Practical Manual

SOIL FERTILITY AND FERTILIZER USE

APS 502 3(2+1)

For

M.Sc. (Ag.) Soil Science

2023

Department of Soil Science

College of Agriculture

Rani Lakshmi Bai Central Agricultural University
Jhansi-284003

Practical manual

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CONTENT

Exercise No.– 1

Objective: Collectionand preparation of representative soil samples for analysis

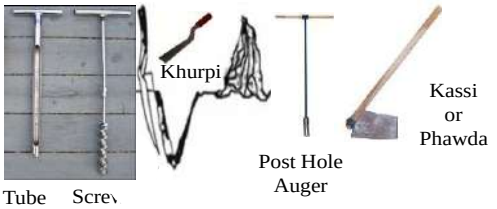
The methods and procedures for obtaining soil samples vary according to the purpose of the sampling. Analysis of soil samples may be needed for agricultural purposesi.e. soil fertility evaluation and fertilizer recommendations for crops. The success or failure of soil analysis, as an aid to fertilizer guidance or any other use is depends on securing a representative soil sample, plus subsequent handling operations. Soil varies to a great extent from place to place and sampling of such soil is quite problematic. Since analysis is done such a sampled soil, it is necessary that the soil sample should be representative of the area.

The error in soil sampling in a field is generally greater than the error in laboratory analysis. The most recommendation call for soil testing of each field is about every three years with more frequent testing on lighter soils. Therefore, it is necessary that the soil sample should be representative of the area.

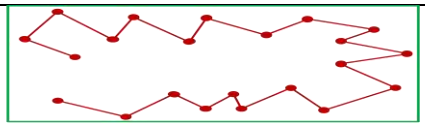
Apparatus and materials:

Khurpi, spade, augers, plastic bowl, scale, wooden roller, mortar and pestle, sieve, polythene/paper/cloth bags, labels, card board cartons and aluminium boxes.

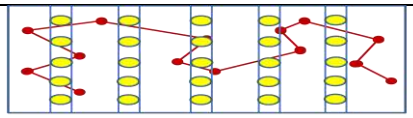
Take separate samples from each field. Also take samples separately from areas or patches looking different in colour, slope, texture, crop performance etc.



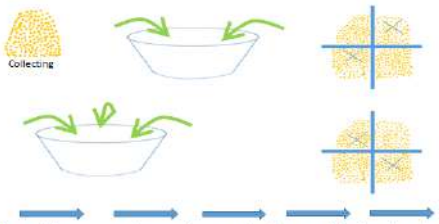
Use appropriate sampling tools. A khurpi or tube auger proves more convenient for sampling from plough layer



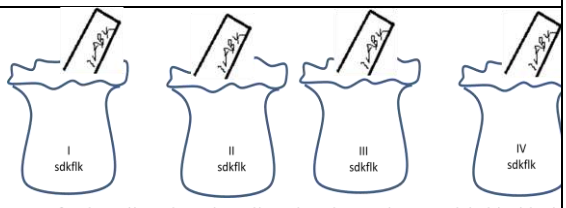
Draw samples from several spots from each field by moving in zigzag manner covering the entire area to prepare composite sample. Leave some margin on all sides.



From field having standing crops, collect soil from several spots in between the rows to prepare a composite sample



Collect the soil at one clean place, mix thoroughly by hand, spread an make four quarters, discard the two opposite ones. Remix the remainin two quarters. Repeat the process to reduce the quantity to about 500g.



Transfer the soil to clean the soil to clean bags. Place one label inside th bag and another pasted outside, indicating sample no., name of owne depth of sampling, identification mark etc. before sending to soil testin laboratory

Figure: Procedure for drawing a representative soil sample

Procedure:

1. Depending on field conditions and the objective of sampling, select proper sampling tool (s).
2. Based on difference in soil type, colour, crop growth or slope, divide the area in different homogenous units.
3. In the uniform field, demarked the sampling points in a zigzag fashion or randomly in such a way that the whole field should be covered i.e. about 22-24 sample per ha.
4. At the sampling site, remove the surface litter with Khurpi or spade. With the help of the sampling tool (auger) or khurpi collect a sample in a plastic bowl.
5. If the soils are hard, make a 'V' shape cut up to 15 cm depth. Remove the soil of the pit. Now scrap or remove 1 cm / 1" soil from the surface up to 15 cm depth from both the side with the help of khurpi. This scraped soil is collected in a plastic bowl. This sample is known as "primary" sample. Such primary samples should be approximately the same weight.
6. After collecting at least 30 – 35 primary samples, mix all the samples in plastic bowl thoroughly and draw about 500 g composite samples by quartering method (All the primary samples thoroughly and divide the whole sample in equal 4 equal parts and discard the 2 opposite portions of the samples and remaining 2 portions are again thoroughly mixed and again divided in to 4 equal parts and 2 opposite parts again discarded. This procedure is continuing until 500 g sample remain in the bowl. This is known as composite sample which is true representative of the area).
7. The most suitable containers for soil samples are polythene bags 6x9'' made of film about 0.13 mm thick, which may be sealed by twisting or tying the neck or by mean of rubber bands or adhesive tape.
8. If the soil is to be kept in moist condition for moisture determination, bacterial count and nitrate estimation etc. air tight containers are preferred.

Information sheet:

The soil sample thus collected must be furnished important information like –

Name address of the farmers	
Address of the farmers	
Village	
Post	
Block	
Tehsil	
District	
State	
Khasra No.	

Identification of the field	
Area of field (hectare/Acre/Bigha)	
Sample depth (0-15 cm / 15-30 cm)	
Local name of the soil	
Date of collecting soil sample	
Name of previous crop (irrigated/ unirrigated)	
Fertilizer used in previous years	
Date of harvest of the previous crop	
Any other problem observed in the field	

Preparation of soil sample for testing:

- Spread sample for drying on clean cloth, plastic or brown paper sheet.
- Remove the stone pieces, roots, leaves & other un-decomposed organic residues from the samples.
- Large lumps of moist soils should be broken.
- After air drying the samples should be crushed gently and sieved through a 2 mm sieve.
- About 250 g of sieved sample should be kept in properly labeled sample bag for testing.

Appropriate time for soil sampling:

An ideal time for soil sampling is just after harvest of the Rabi crops,

Precautions to be taken during collection of soil sampling:

1. Remove all debris from surface before collection of soil sample.
2. Avoid taking sample from upland and low land areas in the same field.
3. Take separate sample from the areas of different appearances.
4. In row crop take sample in between rows.
5. Keep the sample in a clean bag.
6. A sample should not be taken from large area (more than 1-2 ha).
7. Sample for micronutrient analysis must be collected by steel or rust free khurpi/auger and kept in clean polythene bag.
8. Avoid sampling from low – lying spots, manure dumping sites, near trees and from fertilizer placed zones.
9. Use clean bags for sample collection. Do not use bags which had earlier contained fertilizer, manure or plant protection chemicals etc.

Storage:

- The registered and labelled samples in laboratory are finally placed in a cardboard carton. Label the carton properly with the details of soil sample and store in a separate room. The room should be away from direct sunlight/wind or dampness.
- The room exposed to heat or cold or dampness in not advisable.

Guidelines forsampling depth:

S. No.	Crop	Soil sampling depth	
		Inches	cm
1.	Grasses and grasslands	2	5
2.	Rice, finger millet, groundnut, pearl millet, small millets *etc*.(shallow rooted crops)	6	15
3.	Cotton, Red gram, Sugarcane,Banana, Tapioca, Vegetables *etc*.(deep rooted crops)	9	22
4.	Perennial crops, plantations andorchard crops	Three soil samplesat 12, 24 and 36inches	Three soil samplesat 30, 60 and 90 cm

Exercise No.– 2

Objective: To study about the basic principles of the instruments

Most of the analytical methods used for soil and plant analysis involve some kind of instrument. Therefore, it is very important to known about the basic principles of the instruments in order to get the accurate results. The most commonly used instruments in soil and plant analysis are given below:

1. pH meter
2. Conductivity meter
3. Colorimeter / spectrophotometer
4. Flame photometer
5. Atomic absorption spectrometer (AAS)

1. pH meter

Principle:

The pH Meter measures the voltage of an electrochemical cell and based on the temperature sensor determines the pH of a solution. In most pH meters the electrodes and the temperature sensor are fabricated into a single body and are called combination electrodes. The overall potential or the voltage is the algebraic sum of the potentials of the measuring electrode, reference electrode and 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 processes occurs where in some of the hydrogen ions move towards the outer surface of the measuring electrode and replace some of the metal ions inside it. The potential difference of glass electrode and sample solutions will be recorded in the galvanometer.

All three potentials are summed up and measured by high impedance voltmeter. The potential voltage developed across the glass electrode membrane is temperature dependent, with a temperature coefficient of approximately 0.3% per °C. The pH meters have provisions to correct the pH measurements as the temperature changes and it is called as automatic temperature compensation (ATC). The output of the impedance voltmeter is voltage readings.

2. 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 (Bower and Wilcox 1965). The unit of specific conductance is the reciprocal of specific resistance in ohms /cm i.e. mhos /cm. If Cs is the concentration of a solution in gram equivalents L^{-1}, then the volume of solution in ml per equivalent is 1000/Cs, so that conductance = 1000 K/Cs, where, K is the cell constant. 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,

λ^{+} is the conductance of cations, and

λ^{-} is the conductance of anions at infinite dilution

The instrument used for measuring conductivity is also known as conductivity bridge. A typical system consists of an alternating current (AC) Wheatstone bridge, a primary element of conductivity cell and a null balance indicator (as in “Solubridge”) or an electronic "eye” in the conductivity meter. The test solution is filled in a conductivity cell, which is usually made out from two platinum sheets embedded in glass so that the two surfaces facing each other remain exposed. The area and distance between these plates must remain constant. In order to increase the sensitivity of the measurement, one or two stages of amplification are provided before feeding the signal to the electronic eye.

3. Colorimeter / spectrophotometer

Principle:

Colorimeter / spectrophotometeris the determination of the concentration of a substance in a solution by measuring the relative absorption, or transmission of light with respect to a known concentration. Colorimetric analysis is based on the measurement of intensity of radiant energy after it passes through a sample solution. A monochromatic light beam of known intensity is passed through the test solution and the intensity of the transmitted beam is determined with the help of a photo-electric cell. Thus, colorimetry can be considered as the absorption spectrophotometry in the visible range. It is based on Beer's law which states that the intensity of a monochromatic light beam decreases exponentially as the concentration of the absorbing substance increases arithmatically, as expressed below:

$$\log I_o / I_t = kC$$

where,I_o and I_t, are the intensities of the incident radiation and transmitted radiation, respectively, C denotes the concentration in solution, and k is a constant.

The above relationship holds good only when the light is of the wavelength at which its absorption is maximum by the sample. Therefore,monochromation of the incident light through a suitable filter or grating is an essential prerequisite for colorimetry or absorption spectrophotometry (Willard et al. 1965). The Beer-Lambert's law relates the concentration to the logarithm of the ratio of the intensities of incident and transmitted radiations. Hence the absolute intensities need not be determined.

The colorimeter tube acts as a cylindrical lens, converging the light to a sharp line on the photo - cell. If the tube is not exactly circular in cross section, its focal length changes at different angles. This leads to a change in the intensity of the light falling on the photo - cell. A perfectly circular tube when filled with distilled water will not show any deflection of the galvanometer needle at any angle of tube rotation.

4. Flame photometer

Principle:

When a solution of a salt is sprayed into a flame, salt breaks into the component atoms due to high temperature. The atoms released of some specific element take energy from flame and get excited to the higher orbits. Such atoms release energy of a wavelength, which is specific for the elements and is proportional to the concentration of atoms of that element. However, since intensity of emitted light is dependent on temperature, and sensitivity and reproducibility of the analysis vary also with temperature.Each of the alkali and alkaline earth metals has a specific wavelength.

In flame photometry a flame is used for(i) converting the sample from liquid or solid state into the gas phase; (ii) decomposing the sample into atoms, and/or (iii) exciting these atoms into light emission.

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

$I = K \times C^n$

Where: I = Intensity of emitted light

C = Concentration of the element

K = Proportionality constant

At the linear part of the calibration curve n~1,

then $I = K \times C$. In other words, the intensity of emitted light is directly related to the concentration of the sample.

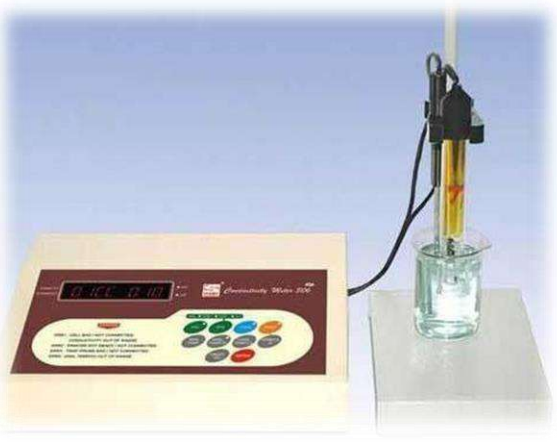
The intensity of the emission is directly proportional to the number of atoms returning to the ground state and the light emitted is in turn proportional to the concentration of the sample.

5. Atomic absorption spectrometer (AAS)

Principle:

Atomic absorption spectrometer (AAS) is based on the principle that when a sample, in the form of a homogenous liquid, is aspirated into a flame where "free" atoms of the element to be analyzed are created. A light source (hollow cathode lamp) is used to excite the free atoms formed in the flames by the absorption of the electromagnetic radiation. The decrease in energy (absorption) is then measured which follows the Lambert- Beers Law that is the absorbance is proportional to the number of free atoms in the ground state.Most of the leading instrument manufacturers have launched tandem models of AAS with interchangeable flame and graphite furnace versions. The graphite furnace has added advantage of analyzing larger number of elements including mercury, arsenic and selenium with greater precision up to parts per billion (ppb) levels. With the help of on-line dilution and calibration in the fully PC-based models, the sample throughput has increased tremendously, while minimizing the human error at various steps.

pH meter



EC meter

Spectrophotometer

Flame photometer

Atomic Absorption Spectrophotometer (AAS)

Exercise No. –3

Objective: Determination of pH in soil sample

The acidity, neutrality or alkalinity of a soil is measured in term of hydrogen activity (active - concentration) of soil – water system.It is defined as negative logarithm of the hydrogen ion activity. Mathematically, it is expressed as:

$$pH = - \log_{10} H^+ \text{ or } - \log_{10} [H^+]$$

Principle:

The pH is usually measured by pH meter, in which the potential of hydrogen ion indicating electrode (glass electrode) is measured potentiometrically against calomel saturated reference electrode which also serves as salt bridge. Now a day, most of the pH meters have single combined electrode. Before measuring the pH of the soil, the instrument has to be calibrated with standard buffer solution of known pH. Since, the pH is also affected by the temperature; hence, the pH meter should be adjusted to the temperature of the solution by temperature correction knob.

Equipment's and Apparatus:

pH meter, beakers (100 ml), glass rods, electrical balance, measuring cylinder, filter paper, washing bottle with distilled water and volumetric flask.

Reagents:

Preparation of standard buffer solution:

1. **pH 4.0:** Transfer one pH 4.0 buffer tablet into 100 ml volumetric flask. Add distilled water to dissolve the tablet and dilute in to 100 ml.
2. **pH 7.0:** Transfer one pH 7.0 buffer tablet into 100 ml volumetric flask and add distilled water to dissolve the tablet and dilute the solution to 100 ml mark with distilled water.
3. **pH 9.2:** Transfer one pH 9.2 buffer tablet into 100 ml volumetric flask. Add distilled water to dissolve the tablet and dilute in to 100 ml.

Procedure:

1. Switch on the instrument and allow it to warm for 10 minutes.
2. Adjust the temperature of pH meter to the temperature of the solution by temperature correction knob.
3. Standardized the pH meter with buffer solution of 4.0, 7.0 and 9.2 pH.
4. Take 20 g soil in 100 ml beaker.
5. Add 50 ml. of distilled water into it.
6. The suspension is stirred at a regular interval for 30 minutes. This time is required for the soil and water to attainequilibrium.

7. After half an hour again stir the soil suspension and measure the pH on a pH-meter and reading taken.

Precautions:

1. Carefully read the instructions of pH meter and follow them.
2. Never allow the lower portion of glass electrode to touch bottom of the beaker.
3. The electrode must be washed with distilled water and dried gently with the help of filter or tissue paper before each measurement.
4. The electrode should be immersed in a beaker of distilled water to avoid drying out of electrodes.
5. Ensure that calomel electrode is clean and filled with saturation KCl solution.
6. Keep the buffer solutions properly stoppered.
7. After use wash the electrode with gentle stream of distilled.

Categories of soil pH values:

Soil pH	**:**	**Interpretation**
< 5.0	:	Strongly Acidic
5.1 – 6-5	:	Slightly Acidic
6.6 – 7.5	:	Neutral
7.6 – 8.0	:	Mild Alkaline
> 8.0	:	Strongly Alkaline

Reference:

- Piper, C.S. (1950). Soil and plant analysis. Intel Science Publisher, Inc. New Yark.

Exercise No.–4

Objective: Determination of electrical conductivity in soil sample

Amount of soluble salts in a sample is expressed in terms of the electrical conductivity (EC) and measured by a conductivity meter. The instrument consists of an AC solubridge or electrical resistance bridge and conductivity cell having electrodes coated with platinum black. The Instrument is also available as an already calibrated assembly (Solubridge) for representing the conductivity of solutions in dSm^{-1} (deci Siemen per meter or milli-mhos per centimetre) at 25^0C.

Principle:

The electrical conductance is measured with the help of solubridge. The instrument is calibrated and cell constant is determined with the help of 0.01 N KCI solutions. This solution gives an electrical conductance of 1.41 m mhos/cm or dSm^{-1} at 25 °C. The solution offers some resistance to the passage of electric current through them depending upon the concentration and the type of ions present. Hence, electrical conductivity of soil – water system increases with increasing content of soluble salts in the soil. Higher the salt content, lesser the resistance to the flow of current. The resistance is defined by the OHM's law as the ratio of electrical potential in volts and strength of the current in amperes.

Resistance in ohms = Potential (Volt) / Current (amperes)

Thus, the measurements of EC give the concentration of soluble salts in the soil at any particular temperature. The reverse of resistance is conduction.

Equipment'sand Apparatus:

EC meter, beakers (100 ml), glass rods, electrical balance, measuring cylinder, filter paper, washing bottle with distilled water and volumetric flask

Reagents:

1. **0.01MPotassium chloride (KCl):** Dissolve 0.7456 g dry potassium chloride (AR) in distilled water and make up the volume to 1000 ml. The electrical conductivity of this solution is 1.41 dSm^{-1} (deci Siemen per meter) at 25^0C.

Procedure:

1. Take 20 g of soil in 100 ml beaker.
2. Add 50 ml of distilled water and shake intermittently for 30 minutes and allow standing until clear supernatant liquid is obtained.
3. Connect the salt bridge to the power supply, switch on the bridge and adjust it to room temperature with the help of temperature setting knob.
4. Calibrate the conductivity bridge with the help of standard KCl solution.

5. Dip the conductivity cell in the supernatant solution so that platinum electrodes are completely immersed in solution.
6. Note down the EC value.

Precautions:

1. Allow the instrument to warm up for 15 minutes.
2. Set the temperature knob to room temperature.
3. Ensure that the conductivity cell should be completely dipped into soil: water suspension or extract.
4. Wash the conductivity cell with gentle stream of distilled water and wipe with tissue paper.
5. Keep the conductivity cell dip in to distilled water when not in use.

Interpretation:

EC (dSm^{-1})	**:**	**Effects**
<1.0	**:**	No deleterious effect on crop
1.0 – 2.0	**:**	Critical for salt sensitive crops
2.0 – 3.0	**:**	Critical for salt tolerant crops
> 3.0	**:**	Injurious to most crops

Reference:

- Piper, C.S. (1950). Soil and plant analysis. Intel Science Publisher, Inc. New Yark.

Exercise No. -5

Objective: Determinationof organic carbon content in soil

Soil organic matter (SOM) is an index of productivity of soil since it is a store house of essential nutrients for plant growth. It is also has a major influence on soil aggregation, nutrient reserve and its availability, moisture retention, aeration of soils and biological activity.The Organic matter on an average contains 58 per cent carbon, the per cent organic matter can be obtained by multiplying per cent organic carbon with 100/58 or 1.724 which is also known as van Bemmlen factor.In soil the chief source of some of the nutrients essential for plant growth is organic matter, such nutrients are N, S and boron is also largely derived from organic matter.The organic matter content of soils is estimated from the organic carbon, determined by titrimetric determination (Walkley and Black, 1934).

Principle:

The organic matter present in soil is oxidized completely with an oxidizing agent potassium dichromatein the presence of concentrated sulphuric acid. After complete oxidation, the excess of potassium dichromateis determined by back titrating it's an against standardreducing agent ferrous ammonium sulphate solution using diphenylamine as indicator.

$$2H_2Cr_2O_7 + 3C + 6H_2SO_4 = 3Cr_2(SO_4)_3 + 3CO_2 + 8H_2O$$

Thus, $2H_2Cr_2O_7$, or $2K_2Cr_2O_7 \equiv 3C$

or 588 g of $K_2Cr_2O_7 \equiv$36 g of C

or 12 litres of 1N $K_2Cr_2O_7 \equiv$36 g of C

or 1 mL of 1N $K_2Cr_2O_7 \equiv$0.003 g of C

Apparatus and Reagents required:

1. 500 ml conical flask
2. Pipette (10 and 20 ml)
3. Burette (50 ml)
4. Sieve (0.5 mm)
5. 1N Potassium dichromate ($K_2Cr_2O_7$): Dissolve 49.04 g of AR grade $K_2Cr_2O_7$ in about 500 mlof distilled water and make the volume to one liter.
6. 0.5N Ferrous ammonium sulphate [$FeSO_4(NH_4)_2SO_4.6H_2O$]: Dissolve 196 g of$FeSO_4(NH_4)_2SO_4.6H_2O$ in distilled water and add 20 ml of concentrated sulphuric acidbefore making final volume to one liter.
7. Diphenylamine indicator: Dissolve 0.5 g of diphenylamine in 20 ml of distilled water and100

ml of concentrated sulphuric acid.

8. Concentrated sulphuric acid.
9. Orthophosphoric acid (86%) or Sodium fluoride (NaF)

Procedure:

1. Weigh 1g soil sample (passed through 0.5 mm sieve) into 500 ml conical flask.
2. Add 10 ml of 1 N $K_2Cr_2O_7$solution and shake gentle to disperse soil in the solution.
3. Add 20 ml of concentrated sulphuric acid (H_2SO_4) carefully from the side of flask, swirl the flask during the addition.
4. Keep the flask on a dry tile or asbestos sheet for 30 minutes at room temperature.
5. Dilute the reaction mixture with 200 ml water and 10 orthophosphoric acids (H_3PO_4) add 10 ml of Sodium fluoride (NaF) solution and 2 ml of diphenylamine indicator and shake vigorously to mix the contents.
6. Take 0.5 N ferrous ammonium sulphate solutions in 50 ml burette and titrate the contents of flask till the colour changes from violet to brilliant green colour.
7. Note the volume of ferrous ammonium sulphate solution used.
8. A blank without soil should be run simultaneously.

Precautions:

1. Add potassium dichromate very carefully so that it does not touching the neck of the flask.
2. Ferrous ammonium sulphate should be standardized daily because of slow oxidation of Fe^{2+} to Fe^{3+}.
3. Be careful while adding sulphuric acid as it can injure the skin and spoil clothes.
4. In case the contents of flask turns green with the addition of indicator before titration,repeat the sample either with double amount of potassium dichromate or with lesser amount of soil.
5. High chloride content, as in case of saline soils, interferes in the estimation of carbon. It can be prevented by adding Ag_2SO_4 @ 1.25% to be concentrated H_2SO_4.

Calculations:

$$\text{Organic Carbon Content in Soil (\%)} = \frac{(B- T) \text{ X N X } 0.003 \text{ X } 100}{\text{Weight of soil (g)}}$$

Where,

- B is blank titer of ferrous ammonium sulphate required for reducing 10 ml of 1N potassium dichromate solution.

- T is sample titer of ferrous ammonium sulphate required for titration of soil sample + 10 ml of 1N potassium dichromate solution.
- N is the Normality of ferrous ammonium sulphate.
- 1 ml of 1 N $K_2Cr_2O_7$ = 0.003g of C

Organic matter in soil:

Assume that organic matter contains 58 % carbon, thus, the organic matter content in soil will be calculated as under:

= Organic Carbon (%) X 100/58
= 1.724

Organic matter (%) in soil = Organic Carbon (%) X 1.724*(* based on 58 % carbon in organic matter)

Actual carbon (%) in soil = Organic Carbon (%) X 100/77

OR

Actual carbon (%) in soil = Organic Carbon (%) X 1.3*(* based on 77 % recovery)

Organic carbon (g/kg) in soil = OC (%) $\times$ 10

Organic carbon (g/ha) in 0-15 cm soil = OC (g/kg) $\times$ 2.24 $\times 10^6$

Interpretation:

Organic carbon (%)	**:**	**Rating**
<0.25	:	Very Low
0.50	:	Low
0.51 – 0.75	:	Medium
0.76 – 1.00	:	High
>1.00	:	Very High

Reference:

- Walkley, A.J. and Black, C.A. (1934). Estimation of soil organic carbon by chromic acid titration method. Soil Science 37: 29-38.
- Jackson, M.L. (1973). Soil Chemical Analysis. Prentice – Hall of India NewDelhi -12.

Exercise No.–6

Objective: Determination of available nitrogen in soil

The major part (more than 90%) of soil nitrogen exists in complex combination in the organic matter (humus) fractions. It becomes available to crop after breakdown to simple forms followed by mineralization. The availability of N is associated with the activity of micro-organisms which develops the organic matter. Distillation of alkaline potassium permanganate solution has often been adopted for estimating the oxidisable and reactive form of soil nitrogen. However, alkaline permanganate method is the quickest of all other methods for the estimation of available nitrogen and has been found to work well even in Indian soils.

The available nitrogen in soil is determined by alkaline permanganate method as per procedure suggested by Subbiah and Asija (1956).

Principle:

A known weight of soil is mixed with alkaline permanganate ($KMnO_4$)solution anddistilled. The organic matter present in soil is oxidized by the nascent oxygen,liberated by $KMnO_4$in the presence of NaOH and ammonia released during oxidation is absorbed in known volume of a boric acid to convert the ammonia to ammonium borate, the excess of which is titrated against standard H_2SO_4.

Equipment and apparatus:

1. KEL PLUS Automatic Nitrogen Estimation System

   The said instrument is used for determination of available nitrogen in soil. It consists of the following:

   - **Automatic Distillation System (Model Classic DX):** It is fully automatic distillation system with programmable auto run digital features, with automatic dilution and addition of boric acid, NaOH and $KMnO_4$. Both modes (auto and manual) are available for distillation reagents addition.
   - **Refrigerated Water Cooling System for Condenser (Model Kel Freeze):** It is refrigerated water cooling system for distillation and condensing system with inbuilt compressor and recirculate pump.
2. Electronic balance
3. Burette of 50 ml
4. Volumetric flask
5. Conical flask of 100, 250 and 500 ml
6. Distilled water
7. Distillation unit

8. Beaker
9. Measuring cylinder of 100 ml

Reagents:

1. **0.32% potassium permanganate solution ($KMnO_4$):** Dissolve 3.20 g ofpotassium permanganate in distilled water and make the volume to 1000 ml.
2. **2.5% sodium hydroxide (NaOH) solution:** Dissolve 25 g of Sodium hydroxide in distilled water and make the volume to 1000 ml.
3. **Mixed indicator:**Dissolve 0.066 g methyl red and 0.099g bromo cresol green dissolve in 100 ml of 95% ethyl alcohol.
4. **2% boric acid solution (H_3BO_3):**Dissolve 20 g ofboric acid in distilled water and make the volume to 1000 ml. Then add 20 ml of mixed indicator into 1000 ml of 2.0% boric acid solution.
5. **0.02 N sulphuric acids (H_2SO_4):**Dissolve 0.55 ml ofsulphuric acid in distilled water and make the volume to 1000 ml.

Procedure:

1. Weight 5 g of processed soil sample and transfer it to digestion tube.
2. First open the supply water and switch on the distillation unit. Set the programme -1 in distillation unit and wait for ready indicator.
3. Set a digestion tube in the distillation unit and other side hose keep 20 ml of 2 % boric acid with mixed indicator containing 250 ml conical flask.
4. Add 25 ml each of potassium permanganate (0.32%) and (2.5%)sodium hydroxidesolution is automatically adding by distillation unit-Programme-1.
5. The sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20 ml of 2 % boric acid containing mixed indicator solution kept in a 250 ml conical flask.
6. With the absorbed of ammonia in boric acid the pinkish colour turns to green and nearly 150 ml of distillate is collected in about 10 minutes.
7. The green colour distillate is titrating with 0.02 N sulphuric acid, the end point colour change from green to original shade (pinkish colour).
8. Simultaneously, a blank titer is to be run without soil.
9. Note the sample & blank reading (ml) and calculate the available nitrogen present in soil.

Calculations:

Available N (Kg/ha) =

$$\frac{\text{Reading of acid (Sample - Blank) X Normality of an acid X 0.014 X 2.24 x } 10^6}{\text{Weight of soil (g)}}$$

Where, Normality of an acid = 0.02 N
Weight of soil = 5 g

Interpretation:

Soil Rating	:	Available N (Kg ha^{-1})
Very low	:	<150
Low	:	151-250
Medium	:	251-400
High	:	401-600
Very high	:	>600

NITROGEN DISTILLATION AND DIGESTION WITH SCRUBBER

Reference:

- Subbiah, B.V. and Asija, G. L. 1956. A rapid procedure for the estimation of nitrogen in soils. Curr. Sci., 25: 259-260.
- AOAC, 1995. Official Methods of Analysis. 16th edn. Association of Official Analytical Chemists, Washington, DC.

Exercise No.–7

Objective: Determination of phosphorous in soil

Next to nitrogen, phosphorous is most critical essential element in influencing plant growth and production throughout the world. Among the more significant functions and qualities of plants on which phosphorous has important effects arephotosynthesis,nitrogen fixation,root development and protein synthesis**.** Thus, it is essential to calculate the available phosphorous present in the soil.

Principle:

Phosphorus is extracted from the soil with 0.5 M $NaHCO_3$ at a nearly constant pH of 8.5. The phosphate ion in solution treated with ascorbic acid in an acidic medium provides a blue colour complex. The intensity of which varies with the P concentration. Measurement of the quantitative determination of phosphorous in soil (Olsen's *et al*., 1954).

Equipment and apparatus:

Spectrophotometer, electrical balance, mechanical shaker, conical flask (100 or 150 ml),funnel, pipettes, volumetric flask (25 ml), reagent bottles and what man No. 1 filter paper.

Reagents:

1. **0.5 M Sodium bicarbonate ($NaHCO_3$) solution:** Dissolve 42.0 g of sodium bicarbonate in about 500 ml of hot distilled water and dilute to1000 ml. If required adjust the pH of solution to 8.5 by adding small quantity dilute NaOH or dilute HCl.
2. **Darco-G 60 (Activated Charcoal):** Use phosphorus free.
3. **5 N Sulphuric acids (H_2SO_4) Solution:** Add 141 ml of conc. H_2SO_4 to make 1000 ml of distilled water.
4. **Reagent- A (Ammonium Paramolybdate) [$(NH_4)_6MO_7O_{24}.4H_2O$]:** Dissolve 12.0 g of AR grade ammonium paramolybdate in 250 ml of distilled water.Dissolve 0.2908 g of potassium antimony tartrate ($KsbO.C_4H_4O_6$) separatelyin 100 ml of distilled water. Mix both of solutions and add 141 ml of concentrated $H_2SO_4$and make up the volume to 2000 ml with distilled water.
5. **Reagent- B (Ascorbic Acid Solution):** Dissolve 1.056 g of ascorbic acid in 200 ml of Reagent – A (This ascorbic acid should be prepared fresh and when required).
6. **Standard phosphate solution:** Weigh 0.4393 g of potassium dihydrogen phosphate (KH_2PO_4) into one litre volumetric flask. Add sufficient distilled water and shake the contents until the salt dissolves. Add 4.8 ml of H_2SO_4 and dilute the solution to 1000 ml with distilled water to get 100 ppm P solution.

7. **Working phosphate solution:** Pipette 5 ml of100 ppm P solution into 250 ml volumetric flask. Add distilled water so as make the volume to 250 ml mark. Itwill give2 ppm P solution.

Preparation of standard curve:

A standard curve shows the relationship between the intensity of a coloured solution of a compound and the per cent transmittance or the absorbance of radiation passing through the solution. It is used to calculate the amount of an element in the unknown sample.

1. To prepare standard curve, pipette 0, 1, 2, 3, 4, 5, etc. ml of 2 ppm standard P solutionin 25 ml volumetric flasks.
2. Add 5 ml of the 0.5M sodium bicarbonate ($NaHCO_3$)extracting solution to each flask, and acidify with 5N H_2SO_4 drop by drop.
3. Add about 10 ml distilled water and 4 ml of reagent 'B', then shake the solution.
4. Make the volume 25 ml by distilled water.
5. Blue colour of varying intensity will be developed in all flasks containing P solution.
6. The intensity of blue colour is read on spectrophotometer at 660 nm wavelengths after 10 minutes.
7. Plot the curve by taking P concentration on X axis and spectrophotometer reading on Y axis. Repeat the process till you get straight line relationship.
8. Calculate the factor i.e. 1 colorimeter reading is equal to how much ppm of phosphorus?

Procedure:

1. Take 2.5 g of soil sample in 100 ml conical flask.
2. Add a pinch of Darco G-60 activated charcoal AR grade.
3. Then add 50 ml of 0.5 M $NaHCO_3$ solution and shake the solution for 30 minute on a platinum type shaker.
4. Similar processes run for a blank without soil.
5. Filter the contents immediately through filter paper (Whatman No. 1).
6. Pipette out 5 ml of filtrate into 25 ml volumetric flask. Add 4 ml of the freshly prepared ascorbic acid solution (Reagent– B) and make volume with distilled water (25 ml) Shake well and keep it for 10 minutes.
7. The intensity of blue colour is read on spectrophotometer at 660 nm wavelengths after 10 minutes.

Observations:

1. Weight of soil sample : 2.5 g
2. Volume of extractant used : 50 ml
3. Volume of aliquot used : 5 ml
4. Absorbency : R

5. Absorbency from standard curve : A
6. Concentration of P for absorbency A : B ppm

Calculation:

$$\textbf{Available P (kg ha}^{-1}\textbf{)} = \frac{\text{F x R (Absorbance) x Volume of extractant x 2.24}}{\text{Volume of aliquot x Weight of soil (g)}}$$

$$= \frac{\text{F x R x 50 x 2.24}}{\text{5 x 2.5}}$$

Where, F (factor) = B / A

Precautions:

1. Darco- G 60 should be used free of phosphorous.
2. The speed of shaker should be constant throughout the duration of shaking.
3. The filtrate should be colorless, otherwise, adds more Darco- G 60 then shake and filter again.
4. Taking the readings within 10-15 minutes after the blue color has been developed, because this colour is not stable for more than 15 minutes.

Interpretation:

Soil Rating	**:**	**Available P (Kg ha^{-1})**
Very low	:	Less than 5
Low	:	5-10
Medium	:	10-20
High	:	20-40
Very high	:	More than 40

Reference:

- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U.S. Dept. Agric. 939: 1-19.

Exercise No.–8

Objective: Determination of potassium in soil

The readily available K constitutes about 1-2% of total K in mineral soils. It consists of soil solution and exchangeable K. The available potassium i.e. exchangeable and water soluble potassium is determined by extracting soil with neutral normal ammonium acetate (CH_3COONH_4)solution. The estimation of potassium is carried out by flame photometer.

Principle:

Ammonium acetate is used to extract exchangeable K in soil as ionic diameter of $NH_4^+$is such that exchangeable K in soil is readily displaced by NH_4^+. K in the extract is atomized into blue flame of a flame photo meter so that it gets excited on gaining energy and emits radiation of a certain wave length in proportion to the concentration of K. The flame photometer employees a relatively low temperature excitation and measures with a photocell the emission intensity which is proportional and to concentration in selected wave length (767 mμ) and for this red filter isused. The amount of which is determined by using a flame photometer by emission spectroscopy.

Apparatus:

Flame photometer with red filter, electrical balance, 100 ml conical flask, funnels, filtration stands, 100 ml volumetric flasks, pipette, Whatman No.1 filter paper and mechanical shaker.

Reagents:

1. **1N Neutral Ammonium Acetate (pH 7.0) solution:**Dissolve 77.0 g of ammonium acetate (NH_4OAC) in distilled water and make volume 1000 ml. Check the pH of the solution, and if necessary add few drops of acetic acid or NH_4OH to adjust the reaction of the solution to 7.0.
2. **Standard Potassium Solution (1000 ppm K):** Dissolve 1.9066 g of pure dry KCl (AR) in distilled water and dilute to one litre. This is 1000 mg kg^{-1}(ppm) K solution.
3. **Working K solution (100 ppm K):** Transfer 100 ml of 1000 ppm K solution into a 1000 ml volumetric flask. Add distilled water to dilute the solution to the 1000 ml mark. Stopper the flask and shake the contents.
4. **Working K solution (40 ppm K):** Transfer 40 ml of 100 ppm K solution into 100 ml volumetric flask and mark volume up to 100 ml. Stopper the flask and shake the contents.

Procedure:

1. Weigh 5.0 g processed soil and transfer it to100 ml conical flask.
2. Add 25 ml of 1N neutralammonium acetate solution.

3. Shake the content for 5 minutes and then filter through Whatman No.1 filter paper.
4. Measure K concentration in the filtrateby flame photometer after calibration.

Observations:

Weight of soil sample	:	5.0 g
Volume of extractant used	:	25 ml
Volume of aliquot taken	:	5 ml

Calculation:

$$\text{Available K (kg/ha)} = C \times \frac{25}{5} \times 2.24$$

$$= C \times 11.2$$

Where,

C is stand for the concentration (mg L^{-1}) of potassium in the sample filtrate obtained on X- axis, against the reading.

Interpretation:

Soil Rating	Very low	Low	Medium	High	Very high
Available K (kg ha^{-1})	<200	200 – 250	250 – 400	400 – 600	> 600

Precautions:

1. The filter should be clear.
2. Warm up the flame photometer for 10-15 minutes before use.
3. These should not be any turbidity or suspended particles in extract, it will chock the capillary feeding tube.
4. The gas and air pressure should be constant.
5. If sample reading goes beyond 100 then dilute the extract.
6. The flame should be soot- free and blue with all the burner cones visible clearly.

References:

- Black, C.A. (1965) Methods of soil analysis Part I Am. Soc. Agron. Inc. Publi. Madison Wisconsin USA.
- Sharma, B. L., Dwivedi, A.K., Sharma, G.D., and Khamparia, R.S. (2010). Technical manual on soil, water and plant testing. Technical Bulletin No. / DRS/JNKVV/ 2010/03.

Exercise No.–9

Objective: Determination of sulphur in soil

Sulphur is present in soils in organic and inorganic forms. Organic S is an important constituent of proteins and amino acids. The major inorganic sources of S include gypsum ($CaSO_4$), and pyrite (Fe_2S). Sulphur is added to soil as fertilizers containing S, such as K_2SO_4 and some pesticides.Plants absorb sulphur in theform of sulphate ion (SO_4^{-2}).

Principle:

Soil is shaken with $CaCl_2$ (0.15 %) solution. Chloride ions displace adsorbed sulphate during extraction. Calcium ions suppress the extraction of soil organic matter and hence eliminate the contamination caused by extractable organic S. The filtrate is analysed for sulphur by turbidimetry method as outlined by Chesin and Yien (1951), in which turbidity produce due to the precipitation ofSO_4^{-2} as $BaSO_4$ when excess $BaCl_2.2H_2O$ is added to a solution is measured on a spectrophotometer at wave length of 420 nm or corresponding to blue filter. Gum acacia is usually included to stabilize the fine suspension of $BaSO_4$.

Apparatus:

1. Colorimeter or spectrophotometer or auto analyzer, mechanical shaker, Erlenmeyer flask, volumetric flask, pipette, electronic balance, conical flask, measuring cylinder, funnel, beaker and burette.

Reagents:

1. **Calcium chloride (0.15 %):** Dissolve 1.4702 g of $CaCl_2.H_2O$ in distilled water and make the volume to 1000 ml.
2. **Salt buffer solution:** Dissolve 40 g magnesium chloride and 4.1 g of potassium nitrate and 28 ml ethanol per litre with distilled water.
3. **Gum acacia solution:** Dissolve 0.5 g of chemically pure gum acacia powder in a mixture of 50 ml of acetic acid and 50 ml of distilled water. Store the solution in a refrigerator to avoid microbial growth.
4. **6N HCl:** Dissolve 500 ml conc. HCl in 500 ml distilled water.
5. **Barium chloride ($BaCl_2.2H_2O$) crystals:** Pass AR grade $BaCl_2$ salt through 30-60 mesh and store for use.
6. Activated charcoal free from sulphur
7. **Standard sulphate–S stock solution (100 ppm):** Dissolve 0.5434 g of dried AR grade potassium sulphate (K_2SO_4) in distilled water and dilute to 1000 ml.

8. **Working standard solution (40 ppm):**Pipette out40 ml of 100 ppm S solution in to 100 ml volumetric flask and mark up to 100 ml.

Extraction of SO_4- S:

- Weight 5.0 g of air dry soil sample in a 250 mlErlenmeyer flask.
- Add 25 mlof 0.15% calcium chloride dihydrate solution and 0.5 g activated charcoal powder free from sulphur.
- Shake for 30 minutes on a reciprocal shaker (180 oscillations per minutes).
- Filter the suspension through Whatman No. 42 filter Paper.

Measurement of SO_4- S:

- Pipette 10 ml aliquot of the extract into a 50 mlErlenmeyer flask.
- Add 1.0 ml each of salt buffer solution, 6N HCl and gum acacia. If S content is more then add more quantity of gum acacia to stabilize turbidity.
- Mix the content and add a pinch (0.5 g) of $BaCl_2$ crystals of 30 to 60 mesh then shake vigorously to dissolve the $BaCl_2$ and obtain a homogeneous suspension.
- After 10 minutes, the turbidity developed is measured in aspectrophotometer at a wave length of 420 nm.

Calculation:

Weight of soil taken	:	5.0 g
Volume of extractant used	:	25 ml
Volume of aliquot of the extract used in analysis	:	10 ml
Absorbance (A) as read from Spectrophotometer	:	A
S from standard curve against absorbance (factor)	:	X mg kg^{-1}

$$S\ (mg\ kg^{-1}) = \frac{\text{Factor X Reading in absorbance X Volume of extractant used}}{\text{Weight of soil taken X Volume of aliquot taken}}$$

Available S (kg ha^{-1}) = S (mg kg^{-1}) X 2.224

Interpretation:

Soil Rating	Very low	Low	Medium	High	Very high
Available S (kg ha^{-1})	< 10	11 – 20	21 – 30	31 – 40	> 40

References:

- Chesnin, L. and Yien, C.N. (1951). Turbidimetric determination of available sulphur. Proc.Soil Sci. Soc. Am. 15: 149.
- Bardsley, C.E. and Lancaster, J.D. (1960). Determination of sulphur and soluble sulphate in soils. Soil Sci. Soc. Amer. 14: 149-151.

Exercise No.–10

Objective: Determination of Zn, Cu, Fe and Mn in soil

The diethylene triamine pentaacetic acid (DTPA) test of Lindsay and Norvell (1978) is commonly used for evaluating fertility status with respect to micronutrient cations, i.e., Zn, Cu,Fe and Mn. The DTPA method is an important and widely used chelating agent, which combines with free metal ions in the solution to form soluble complexes of elements.

Principle:

DTPA, a chelating agent, extracts the easily soluble zinc, iron, copper and manganese by forming soluble complexes. The extracting solution is buffered at pH 7.3 by tri ethanol amine (TEA) and also includes $CaCl_2$ to prevent dissolution of $CaCO_3$. These conditions permit the right amount of Zn, Cu,Fe and Mn to be extracted and $CaCl_2$ to stabilize the pH of the extractant. DTPA extractant has the ability to chelate Zn, Cu,Fe and Mn in competition with Ca^{++} and Mg^{++}. The elements in the DTPA extract are determined by Atomic Absorption Spectrophotometer.

Equipment and Apparatus:

Atomic absorption spectrophotometer,mechanical shaker, Whatman No. 42, volumetric flask, pipette, electronic balance, measuring cylinder, funnel, iodine value flasks, beaker, burette, glass stoppers, plastic storage bottles and conical flask.

Collection and preparation of soil samples:

To avoid contamination, soil samples are to be collected in plastic tub or stainless steel, using rust free instrument or wood and kept in polythene lined cloth bags. Samples are prepared with the help of wooden mortar and pestle and sieved through 2mm nylon screen/mosquito net cloth or stainless steel sieve.

Reagents:

a) **Extracting solution :** (0.005 M DTPA) Dissolve 1.9679g of DTPA (Diethylene tri amine penta acetic acid) + 13.3 ml TEA (Triethanol amine) + 1.47g $CaCl_2$.$2H_2O$ in 200 ml distilled water, dilute to 900 ml, adjust pH 7.3 with 6N HCl while stirring and then make up to 1000 ml and mix thoroughly.

b) **Stock standard solutions:** The standard solutions of different micro-nutrients should preferably be prepared by using their wires. Dissolve 1g wire in a minimum volume of 1:1 nitric acid and dilute to 1000 ml with distilled water to obtain 1000 µg/ml solution of micro-nutrient, or take salts of metals as follows:

Zn- 4.398g l^{-1} $ZnSO_4$, $7H_2O$

Cu- 3.929g l^{-1} $CuSO_4$, $5H_2O$

Fe- 4.977g l^{-1} $FeSO_4$, $7H_2O$

Mn- 3.598g l^{-1} $MnSO_4$, H_2O.

The prepared standards are also available in the market. Out of these standards, prepare working solution of 50 ppm. Then a series of standard solution of 0.5, 1.0, 1.5, 2.0 and 2.5 ppm may be prepared for each metal.

Procedure:

1. Weigh 12.5g soil sample in 100 ml iodine value flasks.
2. Add 25 ml DTPA extraction solution.
3. Cover each flask with polyethylene stoppers.
4. Shake this mixture for 2 hours on shaker at 70 to 80 oscillations per minute.
5. After 2.0 hours of shaking, filter the suspension through Whatman No.42 filter paper.
6. Collect the filtrate in plastic bottles.
7. Determine the content of micronutrients on atomic absorption spectrophotometer.

Precautions:

1. Only double distilled water should be used.
2. Use only corning or borosil glass apparatus and AR grade chemicals.
3. Before feeding the extracts, it should be ensured that they are not turbid otherwise, they may block the capillary of the AAS.

Reference:

- Lindsay, W.L. and Norvell, W.A. (1978). Proc. Soil Sci. Soc. Am.42: 421-428.

Atomic Absorption Spectrophotometer

Exercise No.–11

Objective: Determinationof available boron insoil

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

Principle:

Water soluble boron is the available form of boron. It is extracted from the soil with hot water and subjected to colorimetric estimation, following reaction with azomethine – H reagent. Azomethine-H forms coloured complex with H_3BO_3 in aqueous media.

Equipment's and Apparatus:

Spectrophotometer,boron free glassware- conical flask, volumetric flask, pipette, burette and beaker, poly-propylene tubes 10 ml capacity, dispenser, water bath or heater, Whatman No. 42 filter paper and distilled water.

Reagents:

1. **Buffer solution:** Dissolve 250 g of ammonium acetate (NH_4OAc) and 15 g of ethylene diamine tetra acetic acid (EDTA disodium salt) in 400 ml of distilled water. Slowly add 125 ml of glacial acetic acid and mix.
2. **Azomethine H reagent:** Dissolve 0.45 g of azomethine H in 100 ml of 1% L - ascorbic acid solution. Fresh reagent should be prepared weekly and stored in a refrigerator.
3. **Calcium hydroxide suspension:** Add 0.4g $Ca(OH)_2$ to 100 ml distilled water.
4. **0.1 N HCl:** Add 8.3 ml conc. HCl to 900 ml distilled water, mix, cool to room temperature and make up the volume to 1000 ml.
5. **Calcium chloride 0.01 M:** Dissolve 1.11 g of anhydrous $CaCl_2$ in 900 ml distilled water and make up the volume to 1000 ml.
6. **Boron standard solution:** Dissolve 0.114g of Boric acid (H_3BO_3) in distilled water and adjust the volume to 1000 ml. Each ml contains 20 µg B. Dilute 10, 20, 30, 40 and 50ml of the stock solution to 100 ml with distilled water to have solution with B concentration of 2,4,6,8 and 10 µg of B ml^{-1} respectively. Include a distilled water sample for the 0.0 µg of Bml^{-1}standard solution.

Procedure:

1. Place 20 g air dry soil in 250 ml low B flat bottom flasks.
2. Add 40 ml of 0.01 M $CaCl_2$ solution. Attach water cooled reflux condenser to the flask. Heat the flasks for 5 minutes and then cool and filter the suspension in plastic bottles.

3. Transfer 20 ml aliquot to evaporating dish, add 2 ml $Ca(OH)_2$ suspension and evaporate the solution to dryness. Heat the evaporating dishes gently to destroy organic matter, cool to room temperature, add 5 ml 0.1N HCl. Triturate the residue with rubber policeman to ensure the complete dissolution of the residue (Bingham, 1982).
4. Pipette out 1 ml of aliquot into a 10 ml polypropylene tube.
5. Add 2 ml of buffer solution and mix.
6. Add 2 ml of azomethine- H reagent, mix and after 30 minutes read the absorbance at 420 nm on spectrophotometer.
7. Read the concentration of B against the reading from the standard curve.

Observation:

Volume of Extractant : 40 ml
Volume of aliquot : 1 ml
Weight of soil : 20 g

Where,

R = Reading (Absorbance) of spectrophotometer

F = Factor calculated from the standard

Calculation:

$$B\ (mg\ kg^{-1}) = \frac{F \times R \times 5 \times \text{Volume of Extractant}}{\text{Volume of aliquot} \times 20 \times \text{Weight of soil}}$$

Interpretation:

Soil Rating	:	**Available B (mg kg^{-1})**
Low	:	< 0.50
Medium	:	0.50 – 1.00
High	:	> 1.00

Reference:

- BergerK.C. and Troug E. (1939). Boron determination in soils and plants. Ind. Eng. Chem. Anal. Ed.11: 540-545.

Exercise No. – 12

Objective: Collection and preparation of plant samples for analysis

Plant analysis is used as a diagnostic technique to determine the nutritional status of plants. Mineral composition of plant is influenced by many factors which are also to be considered. Knowledge of nutrient concentration in growing plants can serve as a tool for correcting any deficiencies. Error in plant sampling may results in wrong interpretation of the results for making recommendations. Therefore, selection of right plant part, stage of growing and time of sampling are very important for plant analysis.

Sample preparation:

A sample must be a true representative of the crop or plant part under field conditions. The best way to collect a representative plant sample is to traverse the field diagonally and collect the samples from the deficient and normal plants. These samples should be composited separately representing poor growth and normal growth. Plants which are infected with disease or attack of insects, which are under stress due to excess water or drought and those which are heavily coated with dust should not form a part of the sample. Extraordinary care must be taken to avoid contamination resulting from dust, soil, fertilizer and spray residues etc. The samples so collected should be transferred to paper bags indicating the sample number and/or the field number or other details. In case the sampling site is far away from laboratory and requires longer travel period, the sample may be placed in polyethylene bag and transported in an ice chest. Low temperature will minimize the physiological activity and also check the spoilage of sample due to higher temperature and high humidity.

After collection of plant samples, fresh tissue should be free from dust and other foreign material by washing with detergent solution followed by 0.1N HCl and deionized wateror distilled water. The 0.2% liquid detergent solution will remove waxy coating on the leaf surface. The 0.1N HCl will remove metallic contaminants and deionized water or distilled waterwill wash the previous two solutions.

After washing, place the washed plant sample on filter paper sheets and air dried for 24 hours. Then put the plant samples in new paper bag to be dried in a hot air oven at 65°C ± 2°C for 48 hours.

Grinding and Storage:

The oven dried samples should be group in a grinder, fitted with electricstainless steel blades so as to pass the samples through a 40- mesh sieve. After grinding, the plant sample should be mixed thoroughly and transferred to polyethylene or paper bags, labelled clearly and then stored in the room meant for plant samples. Avoid regular type of grinder as it normally provided contamination.

Precautions:

1. Avoid sampling the plants which are infected with disease and/or insects or those suffering from effects of excess or scarcity of water.
2. Before subjecting the samples for testing, decontaminate the collected samples by thorough washing, and rinsing.
3. Washed samples should be dried as rapidly as possible. The samples should not be packed tightly during drying in an oven and the temperature should not exceed 700°C.
4. The grinder to be used for grinding the samples should have stainless steel blades or hard plastic to avoid contamination and the ground samples must be passed through a 40- mesh sieve to ensure uniform fineness. For iron determination, it is advisable to grind the sample by hand in an agate or porcelain mortar or in a mill made of non-ferrous alloy if hard plastic grinder is not available.
5. The processed samples should be kept in polyethylene or paper bags and stored in room free of dust soil and smoke etc.
6. Care should be taken not to rub the plant tissue acid mixture like any of the washing the samples.

Reference:

- Chapman, H.D. and Pratt, P.F.(1961). Methods of analysis for soils, plants and waters. Univ. of California., 56 - 65.
- Walch, L. and Beaton, J.D. (1973). Soil testing and plant analysis. Soil Sci. Soc. Am. Proc., 3 - 23.

Exercise No. – 13

Objective: Determination of total nitrogen in soil and plant samples

Total nitrogen is estimated by the micro-Kjeldahl method as per procedure suggested by AOAC (1995).

Principle:

Nitrogen present in samples like plant and soil exists in a very complicated bonding structure. During digestion, a known weight of the plant/soil samples in the presence of sulphuric acid with catalyst mixture under high temperature is digested where complicated structures are broken to simple structure, thereby releasing nitrogen in the form of ammonium radical (NH_4^+). During distillation in presence of sodium hydroxide, the released ammonia is condensed and absorbed in known volume of a boric acid with mix indicator to form ammonium borate, the excess of which is titrated with a standard sulphuric acid.

Equipment and apparatus:

Automaticnitrogen estimation system KEL PLUS, electronic balance, burette, pipette, conical flask, measuring cylinder, distilled water, volumetric flask and beaker.

Reagents:

1. **Concentrated sulphuric acid (H_2SO_4).**
2. **Catalyst mixture:** Mix with 250 g potassium sulphate (K_2SO_4), 50 g cupric sulphate ($CuSO_4.5H_2O$) and 5 g metallic selenium powder in the ratio of 50:10:1.
3. **40% sodium hydroxide (NaOH):** Dissolve 400 g of NaOH in 1000 ml distilled water.
4. **4% boric acid (H_3BO_3):** Dissolve 40 g of H_3BO_3in 1000 ml of distilled water after adding 20 - 25 ml of bromo cresol green-methyl redindicator.
5. **Mixed indicator:** 0.066 g methyl red + 0.099 g bromocresol green dissolve in 100 ml of 95% alcohol.
6. **0.1N Hydrochloric acid (HCl).**

The micro-Kjeldahl method consists of the three steps:

I. Digestion
II. Distillation and
III. Titration

Procedure:

I. Digestion:

- Weigh 0.5 g of prepared plant sample or 1 g of soil sample and transfer it to the digestion tube.

- Add 10 ml of concentrated sulphuric acid and 5 g of catalyst mixture to the sample.
- The content of digestion tube is kept in overnight for pre digestion.
- After pre digestion, load the digestion tubes in to the digester and heat the digestion block.
- Switch on the digestion unit and set the initial temperature 100^0C reached.
- Then block temperature is raised to 400^0C. The effective digestion starts only at 360^0C and beyond 410^0C.
- The sample turns light green colour or colourless at the end of the digestion process.

II. Distillation:

- After cooling the digestion tube, load the tube in distillation unit and other side of hose keep 20 ml of 4% boric acid with mixed indicator in 250 ml conical flask.
- 40 ml NaOH (40%) is automatically added by distillation unit programme.
- The digested sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20 ml of 4% boric acid containing mixed indicator solution kept in a 250 ml conical flask.
- With the absorption of ammonia in boric acid, the pinkish colour turns to green.
- Nearly 150 ml of distillate is collected in about 8 minutes.
- Simultaneously, blank sample (without plant/soil) is to be run.

III. Titration:

- The green colour distillate is titrating with 0.1N Hydrochloric acid and the colour changes to original shade (pinkish color).
- Note the blank & sample titer reading (ml) and calculate the total nitrogen content present in plant/soil samples.

Calculations:

$$\textbf{Total N content in plant (\%)} = \frac{\text{R(Sample titre-Blank titre) x Normality of acid x Atomic weight of nitrogen x 100}}{\text{Sample weight (g) x 1000}}$$

$$= \frac{\text{R x 0.1 x 14 x 100}}{\text{0.5 x 1000}}$$

$$\textbf{Factor} = \text{R x 0.28}$$

$$\textbf{Total Ncontent in soil (\%)} = \frac{\text{R(Sample titre-Blank titre) x Normality of acid x Atomic weight of nitrogen x 100}}{\text{Sample weight (g) x 1000}}$$

$$= \frac{R \times 0.1 \times 14 \times 100}{1 \times 1000}$$

Factor=R x 0.14

Crude protein content:

The total nitrogen is estimated by micro-Kjeldahl method as per procedure suggested by AOAC (1995) and the crude protein is calculated by the following formula:

Crude protein content (%) = micro-Kjeldahl nitrogen content (%) x 6.25 (based on the assumptions that nitrogen constitutes 16% of protein).

References:

- AOAC, (1995). Official Methods of Analysis. 16thedn. Association of Official Analytical Chemists, Washington, DC.
- Amma M.K. (1989). Plant and Soil analysis. Rubber REs.Inst. Rubber Board, Kottayam, Kerala.

Exercise No. – 14

Objective: Determination of total phosphorus in plant samples

Total P in plant samples is determined commonly by vanadomolybdo-phosphoric acid yellow colour methods (Koenig and Johnson, 1942).

Principle:

Phosphorus in the acid digest of plant samples can be determined by following the method based on vanadomolybdo-phosphoric acid yellow colour developed by the reduction of heteropoly complex. Heteropoly complex's are thought to be formed by coordination of molybdate ion with phosphorus as a central co-coordinating atom, oxygen of molybdate is substituted for that of phosphate.

Apparatus:

Spectrophotometers, conical flask, volumetric flask, wash bottle, pipette, hot plate and Whatman No. 1 filter paper.

Reagents:

1. **Vanadate-molybdate reagent:** Prepare solution "A" by dissolving 25 g of ammonium molybdate in about 400 ml of warm water.Prepare solution "B" separately by dissolving 1.25 g of ammonium metavanadate in about 300 ml of warm (70°C) water, cool at room temperature and 250 ml of concentrated HNO_3 and cool again at room temperature. Now add solution "A" to solution "B" slowly with continuous stirring and dilute to 1000 ml with distilled water.
2. **6N HCl solution:** Dilute 517 ml of concentrated HCl to 1000 ml.
3. **2, 4 di-nitrophenol indicator (2.5%):** Dilute 2.5 g of 2, 4 di-nitrophenol in 100 ml of 95% ethanol.
4. **Ammonia solution**
5. **Preparation of standard curve:** Dissolve 0.2195 g KH_2PO_4in water, acidifying with 25 ml of 7N H_2SO_4 and dilute to 1000 ml. This solution contains 50 ppm P. From 50 ppm standard P solution, take appropriate volumes to prepare working solution of 0.05, 0.1, 0.2, 0.3, 0.4, 0.8 and 1.00 ppm. Develop the colour in identical manner. Prepare standard curve by plotting P concentration on X-axis and per cent transmittance on Y-axis on a semi-log graph paper.

Procedure:

1. **Digestion of plant material:**
   Weight 0.5 g of plant material in a 50 or 100 ml conical flask. Add 10 ml of di-acid mixture (4:1; Nitric acid: Perchloric acid).Keep it for overnight. Keep on a hot plate and heat gently

at first. Then heat more vigoursly until a clean colorless solution results or till white fumes cease to come out. Do not take it to dryness. Discontinue heating, when the volume is reduced to 3-4 ml. Cool it and transfer to 50 ml volumetric flask and make volume up to the mark by adding distilled water. Filter it through Whatman No. 1 filter paper and use for further analysis.

2. Colour development:

i. Transfer 1-5 ml of aliquot (digested plant material)in a 25 ml volumetric flask.
ii. Add 2-3 drop of2, 4 di-nitrophenol indicator.
iii. Add ammonia solution till yellow colour appears and now add 6N HCl (drop wise) till it become colorless..
iv. Add 5 ml of Vanado-molybdate solution and dilute to 25 ml with distilled water.
v. Mix well and read the intensity of yellow colour on spectrophotometer by using blue filter at 440 nm wavelength.
vi. Run a blank without P solution simultaneously.
vii. Calculate P content of sample using the standard curve.

Calculation:

Weight of plant material taken	:	0.5 g
Volume of the digested material made	:	50 ml
Volume of the digested plant material taken for colour development	:	5.0 ml
Final volume made	:	25 ml
First dilution	:	50/0.5 = 100 times
Second dilution	:	25/5 = 5 times
Total dilution		100 X 5 = 500 times
Transmittance (%) of the test solution	:	T
Concentration of P as read from standard curve against transmittance T	:	A ppm
Total P (ppm) in plant sample	=	A X 500

$$\text{Total P (\%) in plant sample} = \frac{A \times 500}{10{,}000} = A \times 0.05$$

Reference:

- Koenig R.A. and Johnson C.R. (1942). Colorimetric determination of biological materials Ind. Eng. Chem. Analyt. Edn. 14: 155-156.
- Olsen S.R., Cole C.V., Watanabe F.S. and Dean L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U.S. Dept. Agric. 939: 1-19.

Exercise No. – 15

Objective: Determination of potassium in plant samples

Principle:

Potassium in the acid digest of plant sample can be determined by using flame photometer. It is based on the principle that atoms of some specific element absorb energy from flame and get excited to the higher orbit. Such atoms release energy of a characteristics wavelength, which is specific for that element and is proportional to the concentration of atoms of that element in a sample.

Apparatus:

Flame photometer with red filter, electrical balance, 100 ml conical flask, funnels, filtration stands, 100 ml volumetric flasks, pipette, Whatman No.1 filter paper and mechanical shaker.

Reagents:

1. **Concentrated HNO_3**
2. **$HClO_4$**
3. **Standard potassium solution (1000 ppm K):** Dissolve 1.9066 g of pure dry KCl (AR) in distilled water and dilute to one litre. This is 1000 mg kg^{-1}(ppm) K solution.
4. **Working K solution (100 ppm K):** Transfer 100 ml of 1000 ppm K solution into a 1000 ml volumetric flask. Add distilled water to dilute the solution to the 1000 ml mark. Stopper the flask and shake the contents.
5. **Working K solution (40 ppm K):** Transfer 40 ml of 100 ppm K solution into 100 ml volumetric flask and mark volume up to 100 ml. Stopper the flask and shake the contents.

Procedure:

1. Digestion of plant material:
   Weight 0.5 g of plant material in a 50 or 100 ml conical flask. Add 10 ml of di-acid mixture (4:1; Nitric acid: Perchloric acid).Keep it for overnight. Keep on a hot plate and heat gently at first. Then heat more vigoursly until a clean colorless solution results or till white fumes cease to come out. Do not take it to dryness. Discontinue heating, when the volume is reduced to 3-4 ml. Cool it and transfer to 50 ml volumetric flask and make volume up to the mark by adding distilled water. Filter it through Whatman No. 1 filter paper and use for further analysis.
2. Potassium in the acid digest of plant samples can be determined using flame photometer.
3. Depending upon the concentration of K, the digest of plant samples can be used either directly or after dilution for flame photometric determination of K.
4. Calculate the K content of sample using standard curve.

Calculation:

Weight of plant material taken	:	0.5 g
Volume made afterdigestion	:	50 ml
Dilution factor	:	50/0.5 = 100 times
Flame photometer reading	:	20 (Say)
K concentration (ppm) from standard curve against reading	:	Y
Total K (ppm) in plant sample	=	Y X 100

$$\text{Total K (\%) in plant sample} = \frac{\text{Y X 100}}{10{,}000} = \text{A X 0.01}$$

References:

- Black C.A. (1965). Methods of soil analysis Part I Am. Soc. Agron. Inc. Publi. Madison Wisconsin USA.

Exercise No. – 16

Objective: Determination of total sulphur in plantsamples

Principle:

Sulphur in the HNO_3 and $HClO_4$ digest of the plant can be determined by precipitation of sulphate as barium sulphate with the addition of $BaCl_2$ salt (turbid metric method).

Apparatus:

Colorimeter or spectrophotometer,mechanical shaker erlenmeyer flask, volumetric flask, pipette, electronic balance, conical flask, measuring cylinder, funnel, beaker and burette

Reagents:

1. **Di-acid mixture:** Mix concentrated nitric acid andperchloric acid in the ratio of 4:1.
2. **Salt buffer solution:** Dissolve 40 g magnesium chloride and 4.1 g of potassium nitrate and 28 ml ethanol per litre with distilled water.
3. **Gum acacia solution:** Dissolve 0.5 g of chemically pure gum acacia powder in a mixture of 50 ml of acetic acid and 50 ml of distilled water. Store the solution in a refrigerator to avoid microbial growth.
4. **6N HCl:** Dissolve 500 ml conc. HCl in 500 ml distilled water.
5. **Barium chloride ($BaCl_2.2H_2O$) crystals:** Pass AR grade $BaCl_2$ salt through 30-60 mesh and store for use.
6. **Standard sulphate–S stock solution (100 ppm):** Dissolve 0.5434 g of dried AR grade potassium sulphate (K_2SO_4) in distilled water and dilute to 1000 ml.
7. **Working standard solution (40 ppm):** Pipette out 40 ml of 100 ppm S solution in to 100 ml volumetric flask and mark up to 100 ml.

Digestion of plant material:

Take one gram of plant material in digestion flask. Add 10-15 ml of Di-acid (4:1: Nitric acid: Perchloric acid) mixture and swirl the content in 150 ml volumetric flask. Place the content on hot plate till the digestion is over. Filter the solution in 100 ml conical flask and then wash the residue on filter paper several times with the hot water. Make up the volume with distilled water and store the solution in air tight container.

Measurement of SO_4- S:

1. Pipette 10 ml aliquot of the extract into a 50 mlerlenmeyer flask.
2. Add 1.0 ml each of salt buffer solution, 6N HCl and gum acacia. If S content is more then add more quantity of gum acacia to stabilize turbidity.

3. Mix the content and add a pinch (0.5 g) of $BaCl_2$ crystals of 30 to 60 meshes then shake vigorously to dissolve the $BaCl_2$ and obtain a homogeneous suspension.
4. After 10 minutes, the turbidity developed is measured in a spectrophotometer at a wave length of 420 nm.

Calculation:

Weight of plant sample taken	=	1 g
Volume of digested	=	100 ml
Volume of aliquot used in analysis	=	10 ml
Absorbance (A) as read from spectrophotometer	=	A
S from standard curve against absorbance (factor)	=	X mg kg^{-1}

$$S\ (\mu g\ g^{-1}) = \frac{\text{Factor X Reading in absorbance X Volume of digested}}{\text{Weight of plant sample taken X Aliquot taken}}$$

$$S\ (\%) = \frac{S\ (\mu g\ g^{-1}) X\ 100}{10^6}$$

References:

- Chesnin, L. and Yien, C.N. (1951). Turbidimetric determination of available sulphur. Proc. Soil Sci. Soc. Am. 15: 149.
- Bardsley, C.E. and Lancaster, J.D. (1960). Determination of sulphur and soluble sulphate in soils. Soil Sci. Soc. Amer. 14: 149-151.
- Williams, C.H. and Steinberg, A. (1959). Soil sulphur fractions as chemical indices of available sulphur in some Australian soils. Aust. J. Agric. Res. 10: 340-352.

Exercise No.– 17

Objective: Determination of Zn, Cu, Fe and Mn in plantsamples

The diethylene triamine pentaacetic Acid (DTPA) test of Lindsay and Norvell (1978) is commonly used for evaluating fertility status with respect to micronutrient cations, i.e., Zn, Cu,Fe and Mn. The DTPA method is an important and widely used chelating agent, which combines with free metal ions in the solution to form soluble complexes of elements.

Principle:

DTPA, a chelating agent, extracts the easily soluble zinc, iron, copper and manganese by forming soluble complexes. The extracting solution is buffered at pH 7.3 by tri ethanol amine (TEA) and also includes $CaCl_2$ to prevent dissolution of $CaCO_3$. These conditions permit the right amount of Zn, Cu,Fe and Mn to be extracted and $CaCl_2$ to stabilize the pH of the extractant. DTPA extractant has the ability to chelate Zn, Cu,Fe and Mn in competition with Ca^{++} and Mg^{++}. The elements in the DTPA extract are determined by Atomic Absorption Spectrophotometer.

Equipment and Apparatus:

Atomic absorption spectrophotometer,mechanical shaker, Whatman No. 42, volumetric flask, pipette, electronic balance, measuring cylinder, funnel, Iodine value flasks, beaker, burette, glass stoppers, plastic storage bottles and conical flask

Collection and preparation of plant samples:

Plant samples (leaves, grains or straw) should be washed with 0.01N HCl, rinsed with glass distilled water dried in oven at 65°C and crushed with the help of stainless steel scissors.

Reagents:

- Extracting solution: (0.005 M DTPA) Dissolve 1.9679g of DTPA (Diethylene tri amine penta acetic acid) + 13.3 ml TEA (Tri ethanol amine) + 1.47 g $CaCl_2$.$2H_2O$ in 200 ml distilled water, dilute to 900 ml, adjust pH 7.3 with 6N HCl while stirring and then make up to 1000 ml and mix thoroughly.
- Stock standard solutions: The standard solutions of different micro-nutrients should preferably be preparedby using their wires. Dissolve 1.0 g wire in a minimum volume of 1:1 nitric acid and dilute to 1000ml with distilled water to obtain 1000 µg/ml solution of micro-nutrient, or take salts of metals as follows:

  Zn- 4.398g l^{-1} $ZnSO_4$, $7H_2O$

  Cu- 3.929g l^{-1} $CuSO_4$, $5H_2O$

  Fe- 4.977g l^{-1} $FeSO_4$, $7H_2O$

  Mn- 3.598g l^{-1} $MnSO_4$, H_2O

  The prepared standards are also available in the market. Out of these standards, prepare

working solution of 50 ppm. Then a series of standard solution of 0.5, 1.0, 1.5, 2.0 and 2.5 ppm may be prepared for each metal.

Procedure:

1. Weigh 0.5 g plant sample in a conical flask (corning, 100 ml capacity).
2. Add 10 to 12 ml of di acid mixture (1 perchloric + 4 nitric acid) and digest the mixture on hot plate till the residue is colour less.
3. Now take off, cool dilute with distilled water and filter through Whatman No.1 filter paper.
4. Make up the volume of digestate to 50 ml.
5. Read for micronutrient content on atomic absorption spectrophotometer.

Precautions:

1. Only double distilled water should be used.
2. Use only corning or borosil glass apparatus and AR grade chemicals.
3. Before feeding the extracts, it should be ensured that they are not turbid otherwise, they may block the capillary of the AAS.

Reference:

- Lindsay W.L. and Norvell W.A. (1978). Proc. Soil Sci. Soc. Am.42: 421-428.

Exercise No.–18

Objective: Preparation of the glassware's

Requirements:

1. **Glassware (test tube, beaker, conical flask& measuring cylinder):** Corning / borosil glassware that contains arsenic is preferred for most of the laboratory.
2. **Chemicals:** 70% alcohol, potassium dichromate, concentrated H_2SO_4 and HCl.
3. **Distilled water:**Distilled water always used in chemical analysis.The quality of distilled water varies from single distilled to double distilled water depending upon the requirement of analytical techniques e.g. double distilled water is always recommended for micronutrient and heavy metal analysis.
4. **Filter paper:**Several types of filter papers are available in the market depending on their ash content, porosity, and elemental composition. The commonly usedfilter papers areWhatman, munktells, schleicher and schuell. These filter papers are available in different numbers ranging from No. 1 to 60. The pore size decreases with their increasing numbers.
5. **Others:**Balance, detergent, washing tub and brush.

Preparation of cleaning solutions:

1. Chromic acid cleaning solution: Chromic- sulphuric acid cleaning solution is very important for final cleaning of glassware. Visible materials and organic solvents should be removed with water before using cleaning solution. The cleaning solution can be prepared either by dissolving 80 g of $K_2Cr_2O_7$ in about 300 ml of water (with heating) in 2000 ml of corning / borosil beaker. To the beaker, add one litre of technical/commercial grade H_2SO_4. Considerable red chromic oxide (Cr_2O_3) precipitates.
2. Aqua regia: Another cleaning solution used is aqua regia. The aqua regia is prepared by mixing concentration HCl and H_2SO_4 in a ratio of 3:1.

Procedure:

1. Wash the glassware thoroughly with the detergent under tap water.
2. Now rinse the glassware's thoroughly with chromic acid and washes under tap water till the chromic acid removed.
3. Again rinses the glassware's with distilled water and dry them.
4. Keep them in oven at 110°C for biochemical, microbiological and other routine work for one or two hours and then ready for use.
5. For microbiology and biotechnology purpose rinse the glassware's with a piece of cotton dipped in 70% alcohol dry the glassware's in air, wrap them in paper tie with thread and put

them in autoclave.

Precaution:

1. After rinsing the glassware's with chronic acid, it should be washed under tap water thoroughly to remove chronic acid.
2. The glassware should be dried before wiping them with cotton dipped in 70% alcohol.
3. The glassware's must be completely dried before wrapping.

Interpretation:

The glassware's must be washed properly as to make them free of dirt and dust. Proper washing of the glassware's is essential step to be performed before conducting any biochemical, microbiological or biotechnological experiments.

Exercise No.–19

Objective: Basic concept in chemical analysis

Analytical Reagents:

Chemical reagents are supplies in different grades and each grade has a distinct purpose and range of uses. The purest grade is analytical reagent (AR), the second is laboratory reagent (LR), the third is guaranteed reagent (GR) and fourth is technical grade. Second and third form of reagents, have a lot of impurities of micronutrients. Therefore, for the determination of micronutrients, AR grade reagent should be used.

Concentration of acids and bases:

Strength of acids and bases are expressed on the basis of their normality. The strength of some concentrated acids and bases are:

Reagents	Concentration		Approximate specific gravity (g cm^{-3})
	Normality	Per cent by weight	
HCl (Hydrochloric acid)	11.6	37-38	1.19
H_2SO_4 (Sulphuric acid)	35-36	97-100	1.84
HOAC (acetic acid)	17.5	99.5	1.13
HNO_3 (Nitric acid)	16	70-71	1.42
$HClO_4$ (Perchloric acid)	9-11.6	60-70	1.51-1.67
H_3PO_4 (Phosphoric acid)	45	85	1.71
NH_4OH (Ammonium Hydroxide)	15	28-29	0.90
H_2O (Water)	55	100	1.00

Molarity (M):

A one molar solution contains one mole or one molecular weight in grams of a substance in each litre of the solution, whether the substance is in the form of molecules, ions or any other species.

It is number of moles of a compound dissolved in one litre of its solution.

Molarity = No. of moles per litre

$$\text{No. of moles} = \frac{\text{Weight (g)}}{\text{Molecular weight}}$$

Molality (m):

The molality of a solution is the number of moles of the solute per 1000 g of the solvent. Molal solutions are prepared by dissolving gram moles of chemical substance in 1000 g of the solvent. It is usually designated by m.

Molality = No. of moles per 1000 g of the solvent

Normality (N):

The normality of a solution is the number of gram equivalent of the solute per litre of the solution. It is usually designated by N.

$$\text{Normality} = \frac{\text{Molecular weight}}{\text{Valency}}$$

Standard solutions:

Solutions containing known quantity of chemical in per unit volume are known as standard solutions. These solutions are two types:

1. **Primary standard solution**
2. **Secondary standard solution**

Primary standard solution:

Solution prepared by dissolving known quantity of chemical directly, no standardization is required.

Characteristic of primary solution:

a. They are in pure form or their purity is known.
b. They will react only in one way.
c. They are non- hygroscopic in nature and easily come in dry condition.
d. They should have high equivalent weight.
e. Acid or base should be strong in nature.

Selected primary standards:

i. **Acids:** Oxalic acid, Potassium hydrogen pthalet, benzoic acid, constant boiling hydrochloric acid, sulphuric acid and potassium acid iodate.
ii. **Bases:** Sodium carbonate, Mercuric oxide and Borax.
iii. **Oxidizing agents:** Potassium dichromate, potassium bromate and potassium iodate.
iv. **Reduced agents:** Sodium oxalate, arsenious oxide, iron metal and potassium ferrocyanide.
v. **Others:**Sodium chloride andpotassium chloride.

Preparation of primary standard solution:

Dissolve known quantity (for 1N solution- 1 g eq. wt.) of the compound into 1000 ml volumetric flask and make up the volume with distilled water. These solutions were used to standardize secondary standard solutions.

Secondary standard solution:

Solution which required standardization after dissolving known quantity of chemical is known as secondary standard solution.

Preparation of secondary standard solution:

Take 5-10% more quantity than equivalent weight of the compound and dissolve in to distilled water. Transfer the solution into 1000 ml volumetric flask and make up the volume with distilled water. Standardize this solution with the help of primary standard solution of known strength to known the actual strength (normality / molarity) of the solution prepared.