

PRACTICAL MANUAL

Soil, Water and Plant Analysis

HNR 231 2(1+1)

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College of Horticulture & Forestry
Rani Lakshmi Bai Central Agricultural University
Jhansi-284003, Uttar Pradesh

Syllabus: Soil, Water and Plant Analysis 2(1+1)

Introduction to analytical chemistry, Collection and preparation of soil, water and plant samples for analysis. Determination of pH, electrical conductivity, sodium adsorption ratio and exchangeable sodium percentage of soils. Estimation of available macro and micronutrient elements in soils and their contents in plants. Irrigation water quality analysis. Determination of pH and EC in irrigation water samples, Determination of Carbonates and bicarbonates in soil and irrigation water, Determination of Calcium and Magnesium in soil and irrigation water. Determination of N, P, K, Ca, Mg, S and micronutrients in plant samples. Determination of Sodium, Potassium, Chlorine and Boron in irrigation water.

Name of Students

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Batch

Session

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3. Prepare 1 N NaOH and 1 NH₂SO₄ solution. Write the procedures.

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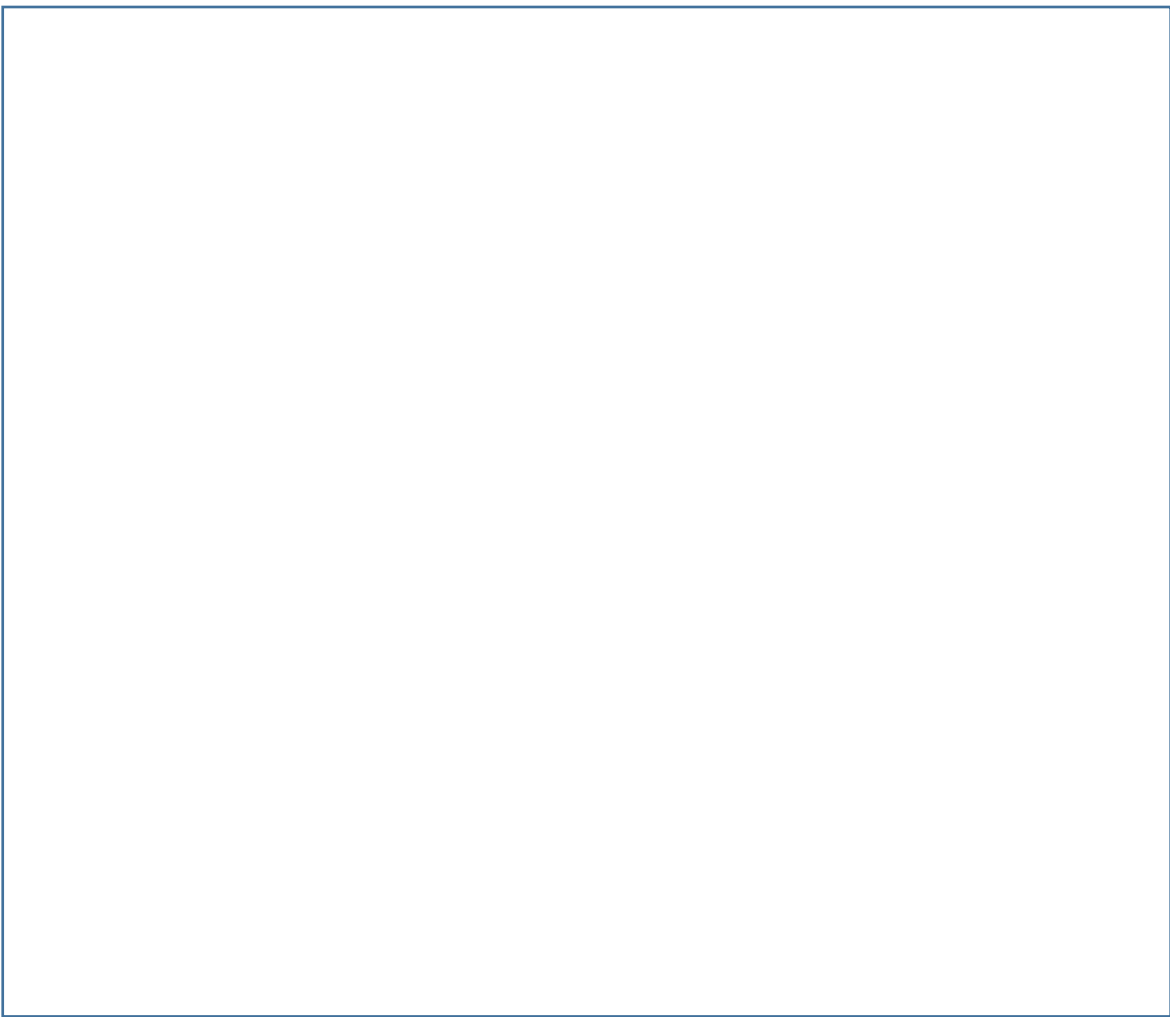
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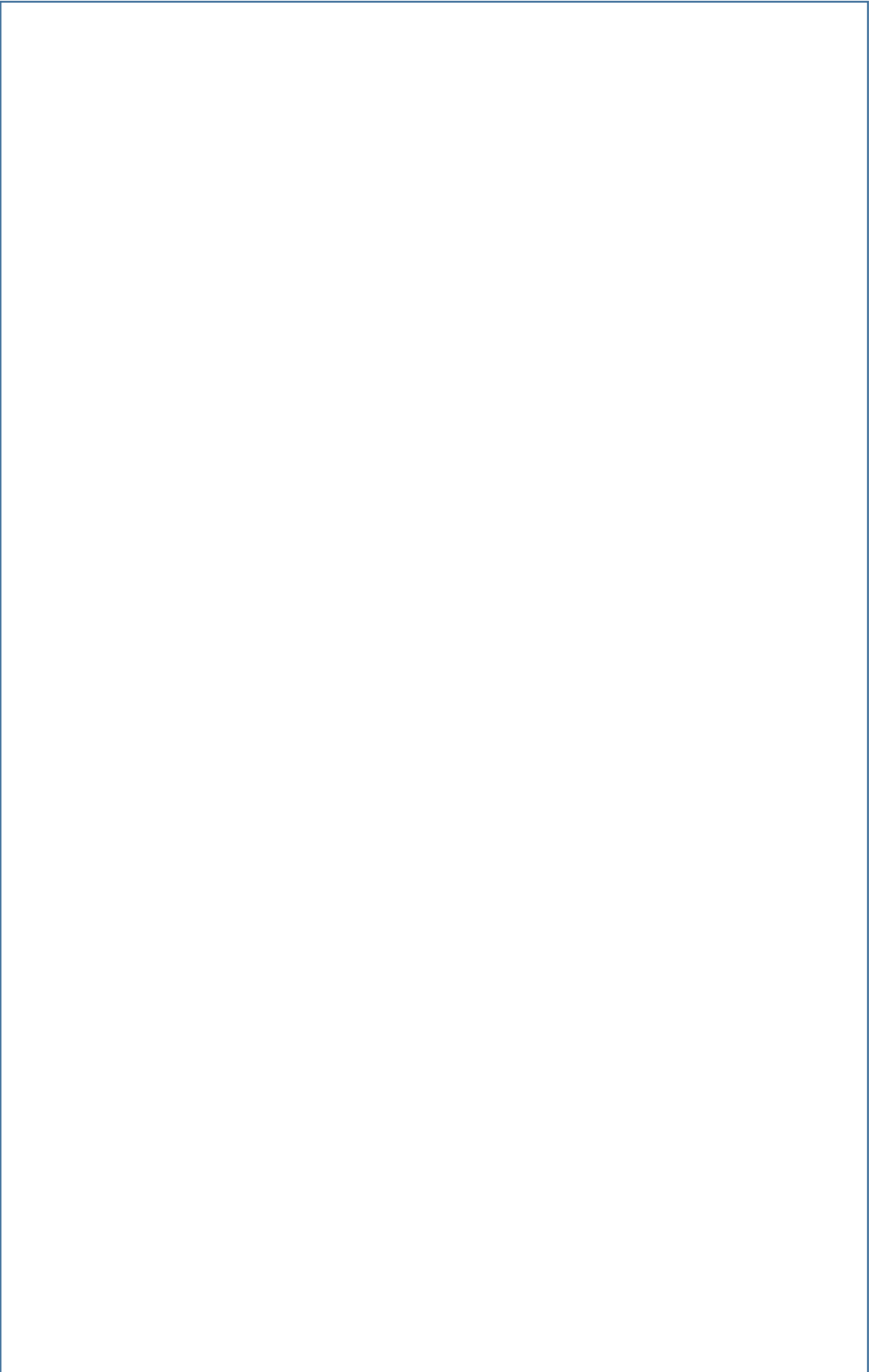
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4. Identify and draw some essential apparatus needed for analytical chemistry.





Experiment No. 2

Objective: Collection and preparation of soil samples for analysis

Each sample collected must be a true representative of the area being sampled. Accuracy of the analyzed sample in laboratory depends on the sampling precision. Hence, collection of large number of samples is advisable so that sample represent the actual condition of the soil.

1. What are the sampling tools required to collect soil samples. Draw some common tools.

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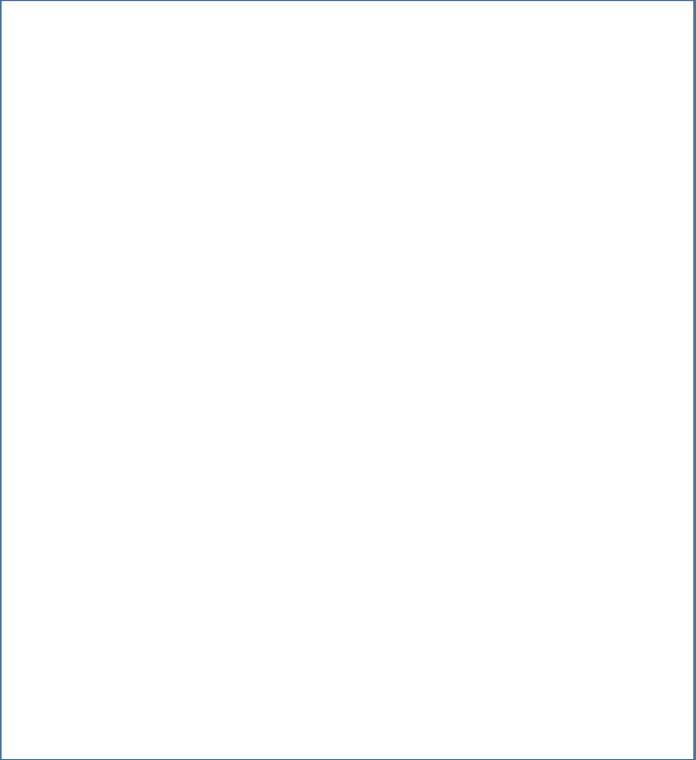
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2. Collect soil sample from the field. Write the procedure of sampling collection and its storage in detail.

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3. What are the precautions one should take while sampling?

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Experiment No. 3

Objective: Collection and preparation of plant samples for analysis

Plant samples are important indicator of nutrient sufficiency. Analyzing plant samples will help in understanding the nutrient availability in plants despite deficiency or sufficiency of nutrients in soil.

1. What are the sampling tools required to collect plant samples.

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2. Collect plant samples for its nutrient analysis. Write procedure you have followed while collecting the sample.

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3. How the collected samples are to be processed for analysis and what are the precautions you need to keep in mind while collecting the sample.

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Experiment No. 6

Objective: Determination of soil organic carbon

Estimation of soil organic carbon (SOC) is done by **WALKLEY-BLACK METHOD**. In this reaction carbon is oxidized by the dichromate ion. Excess dichromate ion is then back titrated with ferrous ion.

Materials required:

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1. Name the reagents required for the analysis of soil organic carbon

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2. Determine soil organic carbon from the given sample. Write procedure with calculation.

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Experiment No. 11

Objective: Determination of available calcium and magnesium from soil sample

Chelating agents like EBT, EDTA has tendency to form complex with metal cations especially with Ca and Mg. Buffer solution is added to increase the pH at 10 so that EBT will form complex with Ca and Mg. EDTA has more tendency to form complex, when the solution is titrated with EDTA, all the complex form with EBT will be broken and replaced by EDTA. Thus, knowing the amount of EDTA needed to form complex will give the amount of Ca and Mg present in the sample.

Materials required:

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1. Name the reagents required for the analysis of available calcium and magnesium.

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2. Determine Av Ca and Mg from the given sample. Write procedure with calculation.

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Experiment No. 12

Objective: Determination of micronutrients from soil sample

Micronutrient cations are extracted by shaking the soil with DTPA extracting solution containing 0.005M DTPA, 0.1M TEA (Triethanol amine) and 0.01M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ buffered at pH 7.3. During this extraction, TEA gets protonated as HTEA^+ as a result of which micronutrient cations from the solid phase come into solution and are chelated by DTPA. Buffering of the extractant in the slightly alkaline pH range and inclusion of soluble Ca^+ through $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ helps avoiding dissolution of CaCO_3 and thus excludes from the estimation of the occluded micronutrients, which do not form a part of the pool that is available for the absorption by plant roots. After that the contents of the micronutrient cations in the soil extract are estimated on atomic absorption spectrophotometer.

Materials required:

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1. Name the reagents required for the analysis of micronutrients like Fe, Mn, Zn and Cu from soil.

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2. Determine concentration (ppm) of Fe, Mn, Zn and Cu from the given sample. Write procedure with calculation.

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3. Determine total N from the given sample. Write procedure with calculation.

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General calculations:

- Weight of material digested = 1g = w
- Vol. made after digestion = 100 ml
- Vol. of aliquot taken after digestion = 5ml
- Vol. 0.02N NaOH used for titration of excess sulphuric acid = Y ml
- Vol. of 0.02N sulphuric acid taken in the receiver = X ml
- Vol. of 0.02N sulphuric acid used for actual absorption of ammonia = X-Y ml = T

$$\text{Total Nitrogen (\%)} = T \times 0.02 \times 14 \times 100/w \times 1000 =$$

4. What are the precautions needed to be taken while analyzing total N?

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Experiment No. 15

Objective: Determination of total phosphorus from plant sample

Phosphorus is a major component in plant DNA and RNA. Phosphorus is also critical in root development, crop maturity and seed production. Total phosphorus in the plant extract is generally determined by **Vanado molybdo phosphoric yellow colour method** in nitric acid system.

Materials required:

1. Name the reagents required for the analysis of total phosphorus from plant.

2. Determine total P from the given sample. Write procedure with calculation.

Experiment No. 17

Objective: Determination of Carbonates and bicarbonates in irrigation water

The analysis is dependent on titrimetric method using an indicator for each carbonates and bicarbonates content in irrigation water.

Materials required:

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1. Name the reagents required for the analysis of carbonates and bicarbonates from irrigation water.

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2. Determine carbonates and bicarbonates from given irrigation water sample. Write procedure with calculation.

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Experiment No. 18

Objective: Determination of Potassium and Sodium in irrigation water

For the determination of K, Na, Ca and Mg cations, flame photometer can be used depending on the principle of its excited atoms due to flame and the energy released during the process. Known concentration of standard solutions can be used to prepare standard curve and therefore, sample concentration can be calculated.

Materials required:

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1. Name the reagents required for the analysis of potassium and sodium from irrigation water.

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2. Determine potassium and sodium from given irrigation water sample. Write procedure with calculation.

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INTRODUCTION TO ANALYTICAL CHEMISTRY

Reagents are manufactured and marketed in different grades of purity. In general, the present reagents are marked as Analytical Reagent or AR or Reagent Grade. These marking ranges from Laboratory Reagents (LR), Chemical Pure (CP), Technical U.S.P. or Commercial Grade etc. for different grades by different manufactures. Generally, the manufactures express the strength of concentrated acids and bases of liquid form in terms of specific gravity because it is used for controlling their strength. Since in laboratory we express strength as normality or molarity.

The strength of any reagent can be calculated by using the following relationship:

$$\text{Strength (N)} = \frac{\text{Volume in one litre} \times \text{Sp. Gravity}}{\text{Equivalent weight}}$$

Buffer solution: The solutions containing a weak acid and its salt or weak base and its salt (e.g. CH_3COONa) and ($\text{NH}_4\text{OH} + \text{NH}_4\text{Cl}$) possess the characteristic property to resist changes in pH when some acid or base is added to them. Such solutions, which resist the changes in their pH values when small amount of an acid or a base is added to them, are referred to as buffer solutions. Following are the important properties of a buffer solution:

- (i) It has a definite pH value.
- (ii) Its pH value does not alter either on keeping for a long time or on dilution.
- (iii) Its pH value is very slightly altered by the addition of a strong acid or a strong base.

Molarity (M): One molar solution contains one mole or one molecular weight in grams of a substance in each litre of solution, whether the substance is in the form of molecules, ions or any other species. Molar method of expressing concentration is useful due to the fact that equal volumes of equimolar solutions contain equal number of molecules. If the concentration of a solution is known in terms of grams of a given solute present in a known volume of solution, the molarity can be calculated.

Molality (m): The molality of a solution is the number of mole of the solute per 1000 g of the solvent. It is usually designated by m.

Normality (N): The normality of a solution is the, number of gram-equivalents of the solute per litre of the solution. It is usually designated by N.

The gram equivalent weight of a substance is the weight in grams which in its reaction corresponds to a gram-atom of hydrogen or of hydroxyl or half a gram atom of oxygen, or a gram-atom of a univalent ion. The equivalent weight: The milliequivalent weight in grams (abbreviated m.eq.) of a substance is the equivalent weight in grams divided by 1000. It is the most convenient value because it is the weight of a substance contained in or equivalent to one ml of a 1N solution.

It is, therefore, a unit which is common to both volumes and weights, making it possible to convert the volume of a solution to its equivalent weight and the weight of substance to its equivalent volume of solution. In applying the meq it is useful to know that the volume of a solution multiplied by its normality gives the number of milliequivalents of the reagent in that volume of solution.

Number of meq = Volume x Normality

Parts per million (ppm) = meq per litre x equivalent weight

COLLECTION AND PREPARATION OF SOIL SAMPLES FOR ANALYSIS

Soil Sampling tools and accessories: Depending upon the purpose and precision required, following tools may be needed for taking soil samples.

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

Sampling procedure

- Prepare a map of the area to be covered in a survey showing different sampling unit boundaries.
- A plan of the number of samples and manner of composite sampling is entered on the map, different fields being designated by letters A, B, C etc. Each area is traverse separately.
- A slice of the plough layer is cut at intervals of 15 to 20 steps or according to the area to be covered.
- Collect at least 10 to 15 samples from each sampling unit and place in a bucket or tray. Generally 10 to 20 spots must be taken for one composite sample depending on the size of the field.
- Scrap away surface liter, obtain a uniform thick slice of soil from the surface to the plough depth from each place. A "V" shaped cut to a depth of 15 cm in the sampling spot using spade to remove 1 to 2cm slice of soil.
- The sample may be collected on the blade of the spade and put in a clean bucket.
- In this way collect samples from all the spots marked for one sampling unit. In case of hard soil, samples are taken with the help of augur from the plough depth and collected in the bucket.
- Reduce bulk sample to about 500g by quartering method in which the entire soil mass is spread, divided into four quarters, two opposite ones are discarded and the remaining two are remixed. Repeat this process until about 500g soil is left.

Processing of soil samples for analysis

- Air dry the soil samples in shade
- Crush the soil clods tightly and grind with the help of pestle and mortar
- Pass the entire quantity through 2 mm sieve
- Discard the plant residues, gravels and other material retained on the sieve
- For certain types of analysis (e.g. Organic carbon), grind the soil further so as to pass it through 0.2 to 0.5 mm sieve
- Remix the entire quantity of sieved soil thoroughly before analysis

Precautions

- Do not sample unusual area like unevenly fertilized, marshy, old path, old channel, old bunds, area near the tree, site of previous compost piles and other unrepresentative sites.
- For a soft and moist soil, the tube auger or spade is considered satisfactory. For harder soil, a screw auger may be more convenient.
- Where crops have been planted in rows, collect samples from the middle of the rows so as to avoid the area where fertilizer has been band placed.
- Avoid any type of contamination at all stages. Soil samples should never be kept in the store along with fertilizer materials and detergents. Contamination is likely when the soil samples are spread out to dry in the vicinity of stored fertilizers or on floor where fertilizers were stored previously.
- Before putting soil samples in bags, they should be examined for cleanliness as well as for strength.
- Information sheet should be clearly written with copying pencil.

COLLECTION AND PREPARATION OF PLANT SAMPLES FOR ANALYSIS

Plant sampling tools: Stainless steel scissors, Knife, Lawn scissors, Pruning shears etc.

Steps for collecting plant sample

- Look for a branch with flowers and or fruits on it and collect a twig of a branch that has good leaves, flowers and or fruits.
- Make at least 3-5 specimens per tree/plant.
- Tag the specimen with a tag and write the collection number on the tag.
- Wrap the specimens in a newspaper and place in a collecting bag
- Describe the general environment in which the plant was collected from, soils, topography, forest type, and elevation and GPS location.
- If live materials are collected, such as seeds, rhizomes, cuttings or wildings for planting, these should be tagged and given the same tag number as that of the specimen voucher.
- Two average samples are taken from the sampling unit using a parallel sampling method, preferably along the design of the sampling unit.
- A sampling unit must always be of the same kind. It is always the variety of the plant that decides the design of the sampling unit.

For orchards

- Collect two leaves from a tree from the two aisles sides of the tree and from the lower third of the foliage.
- Always take the sample from the surface of the foliage (exposed to light) from the centre of the fruiting branches
- One sample consist of 100 leaves on average
- For leaf analysis, collect healthy leaves and if there is a deficiency symptom that affects the whole plantation, then average sample should be taken
- If ill leaves with deficiency symptoms are present only in patches, then exclude these plants from the sample, but later those patches should be sampled separately.

Tree species	Time of sampling	Parts to be sampled
Apple	After growth of shoot is completed	Complete leaves from the middle terminal shoots
Gooseberry, Strawberries	At ripening	Formed leaf without petiole
Plums	When the fruit is taking colour	Complete leaves
Cherries	At Ripening	Complete leaves

Processing of plant samples for analysis

- Wash the plant samples with tap water and then distilled water
- Wipe it using a tissue paper and put in brown paper bag after tagging
- Dry the samples in oven at 60 - 70° C till it becomes totally dried
- Crush/cut the samples into small pieces and store in a sealed polythene bag after tagging properly

Precautions

- Diseased or dead plant material should be avoided.
- Plant tissue damaged by insects and mechanical equipment should also be avoided.
- Do not sample extremely young or old leaves.
- Plant tissue, which has been stressed by excesses of cold, heat, or moisture be avoided.
- Seeds should not be sampled - they do not reflect nutritional status of the whole plant.

COLLECTION AND PREPARATION OF WATER SAMPLES FOR ANALYSIS

Steps for collecting water sample

- Understand the type of analysis needed to be done for collecting a water sample.
- Use only sterile sampling containers. Most labs provide a 100 milliliter container with a snap-lid or screw-capped lid.
- Mark the vessel with the following information: date, time, location, name of the person collecting the sample, storage conditions of the sample, test method and sample code (corresponding to a specific location from where you collected the sample).
- Use sterile single-use gloves to collect the sample.
- Make sure your hands are never immersed in the water, touching the rim of the lid or container, and avoid placing the lid face down and in contact with dirty surfaces.
- Make sure you have prepared a clean insulated cooler that will keep the sample below 10° C (50° F) during storage and transport.

Precautions

- Prevent any debris from entering the sample and container during sample collection and shipment.
- Open the sample container only when you are ready to collect the sample. (Use sterile gloves at all times.)
- Avoid touching the underside of container lid and inside the container rim.
- The container should be inclined in a 45-degree angle to collect the sample with the label facing you.
- Fill the container slowly to minimize any splashing, and avoid filling the container above the marked fill line.
- Recap the container and with clean and sterile gloves, and sanitize the surface of the container using single use chlorine or alcohol wipes. Avoid sanitizer infiltration into the vessel.
- When possible, collect samples in the morning when it's cool and there is less potential variation in the microbial population of ground and surface waters

DETERMINATION OF pH AND ELECTRICAL CONDUCTIVITY IN SOILS AND IRRIGATION WATER

Equipment and Material Required: Mechanical shaker, pH meter, EC meter, Plastic bottles, distilled water, beaker/conical flask, pH buffer solutions, weighing balance, aluminium can box, filtration apparatus, dish, oven, wash bottle, measuring cylinder

Procedure for pH:

- Calibrate the pH meter, using 2 buffer solutions, one should be the buffer with neutral pH (7.0) and the other should be chosen based on the range of pH in the soil.
- Take the buffer solution in the beaker. Insert the electrode alternately in the beakers containing 2 buffer solutions and adjust the pH.
- The instrument indicating pH as per the buffers is ready to test the samples
- Weigh 10g of soil sample/10 ml water into 50 or 100 ml beaker, add 20ml of distilled water
- Allow the soil to absorb water without stirring, then thoroughly stir for 10 second using a glass rod.
- Stir the suspension for 30 minutes and record the pH on the calibrated pH meter.

For EC

- Take 40 g soil/ 40 ml water into 250 ml Erlenmeyer flask, add 80 ml of distilled water, stopper the flask and shake on reciprocating shaker for one hour. Filter through Whatman No.1 filter paper. The filtrate is ready for measurement of conductivity.

- Wash the conductivity electrode with distilled water and rinse with standard KCl solution.
- Pour some KCl solution into a 25 ml beaker and dip the electrode in the solution. Adjust the conductivity meter to read 1.412 mS/cm, corrected to 25°C.
- Wash the electrode and dip it in the soil extract.
- Record the digital display corrected to 25°C. The reading in mS/cm of electrical conductivity is a measure of the soluble salt content in the extract, and an indication of salinity status of this soil. The conductivity can also be expressed as mmhos/cm

Precautions

- Calibration of the instrument is must in order to avoid any error in result
- Handle the electrode carefully as these are made of glass and delicate and sensitive

DETERMINATION OF SOIL ORGANIC CARBON

Equipment and material required: Volumetric flask, Pipette and Burette, Glass rod, Analytical balance, conical flask

Reagents:

- **Potassium dichromate solution:** 1 N. Dissolve 49.04 g pure crystals of potassium dichromate in water and dilute to 1 litre.
- **Ferrous Ammonium sulphate solution:** 0.5 N. Dissolve 139 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in water and add 15 ml conc. H_2SO_4 and dilute to 1 litre. Ferrous ammonium sulphate $\text{Fe}(\text{NH}_4\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ can also be used and if that is to be used dissolve 785 g of the salt in 4 litres of water containing 100 ml conc. H_2SO_4 .
- **Phosphoric acid:** 95%
- **Diphenylamine:** Dissolve 0.5 g diphenylamine in a mixture of 100 ml conc. H_2SO_4 and 20 ml water.
- **Sulphuric acid:** Not less than 96%.

Procedure:

- Weigh 1.0 g soil sample in a 500 ml beaker.
- Add 10 ml 1N $\text{K}_2\text{Cr}_2\text{O}_7$ by means of a pipette and 20 ml concentrated H_2SO_4 by measuring cylinder.
- Mix thoroughly and allow the reaction to proceed for 30 minutes on asbestos sheet.
- Dilute the reaction mixture with 200 ml of water and 10 ml H_3PO_4 by measuring cylinder and add 7-8 drops of diphenylamine indicator.
- Titrate the solution with std. 0.5 N $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ to a brilliant green colour.
- A blank without soil should be run simultaneously.
- Calculate the organic carbon content of a given soil sample.

Precautions

- Handle acids carefully and titration should be carried out effectively as it is sensitive
- Preparation of reagents should be accurate to get accurate result

DETERMINATION OF AVAILABLE NITROGEN FROM SOIL SAMPLE

Equipment and Apparatus Required: Micro-Kjeldahl distillation assembly, Erlenmeyer flask (150 ml capacity), distillation flask, pipettes, burette

Reagents Required: 0.32% KMnO_4 solution, 2.5% NaOH solution, 4% Boric acid, 0.02N H_2SO_4

Procedure:

- Weight 20 g of soil sample of the soil and put into distillation flask.
- Moist the sample with distilled water and fix the distillation assembly.
- Add 100 ml of 0.32% KMnO_4 for solution and 100 ml of 2.5% NaOH solution into distillation flask.
- Take 150 ml conical flask and add 20 ml boric acid and 3-4 drops of mixed indicator. Keep this conical flask at the bottom of receiving tube of distillation assembly.
- The boric acid is back titrated with 0.02N sulphuric acid. At the end point, the blue colour just disappears to pink.
- Blank samples should be run as per above procedure without soil.

Precautions:

1. Always add exactly measured quantity of acid and base.
2. Standards should be prepared accurately
3. The extract should be handled properly

DETERMINATION OF AVAILABLE PHOSPHORUS FROM SOIL SAMPLE

Equipment and Apparatus Required: Erlenmeyer flask (150 ml capacity), mechanical shaker, Whatman No. 1 filter paper, Spectrophotometer.

Reagents required: 0.5 M NaHCO_3 , 1.5% ammonium molybdate solution, Darco G-60, stannous chloride solution

Procedure:

- Weigh 2.5 g soil and transfer it to a 100 ml conical flask.
- Add a pinch of Darco G-60 and 50 ml 0.5 M NaHCO_3 .
- Shake on mechanical shaker for half an hour and then filter it.
- Take 5 ml of filtrate in 25 ml volumetric flask and add 5 ml of ammonium molybdate to it.
- Add 10 ml of distilled water and add 1 ml dilute stannous chloride solution.
- Make volume to the mark by adding distilled water.
- Put the test solution in another colorimetric tube and record per cent transmittance or absorbance.

Precautions

- Always add exactly measured quantity of acid and base.
- Standards should be prepared accurately
- The extract should be handled properly

DETERMINATION OF AVAILABLE POTASSIUM FROM SOIL SAMPLE

Equipment and Apparatus Required: Erlenmeyer flask (150 ml capacity), volumetric flask (1000 ml), funnels, pipettes, Whatman No. 1 filter paper, Flame photometer

Reagents Required: 1N neutral ammonium acetate, standard stock solution of K (1000 ppm K), working standard solution of K (100 ppm).

Procedure:

- Weigh 5 g soil in a 100 ml plastic shaking bottle.
- Add 25 ml of 1 N NH_4OAc (pH 7.0) with a pipette (1:5::soil:solution ratio)
- Keep it as such for about 10-15 minutes.
- Now filter the suspension through Whatman No.1 filter paper in a beaker.
- Prepare a standard curve involving 0, 5, 10, 15 & 20 ppm K solution.
- Take reading of the standard solutions and sample in Flame photometer

Precautions:

1. Always add exactly measured quantity of acid and base.
2. Standards should be prepared accurately
3. The extract should be handled properly

DETERMINATION OF AVAILABLE SULPHUR FROM SOIL SAMPLE

Equipment and material required: Weighing balance, spatula, measuring cylinder, conical flask, volumetric flask, glass rod, magnetic stirrer, beaker, reagent bottle, Wash bottle, filter paper Whatman no.1, colorimeter or spectrophotometer.

Reagents required:

- **Extracting solution:** Dissolve 1.986 g of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in distilled water and dilute to one litre.
- **Barium chloride (BaCl_2):** 30 to 60—mesh crystals: Grind barium chloride crystals until they pass through 30—mesh sieve and are retained on a 60—mesh sieve.
- **Gum acacia solution (0.25%):** Dissolve 0.25 g gum acacia in distilled water and dilute to 100 ml.
- **Standard sulphur solution (100 ppm S):** 0.5434 g of the reagent grade potassium sulphate (K_2SO_4) in distilled water and dilute to one litre.

Procedure:

- Preparation of standard curve:** Pipette out 0.25, 0.5, 1.0, 2.5 and 5.0 ml of 100 ppm S solution in different 25 ml volumetric flasks. Add in every flask 10 ml of 0.15% CaCl_2 solution and 1.0 g of 30 - 60 mesh BaCl_2 crystals. Swirl for one minute to dissolve the crystals and add 1 ml of 0.25% solution of gum acacia. Make up the volume in every flask with distilled water and shake well. Within 5 to 30 minutes after the development of turbidity read the standards on a colorimeter at 420 nm using a blue filter. Plot a standard curve showing relationship between concentration of S and turbidity/absorbance readings.
- Analysis of test sample:** Weigh 10 g air-dried soil and transfer it to a 150-ml conical flask. Add 50 ml of 0.15 % CaCl solution and shake for 30 minutes on an electric shaker. Filter the Suspension through Whatman No.42 filter paper. Pipette out 20 ml of the filtrate in 25-ml volumetric flask and proceed further as in case of standard curve. Run blank with all the chemicals, except the soil. Find out sulphate concentration from the standard curve.

Precautions

- Reagents should be prepared accurately

- Standard working solution should be prepared according to the need

DETERMINATION OF AVAILABLE CALCIUM AND MAGNESIUM FROM SOIL SAMPLE

Equipment and Apparatus required: weighing balance, mechanical shaker, pipettes, burette, Whatman no. 1 filter paper and conical flask

Reagents required:

- **Extractant solution:** 77.08 g Ammonium acetate (NH_4OAC) and make up to 1 litre of distilled water in a vol. flask
- **Buffer solution:** 67.5 g of Ammonium Chloride (NH_4Cl) in 400 ml of distilled water to this and 570 ml of concentrated Ammonia solution and dilute to 1 litre in a vol. flask
- **Erichrome Black T (EBT) indicator:** 1 g of EBT indicator in a 400 ml vol. flask make up using ethanol alcohol
- **0.01 N EDTA:** 2 g of EDTA and 0.05 g of Magnesium Chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) dissolve in water in 1 litre vol. flask

Procedure:

- Weigh 10 g of soil in 150 ml conical flask
- Add 50 ml of Extractant Ammonium Acetate
- Shake the solution in a mechanical shaker for 5 mins and filter using whatman no. 1 filter paper
- Take 10 ml of the aliquot in 150 ml conical flask
- Add 5 ml buffer solution and 1-2 drops of EBT indicator
- Titrate with 0.01 N EDTA till the colour changes from purple to blue

Precautions:

- Use mask as the smell of buffer solution is suffocating
- Titrate carefully as the colour change is very mild
- Prepare reagents carefully

DETERMINATION OF MICRONUTRIENTS FROM SOIL SAMPLE

Equipment and Apparatus Required: Weighing balance, mechanical shaker, pipettes, burette, conical flask and Whatman No. 42 filter paper

Reagents Required: DTPA extracting solution i.e. 0.005 M DTPA, 0.01M CaCl_2 , 0.1 M TEA

Procedure:

- Weigh 20 g soil in a 100 ml conical flask.
- Add 40 ml of DTPA extracting solution (pH 7.3) in 1:2 :: soil : solution ratio
- Shake for 30 minutes in a mechanical shaker
- Now filter the suspension through Whatman No.42 filter paper in a beaker/plastic bottle.
- Prepare a standard curve involving 0, 5, 10, 15 & 20 ppm Fe, Mn, Zn, Cu working solution from the given stock solution of 1000 ppm
- Take the reading in AAS

Precautions

- Shaking should be done properly and filtration should be done immediately after shaking
- Filtration should be done with filter paper of Whatman No. 42 as AAS is very sensitive

- Handle the instrument carefully as it contains flame

DETERMINATION OF SODIUM ADSORPTION RATIO OF SOILS

Equipments and materials required: Flame photometer, Weighing balance, Mechanical shaker, Burette, pipette, conical flask, volumetric flask

Reagents required:

- **1N Neutral Ammonium Acetate**
- **Stock solution of Na** – Dissolve 5.845 g of AR grade dried NaCl in water and make up the volume to 1 L.
- **Working standard of Na-** Dilute 5, 10, 15, 20, 30, 40 and 50 ml portion of the stock solution to 100 ml in volumetric flasks to get working standards of 5, 10, 15, 20, 30, 40 and 50 meq /L Na concentrations.

Procedure:

- Weigh 5 g soil in a 100 ml plastic shaking bottle.
- Add 25 ml of 1 N NH₄OAc (pH 7.0) with a pipette (1:5::soil:solution ratio)
- Keep it as such for about 10-15 minutes.
- Now filter the suspension through Whatman No.1 filter paper in a beaker.
- Prepare a standard curve involving 5, 10, 15, 20, 30, 40 and 50 ppm Na solution.
- Take reading of the standard solutions and sample in Flame photometer
- Draw a standard curve by plotting the readings against Na concentrations.

Precautions

- Prepare the reagents carefully
- Filtration should be done immediately after shaking
- Take the reading when the flame is blue

DETERMINATION OF TOTAL NITROGEN FROM PLANT SAMPLE

Equipment and Apparatus Required: Pestle mortar, Distillation flask, pipettes, burette, heater and Whatman No. 1 filter paper

Reagents Required: Conc. H₂SO₄, digestion mixture, NaOH solution, H₂SO₄, methyl red indicator

Procedure:

1. Transfer 1g of prepared plant material wrapped in a piece of filter paper, to a 300 ml kjeldahl's digestion flask.
2. Add to it 10 g of catalyst mixture and 25-30 ml of concentrated sulphuric acid.
3. Mix the contents of the flask by swirling with care not to through the samples on the side.
4. Start digesting the contents of the flask on digestion heater for 20-30 min until frothing stops. 5. Continue heating until the organic matter is destroyed and the solution is clear light yellow or grey colour. Cool and make the volume 100ml with distilled water.
5. Pipette out 10 ml of 0.02N sulphuric acid in a 150 ml conical flask, add 2 -3 drops of methyl red indicator.
6. Take 5 ml of aliquot in distillation flask and connect it to the mouth of the distillation flask.
7. Now pour 25ml of 45% NaOH in distillation flask through the funnel attached to the distillation apparatus.
8. Collect about 30ml the distillate.
9. Titrate the excess of 0.02 N sulphuric acid in a conical flask against 0.02N NaOH. The end point is change in colour from pink to yellow.

Precautions

- Prepare reagents carefully
- Handle distillation chamber carefully as it will boil at high temperature
- Titration should be accurate as the amount is very less

DETERMINATION OF TOTAL PHOSPHORUS FROM PLANT SAMPLE

Equipment and Apparatus Required: Pestle mortar, Distillation flask, pipettes, burette, heater and Whatman No. 1 filter paper

Reagents Required: 4N Sodium bicarbonate, 6N Hydrochloric acid, 2,4 dinitrophenol indicator, Nitric acid – vandate – molybdate reagent, Phosphate standard

Preparation of plant digest

- Wash the samples with distilled water and air dry them or put a filter paper sheet over them to absorb the excess moisture.
- After this initial drying, place the samples in the brown paper bags and place them in hot air oven. Set the temperature of oven at 65°C and let the samples dry overnight or until all moisture in them is completely lost.
- Take these dry leaf samples and grind them into fine powder or small pieces using a grinder or scissor.
- Take 1 gram leaf sample powder in a kjeldahl/conical flask.
- Prepare a Di-Acid solution of nitric acid and perchloric acid in the 4:1 ratio *i.e.* 400ml of nitric acid and 100ml of perchloric acid. Add 20 ml of this Di-Acid solution to the kjeldahl/conical flask containing leaf sample powder/pieces. This acid solution is a strong oxidizing agent which will extract all the plant nutrients into an extract form.
- Gently swirl the contents of the conical flask and cover their mouth of the flask. Let the contents of the flask undisturbed for 10-12 hours.
- Put the conical flask on a hot plate and heat the contents of the flask in a well- ventilated place.
- The fumes initially have a light brown colour. As it tends to reach the end point of the heating, the fumes start to get accumulated in the neck of the conical flask which are purely white. At this point stop the heating process.
- The correct proportion of the extract at the end of the heating is about 1-2 ml of the extract.
- After complete cooling of the flask and the extract, collect the extract in a 25 or 50 ml conical flask and makeup the volume to 100 ml with distilled water in a vol. flask. Use this diluted plant extract for further analysis as practiced for soil analysis of K, P, S, Zn etc

Procedure:

- Take 5 ml of plant digest in 25ml volumetric flask.
- Add 1-2 drops of 2-4 dinitrophenol indicator and 4N NaHCO₃ solution drop wise till yellow color appears.
- Now add 6N HCl drop wise till yellow color disappears.
- Add 2.0ml of 6N HCl in excess to get required pH of 4.8.
- At this stage add 5ml vanadate molybdate reagent and make up the volume upto 25ml. the colour develops in several minutes and is stable for 2 months at high P concentrations, but at P concentration of 5 ppm it is stable for only 2 weeks.
- Prepare a blank in the similar way. Read the intensity of yellow color formed on a spectrophotometer at a wavelength of 880 nm and make up the volume upto 25ml. the color develops in several minutes and is stable for 2 months at high P concentrations, but at P concentration of 5 ppm it is stable for only 2 weeks.
- Read the intensity of yellow color spectrophotometer at a wavelength of 880 nm.

Precautions

- Carefully handle the digestion and distillation chamber as it is maintained at very high temperature
- Concentrated acids are used, need to be careful while handling

DETERMINATION OF TOTAL POTASSIUM FROM PLANT SAMPLE

Equipment and Apparatus Required: Flame photometer, Pestle mortar, Distillation flask, pipettes, burette, heater and Whatman No. 1 filter paper

Reagents Required: Acid for digestion, Distill water, Potassium standard solutions

Procedure:

1. Digestion of plant samples (1 g) is carried out in digestion chamber using di acid.
2. Take 1 ml of plant extract in 50 ml of volumetric flask.
3. Make the final volume upto 50 ml with distilled water.
4. Now feed the solution to the atomizer assembly of the flame photometer, the galvanometer of which has already been adjusted with the standard K solutions and note down the reading.

Precautions:

- Carefully handle the digestion and distillation chamber as it is maintained at very high temperature
- Concentrated acids are used, need to be careful while handling

DETERMINATION OF CARBONATES AND BICARBONATES IN IRRIGATION WATER

Materials Required: Burette, pipette and conical flask, H₂SO₄ (0.1 N), Phenolphthalein and Methyl orange indicator.

Procedure:

- Pipette out 10 ml of irrigation water sample into a conical flask
- Add 1 or 2 drops of phenolphthalein indicator; the solution will turn pink.
- Titrate the contents against the 0.01 N H₂SO₄ filled in burette; the end point is indicated by the disappearance of pink color. Record the burette reading.
- If on addition of phenolphthalein the sample does not turn pink, it indicates the absence of CO₃. In such case add methyl orange indicator to the same aliquot and titrate against 0.01N H₂SO₄ till the straw yellow colour changes to rose red. Note down the burette reading.

Precautions:

- While titrating, the H₂SO₄ solution should be added drop-wise
- Prepare reagents carefully and accurately

DETERMINATION OF POTASSIUM AND SODIUM IN IRRIGATION WATER

Equipment and materials required: Flame photometer, Burette, pipette, conical flask, volumetric flask, Whatman No. 1 filter paper

Stock solution of K and Na –

- 1 N neutral Ammonium acetate for K (1000 ppm)
- Dissolve 5.845 g of AR grade dried NaCl in water and make up the volume to 1 L for Na.

Working standard of K and Na -

- K - 0, 5, 10, 15 & 20 ppm K solution from 1000 ppm K stock solution
- Na - Dilute 5, 10, 15, 20, 30, 40 and 50 ml portion of the stock solution to 100 ml in volumetric flasks to get working standards of 5, 10, 15, 20, 30, 40 and 50 ppm Na concentrations.

Procedure:

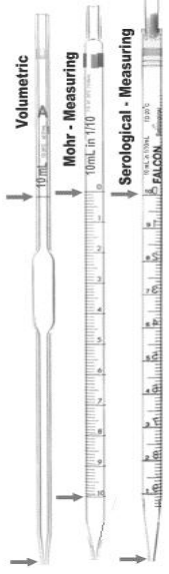






- Filter a portion of the water sample if suspended material is visible. Filtration is desirable as it prevents choking of the capillary tube of the flame photometer.
- Take the working standard solutions and record the flame photometer reading.
- Feed the test sample and record the reading.









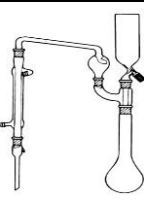
- Draw a standard curve by plotting the readings against Na concentrations.
- The reading given by the flame photometer is in ppm

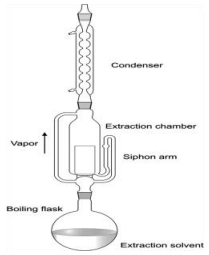





Precautions: Handle flame photometer carefully as it includes flame during analysis. Filtration should be done properly

Appendix

IMPORTANT LABORATORY GLASSWARE AND EQUIPMENTS

1.	<p>Pipettes: Laboratory instrument/ glassware used to transport a measured volume of liquid. Three types of glass pipets used in the laboratory</p> <ul style="list-style-type: none"> ▪ Volumetric pipets Designed to transfer a fixed amount of liquid when filled to the mark, e.g. 10 mL and only 10 mL. There is generally only one "fill-line" on a volumetric pipet. ▪ Serological pipets are TD = to deliver. To accurately dispense the measured volume the last bit must be blown out. ▪ Mohr pipets are TC = to contain. These pipets are designed to dispense the correctly measured volume, so there will be a minute amount of liquid left in the tip. 	
2.	<p>Burette: Burette also spelled Burette, laboratory apparatus used in quantitative chemical analysis to measure the volume of a liquid or a gas. It consists of a graduated glass tube with a stopcock (turning plug, or spigot) at one end.</p>	
3.	<p>Beaker: Used for transferring liquid to another container or to transfer a small amount of reagent for use in procedures. Volume is not accurate, just an estimate.</p>	
4.	<p>Erlenmeyer Flask or Conical flask : Features a conical base, a cylindrical neck and a flat bottom. They are marked on the side (<i>graduated</i>) to indicate the approximate volume of their contents.</p>	
5.	<p>Graduated Cylinder or Measuring cylinder For rapid measurement of liquid volume. They are generally more accurate and precise for this purpose than flasks.</p>	
6.	<p>Volumetric Flask: A <i>volumetric flask</i> is used to measure very precisely one specific volume of liquid. This flask is used to prepare a solution of known concentration.</p>	
7.	<p>Funnel: A funnel is a pipe with a wide (often conical) mouth and a narrow stem. It is used to channel liquid or fine-grained substances into containers with a small opening.</p>	

8.	<p>Separatory Funnel</p> <p>A separatory funnel, also known as separation funnel or separating funnel, is a laboratory glassware used in liquid-liquid extractions to separate (partition) the components of a mixture into two immiscible solvent phases of different densities.</p>	
9.	<p>Desiccator</p> <p>Desiccators are sealable enclosures containing desiccants used for preserving moisture-sensitive items. A common use for desiccators is to protect chemicals which are hygroscopic or which react with water from humidity.</p>	
10.	<p>Test tubes:</p> <p>Test tubes are used by chemists to handle chemicals, especially for qualitative experiments and assays. Their spherical bottom and vertical sides reduce mass loss when pouring, make them easier to wash out, & allow convenient monitoring of the contents.</p>	
11.	<p>Centrifuge tubes</p> <p>Centrifuge tubes are used in laboratory centrifuges, machines that spin samples in order to separate solids out of liquid chemical solutions. The centrifuge tubes can be made of glass or plastic, and resemble miniature test tubes with tapered tips</p>	
12.	<p>Kjeldahl flask</p> <p>Kjeldahl flask is a round bottom flask with a long wide neck that is used in the determination of nitrogen by Kjeldahl's method.</p>	
13.	<p>Reagent bottles, also known as media bottles or graduated bottles, are containers made of glass, plastic, borosilicate or related substances, and topped by special caps or stoppers and are intended to contain chemicals in liquid or powder form for laboratories.</p>	
14.	<p>Wash bottle</p> <p>A wash bottle is a squeeze bottle with a nozzle, used to rinse various pieces of laboratory glassware, such as test tubes and round bottom flasks.</p> <p>Wash bottles are sealed with a screw-top lid. When hand pressure is applied to the bottle, the liquid inside becomes pressurized and is forced out of the nozzle into a narrow stream of liquid.</p>	
15.	<p>Dropper or Pasteur pipette</p> <p>Pasteur pipettes, also known as droppers are used to transfer small quantities of liquids. They are usually glass tubes tapered to a narrow point, and fitted with a rubber bulb at the top.</p>	
16.	<p>Nitrogen distillation unit</p> <p>The distillation unit is used to perform nitrogen analysis and protein determination according to Kjeldahl method in food & feed industry and other applications in environmental and chemical industries after having digested the sample accurately.</p>	

17.	<p>Soxhlet extraction</p> <p>A Soxhlet extractor is a kind of laboratory equipment. It is made of glass. Franz von Soxhlet invented it in 1879. It has a flask, an extraction chamber, and a condenser. It can be used for solid-liquid extractions.</p>	
18.	<p>Heating mantle</p> <p>A heating mantle, or isomantle, is a piece of laboratory equipment used to apply heat to containers, as an alternative to other forms of heated bath.</p>	
19.	<p>Centrifuge</p> <p>A centrifuge is a laboratory device that is used for the separation of fluids or liquid, based on density. Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier materials to the outside of the vessel.</p>	
20.	<p>Hot air Oven</p> <p>Hot air ovens are electrical devices which use dry heat to sterilize. They were originally developed by Pasteur. Generally, they can be operated from 50 to 300 °C, using a thermostat to control the temperature.</p>	
21.	<p>pH meter</p> <p>pH meter, electric device used to measure hydrogen-ion activity (acidity or alkalinity) in solution. Fundamentally, a pH meter consists of a voltmeter attached to a pH-responsive electrode and a reference (unvarying) electrode.</p>	
22.	<p>Water bath</p> <p>A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature over a long period of time.</p>	
23.	<p>Analytical balances</p> <p>The Analytical balances in the General Chemistry labs are very sensitive instruments used for weighing substances to the milligram (0.001 g) level.</p>	