DTPA extracting solution.
- Cork the bottles or flasks and place them upright on a horizontal shaker. Shaker for 2 hours with a speed of 120 cycles per minute. Filter, the suspension with Whatman 42 filter paper.
- Keep the filtrate in polypropylene bottles to be analyzed for Zn, Cu, Mn and Fe with atomic absorption spectrophotometer.
- When the sample needs dilution before measurement, they should be diluted with DTPA solution to maintain a constant matrix.

## TOTAL NITROGEN IN PLANT SAMPLE

### Reagents

- Catalyst mixture/ Digestion accelerator, 10 g anhydrous sodium sulphate and 1,0 g of pure dry copper sulphate pentahydrate per sample.
- Mixed indicator: Dissolve 0.1 g bromo-cresol green and 0.07 g methyl red indicator in 100 ml of 95% ethyl alcohol.
- Boric acid solution: Dissolve 40 g $H_3BO_3$, per lire of distilled water. As a general practice 5 ml mixed indicator solution is added to each litre of boric acid solution
- Sodium hydroxide solution: 40% NaOH solution using N free sodium hydroxide flakes. This solution should be allowed to stand for 24-48 hours so as to precipitate out sodium carbonate and other impurities.
- Concentrated sulphuric acid, sp. gr. 1.84, C.P.
- Salicylic acid.
- Sodium thiosulphate.
- 0.1 N $H_2SO_4$

### Reactions:

**(i) Digestion:**

$$(C_6H_{10}O_5)n + 2nH_2SO_4 = 6n\ CO_2 + 7n\ H_20 + 2n\ SO_2$$

$$2[R - COONH_2) + 2\ H_2SO_4 + 5\ H_2 = 2\ (NH_4)_2\ SO_4 + 2\ [R - COOH)$$

**(ii) Distillation:**

$$2(NH_4)2SO_4 + 4NaOH = 2Na\ SO_4 + 4H_2O + 2NH_3$$

$$2NH_3 + 2\ H_20 = 2\ NH_4OH$$

$$NH_4OH + H_3\ BO_3 = NH_4\ [B\ (OH)_4]$$

**(iii) Titration:**

$$2\ NH_4\ [B(OH)_4] + H_2SO_4 = (NH_4)_2\ SO_4 + 2\ H_3\ BO_3 + 2\ H_2O$$

### Procedure

- Take a 0.25-0,5 g finely ground plant sample to pass 40-mesh sieve in a piece of butter paper or any thin paper and after properly wrapping it, transfer to a 500 ml Kjeldahl flask.
- Add 25 ml of concentrated $H_2SO_4$, and allow to stand
- Allow to stand for 30 minutes and then add 5 g sodium thiosulphate.
- Again, allow to stand for 30 minutes. add catalyst mixture and heat Kjeldahl flask first slowly till frothing continues after that heat it briskly. Continue digestion for half an hour after the digest gets clean
- Cool and add 150 ml of water carefully.
- Cool again. Add 120 ml 40 % NaOH solution along the sides of Kjeldahl flask, few glass beads and a drop of a mineral oil and immediately connect it to distillation bulb in the distillation unit.
- Distillation should be continued till 150 ml distillate is collected.
- Titration of ammonia evolved against standard sulphuric acid.
- Run a blank simultaneously with a piece paper (same size as used for wrapping of sample) and other reagents.

## TOTAL P IN PLANT SAMPLE

### Digestion of plant material

### Reagents

- Concentrated nitric acid (AR Grade)
- 60% $HClO_4$ (AR Grade)
- Approximately 2 NHCl (AR Grade)

**Wet digestion (Di-acid digestion):** Wet ashing of plant samples can be done by a tri-acid mixture ($HClO_4$ + $H_2SO_4$, + $HNO_3$; 3:1:10) or a di-acid ($HClO_4$ + $HNO_3$; 3:10) digestion excluding $H_2SO_4$, Exclusion of $H_2SO_4$, permits the use of same extract for S

### Procedure

- Weigh 0.5 to 1.0 g of dried plant sample in a 100 ml Erlenmeyer flask.
- Add 10 ml of concentrated $HNO_3$, place a funnel on the Erlenmeyer flask and keep for about 6-8 hours for pre-digestion.
- Add 10 ml of $HNO_3$ and 2-3 ml of $HClO_4$,
- Place the Erlenmeyer flask on a hot plate kept in an acid proof digestion chamber having a fume exhaust system, heat at 100°C for 1 hour and then raise the temperature to 200°C.
- Continue digestion until the contents become colourless and only dense fumes appear.
- Continue heating at 200°C until the contents are reduced to 2-3 ml. Do not allow to dry up

- Remove the Erlenmeyer flask from hot plate, cool and add 10 ml of 2 N HCL.
- Warm the contents of the Erlenmeyer flask and filter through Whatman nor 42 filter paper into a 100 ml volumetric flask. Give 3-4 washings of 15-20 ml.
- Make up the volume to 100 ml.
- Estimate P content in the digest as per section

**For Phosphorus estimation**

**Reagents**

- Ammonium metavanadate solution (0.25%): Dissolve 2.5 g of ammonium metavanadate in 500 ml of boiling water, add 20 ml of concentrated $HNO_3$ and dilute it to 1 litre and store in an amber glass bottle.
- Ammonium molybdate solution (5%): Dissolve 25 g of ammonium molybdate in 400 ml distilled water (warmed to 50°C). Cool the solution and make up the volume to 500 ml and filter if cloudy. Store in an amber color glass bottle.
- Nitric acid ($HNO_3$) solution (5M): Dilute 530 ml concentrated $HNO_3$ of Sp. 1.42 to 1 litre.

or

Mixed reagent: Dissolve 50.0 g ammonium molybdate and 2.5 g ammonium metavandate in 500 ml of warm distilled water separately in two beakers. Cool them and mix in a two litre volumetric flask and add 500 ml concentrated $HNO_3$ cool it to make up the volume with distilled water to two litres.

- Standard P solution (100 ppm): Dissolve 0.4390 g dried $KH_2.PO_4$ in 400 ml distilled water, add 25 ml of 7 N $H_2SO_4$ and make upto 1 litre.

**Procedure**

- Take 5 ml extract in a 50 ml volumetric flask (0, 2.5,5.0,7.5 and 10 ml of standard p solution),
- Add 5 ml 5 N $HNO_3$ and 5 ml 0.25% ammonium metavanadate solution and mix thoroughly.
- Now add 5 ml of 5% ammonium molybdate solution, mix and make up the volume. (Or add 10 ml of mixed reagent).
- Read the transmittance or absorbance at 470 nm wavelengths on a spectrophotometer set at zero with reagent blank.
- Find out the concentration of P in solution at the spectrophotometer reading so obtained from the standard curve prepared from the readings for standard solution.
- Run a reagent blank and make necessary adjustments.

## TOTAL K IN PLANT SAMPLE

**Reagents**

- K stock solution (1,000 ppm): Dissolve 1.908 g AR grade potassium chloride in about 200 ml water and make up the volume with distilled water to 1 litre.
- K working solution (100 ppm): Take 100 ml of 1000 ppm K solution in a 1 litre volumetric flask and make up the volume with distilled water to 1 litre.
- K working solutions (25 and 50 ppm): Take 25 and 50 ml of 100 ppm K solution in 100 ml flasks and make up the volume with distilled water to 100 ml.

**Procedure**

- Take 50 ml of di-acid digest made for total P analysis in porcelain dish and evaporate to dryness on water bath.
- Wash the sides of the dish with water and again evaporate to dryness. Dissolve the residue left in water and transfer to 100 ml volumetric flask.
- Make up the volume.
- Feed the solution to the capillary of the flame photometer and note the readings as ppm of K.

## TO DETERMINE THE TOTAL S IN PLANT SAMPLE

**Reagents**

- Barium chloride dihydrate ($BaCl_2.2H_2O$): 20-60 mesh crystals, analytical grade. Pass $BaCl_2.2H_2O$ crystals through 20 mesh sieve and retain them on 60 mesh sieves.
- Gum acacia solution: Dissolve 0.5 g powdered gum acacia in a mixture containing 50 ml glacial acetic acid and 50 ml distilled water. Store the solution in a refrigerator in order to avoid growth of micro-organisms.
- Salt buffer solution: Dissolve 50 g $MgCl_2.6H_2O$, 4.1 g $KNO_3$ and 28 ml ethanol in a 1000 ml volumetric flask and make up the volume upto the mark.
- 6N HCl: It is approximately 1:1 acid: water ratio.
- Standard S solution: Dissolve 0.5434 g $K_2SO_4$ (AR grade) in 500 ml distilled water and make up the volume to 1 litre (This is 100 ppm $SO_4^{2-}$ -S solution).

**Procedure**

- Take 0, 10, 20, 30, 40, 50 ml of 100 ppm of $SO_4^{2-}$ -S solution in 250 ml volumetric flasks.
- Add 25 ml salt buffer solution to each. Make up the volume with DW. The solutions provide 0, 4, 8, 12, 16 and 20 ppm $SO_4^{2-}$ -S, respectively.
- Transfer 10 ml of digested solution to 50 ml Erlenmeyer flask. Add 1 ml 6 N HCl and 1 ml 0.5% gum acacia solution. Mix the contents by swirling and add 0.58 $BaCl_2.2H_20$ crystals. Allow the flask to stand for a minute and then swirl the contents gently until the $BaCl_2.2H_20$ crystals are dissolved.
- Read the turbidity on a colorimeter using blue filter or on a spectrophotometer using 420 nm wavelengths. Plot the absorbance against the concentrations and draw a standard curve.