Course- Soil, Plant, Water and Seed Testing (ELP)
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Practical Manual

THE NEOTIA UNIVERSITY
ज्ञानम् आत्म प्रदीपाय

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Study of soil sampling tools, collection of representative soil sample, its processing and storage

Soil sampling tools:

Soil Sampling Tools: Core Sampler, Posthole Auger, Tube Auger, Screw Auger, spade pickaxe, khurpi, bucket, cloth bag, scale.

AUGERS:

Auger is an instrument used for taking soil sample in the field. Whenever soil samples are to be collected by easy and quick method the augers are used. Select the place from where the samples are to be collected and then work with auger.

In general when the soil is completely dry, it is difficult to work with auger, but if the soil is moist sampling is easy. There are various types of augers used, depending upon the type of the soil depth of sampling and such other conditions.

1) Screw auger :

It works like a screw in the field. The soil adheres in the grooves. Soil at any depth can be taken up by making mark on the stem and working the auger upto the mark. It is taken up straight way and the soil is collected from the grooves. This auger is useful even if there is a crop in the field.

This auger consists of three parts.

1. Handle : It is a wooden/iron portion.
2. Stem : It is of iron-length 35 cm. or desired length.
3. Grooves : It is of iron-distance between two grooves is 2.5 cm.

2. Dr.Leathers Auger :

It consists of a strong hallow steel cylinder with a sharp cutting edge. It is useful for taking surface sample only. Twenty-five cm. Column of soil is collected in the cylinder.

This auger : consists of three parts :

1.Handle : It is a wooden/iron portion.

2.Stem : It is of iron

3.Cylinder : It is of Iron.

3. American Soil Sampler :

The flank pointed out cuts the soil at the desired depth upto 20 cm. Then by turning the handle the soil enters into the cavity and accumulates there. Then by turning it in a reverse way, slit is closed and the auger is taken out. The soil collected in the cavity is not allowed to fall down. This auger is useful in sandy soil. It works better in rice field and in water logged field.

This auger consists of three parts :

1. Handle : It is of Iron.
2. Stem : It is of iron
3. Cylinder : It is of Iron.

Cylinder consists of three parts:

1 . Sliding covers
2. Flank,
3. Slit.

4. Lyalpur Auger :

The point of the auger enters into the soil. The soil is collected in the cylinder. It can work upto the desired depth. The cutter piece at the end cuts the soil which is collected in the cylinder. Then the soil is removed from the cylinder. It is useful for taking the soil samples in irrigated conditions.

This auger consists of three parts :

1.Handle : It is of Iron
2.Stem : It is Iron
3.Cylinder : It is of iron, length 13" (30 cm) dist.

In the cylinder, there is a slit of cavity of length 6" (15cm) and breadth ½" (1.5 cm.)

5. Post hole auger:

A chisel shaped point enters in the soil and the soil enters in the cylinder. While taking it out the tongue does not allow the soil to fall down. The auger works at any desired depth. It gives samples of surface, soil up to 25 cm. In case if the soil is sufficiently deep, the deeper layers sample a may also be collected.

This auger consists of three parts:

1. Handle : It is of wooden portion.
2. Stem : It is of iron.
3. Cylinder : It is of iron consisting of side plates and tongue. The distance between two side plates is 6 cm.

The different soil sampling tools are used for soil sampling

1. Soil tube auger
2. Screw auger
3. Post hole auger
4. Spade
5. Khurpi/dager
6. Bucket/ghamela
    - For sampling of soft and moist soil tube auger, spade or khurpi can be used satisfactory .
    - A screw type of auger may prove more convenient on hard/dry soil
    - Post hole auger useful for sampling of wet area like rice field
    - Tools for collecting the samples should be free from or any foreign materials which may contaminant the samples.
    - Bucket or Ghamela are used for collecting and mixing the soil sample

Collection of soil samples :

Purpose of collection of soil sample: Soil samples are collected from the field to study physical, chemical and biological properties of the soil.

Procedure: -

1. First divide the field according to the slope, colour of soil, depth, texture, management and cropping pattern, after demarcation of field into uniform portions each of which must be sampled separately.
2. Then divide each portion into two parts, draw the Zigzag line having about 8 to 10 corners on both the sides of middle line so that it will cover the whole area.
3. Where the crops have been planted, collect the soil samples between the lines.

4. Do not sample unusual area. Avoid area recently fertilized, old bunds, marshy spots, near trees, compost heaps or other than non-representative locations.
5. Use proper sampling tools like auger soils, tube, phawda (spade) or Khurpi (Trowel) crop sampler.
6. Before taking the sample scrap away surface litter or any stone etc. Collect the soil samples from 10 to 20 spots in the field depending upon the area. At each corner of the zigzag line take the samples by auger at the depth of 20-30 cm or with the help of trowel and spade by digging the "V" shaped pit up to plough depth. Then cut out uniform thick 2 cm. Slice of soil from top to bottom (0-30 cm) of the exposed soil surface of "V" shaped pit. Collect the sample on the blade or in your hand and place it in clean bucket. Collect all the samples from the uniform area into the same bucket
7. Pour the soil from the bucket on the piece of clean paper or cloth and mix thoroughly. Discard by quartering, excess soil and collect approximately one kg. of soil. To quarter the sample, mix well, divide into four equal parts and reject opposite quarters. Mix the remaining two potions and repeat the procedure as many times as necessary to arrive at the desired size of sample. If the sample is wet or moist, dry it in the shade before putting into a plastic bag. Fill the sample into a plastic bag and put the plastic bag into a cloth bag.
8. Fill out the information sheet completely and may be place inside the sample bag and put one outside the bag and send the sample to laboratory immediately for analysis.

Sample Information Sheet

1. Sample No.
2. Date of collection of sample
3. Name of the cultivator
4. Address: Village
5. Type of soil
6. Survey No.
7. Proposed crop / crops & Variety
8. History of the field for the last 3 years crop, kind of fertilizer and quantity applied.
9. Depth of sampling
10. Field condition: Dry / Wet.
11. Extent of sampled area in ha.

1. Number of samples taken to make a composite sample.

Preparation of Soil Sample :

i. When air dry, break the lumps with a wooden pestle in a wooden mortar so that the aggregate particles are crushed but no actual grinding takes place.

ii. Shift the soil through a sieve with round holes of 2mm (10 meshes) diameter sieve. Use wooden pestle and mortar for further crushing. Repeat the screening till the aggregate particles are fine enough to pass through the sieve and only stones and gravels remain. Before discarding this residue, weigh it and report as gravel and stones (detritus)

iii. For special determination, it is necessary to weigh out small amount of soil i.e. organic matter, $CaCO_3$ and chemical analysis, it is desirable to grind a representative sub sample and sieve it through 0.5 mm. Sieve (32 mesh)

iv. For micronutrients like copper, iron, manganese and zinc, a brass sieve should be avoided and aluminum or plastic sieve with nylon netting should be used.

v. Nitrate, ammonia, bacterial count and moisture determination must be carried out on soils obtained straight from the field. Air-drying of these samples is not permissible and not desired.

vi. The processed samples are analyzed for texture, carbonate content, pH, EC and available nutrients.

Storage of soil samples:

Collected soil sample should be store in well labeled cloth bag size 13cm X 25 cm or plastic bottle (1Kg)

Questions :

1. Explain the procedure of collection of representative soil samples.
2. State the importance of soil sampling.
3. What is auger? Give the name various types of augers.
4. What are the precautions to be followed during soil sampling?
5. Why do you dry the soil sample in shade?
6. Is it desirable to sample the field in each season?
7. What is the relation between mesh and pre size in mm in sieve ?

References :

1) Perur, N.G. Subramanian, C.K, Muhr, G.R. and Ray H.E. (1973): Soil fertility evaluation to

serve Indian farmers.

2) Jackson, M.L. Soil chemical analysis (1973) prentice hall of India, Pvt. Ltd. New Delhi.
3) Gupta P.K. (2007) Soil Plant Water and Fertilizer analysis , Agrobios (India) Jodhpour

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Determination of bulk density of soil

Bulk density of soil is expressed as the ratio of the mass (weight) of soil particle to their total volume including the pore space between the soil particles. Alternately, it is the weight of unit volume of dry soil. It is usually expressed in the unit of gram per cubic centimeter (g/cc) or mega gram per cubic meter (Mg/m^3). In fine textured soil bulk density vanes from 1.00 to 1.60 g/cc, whereas in coarse textured soil from 1.20 to 1.80 g/cc.

$$Bulk\ Density = \frac{Weight\ of\ soil}{Voliume\ of\ soil}$$

Here, the volume of soil means as it exists in situ i.e. in field condition.

Equipments and Materials

Core sampler consists of two cylinders fitted one inside the other (The inside cylinder is the sample holder; the outer core extends at both the ends; upper end to accept the hammer and lower end to form the cutting edge), knife, slide caliper, aluminium moisture box analytical balance, oven, desiccators.

Procedure

- Drive the sampler into a vertical soil surface slightly enough to fill the sampler but avoiding the compression of the soil in the confined space of sampler.
- Using spade dig out the sampler without disturbing the natural position of soil within the sampler.
- Separate the two cylinders retaining the soil mass undisturbed in the sample holder (inner core).
- Trim the soil extending beyond each end of sampler with the help of sharp knife. Now the volume of sample holder is equal to the volume of soil.
- Transfer the total soil from sample holder to a previously weighed moisture box and weigh it.
- Dry it in the oven at 105°C until a constant weight is reached (10 to 15 hours).
- Cool the moisture box and its content at room temperature in the desiccators and weigh it again.

- Determine length and inner diameter of the sampler with the help of slide caliper.

Calculation

Say,

Weight of aluminium box = W_1 g

Weight of aluminium box + field moist soil = W_2 g

Weight of aluminium box + oven dry soil = W_3 g

Weight of oven dry soil = (W_3 - W_3) g = Y g

Weight of water in soil = (W_2- W_3) g = Z g

Thus,

$$\text{Bulk Density of soil (g/cc)} = \frac{Y}{\frac{\pi d^2 h}{4}} = \frac{4Y}{\pi d^2 h}$$

Where, d = inner diameter of the sampler, in cm h = height of the sampler, in cm

Questions:

1) Define bulk density of soil and state its significance.
2) State the average bulk density of clay, sandy and silt soils of West Bengal.
3) State reasons for variation in density of soil.

Reference URL

- https://www.youtube.com/watch?v=qcm1WTGlric

Determination of particle density of soil by pycnometer method

Principle :

Particle density of soil is the ratio of mass (weight) of solid particles to their total volume, excluding the pore space between particles. Bulk density is usually expressed in the unit of gram per cubit centimeter (g/cc) or mega gram per cubic meter (Mg/m^3). For mineral soils the value usually varies from 2.60 - 2.75 g/cc. For general calculation average particle density of arable soil is considered to be 2.65 g/cc Particle density of organic matter (1.2 -1.7 g/cc) is much less than that of mineral soil.

$$Particle\ Density = \frac{Mass\ of\ soilid\ particles\ of\ soil}{Voliume\ of\ solid\ particle\ of\ soil}$$

Volume of solid particles is calculated indirectly by volume of fluid (usually water) displaced by the solid particles. Again, volume of water is calculated from the mass and density of water.

Equipments and Materials

Pycnometer fitted with a glass stopper having a vertical capillary opening (Fig. below), analytical balance, hot plate.

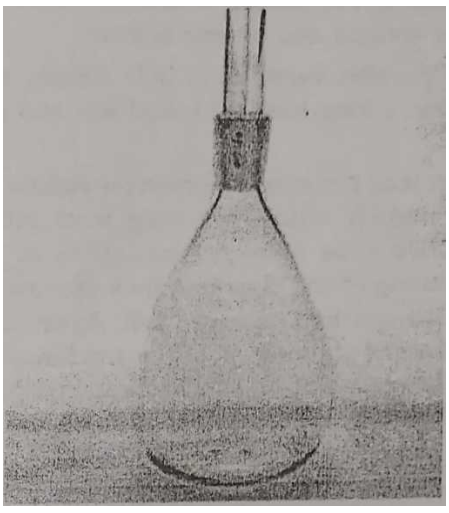

Fig. Pycnometer

Procedure

- Weigh a clean, dry pycnometer and pour oven dry soil to nearly one quarter of its volume.

- Weigh the pycnometer with soil after properly cleaning the outside of the pycnometer with dry cloth duster.
- Fill about half of the volume of pycnometer with distilled water.
- To expel entrapped air within the suspension gently boil the water for few minutes (5 minutes).
- Cool the pycnometer and its content to room temperature.
- Fill the total volume of pycnometer with preboiled cooled distilled water.
- « Insert the stopper carefully and clean outside of pycnometer with dry cloth.
- Weigh the pycnometer with its content.
- Remove soil from pycnometer and fill it with preboiled cooled distilled water and weigh it.
- Record the room temperature.

Calculation

Weight of empty pycnometer = W_A g
Weight of pycnometer + oven dry soil = W_sg
Weight of pycnometer + soil + water = W_{sw} g
Weight of water filled pycnometer = W_w g
Density of water at room temperature = D_{vv} g/cc

$$\text{Particle Density of soil } \left(\frac{\text{g}}{\text{cc}}\right) = \frac{W_S - W_A}{\left\{\frac{(W_W - W_A) - (W_{SW} - W_S)}{D_W}\right\}}$$

$$= \frac{D_W(W_S - W_A)}{(W_W - W_A) - (W_{SW} - W_S)}$$

Questions :

1) Define particle density.
2) What is the significance of particle density

Reference URL

- https://www.youtube.com/watch?v=-PcD2XoBcA8&t=206s

Determination of Porosity of Soil by Indirect Method

Porosity or total pore space of soil is the fraction of soil volume not occupied by soil particles. Mathematically, it is the ratio of volume of pore space to total volume of soil. It is governed by the orientation of the soil solids. Porosity gives idea about the storage capacity of fluid (air and water).

$$\%\text{Pore Space(f)} = 100 - \left(100\frac{D_b}{D_p}\right) = \left(1 - \frac{D_b}{D_p}\right)100$$

Where, D_b and are the bulk density and particle density of soil respectively.

Procedure

- Determine bulk density and particle density of soil as Exp no 5 and 6
- Calculate the porosity of soil using the above equation

Result

Questions :

1. State the significance of porosity of soil in relation to plant growth.
2. State the types of soil pores.

Reference:

1) A textbook of Soil Analysis by T.C. Baruah. H. P. Barthakar, Vikas publishing House Pvt. Ltd. 1997. Pp.11-12.

2) Soil Physical Analysis by R.A. Singh, 1980. pp. 62-63.

Determination of moisture content in soil by gravimetric method

Water plays a very significant role in soil-plant growth relationship. In fact, the soil water is a regulator of physical, chemical and biological activities in the soil.
Water in a soil may be measured in a number of ways, viz.

1. Gravimetric method (The standard method)
2. Neutron scattering
3. Gamma ray alternation,
4. Soil moisture tension, and
5. Electrical conductivity (Gypsum block).

Gravimetric Method

Principle : Weighed soil sample is placed in an oven at 105^0C and it is died to constant weight. The weight difference is considered to be water present in soil sample.

Apparatus :
Sampling auger, Moisture cans / Aluminium box, Hot air oven, Balance

Procedure :

1. Weight the empty moisture boxes.
2. Used the sampling to take a soil sample of 40-50 g. from the required depth. Put the soil immediately in the moisture box and close it to prevent loss of moisture by evaporation.
3. Bring the boxes containing the moist soil to the laboratory and weight them immediately. Remove the lids and place the moisture boxes in oven at 105^0 C for 24 hours.
4. Allow the sample to cool for sometime in oven. Then close the boxes and put them in the desiccators for further cooling. After the completion of this step, weight the closed boxes with the oven dry soil.

Observations :

1. Weight of empty box - A
2. Weight of box + moist soil - B
3. Weight of box + oven dry soil - C
4. Moisture content in soil - B-C
5. Weight of oven dry soil - C-A

Calculations :

$$\text{Per cent moisture in soil} = \frac{\textbf{(B-C)}}{\textbf{(C-A)}} \times 100$$

i.e $\frac{\textbf{Loss in wt.}}{\text{Oven dry wt. of soil}} 100$

Questions:

1. What do you mean by oven dry soil?
2. What do you mean by constant weight?

References:

1. Khanna S.S. and Yadav, D.V. 1979. Practical Manual for Introductory Courses in Soils. P. 45- 4

Reference URL

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Determination of soil pH

A) Soil pH

One of the most enlightening attributes of a soil is its pH. Whether a soil is acidic or basic has much to do with the solubility of various components the relative bonding of ions on exchanges sites and activity of various microorganisms. The plant nutrient is 6.5 to 7.5. Thomas (1957) noted that the three-soil pH availability is influenced by soil pH. The ideal pH range for availability of nutrients is 6.5 to 7.5. Thomas (1957) noted that three soil pH ranges are particularly informative pH less than 4 indicates the presence of free acid generally from association of sulphides: a pH below 5.5 suggests the likely occurrence of exchangeable Al and pH from 7.8 to 8.2 indicates the presence of $CaCO_3$.

I] Equipment for measurement of soil pH :

The pH meter consists of two electrodes viz. (i) Glass electrode and (ii) Calomel electrode (reference electrode)

A. Glass electrode:

1. The glass electrode consists of thin-walled bulb of pH sensitive glass, sealed to a stem of high resistance glass. It is better to choose a lower resistance electrode 9 (pH range 0 to 12) with a more repaid response and to tolerate the possibility of small errors caused by reaction between the electrode surface and the film of soil suspension in contact with it.
   New electrodes should be checked in at least three standard buffers, say near pH 4,7 and 9 for linearity of response.
2. The useful life of glass electrode is extended if it is kept most when not in use. Combined glass and reference electrode should also be stored in a buffer solution, but separate reference electrodes should have their liquid junctions immersed in nearly saturated KCl solution protected from evaporation.
3. With continued use, the performance of glass electrode gradually worsens. Electrode with poor performance should be replaced.
   Erratic off scale readings indicate very high electrical impedance in the electrical circuit.

Air bubbles interrupt the path between the glass bulb and the internal reference electrode. Gentle tapping and shaking usually dislodge these bubbles.

B. Reference electrode:

a. These electrodes are usually the calomel type with saturated KCl electrolyte. But Ag-AgCl electrodes give quite satisfactory service and have an advantage in being easily repaired or even constructed in a laboratory.

b. Calomel electrodes must not be heated above 70 ^{0}C . They should be closely inspected regularly to see that no air gaps have developed.

c. The liquid junction between the reference electrode and the test liquid usually made with KCl solution, which is also the reference electrolyte. The liquid junction potential with soil suspensions is not the same with other electrolytes. The KCl solution used should not be saturated at any temperature above the minimum to which the electrode will be subjected. For example, a solution of 32 g KCl in 100ml water, is just undersalurated at 15 ^{0}C.

d. The KCl solution should flow through the liquid junction at a very low but detectable rate.

e. The liquid junction of reference electrode should just enter the surface of the soil suspension in order to be in a zone of minimum clay concentration. This makes the junction potential as small as possible and closest to that in the standardizing buffer solution. The difference in the junction potential in buffer and suspension is included in the pH shown by the meter.

II] Reagents:

A. Standard buffer solution, pH 4.00. Prepare stock solution of 0.3 M potassium hydrogen phthalate by dissolving 15.3g of the analytical grade salt in about 225 ml of hot water. cooling the solution, and diluting it to 250 ml .Add a drop of toluene to discourage growth of micro organisms. For the standard buffer pH.4.0 mix 100 ml of the stock

solution with 500ml water. Prepare the fresh solution every week.

B. Standard buffer solution, pH 9.2 : Dissolve 3.81g sodium tetraborate (A.R) in water and dilute to 1000 ml.

C. 1.0 N Potassium Chloride solution (A.R)

III] Apparatus :

Electrometric pH meter with glass and calomel electrodes.

IV] Procedure :

1. Weight 20g air dry soil in to breaker and add 50 ml distilled water. Stir at regular intervals for one hour.
2. In the mean time turn the pH meter on, allow to warm up, and standardize the glass electrode using both the standard buffers. Remember to adjust the temperature compensation knob to the temperature of the solution. Measure the pH of the sample suspension, stirring the suspension well just before introducing the electrodes and note down the reading.
3. Rinse the electrodes after each determination and carefully blot them dry with filter paper before the next determination. Standardize the glass electrode after every ten determinations.

Result:

The pH of given soil sample is …….

Questions:

What p and H stand for?

What is the soil: water ratio used in the determination of soil pH?

What is the utility of soil pH?

What are the factors affecting soil pH?

Reference:

1. Hesse. P.R. 1971. A textbook of Soil chemical analysis. John Murray. Pubi Ltd.50 Albemarle, Street, London
2. Jackson, M.L.1967. Soil chemical analysis. Practice-Hall of India Pvt. Ltd. New Delhi.

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Determination of electrical conductivity of soil

Principle :

The method is based upon the principle that conductivity of soil is nearly proportional to salts concentration.

Reagents :

Standard potassium chloride solution: 0.7456g of dry reagent grade potassium chloride is dissolved in freshly prepared double distilled water and made to one litre. At 25 ^{0}C it gives an electrical conductivity of 1411.8 x 10^{-4} (0.0014118) mhos/cm or 1.41 mmhos/cm . The conductivity bridge is to be calibrated and cell constant determined with the help of this solution. Even if the scale is marked directly in mhos/cm it is necessary to check and calibrate the instrument with the KCl solution.

Procedure :

10 g of soil is shaken intermittently with 25ml of distilled water in a 150 ml Erlenmeyer (conical) flask for one hour and allowed to stand. Alternatively, the clear extract after pH determination can be used for electrical conductivity measurement. The conductivity of the supernatant liquid is determined with the help of the salt (conductivity) bridge. The measurement of EC (expressed in mmhos/cm or dS/m.) is to be adjusted for known temperature (usually 25 ^{0}C) of the solution by setting the knob provided for this purpose.

Conversion factors:

TDS (m.eq/L) = EC (dS/m) ×10

TDS (ppm) = EC (dS/m) ×640

TDS (%) = EC (dS/m) ×0.064

Osmotic pressure (bars) = EC (dS/m) ×0.36

Result : The given soil sample contains,

EC (dS/m)	
TDS (m.eq/L)	
TDS (ppm)	
TDS (%)	
Osmotic pressure (bars)	

Inference: The given soil sample is……..

Questions

1) Why it is essential to measure the electrical conductivity of soil?
2) How will you measure the E.C.?
3) Name the instrument used for conductivity measurement?
4) What precautions will you take while measuring the E.C. ?

References:

Method of Soil Analysis : Part II (1965) Ed.C.A.Black Americal Socy of agronomy Madison , U.S.A.

Reference URL

- https://www.youtube.com/watch?v=C47w6B9NgL8

Determination of Cation Exchange Capacity of soil (by ammonium acetate method)

Principle :-

Soil is first saturated with normal ammonium acetate (pH 7.0). Excess ammonium (NH_4^+) ions and displaced cations are then removed by washing with alcohol. The amount of NH_4^+ ions retained by the soil is measured by steam distillation of NH4-saturated soil with MgO. During distillation evolved ammonia is absorbed in a boric acid solution as ammonium borate and the amount ammonium borate formed is determined by titration with standard sulphuric acid in presence of mixed indicator.

Equipments and Materials

Kjeldahl distillation set, shaker, Buchner funnel, Whatman No. 42 filter paper, balance, 800 ml Kjeldahl flask, 250 ml conical flask, 50 ml measuring cylinder.

Reagents :

- 1N ammonium acetate (pH 7.0): Either dilute 57 ml of glacial acetic acid (99.5%) to 800 ml with water containing 70 ml concentrated ammonia solution or dissolve 77.08g ammonium acetate (NH_400CCH_3) crystal in about 800 ml water. Cool and adjust pH to 7.0 with dilute acetic acid or ammonia solution. Make up volume to 1 liter.
- Ethanol, 60%: Dilute 630 ml of absolute alcohol (95%) to 1 liter with distilled water.
- Ammonium chloride crystal
- Magnesium oxide powder (MgO)
- Mixed indicator: Dissolve 0.5 g bromo cresol green and 0.1 g methyl red in 100 ml of 95% ethanol. Adjust the solution to the bluish purple midcolour at pH 4.5 with dilute NaOH or HC1. This indicator is pink at pH 4.2 or lower and bluish green as pH rises to 4.9 and above.
- Boric acid- indicator solution, 2%: Dissolve 20 g boric acid in about 800 ml hot distilled water. Cool, then add 20 ml of mixed indicator and adjust the pH of the solution by dilute NaOH or HCl until the bluish colour of the indicator weakens toward pink. Make up volume to 1 liter.

- Standard sulphuric acid, O.'IN: Dilute 2.8 rnl of concentrated sulphuric acid to I liter with distilled water and standardize against standard sodium carbonate (0.1 N) using methyl red indicator.
- Methyl red indicator, 0.5%: Dissolve 0.5 g indicator in 100 mi of 05% ethanol.
- Silver nitrate solution, 0.1 M: Dissolve 8.5 g silver nitrate (AgN03) in 500 ml distilled water and add 2 ml concentrated HNO_3 to it.

Procedure

- Take 10 g air dry processed soil in a 250 ml conical flask and add 50 ml of 1 N ammonium acetate solution.
- Shake the suspension for an hour and leave it for overnight.
- Filter the content through Whatman No. 42 filter paper fitted on Buchner funnel under suction. Transfer the soil completely to the filter paper.
- Leach the soil with neutral normal ammonium acetate solution for about ten times. Before adding fresh aliquot (20 ml) allow the leachate to be drained out completely.
- Leachate is preserved for determination of individual exchangeable cations.The soil left on the filter paper will be required for CEC determination.
- Wash the soil with 60% ethanol to remove excess ammonium acetate. To be confirmed add a pinch of ammonium chloride to the soil on the filter paper and continue washing until the leachate becomes free from chloride (test with $AgN0_3$).
- Transfer the soil with filter paper to an 800 ml distillation flask; add about 200 ml of water, little liquid paraffin and one spoonful of MgO.
- Distill and collect ammonia by absorbing in 50 ml of 2% boric acid mixed indicator solution taken in a 250 ml conical flask. Collect about 150 ml distillate.
- Titrate the distillate with standard sulphuric acid (0.1N) till pink colour appears.
- Run a blank distillation in a similar way without soil.

Calculations

Say,

Weight of soil taken = W g

Volume of standard sulphuric acid required for sample titration — V_s ml

Volume of standard sulphuric acid required for blank dtiation -$V_B$ml

Strength (N) of standard sulphuric acid - S

Cation exchange capacity, meq/100g = $(V_s - V_B) \times S \times (100/W)$

[As 1000 ml 1 N of any acid or alkali =1 equivalent of any cation

Or 1 ml 1 N of any acid or alkali = 1 milliequivalent of any cation]

Rating

Soil Class on CEC	**CEC** (meq/**100 g soil)**
Low	<10
Medium	10-25
High	25-45
Very high	>45

Result

Questions :

1. Define CEC, Explain factors affecting it.
2. Explain the significance of soil CEC in agriculture.
3. Describe the role of Neutral normal ammonium acetate and 60% alcohol in determination CEC.

Reference:

1. Laboratory manual for agricultural chemistry – A.Sankaram. Methods of soil analysis (1965) Part-II Ed. C.A. Blank, American Society of Agronomy Madison, U.S.A.

Reference URL

- https://www.youtube.com/watch?v=7rMmH5PI93Q&t=380s

Estimation of organic carbon and organic matter content in soil (Walkley and Black's method)

Principle

The organic carbon in organic matter is oxidized by known but excess of chromic acid ($K_2Cr_2O7 + H_2SO_4$). The excess chromic acid not reduced by organic matter is determined by back titration with std. $FeSO_4$ solution (redo titration), using diphenylamine or Ferroin indicator. The organic carbon content in soil is calculated from the chromic acid utilized (reduced) by it.

Reagents

1. Potassium dichromate solution 1N : Dissolve 49.04 g of dried regent grade potassium dichromate in distilled water and dilute to 1 liter.
2. Ferrous sulphate solution (0.5 N) : Dissolve 139g of reagent grade $FeSO_47H_2O$ in distilled water and add 15 ml of concentrated H_2SO_4 cool and dilute to 1 liter. Ferrous ammonium sulphate $Fe(NH_4)_2(SO_4)_2.6H_2O$ can also be used by dissolving 196.19 g of salt in 800 ml of water containing 20 ml of concentrated H_2SO_4 and making up to one liter and standardize the solution.
3. Diphenyl amine indicator: Dissolve 0.5 g of DPA in 100mL of conc. H_2SO_4.
4. Concentrated sulphuric acid : (Note less than 96%)
5. Phosphoric acid H_3PO_4 (85%)
6. Sodium fluoride NaF, (Solid).

Procedure

Transfer without loss 1g of soil sample finely ground and passed through 0.5 mm sieve into 500 ml Erlenmeyer flask. Add by means of a pipette 10 ml of 1 N Potassium dichromate solution and swirl the flask gently. Add 20 ml of conc. H_2SO_4 by measuring cylinder. Swirl the flask by hand for a minute or two and set aside on an asbestos pad for exactly half an hour. At the end of half an hour, add 200 ml of distilled water, 10 ml of H_3PO_4, 0.2 g of NaF and 1 ml of diphenylamine indicator and shake the content of flask. Titrate the contents of flask against 0.5 N ferrous sulphate or famous ammonium sulphate solution till the violet blue color changes to green. Note the burette reading at this point.

Reactions

Oxidation

i. $2K_2Cr_2O_7 + 8H_2SO_4$ ---------- $2K_2SO_4 + 2Cr_2(SO_4)_3 + 8H_2O + 60$

ii. $3C + 60 = 3CO_2$

iii. $K_2Cr_2O_7 + 6FeSO_4 + 7H_2SO_4$---------- $K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O$

Observation table to be recorded

Sr. No.	Observations	Value
1.	Name of the soil	
2.	Wt. of soil taken	
3.	Wt. of oven dry soil	
4.	Vol. of 1 NK_2Cr_2O7 added	
5.	Vo1. of 0.5 N $FeSO_4$ required for Blank (B)	
6.	Vo1. of 0.5 N $FeSO_4$ required for sample (T)	

Calculations

$$\text{Percent organic carbon} = (B - T) \times N \times 0.003 \times \frac{100}{\text{Wt. of soil (oven dry)}}$$

In this method recovery of organic carbon is 77% hence a correction factor of 100/77 = 1.3 is used to get correct value. (Express results on oven dry basis)

Percent organic matter = % organic carbon x 1.724.

Rating of soil for organic carbon :

1. Very low	Less than 0.20%
2. Low	0.21 to 0.40%
3. Moderate	0.41 to 0.60%
4. Moderately high	0.61 to 0.80%
5. High	0.81 to 1.00%
6. Very high	Greater than 1.0%

Result

Questions

1. Explain the principle involved in the estimation of organic carbon determination by Walk Ely and Black method.
2. Explain the role of the following regents in the estimation of organic carbon.
    a) K_2Cr_2O7 b) Ferrous sulphate c) Conc. H_2SO_4
3. Why per cent of organic carbon is multiplied by 1.724 to get per cent of organic matter?
4. Why is it necessary to multiply 1.3 to get exact amount organic carbon in soil?
5. Which indicator is used in organic carbon estimation?

References

1. Jackson, M.L. (1967). Soil Chemical Analysis. Prentice Hall of India, New Delhi, pp. 214-221.
2. Khanna, S.S. and D.V. Yadav, (1979). Practical Manual for introductory Courses in Soils. HAU, Hisser.
3. Hesse.P.R. (1971). A Text of Soil Chemical Analysis. John Murray, Publ Ltd. 50 Albemarle Street, London, WLX-4BD.

Reference URL

- https://www.youtube.com/watch?v=JCQxGqD--GE

Estimation of Mineralizable Nitrogen in Soil (Subbiah and Asija, 1956)

Principle:

The easily mineralizable nitrogen is estimated using alkaline KMnO4, which oxidizes the organic matter present in the soil and hydrolyzes the liberated ammonia which is condensed and absorbed in boric acid, and titrated against standard acid. The method has been widely adopted to get a reliable index of nitrogen availability in soil due to its rapidity and reproducibility. The process of oxidative hydrolysis is, however, a progressive one and hence a uniform time and heating temperature should be allowed for best results. Use of glass beads checks bumping while liquid paraffin checks frothing during heating.

Apparatus:

a) Nitrogen distillation unit or Kelplus distillation unit.
b) Distillation tube, conical flasks, pipettes, burette etc.

Reagents:

1) 0.32% KMnO4: Dissolve 3.2 g of $KMnO_4$ in distilled water and make the volume to one litre.
2) 2.5% NaOH: Dissolve 25 g of sodium hydroxide pellets in water and make the volume to one litre.
3) 2% Boric acid: Dissolve 20 g of boric acid powder in warm water by stirring and dilute to one litre.
4) Mixed Indicator: Dissolve 0.066 g of methyl red and 0.099 g of bromocresol green in 100 mL of ethyl alcohol. Add 20 mL of this mixed indicator to each litre of 2% boric acid solution.
5) 0.1M Potassium Hydrogen Phthalate: Dissolve 20.422 g of the salt in distilled water and dilute to one litre. This is a primary standard and does not require standardization.
6) 0.02M H_2SO_4: Prepare approximately 0.1M H_2SO_4 by adding 5.6 mL of conc. H_2SO_4 to about one litre of distilled water. From this, prepare 0.02M H_2SO_4 by diluting a suitable

volume (20 mL made to 100 mL) with distilled water. Standardize it against 0.1M NaOH solution.

7) 0.1M NaOH: Dissolve 4g NaOH in 100 mL distilled water. Standardize against potassium hydrogen phthalate.

Procedure:

1) Measure 20 mL of 2% boric acid containing mixed indicator in a 250 mL conical flask and place it under the receiver tube. Carefully dip the receiver tube in the boric acid solution.
2) Take 2.5 g of soil sample in a 350mL distillation tube (run a blank without soil in a 350 mL distillation tube before started sample).
3) Moisten the soil with about 10 mL of distilled water, wash down the soil, if any, adhering to the neck of the flask.
4) Add 25 mL (Soil:0.32% $KMnO_4$::1:5) of 0.32% of $KMnO_4$ solution in the distillation tube manually or instrumentally.
5) Add a few glass beads or broken pieces of glass rod.
6) Add 2-3 mL of paraffin liquid, avoiding contact with upper part of the neck of the flask.
7) Add 25 mL of 2.5% NaOH solution and immediately attach to the rubber stopper fitted in the alkali trap.
8) Press the run switch and continue distillation until about 100 mL of distillate is collected.
9) First remove the conical flask containing distillate and then remove the distillation tube to avoid back suction.
10) Titrate the distillate against 0.02M H_2SO_4 taken in burette until pink colour starts appearing.
11) Carefully remove the distillation tube and drain the contents in the sink.

**If brown colour not appearing in distillation tube at the time of distillation (after 6-7 min) then add 5-10 mL of 2.5% NaOH solution

Precautions:

a) Check the tap water and distilled water.

b) Dip the delivery tube end in the receiver containing standard boric acid solution before adding NaOH solution in the distillation flask.

c) Close the distillation tube to the distillation apparatus tightly then add NaOH to avoid the loss of ammonia.

d) During distillation, first remove the receiver flask and then distillation tube.

e) Collect about 100 mL of distillate in 10 minutes steady distillation.

Observation:

Sl No.	**Initial burette reading (mL)**	**Final burette reading (mL)**	**mL of (0.02N H2SO4)**

Calculation:

Mineralizable N (kg/ha) = Volume of acid used to neutralize ammonia in the sample = (A – B) mL

Where,

A = Volume of 0.02N H_2SO_4 used in titration of soil sample against ammonia absorbed in boric acid.

B = Volume of 0.02N sulphuric acid used in blank titration.

N= Normality of sulphuric acid

1 mL of 0.02N sulphuric acid = 0.56 mg N (1000 mL of 1N H_2SO_4 = 14 g Nitrogen).

****[Rating: Low = < 280 kg/ha, Medium= 280-560 kg/ha, High= > 560 kg/ha]**

Result:

The available nitrogen status of the soil is _____ kg /ha.

Video Link:

https://www.youtube.com/watch?v=l10NBPglZzI

Estimation of Available Phosphorus in Acid Soil

Principle:

In soil, phosphorus exists in the form of various types of orthophosphates. A very small fraction of these is available to plants at a given time. Available phosphorus content of soil consists mainly of Ca-, Al-, and Fe-P. In the neutral and alkaline soils particularly, Ca-P is the dominant fraction. Organic -P fraction is also in considerable amount, but is usually not included in the determination of available phosphorus. A large number of extraction reagents from Dyer's 1% citric acid to some of the multi nutrient extractants, buffer solutions, acids and chelating agents have been suggested for available phosphorus from time to time. However, no single extractants appears to be suitable for all types of soils. Two types of extraction methods are more popularly adopted. Under acidic conditions, Bray's P-1 (or Bray No. 1), which involves soil extraction with a solution consisting of 0.03 (N) NH_4F and 0.025 (N) HCl is widely followed. The fluoride complexes Al and Fe in soil, thus releasing some bound P besides the easily acid soluble P (largely Ca-P). This extractant is suitable for soils containing less than 2% calcite or dolomite because in calcareous soils, carbonates quickly neutralize the acid, resulting in less extraction of P.

Bray's P-1 (Bray and Kurtz, 1945)

Apparatus and Instruments:

a) Spectrophotometer
b) Shaker
c) Pipettes
d) Beakers
e) Conical flasks
f) Volumetric flasks

Reagents:

1. Bray Extractant No 1 (0.03M NH_4F in 0.025M HCl): Dissolve 1.11 g of NH_4F (AR) in one litre of 0.025N HCl.

2. Dickman Bray's reagent (Molybdate reagent): Dissolve 15.0 g $(NH_4)_2MoO_4$ in 300 mL warm distilled water, cool and add the solution to 350 mL of 10 N HCl solution gradually with stirring. Dilute to one litre with distilled water.
3. Stannous chloride solution (40 % Stock Solution): Dissolve 10 g $SnCl_2.2H_2O$ in 25 mL of concentrated HCl. Add a piece of pure metallic tin and store the solution in a glass stoppered bottle.
4. Stannous chloride solution (Working Solution): Dilute 1 mL of the stock solution of stannous chloride to 66.0 mL with distilled after just before use. Prepare fresh dilute solution every working day.

Preparation of the Standard Curve:

Dissolve 0.439 g of pure dry KH_2PO_4 in about half a litre of distilled water. About 25 mL 7N H_2SO_4 is added and made up to one litre with distilled water. This solution contains 100 ppm stock solution of P (100 mg P /L). Preserve this as a stock standard solution of phosphate. From this, a 2 ppm P 12 solution is made. Take 0,1, 2, 3, 4, 5 and 6 mL of this 2ppm solution to 25 mL volumetric flasks. To these 5 mL of extracting reagent (Bray's) is added as described above by adding Bray's No. 1, 5 mL of Dickman Bray's reagent, 1 mL $SnCl_2$ and make the volume with distilled water and take reading as per sample readings.

Procedure:

a) Take 2.5 g of soil and 25 mL of the Bray's reagent (1:10 Soil: Solution) are shaken for 5 minutes in 250 mL conical flask and filtered.
b) Take 5 mL of the filtered soil extract with a bulb pipette in a 25 mL volumetric flask. To avoid interference of fluoride 7.5 mL of 0.8 M boric acid (50g H_3BO_3 per litre) can be added to 5 mL of the extract.
c) Add 5 mL of the molybdate reagent and add about 10 mL distilled water, shake and add 1 mL of the dilute $SnCl_2$ solution with a pipette. Make up the volume with distilled water and shake thoroughly.
d) Read the blue colour after 10 minutes on the spectrophotometer at 660 nm wavelength after setting the instrument to zero with the blank prepared similarly.

e) **Phosphorus is determined spectrophotometrically by Dickman and Bray's (Dickman and Bray, 1940) method.

Estimation of Available Phosphorus in Alkaline soil

Principle:

The other and most widely used extractant is the 0.5 (M) $NaHCO_3$ solution at pH 8.5. The reagent is most suitable for neutral to alkaline soils and is designed to control the ionic activity of calcium through solubility product of $CaCO_3$, thus extracting the most reactive forms of P from Al-, Fe-, and Ca- phosphates. Phosphorus in the extract can be determined using suitable method of colour development and measuring the colour intensity at an appropriate wavelength.

Olsen's Method (Olsen et al., 1954)

Apparatus and Instruments:

a) Spectrophotometer
b) Shaker
c) Pipettes
d) Beakers
e) Conical flasks
f) Volumetric flasks

Reagents:

a) 0.5 M $NaHCO_3$: Dissolve 42 g Sodium bicarbonate in 1 litre of distilled water and adjust the pH to 8.5 by addition of dilute NaOH or HCl. Filter it, if necessary.
b) Activated carbon –Darco G 60 or P free charcoal.
c) Molybdate reagent: Same as for the Bray's Method No. 1 except that use 400mL of 10 N HCl instead of 350 mL/litre.
d) Stannous chloride solution: Same as in Bray's Method No. 1.

Preparation of the Standard Curve:

Dissolve 0.439 g of pure dry KH_2PO_4 in about half a litre of distilled water. About 25 mL 7 N H_2SO_4 is added and made up to 1 litre with distilled water. This solution contains 100 ppm stock solution of P (100 mg P /L). Preserve this as a stock standard solution of phosphate. From this, a 2 ppm P solution is made. Take 0,1, 2, 3, 4, 5 and 6 mL of this 2-ppm solution to 25 mL volumetric flasks. To these 5 mL of extracting reagent (Olsen's) is added as described above by

adding, 5 mL of molybdate reagent (for Olsen's method), 1 mL $SnCl_2$ and make the volume with distilled water and take the colour reading on the spectrophotometer at 660 nm wavelength. Plot the absorbance reading against P mg/L and prepare the standard curve by adjoining points.

Procedure:

1) Weigh 2.5 g soil sample in a 250 mL conical flask.
2) Add 1-2 g of Darco G 60 or P free charcoal and 50 mL of the bicarbonate extractant (0.5M $NaHCO_3$, pH8.5).
3) Shake for 30 minutes on the mechanical shaker and filter through Whatman No. 42 filter paper.
4) Transfer 5 mL of filtrate in to 25 mL volumetric flask and gradually add 5 mL of ammonium molybdate containing 400 mL of 10 N HCl.
5) Stir slowly and carefully to drive out the CO_2 evolved.
6) After stop of bubbliness, add distilled water washing down the sides and bring the volume to 22 mL
7) Add 1 mL of freshly prepared diluted $SnCl_2$, shake a little and make up the volume.
8) Run a blank without soil in similar manner.
9) Read the blue colour after 10 minutes on the spectrophotometer at 660 nm wavelength (or red filter for colorimeter) after setting the instrument to zero with the blank prepared similarly.

Precautions:

a) Clean all glassware with distilled water.

b) Start filtration quickly after shaking

c) Before taking reading, warm up the instrument minimum 30 min.

d) Take reading after 10 min.

Observation:

Sl No.	**Spectrophotometer reading (Abs)**

Calculation:

Available P (kg/ha) = R × dilution factor × 2.24

Where,

R = ppm P in the sample (read from standard curve)

Dilution factor = 50/2.5 × 25/5 = 100 (Weight of the soil taken = 2.5 g,

Volume of the extract = 50 mL;

Volume of the aliquot taken for estimation = 5 mL and

Volume made for estimation (dilution = 5 times) = 25 mL)

Rating:

P kg/ha	Low	Medium	High
Bray's Method	< 30	30-60	>60
Olsen's Method	< 15	15-30	>30

Conversion factor: P × 2.29 = P2O5

Result:

The available phosphorus status of the soil is _____ kg /ha

Video link:

https://www.youtube.com/watch?v=y2FsPH5ZDXY

https://www.youtube.com/watch?v=R1lFrMjoraE

Estimation of available Potassium in Soils (Jackson, 1973)

Principle:

Potassium in soil exists as water soluble, exchangeable, non-exchangeable (fixed) and lattice-K. The first two forms constitute only a small part, normally not more than 1% of the total content and are considered to be easily available to plants. On amount basis, the exchangeable form (K^+ ions adsorbed on exchange sites) far exceeds water-soluble fraction and the two are in equilibrium with each other. Only when these two forms are depleted, a part of the non-exchangeable K moves to the exchange sites and soil solution. This movement is a slow process and not sufficient to meet K requirement of plants. Therefore, most of the methods suggested are based on the determination of easily available fractions *ie.* water soluble and exchangeable K. attempts, however have been made to include a part of the non-exchangeable fraction in certain methods to increase the prediction value. Thus, methods for both available and non-exchangeable k have been in use. For all practical purpose, the extraction and estimation of exchangeable K along with water soluble K is good enough. The ammonium ions are very close in size to K^+ and replace the latter efficiently. During the estimation, accurate burns clearly and does not leave any residue on the burner of the flame photometer.

Instruments:

a) Flame Photometer
b) Glass electrode pH meter

Reagents:

1. 1(N) ammonium acetate solution: Dissolve 77.08 g of ammonium acetate ($NH_4C_2H_3O_2$) in about 800 mL of distilled water and adding to its 57 mL of glacial acetic acid and 68 mL of ammonium solution (sp.gr. 0.91) followed by dilution to one litre and adjusting pH 7.0 after cooling.
2. Standard potassium solution: Dissolve 1.908 g pure KCl (oven dried) in one litre of distilled water. This solution contains 1000 mg KCl. Dilute suitable volumes of this solution to get 100mL of working standards containing 0, 5, 10, 15, 20, 25 and 30 mg KCl. The working standards should be making up the volume with ammonium acetate solution.

Procedure:

a) Weigh 5 g of soil sample in 250 mL conical flask and add 25 mL of 1 N ammonium acetate solution and shake for 5minutes.
b) Filter through Whatman No. 1 filter paper.
c) Measure K concentration in the filtrate flame photometer.

Preparation of the Standard Curve:

Set up the flame photometer by atomizing 0 and 20 mg K/mL solutions alternatively to 0 and 100 reading. Atomize intermediate working standard solutions and record the readings. Plot these readings against the respective potassium contents and connect the points with a straight line to obtain a standard curve.

Precautions:

1. Start filtration quickly after shaking

2. Before taking reading, warm up the instrument minimum 30 min

3. Check the flame level

Observation:

Sl No.	**Flame photometer Reading (ppm)**

Calculation:

K (kg/ ha) = R × dilution factor × 2.24

Where,

Dilution factor = 25/5 (1:5:: Soil : Solution)

R = content of K (mg) in the sample, as read from the standard curve.

****[Rating (K kg/ha): Low = < 120 kg/ha, Medium= 120-280 kg/ha, High= > 280 kg/ha Conversion factor: %K × 1.2047 = %K2O]**

Result:

The available potassium status of the soil is _____ kg /ha.

Video link:

https://www.youtube.com/watch?v=IxK_I-Q2e8w

Estimation of available Ca and Mg in Soils

Principle:

The method described here was developed by Lavkulich (1981) for standard analysis of a wide range of soil types. It involves fewer steps than some other similar methods such as that of Mc Keague (1978). Problems with this approach to measuring exchangeable cations and CEC have been discussed extensively in the literature (Chapman 1965; Bache 1976; Rhoades 1982; Thomas 1982) but we agree with the conclusion of Thomas (1982) that "there is no evidence at the present time that cations other than NH_4^+ give results that are less arbitrary than those obtained using NH_4^+." Errors due to the dissolution of $CaCO_3$ and gypsum will result in an excess of Ca^{2+} being extracted by NH4+ and a decrease in the amount of NH_4^+ retained due to competition between Ca^{2+} and NH_4^+ during equilibration in the saturating step. In soils containing these minerals, exchangeable Ca will be too high and total CEC too low. The former problem cannot easily be corrected (Thomas 1982); however, more accurate measurement of CEC in this type of soil can be obtained by using the method described by Rhoades (1982). Fixation of K^+ and NH_4^+ in phyllosilicates can result in either an over- or underestimation of exchangeable K^+ when NH_4^+ is used as an extractant depending on whether the NH_4^+ moves through the interlayer positions replacing the K^+ or whether it causes the collapse of the edges preventing further exchange. Compared to the other methods presented in this chapter, this method uses a larger sample size, which helps to decrease the sample to sample variability. Another advantage of this procedure is that there are no decantation steps that can cause the loss of sample, particularly in the case of organic soils. The method described below can be used to measure either exchangeable cations and CEC or just exchangeable cations. In the latter case, the sum of exchangeable cations (including Al) could be 26 used as an estimate of CEC. Due to the high pH of the extracting solution, the amount of Al measured will usually be lower than that displaced by $BaCl_2$ or KCl.

1. **Calcium by Versenate (EDTA) method:**

Apparatus:

a) Shaker

b) Porcelain dish
c) Beakers
d) Volumetric/conical flask

Reagents:

a) Ammonium chloride: Ammonium hydroxide buffer solution: Dissolve 67.5 g ammonium chloride in 570 ml of conc. ammonium hydroxide and make to 1 litre.
b) Standard 0.01N calcium solution: Take accurately 0.5 g of pure calcium carbonate and dissolve it in 10 ml of 3N HCl. Boil to expel CO_2 and then make the volume to 1 litre with distilled water.
c) EDTA solution (0.01N): Take 2.0 g of versenate, dissolve in distilled water and make the volume to 1 litre. Titrate it with 0.01N calcium solution and make necessary dilution so that its normality is exactly equal to 0.01N.
d) Muroxide indicator powder: Take 0.2 g of muroxide (also known as ammonium purpurate) and mix it with 40 g of powdered potassium sulphate. This indicator should not be stored in the form of solution, otherwise it gets oxidized.
e) Sodium diethyl dithiocarbamate crystals: It is used to remove the interference of other metalions.
f) Sodium hydroxide 4 (N): Prepare 16% soda solution by dissolving 160 g of pure sodium hydroxide in water and make the volume to 1 litre. This will give pH 12.

Procedure:

1. Take 5 g air dried soil sample in 150 ml conical flask and add 25 ml of neutral normal ammonium acetate. Shake on mechanical shaker for 5 minutes and filter through Whatman No.1 filter paper.
2. Take a suitable aliquot (5 or 10 ml) and add 2-3 crystals of carbamate and 5 ml of 16% NaOH solution.
3. Add 40-50 mg of the indicator powder. Titrate it with 0.01N EDTA solution till the colour gradually changes from orange red to reddish violet (purple). It is advised to add a drop of EDTA solution at an interval of 5 to 10 seconds, as the change of colour is not instantaneous.

4. The end point must be compared with a blank reading. If the solution is over titrated, it should be back titrated with standard calcium solution and exact volume used is thus found.
5. Note the volume of EDTA used for titration.

Observation:

Sl. No.	**Initial burette reading (mL)**	**Final burette reading (mL)**	**Final volume (mL)**

Calculation:

If N_1 is normality of Ca^{2+} and V_1 is volume of aliquot taken and N_2V_2 are the normality and volume of EDTA used, respectively, then

$N_1V_1 = N_2V_2$

Or, $N_1 = N_2V_2/V_1$ =Normality of EDTA X Volume of EDTA/ml of aliquot taken

Hence, N_1 (Normality) = equivalent of Ca^{2+} present in one litre of aliquot

Hence, Ca^{2+} (me/litre) = Normality of EDTA X Volume of EDTA X 1000/ ml of aliquot taken

When expressed in soil weight basis,

Ca^{2+} me/100g soil = 100/wt. of soil X extract volume/1000 X Ca as me/litre

Result:

The Ca content in soil is _____ me/100 g soil.

Video link:

https://www.youtube.com/watch?v=hTy9JBllUVg

2. Calcium plus Magnesium by Versenate (EDTA) method

Magnesium in solution can be titrated with 0.01N EDTA using Eriochrome black T dye as indicator at pH 10 in the presence of ammonium chloride and ammonium hydroxide buffer. At the end point, colour changes from wine red to blue or green. When calcium is also present in the solution this titration will estimate both calcium and magnesium. Beyond pH 10 magnesium is not bound strongly to Erichrome black-T indicator to give a distinct endpoint.

Apparatus:

a) Shaker
b) Porcelain dish
c) Beakers
d) Volumetric/conical flask

Reagents:

1. EDTA or Versenate solution (0.01N): Same as in calcium determination.
2. Ammonium chloride ammonium hydroxide buffer solution: Same as in calcium determination.
3. Eriochrome black T indicator: Take 100 ml of ethanol and dissolve 4.5 g of hydroxyl amine hydrochloride in it. Add 0.5 g of the indicator and prepare solution.
4. Hydroxylamine hydrochloride removes the interference of manganese by keeping it in lower valency state (Mn2+). Or mix thoroughly 0.5 g of the indicator with 50 g of ammonium chloride.

5. Sodium cyanide solution (2%) or sodium diethyl dithiocarbamate crystals: This is used to remove the interference of copper, cobalt and nickel.

Procedure:

a) Take 5 g air dried soil in 150 ml flask, add 25 ml of neutral normal ammonium acetate solution and shake on a mechanical shaker for 5 minutes and filter through Whatman No.1 filter paper.
b) Pipette out 5 ml of aliquot containing not more than 0.1 me of Ca plus Mg. If the solution has a higher concentration, it should be diluted.
c) Add 2 to 5 crystals of carbamate and 5 ml of ammonium chloride-ammonium hydroxide buffer solution. Add 3-4 drops of Eriochrome black-T indicator.
d) Titrate this solution with 0.01N versenate till the colour changes to bright blue or green and no tinge of wine-red colour remains.

Calculation:

If N_1 and V_1 are normally (concentration of Ca^{2+} and Mg^{2+}) and volume of aliquot taken and N_2V_2 are the normality and volume of EDTA used respectively, then

$N_1V_1 = N_2V_2$

Or, $N_1 = N_2V_2/V_1$ = Normality of EDTA X Volume of EDTA/ ml of aliquot taken

Here, N_1 (Normality) = equivalents of Ca^{2+} plus Mg^{2+} me/litre = Normality of EDTA X Volume of EDTA X 1000/ ml of aliquot taken

Milliequivalent (me) of Mg^{2+} = me (Ca^{2+} + M^{2+}) – me of Ca^{2+}

When expressed on soil weight basis

Ca^{2+} + Mg^{2+} me/100g soil = 100/wt. of soil X extract volume/ 1000 X Ca^{2+} + Mg^{2+} me/litre

Observation:

Sl No.	**Initial burette reading (mL)**	**Final burette reading (mL)**	**Final volume (mL)**

Result:

The Ca + Mg content in soil _____ me/100 g soil

Video link:

https://www.youtube.com/watch?v=yDGMgYwHoVA

Estimation of Available Sulphur in Soils

Principle:

Besides some amount in the soil solution, available sulphur in mineral soils mainly adsorbed SO42- ions. Both CaCl2 and phosphate solutions (as monocalcium phosphate) are generally used for replacement of the adsorbed SO_4^{2-} . Use of Ca salts have a distinct advantage over those Na or K, as Ca prevents deflocculation in heavy textured soils and leads to easy filteration. SO42- in the extract can be estimated turbidimetrically using a colorimeter/ spectrophotometer/ autoanalyzer. A major problem arises when the amount of extracted sulphur is too low to be measured precisely.

Apparatus and Instruments:

a) Spectrophotometer
b) Mechanical Shaker
c) Conical flasks
d) Volumetric flask

Reagents:

1. 0.15% $CaCl_2$: Take 1.5 g $CaCl_2$ in a volumetric flask and makeup volume 1000 mL mark with distilled water.
2. Sodium acetate acetic acid buffer (pH 4.8) [CH_3COONa-CH_3COOH]: 1000 mL volumetric flask add 100 g sodium acetate, add distilled water 500 mL to mixed, add 30 mL 99.5% acetic acid, shake to dissolved sodium acetate the makeup volume 1000 mL mark.
3. 0.25% Gum acacia solution: Dissolve 0.25g of chemically pure gum acacia powder in 100 mL of hot water and filter in hot condition through Whatman No.42 filter paper. Cool and and keep in refrigerator.
4. Barium chloride crystal AR grade ($BaCl_2.2H_2O$): Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.
5. 100 ppm S solution: Take 0.5434 g of K_2SO_4 and add 500mL distilled water to dissolved then make up to 1000 mL mark of volumetric flask

Procedure:

a) Weigh 5 g soil sample in a 250 mL conical flask.
b) Add 25 mL of 0.15%$CaCl_2$.
c) Shake with 230 rpm for 30 minutes on the mechanical shaker and filter through Whatman No. 42 filter paper.
d) Transfer 10 mL of filtrate in to 25 mL volumetric flask and gradually add 10 mL of sodium acetate acetic acid buffer.
e) Add 1 g $BaCl_2.2H_2O$ powder and shake well.
f) Then add 1mL gum acacia and make up the volume with distilled water.
g) Run a blank without soil in similar manner.
h) Measure the turbidity intensity at 440 nm (blue filter).
i) Run a blank side by side.

Preparation of standard curve:

Put 0, 1.25 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 mL of the working standard solution (10 mg S/litre) into a series of 25 mL volumetric flasks to obtain 0, 0.5, 1.0, 2.0,3.0, 4.0, 5.0 and 6.0 ppm. Develop turbidity as described above for sample aliquots. Read the turbidity intensity and prepare the curve by plotting readings against sulphur concentrations.

Precautions:

1. Before taking reading, warm up the instrument minimum 30min
2. Take reading within 30min

Observation:

Sl No.	**Spectrophotometer (Abs)**

Calculation:

Available S in soil (mg/kg) = R × 25/5 × 25/10 = R × 12.5

Where,

R stands for the quantity of sulphur in mg as obtained on X-axis against an absorbance reading (Y-axis) on standard curve.

5 is the weight of the soil sample in g and 25 is the volume of the extractant in mL. 10 is the volume of filtrate solution in mL in which turbidity is developed and make up the volume to 25mL.

Result:

The available sulphur status of the soil is _____ mg/kg or ppm.

Video link:

https://www.youtube.com/watch?v=8FsnaWA1Jp4

Estimation of Total Nitrogen in Plants (Kjeldahl method)

Principle:

Nitrogen mostly present in soil in organic form. Relatively small amount of nitrogen usually occurs in ammonium and nitrate form, the available form. The Kjeldahl method of total N determination includes both organic and ammonium form and with modification nitrate form of N can also be included.

Plant samples for N determination are digested in sulphuric acid at a temperature below 360 and 410^{o}C. Below 360^{o}C, the digestion process is very slow or incomplete but above 410^{o}C some of the ammonia may be lost. The rate of digestion is accelerated by using copper sulphate as a catalyst and anhydrous sodium sulphate or potassium sulphate to raise the boiling temperature of H_2SO_4. The temperature of digestion is carefully regulated for best and complete digestion, which normally takes less than 2 hours. On completion of digestion, the samples are cooled and diluted as concentrated alkali is to be added to H_2SO_4 digest for distillation. The distilled ammonia is quantitatively adsorbed in boric acid and titrated against standard acid.

Apparatus:

1. Kelplus digestion and distillation unit.
2. Distillation tube
3. Conical flasks
4. Burettes
5. Pipettes

Reagents:

1. Sulphuric acid – H_2SO_4 (93-98%)
2. 40% sodium hydroxide solution: Dissolve 350 g solid NaOH in water and dilute to one litre
3. 4% Boric acid: Dissolve 40 g of boric acid powder in warm water by stirring and dilute to one litre

4. Mixed Indicator: Dissolve 0.066 g of methyl red and 0.099 g of bromocresol green in 100 mL of ethyl alcohol. Add 20 mL of this mixed indicator to each litre of 4% boric acid solution
5. 0.2N H_2SO_4: Prepare approximately 0.2N acid solution and standardize against 0.1N sodium carbonate
6. Salicyclic acid or Devarda's alloy: Using for reducing NO_3 to NH_4^+, if present in the sample
7. Digestion mixture: 10 g Potassium sulphate (K_2SO_4) or anhydrous sodium sulphate (AR grade) + 1 g catalyst mixture (20 parts of Copper sulphate [$CuSO_4.H_2O$ (AR grade)] powder and 1part metallic selenium powder.

Procedure:

a) Take 1 g soil or 0.5 g plant/seed sample in distillation tube.
b) Add 3-5 g digestion mixture and add 10 mL concentrated H_2SO_4.
c) Heat low temperature for 30 min. and then increased heat up to 410 °C and digest for 1-2 h or until solution is crystal clear green colour and then stop the digestion. If necessary, add small amount of paraffin or glass beads to reduce frothing.
d) Remove the flask from the heater and cool, add 50 mL water and transfer to distilling flask for distillation.
e) At the time of distillation, add 30 mL of 40% NaOH in the distilling flask in such a way that the contents do not mix.
f) The contents are distilled for 5 minutes by pressing run bottom of Kelplus distillation unit and the liberated ammonia collected in a conical flask (250 mL) containing 20 mL of 4% boric acid solution with mixed indicator.
g) First remove the conical flask containing distillate and then remove the distillation tube to avoid back suction.
h) Titrate the distillate against 0.2N H_2SO_4 taken in burette until pink colour starts appearing.
i) Carefully remove the distillation tube and drain the contents in the sink.

Precautions:

1. The material after digestion should not solidify.
2. No NH_4 should be lost during distillation.
3. If the indicator changes colour during distillation, determination must be repeated using either a smaller sample weight or a larger volume of standard acid.
4. Standard the 0.2N H_2SO_4 by using standard solution.
5. Check the tap water and distilled water.
6. Dip the delivery tube end in the receiver containing standard boric acid solution before adding NaOH solution in the distillation flask.
7. Close the distillation tube to the distillation apparatus tightly then add NaOH to avoid the loss of ammonia.
8. During distillation, first remove the receiver flask and then distillation tube.
9. Collect about 100 mL of distillate in 10 minutes steady distillation.

Observation:

Sl No.	**Initial burette reading**	**Final burette reading**	**mL of (0.2N H_2SO_4)**

Calculation:

N (%) = (A- B) X Normality of H2SO4 X 0.014 X 102/ Wt. of the sample

Volume of acid used to neutralize ammonia in the sample = (A – B) mL

Where,

A = Volume of 0.2N H2SO4 used in titration of soil sample against ammonia absorbed in boric acid.

B = Volume of 0.2N sulphuric acid used in blank titration.

Normality of sulphuric acid = 0.2

(1000 mL of 1N H2SO4 = 14 g Nitrogen).

Protein Content:

Crude protein was determined by multiplying percentage of nitrogen content in seeds of crops with a factor of 6.25 (Tai and Young 1974).

% Crude Protein = % N x 6.25

Result:

The total nitrogen content in seed/plant is _____ %.

Video link:

https://www.youtube.com/watch?v=3uQOezl_Uos

Estimation of Phosphorus in Plants

Sample digestion for plant analysis of P, K and S:

For the release of mineral elements from plant tissues, dry ashing and wet oxidation are the two widely adopted methods. Dry ashing is carried out usually at an ignition temperature of 550 to 600^{o}C followed by its extraction in dil. HCl or H_2SO_4 for determining various elements. Ashing at temperature exceeding 600^{o}C leads to considerable volatilization loss of P and K. to prevent this loss of P, dry ashing is carried out in presence of an alkali like Mg (NO_3) in alcohol. Volatilization loss of S and Cl also takes place during ignition, which can be prevented by adding Na_2CO_3. A part of P also gets occluded, causing lot of error. For these reasons and for being comparatively more time taking, dry ashing is only occasionally adopted. Wet oxidation employs oxidizing acids like HNO_3- H_2SO_4- $HClO_4$ tri- acid mixture or HNO_3- $HClO_4$ di- acid. Use of $HClO_4$ avoids the volatilization loss of K and provides a clear solution while H_2SO_4 helps in completing oxidation. $HClO_4$ on heating produces anhydrous $HClO_4$ which dissociates into nascent chlorine and oxygen, increasing the oxidation efficiently at high temperature. Direct contact with $HClO_4$ with plant samples might lead to explosion and fire, hence the pre-digestion of samples in HNO_3 is preferred. Digestion with HNO_3- $HClO_4$ instead of the tri-acid mixture is also adopted specially when S is also to be determined in the same digest.

Principle:

Phosphorus in the aliquot can be determined using the methods based on molybdophosphoric blue colour developed by reduction of the hetero- poly complex or by vanadomolybdophosphoric yellow colour method. The vanadomolybdophosphoric yellow colour method is suitable for P determination in plant extracts.

Apparatus:

a) Spectrophotometer
b) Volumetric flask
c) Pipette
d) Conical flasks
e) Beaker

Reagents:

1. Triacid mixture: Look over the procedure of sample digestion
2. Ammonium molybdate ammonium vanadate solution: Dissolve 22.5 g of $(NH_4)_6\ MO_3O_7\ .4H_2O$ in 400 mL of distilled water in a beaker. Take 1.25 g of ammonium vanadate in another beaker, add 300 mL distilled water and boil it. Add ammonium vanadate solution to the ammonium molybdate solution and cool the contents. Add 250 mL of concentrated HNO_3 and dilute it to 1 litre.
3. Phosphate standard solution: Take 0.22 g of AR grade KH_2PO_4 in a beaker and dissolve in distilled water, transfer the solution to a 1 litre volumetric flask and make up the volume. This solution contains 50 ppm of P.

Procedure:

a) After digestion (see digestion of Seed and Plant samples), take 10 mL filtered solution into a 25 mL volumetric flask. Add 10 mL ammonium molybdate-ammonium vanadate solution and make the volume to 25 mL with distilled water. Yellow colour will develop after 15-20minutes.

b) From the 50ppm standard solution of P pipette out 0, 2, 4, 6, 8 and 10 mL and transfer in 50 mL volumetric flaks then add 10 mL of ammonium molybdate ammonium vanadate solution and make the volume. This will contain 0, 2, 4, 6, 8 and 10 ppm P.

c) Measure the colour intensity of standard solutions at a wave length of 470 nm or by using blue filter in a Spectrophotometer. Prepare the standard curve by plotting concentration of P on X-axis and Spectrophotometer readings on Y-axis on a graph paper.

d) Take readings of seed/plant samples in the similar manner that of standard.

Precautions:

1. The temperature of the digestion mixture during digestion should not exceed 230^0C.
2. The reading of the P in solution should be taken after 30 minutes from the development of yellow colour.
3. Ammonium molybdate-ammonium vanadate solution should be stored in a coloured bottle to prevent oxidation.

4. A clear and white residue in flask should remain after digestion. In case of incomplete digestion, the material should be again digested after addition of 5 mL of triacid.

Observation:

Sl No.	**Spectrophotometer Reading (Abs)**

Calculation:

P (mg/kg) in plant sample = R × d.f.

Where,

R = ppm reading of plant from standard curve

Dilution factor (d. f.) = Volume of extractant/ Weight of soil X Final volume of solution/ Volume of digest extract

= 100 X 25/ 0.5 X 10 = 500

Where,

Weight of plant sample = 0.5 g, Volume of digested extract prepared = 100 mL, Volume of digested extract taken = 10 mL, Volume of final coloured extract prepared = 25 mL

Results:

P content in sees/plant _____ mg/kg.

** **% P = P ppm / 10000**

Video link:

https://www.youtube.com/watch?v=y2FsPH5ZDXY

Estimation of Potassium in Plants

Principle:

Potassium in the acid digest of plant samples can be determined using flame photometer. Depending on the concentration of K in the plant sample, the digest can be used either directly or after dilution foe flame photometric determination.

Apparatus:

a) Flame photometer
b) Volumetric flask
c) Pipette
d) Beaker
e) Conical flask
f) Funnel
g) Hot plate

Reagents:

1. Triacid digestion: see digestion of Seed and Plant samples.
2. Potassium standard solution: Dissolve 1.9103 g of AR grade KCl in distilled water. Transfer it to 1 litre volumetric flask and make the volume. This solution contains 1000 ppm of K. To prepare 100 ppm solution takes 10 mL of 100 ppm K solution in a 100 mL volumetric flask and make up its volume.

Procedure:

a) Digest the plant sample with triacid as per the method given in digestion of Seed and Plant samples and filtered the solution and make the volume in 100 mL volumetric flask.
b) Prepare 0, 2, 4, 6, 8 and 10 ppm K solution by taking 0, 2, 4, 6, 8 and 10 mL of 100 ppm K solution in 100 mL flask respectively and making up their volume.
c) Feed the standard in flame photometer and standardized the instrument on low and high level of K content and then take the readings in ppm of K content.
d) If the readings of sample are showed over calibration then dilute the extract 5 to 10 times or more as required.

Precautions:

1. Extract should be clear and it should be prepared from double distilled water otherwise it clogs the sucking capillary.
2. The air pressure should be maintained steadily at 0.48 kg/cm^2 to get uniform flame and proper atomization.
3. Do not feed K solutions having higher concentration than that prescribed for the instrument.
4. Be sure that the filter used in flame photometer is of potassium.
5. After taking 8-10 readings feed distilled water and then again take the 43 sample readings.
6. After completion of reading close the gas then after some time stop the air supply.

Observation:

Sl No.	**Flame Photometer Reading (ppm)**

Calculation:

K (mg/kg) content in seeds/plant = R × d.f

Where,

R = ppm reading of from standard curve

Dilution Factor (d. f.) = Volume of extractant/ Volume of digested extract

= 100/ 0.5 = 200

Where,

Weight of plant sample = 0.5 g,

Volume of digested extract prepared = 100 mL.

Results:

K content in seeds/plant is _____ ppm.

**** % K = K ppm / 10000**

Video Link:

https://www.youtube.com/watch?v=IxK_I-Q2e8w

Estimation of Total Sulphur in Plants

Principle:

Since, dry ashing leads to volatilization loss of S present in the organic combination, and the wet oxidation based on tri- acid mixture includes H_2SO_4, both of these methods cannot be used for S determination in plant samples. Therefore, HNO_3- $HClO_4$ digest of plant is conveniently used. Turbidimetric method as described for soil sulphur or the barium chromate colorimetric method can be used for determination of S in the digest. Sulphur estimated by Turbidimetric method by using VIS-spectrophotometer at 490 nm (Chesnin and Yien 1950) and expressed the concentration in percentage.

Apparatus:

a) Spectrophotometer
b) Volumetric flask
c) Pipette
d) Conical flasks
e) Beaker

Reagents:

1. Triacid mixture: see digestion of Seed and Plant samples.
2. Sodium acetate acetic acid buffer (pH 4.8) [CH_3COONa-CH_3COOH]: 1000 mL volumetric flask add 100 g sodium acetate, add distilled water 500mL to mixed, add 30 mL 99.5% acetic acid, shake to dissolved sodium acetate the makeup volume 1000mL mark.
3. 0.25% Gum acacia solution: Dissolve 0.25g of chemically pure gum acacia powder in 100 mL of hot water and filter in hot condition through Whatman No.42 filter paper. Cool and keep in refrigerator.
4. Barium chloride crystal AR grade ($BaCl_2.2H_2O$): Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.
5. 100 ppm S solution: Take 0.5434 g of K_2SO_4 and add 500mL distilled water to dissolved then make up to 1000 mL mark of volumetric flask.

Procedure:

a) After digestion (see digestion of Seed and Plant samples), Transfer 10 mL of digest filtrate in to 25 mL volumetric flask and gradually add 10 mL of sodium acetate acetic acid buffer.
b) Add 1 g $BaCl_2.2H_2O$ powder and shake well.
c) Then add 1mL gum acacia and make up the volume with distilled water.
d) Run a blank without soil in similar manner.
e) Measure the turbidity intensity at 490 nm
f) Run a blank side by side.

Preparation of standard curve:

Put 0, 1.25 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 mL of the working standard solution (10 mg S/litre) into 47 a series of 25 mL volumetric flasks to obtain 0, 0.5, 1.0, 2.0,3.0, 4.0, 5.0 and 6.0 ppm. Develop turbidity as described above for sample aliquots. Read the turbidity intensity and prepare the curve by plotting readings against sulphur concentrations.

Precautions:

1. The temperature of the digestion mixture during digestion should not exceed 230^0C.
2. The reading of the S in solution should be taken within 30 minutes from the development of turbidity.
3. A clear and white residue in flask should remain after digestion. In case of incomplete digestion, the material should be again digested after addition of 5 mL of tri acid.

Observation:

Sl No.	**Spectrophotometer Reading (ppm)**

Calculation:

S (mg/kg) in plant sample = R × d.f.

Where,

R = ppm reading of plant from standard curve

Dilution Factor (d. f.) = Volume of extractant / Wt. of soil X Final Volume of solution/ Volume of digested extract

= 100 X 25/ 0.5 X 10 = 500

Where,

Weight of plant sample = 0.5 g, Volume of digested extract prepared = 100 mL, Volume of digested extract taken = 10 mL, Volume of final coloured extract prepared = 25 mL

Results:

S content in sees/plant _____ ppm.

**** % S = S ppm / 10000**

Video link:

https://www.youtube.com/watch?v=nDW3vW6PnR0

Business idea: <u>Development of irrigation water testing laboratory</u>

Collection of Water Samples

Irrigation is an important activity in crop production. Long term application of this water affects the soil property depending upon the quality of water used for irrigation. Concentration of soluble salt, alkalinity or excess of any particular element leads to the accumulation and related problem in crop production. Hence, quality should be determine and its suitability should be established before planning for long term use of any water resource.

Water sampling is a vital part in analysis of the water for its quality appraisal for agricultural and environmental applications. Therefore, water sampling program starts with collections of samples which accurately represent the characteristics of the bulk material and handled conveniently in the laboratory while still providing test results. The major source of error in the whole process of obtaining water quality information often occurs during sampling. Over 50 % of the faulty data that occur in laboratory test results are due to sampling error, rather than during laboratory analysis. Adoption of the standard recommended practices helps in minimization of the sampling error.

Selection of the sampling containers

- Containers should be examined for cleanliness, ensuring it is strong and durable, so that it will not break in transit and that the cap does not leak once it is secured.
- Containers must not contain any of the compounds that samples are to be analyzed for.
- Container must be of the appropriate size.
- Containers must be high density polyethylene or glass containers with Teflon® lid liners for most analyses.
- Rubber and cork stoppers must not be used so as to avoid the risk of contaminating the sample.

Water sampling techniques

For collection of water sample following method may be adopted:

- Glass or plastic bottles thoroughly cleaned and rinsed 3-4 times with the water to be tested should be used for collection of samples. The bottle should not be washed with detergents or soaps.

- Take about 500 mL of sample after runing the tube well or hand pump for about 15-20 min. to drain out the water retained in the pipe.
- If a new tube well boring is in progress, collect water samples at different depths at intervals of about 3-4 m. Since the water in such a situation is always turbid, with suspended impurities, it should be collected as such without caring for turbidity, as it does not affect the test results.
- From a tank or pond collect the sample from at least 5 to 10 m away from the boundaries after displacing surface water, which might contain organic material floating over it.
- From a well, the sample can be drawn either during irrigation, just before the water falls in the channel or by drawing it with the help of a bucket or any other clean container using a rope. The water surface should be disturbed a little to remove any floating material before collection of the sample.
- Place the cap on the bottle tightly.
- Write the name, address, sample no., identification mark etc. on the bottle.
- Separately provide following information on the sheet of paper:
  - Name of the crop to be grown
  - Texture of soil to be irrigated,
  - Previous experience about the effect of the water on soil surface,
  - Previous test results if any,
  - Other sources available for irrigation, and
  - Crop performance so far.
- Sample should be analysed preferably within 2-3 days of collection of the sample.
- It is desirable to send both soil and water samples together for testing, as it helps in making the test reports and recommendations more fruitful.

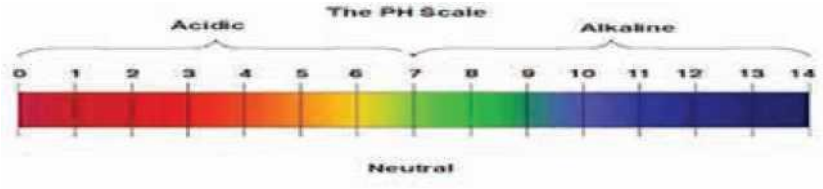
Estimation of irrigation water pH

pH of saline irrigation water, is not very much important since presence of neutral salts keeps it around 7.0. However the pH of bicarbonate waters is usually more than 7.5, its determination is very important because it may reflects the degree of sodicity in the water samples.

Principle pH is the negative logarithm of hydrogen ions concentration. It is expressed as: pH = -log (H+)

If pH below 7, the H+ ion concentration is exceeds to OH^- and range is acidic. When the OH- concentration is more than H+, pH lies between 7 to 14 (alkaline range).

The scale of measurement is from 1 to 14 on a pH scale:



Equipment and Apparatus

pH meter and 100 mL beakers

Reagents

- **Buffer solutions (pH 4 and 9.2)**

pH 4.0: Prepare stock solution of 0.3 *M* potassium hydrogen phthalate by dissolving 15.3 g of

the analytical grade salt in about 225 mL of hot water, cool the solution and dilut to 250 mL. Add a drop of toluene to discourage growth of micro-organisms. For the standard buffer pH 4.0 mix 100 mL of the stock solution with 500 mL water. Prepare a fresh solution every week.

pH 9.2 : Dissolve 3.81 g sodium tetraborate (AR) in water and dilute to 1000 mL.

Procedure

1. Calibrate the pH meter
2. Take a 50 mL water sample in 100 mL flask, Put the combined electrode in the water sample and take the reading after 30 seconds.
3. Remove the combined electrode from the sample and rinse thoroughly with distilled (DI) water in a separate beaker and carefully dry excess water with a tissue.

Practical suggestions

- Make sure that the combined electrode contains saturated KCl solution and some solid KCl.
- Allow the pH meter to warm for 10 minutes before recording the pH.
- Never allow the lower portion of glass electrode to touch the bottom of the beaker.

Estimation of irrigation water Electrical Conductivity (EC)

Electrical Conductivity (EC) is the measure of the ability of a solution to carry an electric current or the concentration of soluble salts in the sample at any particular temperature. The EC measurement is affected by dissolved CO_2, turbidity, temperature and the nature of various ions and their relative concentration.

Equipment and apparatus

Electrical conductivity meter, beakers

Reagent

- **Potassium chloride (KCl) 0.01 M:** Dissolve 0.7456 g of KCl in distilled water and make up the volume to 1 L at 25 ^{0}C. This is standard reference solution. At 25 ^{0}C it has an electrical conductivity of 1.412 dS m^{-1}.

Procedure

1. Take about 75 mL water sample in a 100 mL beaker, and then put the clean and dried conductivity cell in beaker.
2. Take the reading the display will also need some time to stabilize before the reading.
3. Remove the conductivity cell from the glass beaker, rinse thoroughly with distilled water, and

carefully dry excess water with tissue paper.

Calculation

The EC value can either be used as such for categorizing the water on salinity basis or may be used to get the concentration of as given below;

Total soluble salt content (mg L^{-1}) =EC (dS m^{-1}) at 25 ºC x 640 Total salt content me L^{-1} (approximately) =EC (dS m^{-1}) at 25 ºC x 10

Practical suggestions

- Cleaning of the conductivity cell is needed if contaminated.
- The reference temperature should be 25 °C, and the result expressed in dS m^{-1}. If the measurement is carried out at a different temperature, the result should be corrected to 25 °C. Check accuracy of the EC meter using a 0.01 *M* KCl solution, which should give a reading of 1.413 dSm^{-1} at 25 °C.
- The use of the unit deci-Siemens per meter is preferred over the unit milli-mhos. Both units are equal, that is, 1 dSm^{-1} = 1 $mmhocm^{-1}$.

Estimation of Total Dissolved Solids (TDS)

TDS is defined as the substances remaining after evaporation and drying of a water samples. The remaining fraction is approximately equivalent to the total content of the dissolved and suspended matter in the water sample. Non-filterable residue corresponds to the total suspended solids (TSS) and the filterable residue is the TDS. This is accomplished by comparing the value of calculated TDS with the measured value. Ion concentration, in mgL^{-1} of constituents, required to calculate the TDS are as follow:

Calculated TDS ($g\ L^{-1}$) = $Na+K+Ca+Mg+Cl+SO_4+SiO_3+ (NO_3-N)+F$

Principle

A well mixed, measured portion of a sample is filtered through a standard glass-fiber filter and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180 ^oC. The increase in dish weight represents the total dissolved solids.

Apparatus

- Evaporating dishes made porcelain
- Platinum or high-silica glass
- Steam bath
- Desiccators provided with a desiccant containing a colour indicator for moisture concentration
- Glass-fiber filters
- Suction flask
- Analytical balance

Procedure

1. Take an aliquot of sample to yield between 2.5 and 200 mg dried residue.
2. Filter measured volume of well mixed water sample through the glass-fiber filter;
3. Wash with 3 successive 10 mL volumes of distilled water, allowing complete draining between washing.
4. Continue suction for about 3 minutes after filtration is complete.
5. Transfer filtrate to a weighed evaporating dish *(Wtd)* and evaporate to dryness on a steam bath.

6. Dry for at least 1 hour in an oven at 180 ^{o}C, cool in desiccators, and weigh (*Wtd+s).*
7. Repeat the cycle of drying, desiccating and weighing until a constant weight is obtained or until weight loss between successive weighing is less than 4 % or 0.5 mg which is less.

Calculation

$$\text{TDS (mg L}^{-1}) = \frac{(Wt_{d+S} - Wt_{d}) \times 1000}{V}$$

Where:

Wt_{d+S}= Weight of dish plus solids (mg)

Wtd= Weight of dish before use (mg)

V= Volume of water sample used for measurement (mL)

Estimation of Total Suspended Solids

Total Suspended Solids (TSS) applies to dry weight of the material that is removed from a measured volume of water sample by filtration with a standard filter. The test is basically empirical and is not subject to usual criteria of accuracy. To achieve reproducibility and comparability of results requires close attention to procedural details, especially filter characteristics and time and temperature of drying.

Apparatus

- Desiccators provided with a desiccant containing a colour indicator for moisture concentration
- Analytical balance capable of weighing to 0.001 g
- Glass-fiber filter disc, Whatman GF/C or equivalent
- Drying oven
- Buchner funnels
- Vacuum pump

Pre-treatment of filter disc and crucible

1. Place a filter disc on the filter holder. Assemble filter holder in suction flask apparatus, connect to vacuum source and apply vacuum.
2. Wash the filter disc with 3 successive 20 mL portions of distilled water. Continue to apply vacuum for 2-3 minutes after the water has passed through the filter. Discard the filtrate.
3. Remove the filter paper from the membrane filter funnel or the Buchner funnel and place it on a supporting surface in drying oven.

4. Place the crucible (s) in the drying oven. The oven should be maintained at 105 ^{o}C and drying should be continued for at least 1 hour.
5. Cool the filter (s) and crucible (s) in desiccators and weigh it on an analytical balance.
6. Repeat the cycle of drying, desiccating and weighing until the weight loss between two successive series operations is less than
    0. 5 mg.
7. Store filter (s) and crucible (s) in desiccators until required.

Procedure

1. Remove the filter disc and crucible from the desiccators, and weigh (*Wt_f+c).*
2. Place the filter in the filter holder and assemble the filter holder in the suction flask apparatus. Connect to the vacuum source and apply vacuum.
3. Wet the filter with a few drops of distilled water to seat the filter.
4. Shake the sample vigorously and measure out 100 mL in a 100 mL graduated cylinder or volume flask. Pour this portion of the sample into the filter funnel (be careful not to disturb the placing of the filter disc).
5. Rinse out the measuring flask or cylinder with a small quantity of distilled water. If the sample is very low in suspended material, a large volume of sample may be used.
6. When filtration is complete, carefully remove the filter disc from the filter holder with tweezers (or remove the crucible from its supporting socket with a pair of tongs), and place it in the drying oven.
7. Dry for at least 1 hour at 105 ^{o}C. Cool in desiccators, and weigh (*Wt+c+s).*
8. Repeat the drying, desiccating and weighing cycle until the weight loss between 2 successive weighing is less than 0.5 mg.
9. Record the final weight obtained

Calculation

$$\textbf{TSS (mg L}^{x}\textbf{)} = \frac{(Mt_{fc+s} - Wt_{f+C}) \times 1000}{V}$$

Where:

$Wt_f + c + s$ = Weight of filter and crucible plus solids (mg)

$Wt_f + c$ = Weight of filter and crucible before use (mg)

V = Volume of water sample used for measurement (mL)

Estimation of Biological Oxygen Demand

Microorganisms such as bacteria are responsible for decomposing organic waste. When organic matter is discharged into a watercourse it serves as a food source for the bacteria present there. If organic matter such as dead plants, leaves, grass clippings, manure, sewage, or even food waste is present in a water supply, the bacteria will begin the process of breaking down this waste. Biological Oxygen Demand (BOD) is a measure of the oxygen used by microorganisms to decompose this waste. BOD refers to the amount of oxygen that would be consumed if all the organics in one litre of water were oxidized by bacteria and protozoa. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste.

Principle

This test is based on Winkler's Method. Oxygen combines with Mangnous hydroxide to form higher hydroxides, which on acidification liberate iodine equivalent to that of oxygen fixed. This iodine is titrated by standard Sodium thiosulfate solution using starch as an indicator.

Test Procedure

The BOD test takes 5 days to completeand is performed using a dissolved oxygen (DO) test procedure. The BOD level is determined by comparing the DO level of a water sample taken immediately with the DO level of a water sample that has been incubated in a dark location for 5 days. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level.

Reagents:

- **0.0250** ***M*** **Sodium thiosulfate:** 3.1 g sodium thiosulfate pentahydrate ($Na_2S_2O_3$ ^5H_2O) in reagent water. Add 0.2 g sodium hydroxide (NaOH, 1 to 2 pellets) and dilute to 500 mL. Alternate: Dissolve exactly 6.206 grams sodium thiosulfate crystals in freshly boiled and cooled reagent water and make up water to a volume of 1 liter. For preservation, add 0.4 gm or 1 pellet of sodium hydroxide. Solutions of "thio" should be used within two weeks to avoid loss of accuracy because of decomposition of the solution. Alternate: Phenylarsine Oxide solution

(PAO) may be used instead of “thio” [0.025 *N* PAO available and standardized from commercial sources.]

- **Alkaline Iodine Azide (AIA) solution:** 50 g sodium hydroxide (NaOH) and 15 g potassium iodide (KI) are dissolved in reagent water and diluted to 100 mL. Add 1 g sodium azide (NaN_3) dissolved in 4 mL distilled water.
- **starch solution:** 2 g starch and 0.2 g salicylic acid dissolved in 100 mL hot water.
- **Manganous Sulfate solution:** Dissolve 40 g Manganoussulfatedihydrate ($MnSO_4 \cdot 2H_2O$) in reagent water, filter and dilute to 100 mL.
- **Sulfuric acid:** Use concentrated reagent grade acid (H_2SO_4). Handle carefully, since this material will burn skin and clothes.
- **Potassium Iodide (KI) crystals**

Standardization (Procedure):

1. Dissolve 2 g potassium iodide in approximately 100 mL Erlenmeyer flask.
2. Add 1.0 mL concentrated Sulfuric acid.
3. Add a magnetic stir bar.
4. Fill burette with the sodium thiosulfate solution and lower level to 0.0 marks. Be sure that the tip of the buret is filled with solution and not air.
5. Begin stirring and add sodium thiosulfate solution to the flask until a light yellow colordeveloped.
6. Add about 2 mL of starch solution. The color will change to deep blue or purple.
7. Continue addition of sodium thiosulfate until the solution clears permanently. Stop the titration and record the volume of of sodium thiosulfate used.
8. If exactly 20 mL of sodium thiosulfate solution is used it can be recorded as standardized at 0.0250 M. If less than 20 mL are needed, add some reagent water to the sodium thiosulfate solution (5 mL reagent water to 100 mL titrant for each 1 mL that the titrant is low). If more than 20 mL are required, add solid sodium thiosulfate to the titrant solution (310 mg per 1000 mL for every 1.00 mL the titrant is high). Recheck the standardization.

9. Repeat the standardization and record both determinations and the average in the notebook.

Procedure

1. Collect samples in 300 mL BOD bottle taking special care to avoid aeration of the liquid being collected. Fill bottle completely (no air under cap). Samples should be taken in triplicate. Keep one bottle of each set in an incubator at 20°C for 5 days.
2. Insert stopper in bottle prepared for DO, then remove.
3. Add, under the surface of the sample, 1-2 mL Manganous Sulfate solution.
4. Immediately add 1-2 mL Alkaline Iodide Azide solution below the surface of the liquid.
5. Insert stopper in bottle, avoid trapping air bubbles, and invert it until it mix well. Repeat this shaking after the flock has settled halfway.
6. Allow flock to settle to half the volume of the bottle a second time, then open and add 1-2 mL concentrated Sulfuric acid by allowing the acid to run down the neck of the bottle above the surface of the liquid.
7. Insert stopper and invert to mix until the flock dissolves.
8. Transfer the solution to an Erlenmeyer flask with magnetic stir bar for the titration procedure.
9. Titrate with the 0.0250 M sodium thiosulfate solution until the solution is pale yellow.
10. Add 2 mL starch and continue titration until the color is permanently discharged. Record the volume of sodium thiosulfate used.Each 1.00 mL of 0.025 M sodium thiosulfate used is equivalent to 1.00 mg L^{-1} DO.
11. Repeat same steps with other samples earlier kept in incubator and record DO.

Calculation:

$$\textbf{DO mg equivalent} = \frac{\text{Vol. of } Na_7S_70_7 \text{ sol.Used xW of } Na_7S_7O_3 \text{ x8000}}{\text{Vol. of taken}}$$

$$\text{BOD (mg } L^{-1}) = \frac{DO_j - DO_f}{P}$$

Where,

DO_i. and DO_f are initial and final values of DO.

P is the decimal fraction of sample diluted in the 300 mL BOD bottle.

Ranges for typical BOD levels in water bodies are

- < 5 mg L^{-1} in natural, unpolluted waterways
- 20-30 mg $L^{-1}$for well-treated sewage
- 150-300 mg $L^{-1}$for raw sewage
- 100-500 mg $L^{-1}$for urban storm water runoff.

Precautions

- Test should be done as early as possible after sample collection.
- If test is not started within two hours of collection, it should be kept at 4 °C up to 6 hours.
- Do not open BOD bottles before analysis.
- The pH of samples should be adjusted between 6.5 - 7.5 using sulfuric acid or sodium hydroxide.

Estimation of different forms of Nitrogen in water

Nitrogen generally occurs in trace amount in surface (river, canal) water but can be present in higher concentration in some ground waters (tubewells). Beneficial effect of nitrogen on crop production has been widely reported. The presence of K+ and NO_3^- ions in appreciable amounts in irrigation water has been found to partially counteract the adverse effect of salinity and sodicity on plant growth. In drinking waters, nitrates content higher than 10 mg L^{-1} it causes illness called methaeglobinaemia or blue baby syndrome in infants. Water may contain nitrogen in different forms such as nitrate, nitrite, ammonia and organic nitrogen. Total nitrogen includes all forms of nitrogen. The concentration of nitrogen in water samples can be used to assess nutrient status of the water resources or as indicator water pollution due to enrichment from various sources such as fertilization of crop lands, animal wastes, sewage and growth of nitrogen fixing plants. Depending upon the objective, the water samples can be analysed for total as well as water soluble inorganic nitrogen.

A. Water soluble inorganic nitrogen

Principle

Ammonium nitrogen and NO_3^- N plus NO_2^- N are determined by steam distillation, using heavy MgO for NH_4 and Devarda's Alloy for NO_3 .The distillate is collected in saturated H_3BO_3 and titrated to pH 5.0 with dilute H_2SO_4.

Apparatus

- Distillation unit
- Automatic titrator connected to a pH-meter
- Stirrer

Reagents

- Magnesium Oxide (MgO), powder: Heat heavy magnesium oxidein a muffle furnace at 600-700 °C for 2 hours, and cool in a desiccators containing KOH pellets, and store in a tightly stoppered bottle.
- Devarda's Alloy (50 Cu: 45 Al: 5 Zn):Ball-mill reagent-grade Devarda's Alloyuntil the product will pass a 100-mesh sieve (0.150 mm) and at least 75% will pass a 300-mesh sieve (0.05 mm).
- Boric Acid Solution (H_3BO_3), saturated: Add 500 g H_3BO_3into a 5-L volume. Add 3 L distilled water (DI), and swirl vigorously and leave overnight.
- Tris Solution (hydroxymethylaminomethane) ($C_4H_uNO_3$), 0.01 *N:* Dry reagent-grade Trisin an oven at 80 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- Sulfuric Acid Solution (H_2SO_4), 0.01 *N* : Add 28 mL concentrated H_2SO_4, to about 600-800 mL distilled water in a 1L flask, mix well, let it cool, and bring to 1 L volume. This solution contains 1 *N* H_2SO_4 solution (Stock Solution). Pipette 10 mL Stock Solutionto 1 L flask, and bring to volume with distilled water. This solution contains 0.01 *N* H_2SO_4.
- Standard Stock Solution

• Dry reagent-grade ammonium sulfate $(NH_4)_2SO_4$, and potassium nitrate (KNO_3) in an oven at 100 °C for 2 h, cool in a desiccators, and store in a tightly stoppered bottle.

- Dissolve 5.6605 g $(NH_4)_2$ SO_4 and 8.6624 g KNO_3 in distilled water, and bring to 1 L volume. This solution contains (1.2 g NH_4-N, and 1.2 g NO_3^-N) L^{-1} (Stock Solution).
- Prepare a Standard Solution from the Stock Solution as follows: Dilute 50 mL Stock Solution to 1 L volume by adding 2 MKCl solution (Diluted Stock Solution).

- A 20 mL aliquot of Diluted Stock Solution contains 1.2 mg NH_4 N and 1.2 mg NO_3 N.

Procedure

Pre-treatment of the distillation unit

1. The distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7-8 mL distillate/minute.
2. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.

Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 - 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.

2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the 0.01 *N* H_2SO_4 in the Auto-Titrator by titrating three separate 10 mL aliquots of the primary standard, 0.01 *N* Tris solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found. The H_2SO_4 normality is:

$$N\ H_2S0_4 = \frac{10 \times N_{Tris}}{VH2SO_4}$$

Carry out distillations as follows

To determine NH_4-N

1. Pipette 20 mL water or wastewater sample into a 100 mL distillation flask.
2. Pipette 1 mL saturated H_3BO_3 solution and 1 mL distilled water into a 50 mL beaker (duplicate beakers).
3. Place the first beaker underneath the condenser tip, with the tip touching the solution surface.
4. Add about 0.2 g heavy MgO, with a calibrated spoon, to the distillation flask.
5. Immediately, attach the distillation flask to the distillation unit with a clamp.
6. Start distillation, and continue for 3 minutes, then lower the dish to allow distillate to drain freely

into the Pyrex evaporating dish or beaker.

7. After 4 minutes, when 35 mL distillate or more is collected, turn off the steam supply and remove the distillation flask (first distillate).
8. Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NH_4 N from Diluted Stock Solution) and two blanks (pipette 20 mL 2 KCl solution). Recovery of NH_4 N should be at least 96 %.

To determine $N0_3^-$N and $N0_2^-$N

1. Place the second beaker underneath the condenser tip, with the tip touching the solution surface.
2. Immediately, add 0.2 g Devarda's alloy, with a calibrated spoon, to the same distillation flask, then attach back to distillation unit with a clamp, and start distilling.
3. After 4 minutes, when 35 mL distillate or more is collected, turn off the steam supply and remove the distillation flask (second distillate).
4. Wash tip of the condenser into Pyrex evaporating dish or the beaker with a small amount of distilled water.
5. Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NO_3^- N from Diluted Stock Solution) and two blanks (pipette 20 mL distilled water). Recovery of NO_3-N should be at least 96 %.

Titration

Titrate the first distillate (for ammonia) and the second distillate (for nitrate), separately, to pH 5with standardized 0.01 *N* H_2SO_4using an Auto-Titrator.

Calculation

$$NH_4 \text{ or } NO_3 \left(\text{mg L}^{-1}\right) = \frac{(V - B) \times N \times 14.01 \times 1000}{V_1}$$

Where:
V= Volume of 0.01 *N* H_2SO_4 titrated for the water sample (mL)
B = Distillate blank titration volume (mL)
N = Normality of H_2SO_4 solution
VI= Volume of water sample used for distillation (mL)
14.1= Atomic weight of N

Technical remarks

- If possible, NH_4+ N and NO_3^- N should be determined immediately after sampling. If the analysis cannot be done immediately, water and wastewater samples may be kept refrigerated (4 °C).
- All water for reagents and dilution should be NH_3free. Distilled water is usually satisfactorily.
- Filter paper may contain traces of NH_4+, so the first 20-25 mL of filtrate should be discarded.
- Never use acid preservation for samples to be analyzed for NO_3^- or NH_4+.

Total Nitrogen

For the total nitrogen water samples can be analyzed after digesting the samples by persulphate digestion followed by colourimetric estimation of nitrate nitrogen. Alternatively water samples can also be analysed for total Kejldahl nitrogen.

Persulphate oxidizable total nitrogen

Alternately In this method salicylic acid is nitrated in an alkaline solution and light absorbance is measured at 410 nm

Apparatus and equipments:

Spectrophotometer, test tube shaker, autoclave, glass culture tube with Teflon lined screw cap.

Reagents

- Oxidizing agent: oxidizing agent is prepared by adding 25 g potassium persulphate AR grade ($K_2S_2O_8$) and 15 g of boric acid AR grade (H_3BO_4) in 50 mL of 3.75 M NaOH solution and the volume is made up to 500 mL with double distilled water. The reagent can be stored for upto 1 week at room temperature in dark bottle.
- 3.75 M NaOH:Dissolve 75 g NaOH in 500 mL distilled water
- 4 M NaOH solution: Dissolve 160 g NaOH cakes in 600 mL distilled water, make up to 1000 mL and mix well
- 5% Salicylic Acid: Dissolve 5 g salicyclic acid in 95 mL concentrated H_2SO_4 (95-97%).
- 1000 mg NO_3^- L^{-1} solution: Dissolve 7.223 g dry potassium nitrate in distilled water and make up to volume in a 1000 mL volumetric flask
- Working standards: Pipette 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of stock solution in 100 mL volumetric flaks and make up to volume to give the 0, 2, 4, 6, 8 and 10 mg NO_3^- L^{-1} working standards.

Procedure

1. Take 5 mL water sample in glass culture tube to this add 5 mL of the oxidizing agent, and immediately sealed the culture tube with screw caps containing Teflon liners.
2. Set a blank with 5 mL distilled water in place of aliquot

3. Autoclave the culture tube at 120 °C for 50 min.
4. After autoclaving cool the culture tube
5. Pipette 0.5 aliquot and each of the working standards in test tube for NO_3^- N determination
6. Add 1 mL of 5% salicylic acid slowely with stirring and wait for 30 min
7. Now add 10 mL of 4 *M* NaOH solution slowely and leave for 1 h for colour development
8. Mix the reagents properly using cyclomixer
9. Read the absorbance of the yellow colur at 410 nm using spectrophotomer
10. Plot the standard curve from absorbance versus concentration and find out the sample and blank concentration

Calculation:

NO_3^- N (mg L^{-1}) = (S-B) x 2

Where S= concentration of NO_3^- N (mg L^{-1}) in sample B = concentration of NO_3^- N (mg L^{-1}) in blank Factor “2” is the dilution factor during digestion of the sample

Total Kejldahl nitrogen

The Kjeldahl procedureis a good estimate of N content in the water or waste water samples. This procedure involves digestion and distillation. The water or waste water sample is digested in concentrated H_2SO_4 with a catalyst mixture to raise the boiling temperature and to promote the conversion from organic-N to ammonium-N, which is obtained by steam distillation, using excess NaOH to raise the pH. The distillate is collected in saturated H_3BO_3; and then titrated with dilute H_2SO_4 to pH 5.0.

Apparatus

- Block-digester
- Distillation unit
- Automatic titrator connected to a pH-meter
- Vortex tube stirrer

Reagents

- Catalyst Mixture (K_2S0_4 - Se), 100: 1 w/ w ratio: Grind reagent-grade chemicals separately and mix. If caked, grind the mixture in a porcelain pestle and mortar to pass a 60-mesh screen (0.250 mm), taking care not to breath *Se* dust or allow *Se* to come in contact with skin.
- Sulfuric Acid (H_2SO_4), concentrated (98 %, sp. gr. 1.84)
- Sodium Hydroxide Solution (NaOH), 10 N:Dissolve 400 g NaOH in distilled water, transfer to a 1 L heavy-walled Pyrex flask, let it cool, and bring to volume.
- Boric Acid Solution (H_3BO_3), saturated:Add 500 g H_3BO^into a 5 L volume. Add 3 L DI water, and swirl vigorously. Leave overnight.
- Tris Solution [hydroxymethylaminomethane] ($C_4H_{11}NO_3$), 0.01 N:Dry reagent-grade Tris in an oven at 80 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle. Dissolve 1.2114 g Trisin distilled water, and bring to 1 L volume
- Sulfuric Acid Solution (H_2SO_4), 0.01 N:Add 28 mL concentrated H_2SO_4 to about 600 - 800 mL distilled water in a 1 L flask, mix well, let it cool, and bring to 1-L volume. This solution contains 1 *N* H_2SO_4 solution (Stock Solution). Pipette 10 mL Stock Solutionto 1-L flask, and

bring to volume with DI water. This solution contains 0.01 *N* H_2SO_4.

- Standard Stock Solution:Dry reagent-grade ammonium sulfate $(NH_4)_2SO_4$in an oven at 100 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle. Dissolve 5.6605 g dried $(NH_4)_2SO_4$in distilled water, and bring to 1 L volume. This solution contains 1.2 g NH_4 N L^{-1} (Stock Solution).

Procedure A. Digestion

1. Pipette 20 mL aliquot of the water sample into a 100 mL calibrated digestion tube.
2. Add about 3.0 - 3.5 g catalyst mixture, a few pumice boiling granules, add 10 mL concentrated H_2SO_4 (in the fume hood) and then swirl carefully. Place the tubes in the rack and put a glass funnel in the neck of the tube.
3. Place the tubes rack in the block-digester, and slowly increase temperature setting to about 370 - 380 °C. The H_2SO_4 should condense about half-way up the tube neck; when solution clears, continue heating for about 3 hours.
4. Lift the tubes rack out of the block-digester, carefully place on a rack holder, and let tubes cool to room temperature.
5. Slowly add about 15 mL distilled water to the tubes, cool, and bring to volume with distilled water.
6. Each batch of samples for digestion should contain at least one reagent blank (distilled water sample), and one chemical standard (distilled water sample, pipette 1 mL Stock Solution).

Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7-8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the 0.01 *N* H_2SO_4 in the Auto-Titrator by titrating three separate 10 mL aliquots of the primary standard, 0.01 *N* Tris solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found.

The H_2SO_4 normality is: $N\,H_2SO_4 = \frac{10 \times N_{Tris}}{V\,H_2SO_4}$

Carry out distillations as follows:

1. Dispense 1 mL saturated H_3BO_3 solution and 1 mL distilled water into a 100 mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.
2. Pipette 20 mL aliquot into a 100 mL distillation flask, and add 10 mL 10N NaOH solution.
3. Immediately attach the flask to the distillation unit with a clamp, start distillation, and continue for 3 minutes, lower the dish to allow distillate to drain freely into the dish.
4. After 4 minutes when about 35 mL distillate is collected, turn off the steam supply, and wash tip of the condenser into the evaporating dish with a small amount of DI water.
5. Titrate the distillate to pH 5.0 with standardized 0.01 *N* H_2SO_4 using an Auto-Titrator.
6. Each distillation should contain at least two standards (pipette 10 mL digested water sample) and two blanks (pipette 10 mL digested water blank). Recovery of NH_4-N should be at least 96 %.

Notes

- After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish.
- Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100 mL empty distillation flask to distillation unit. Place a 100 mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds.

Calculation:

$$\textbf{\% Recovery} = \frac{(V - B)\ x / V\ x\ 14.01\ x\ 100}{V_3\ x\ C}$$

$$\textbf{N \%} = \frac{(V - B) \times N \times V_1 \times 14.01 \times 100}{V_2 \times 1000}$$

Where:

V = Volume of 0.01 N H_2SO_4 titrated for sample (mL) V1= Total volume of the digest (mL)

V2= Volume of water digest used for distillation (mL)

B = Digested blank titration volume (mL)

N = Normality of H_2SO_4 solution

14.1 = Atomic weight of N

V3 = Volume of NH_4-N standard solution (mL)

C = Concentration of NH_4-N standard solution (pg mL^{-1})

Estimation of different forms of Phosphorus in water

Phosphorus compounds are present in fertilizers and in many detergents. High concentration of P compounds may produce a secondary problem in water bodies. In such situations, the presence of additional P compounds can stimulate algal growth and enhances eutrophication.Groundwater rarely contains more than 0.1 mgL^{-1} phosphate if not polluted by organic matter. Phosphorus is essential to the growth of organisms and can be the nutrient that limits biological growth in water bodies. The water samples are analysed for the total as well as soluble reactive phosphorus

A. Soluble reactive phosphorus (SRP)

Soluble reactive phosphorus (SRP) describes the dissolved phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample and are termed 'reactive phosphorus'. Reactive phosphorus is largely a measure of orthophosphate (PO_4^{3-}); however, a small fraction of any condensed phosphate present is usually hydrolysed unavoidably in the analytical procedure. Reactive phosphorus occurs as both dissolved and suspended phosphorus.

Principle

SRP can be determined by vanadomolybdo phosphoric acid(colorimetric methods), where are the ammonium molybdate and potassium antimony tartrate react in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid- that is reduced to an intensely colored molybdenum blue by ascorbic acid.

Apparatus

- Spectrophotometer or colorimeter
- Standard laboratory glassware: beakers, volumetric flasks, pipettes, funnels

Reagents

- Sulfuric Acid Solution (H_2SO_4), 5 MDilute 148 mL *concentrated* H_2SO_4 (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume.
- p-nitrophenol Indicator, 0.25 % w/v
- Reagent A:Dissolve 12 g ammonium heptamolybdate$(NH_4)6Mo7O_{24}.4H_2O$ in 250 mL distilled water (a).Dissolve 0.2908 g antimony potassium tartrate$(KSbO.C_4H_4O_6)$ in 100 mL distilled water (b).Add both dissolved Reagents (a) and (b) to a 2 L flask. Slowly add 1-L 5 *N* $H_2SO_4$to the mixture. Mix thoroughly, and dilute to 2-L volume.Store in a dark Pyrex bottle in cool place.
- Reagent B:Dissolve 1.056 g L-Ascorbic acid $(C_6H_8O_6)$ in 200 mL Reagent A, mix well. This reagent should be prepared as required because it does not keep for more than 24 hours.
- Standard Stock Solution:Dry about 2.5 g potassium dihydrogen phosphate(KH_2PO_4) in an oven at 105 °C for 1 hour. Cool in desiccator, and store in a tightly stoppered bottle. Dissolve 2.197 g dried KH_2PO_4in distilled water, and bring to 1 L volume .This solution contains 500 ppm P (Stock Solution). Dilute 50 mL stock solutionto 250 mL volume by adding distilled water. This solution contains 100 ppm P (Diluted Stock Solution). Prepare a series of working standard solutions from the diluted stock solutionas follows:

  Dilute 5, 10, 15, 20 and 25 mL Diluted Stock Solutionto 500 mL numbered flasks by adding distilled water, and then bring to volume. These solutions contain 1, 2, 3, 4, and 5 ppm P, respectively.

Procedure

1. Pipette a suitable aliquot of clear filter water sample (10 mL natural water sample) into a 50 mL Erlenmeyer volumetric flask add few drops of p-nitrophenol indicator
2. Add the required acid or base to all the water samples to bring the solution pH to 5.0.
3. Add 8 mL Reagent B, and dilute to 50 mL volume with distilled water, mix well.
4. Prepare a standard curve by pipetting 2 mL of each standard (1-5 ppm), and proceed as for the samples. Also make a blank with only distilled water, and proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 10 minutes on the Spectrophotometer at 882 nm wavelength.
6. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
7. Read P concentration in the unknown samples from the calibration curve.

Calculation

P (mg L^{-1}) = P mg L^{-1} from standard curve x $\frac{V_1}{V}$

Where:
V= Volume of water sample used for measurement (mL)
VI= Volume of flask used for measurement (mL)

B. Total Phosphorus

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. In water it may be in several forms like orthophosphates (PO_4^{3-}), condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particle or detritus, or in the bodies of aquatic organisms. Phosphorus enrichment in water bodies may be from detergents in sewage and waste waters, fertilizers, animal wastes, sewage and some industrial wastes. High levels of phosphorus and/or other key nutrients may lead to eutrophication of the water bodies. Total phosphorus in water samples can be analyzed after digesting the samples by persulphate digestion followed by colourimetric estimation of phosphorus as given in section.

Reagents

1. Oxidizing agent: oxidizing agent is prepared by adding 10 g potassium persulphate AR grade ($K_2S_2O_8$) and 1.5 g of NaOH in 500 mL volumetric flask and the volume is made up to 500 mL with double distilled water. The reagent can be stored for upto 1 week at room temperature in dark bottle.
2. Reagents of the P estimation as given SRP estimation

Procedure

1. Take 5 mL water sample in glass culture tube to this add 5 mL of the oxidizing agent, and immediately sealed the culture tube with screw caps containing Teflon liners.
2. Autoclave the culture tube at 120°C for 30 min.
3. After autoclaving cool the culture tube and
4. Take suitable volume of aliquot and develop the colour as mentioned in soluble reactive phosphorus estimation section
5. Read the absorbance using spectrophotometer.

Calculation:

P mg L^{-1} = 2 **x** P mg L^{-1} from standard curve x v_1/v

Estimation of Potassium in irrigation water

Although potassium (K) is a relatively abundant element, its concentration in natural fresh waters is usually less than 20 mgL^{-1}. Brines and seawater, however, may contain as much as 400 mgL^{-1} K or more. Potassium in water can be determined by flame photometry.

Principle

The estimation of K is based on the emission spectroscopy, which deals with excitation of electrons from ground state to a higher energy state and coming back to its original state with the emission of light.

Apparatus

- Flame photometer with accessories
- Beakers
- Pipettes and volumetric flasks, as required for dilution and tests of interference effects

Reagents

Standard Stock Solution

- Dry 3-5 g potassium chloride (KCl) in an oven at 120 °C for 1 -2 hours and cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 1.907 g dried KClin distilled water, and bring to 1-L volume. This solution contains 1000 ppm K (Stock Solution).

- Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 2, 4, 6, 8, 10, 15 and 20 mL Stock Solution to 100 mL numbered flasks by adding distilled water, and then bring to volume. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm K, respectively.

Procedure

1. Filter a portion of water sample through Whatman no. 42 filter paper.
2. Calibrate Flame Photometer with a series of suitable K standards with distilled water as blank sample.
3. Measure the water samples, take the emission reading on the Flame Photometer at 767-nm wavelength, and record the readings.
4. Draw a calibration curve.
5. Calculate K concentrations according to the calibration curve.

Calculations

K (meL^{-1}) = K(ppm) from standard curve/39.1

Where:

39.1= Atomic weight of K

Practical suggestions

- Check the performance of the photometer at frequent intervals by spraying some of the standard solutions and adjust the sensitivity as necessary.
- If K concentration is higher than the top standard, make an appropriate dilution. If this is a dilution of the original sample, multiply by the appropriate factor.

Estimation of Chlorides in irrigation water

Important anions from water quality point of view are chloride, carbonate and biocarbonate, sulphates and nitrate. Concentration of chloride generally increases with increasing in electrical conductivity (EC) of waters. Therefore, magnitude of total salt may be predicted if chloride concentration is known.

Chloride (Cl^-) anions are usually present in natural waters. A high Cl^- concentration occurs in waters that have been in contact with Cl^-containing geological formations. Otherwise, high Cl^- content may indicate pollution by sewage or industrial wastes or by intrusion of seawater or saline water into a freshwater body or aquifer. A salty taste in water dependents on the ions with which the $Cl^-$are associated. With Na ions the taste is detectable at about 250 mgL^{-1}, but with Ca or Mg the taste may be undetectable at 1,000 mg L^{-1}. Chlorides being highly soluble is present in all waters but the amount is often very low in natural waters.

Principle

The determination of Cl^- is done by $AgNO_3$ (Mohrs's titration) method, which is based upon the fact that in solution containing Cl^- and chromate. Silver reacts with all the Cl^- and precipitates before the reaction with chromate begins. The appearance of the brick-red colour of the silver chromate precipitate is the end-point of the titration.

Apparatus

Burette and stand, volumetric flask and Beakers

Reagents

- Potassium Chromate Solution (K_2Cr0_4), 5% in water: Dissolve 5 g K_2CrO_4 in 50 mL distilled water. Add dropwise 1 *N* silver nitrate ($AgNO_3$) until a slight permanent red precipitate is formed. Filter, and bring to 100 mL volume with distilled water.
- Silver Nitrate Solution ($AgNO_3$), 0.01 N; Dry about 3 g $AgNO_3$ in an oven at 105 °C for 2 hours, cool in a desiccators, and store in a tightly stoppered and brown bottle. Dissolve 1.696 g dried $AgNO_3$in distilled water, and bring to 1 L volume.
- Sodium Chloride Solution (NaCl), 0.01 N:Dry about 3 g NaCl in an oven at 140 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered and brown bottle. Dissolve 0.585 g NaCl in

distilled water, and bring to 1 L volume.

Procedure

1. Pipette a suitable aliquot of water sample (10 mL natural water sample) into a 250 mL Erlenmeyer flask.
2. Add 4 drops potassium chromate solution.
3. Titrate against $AgNO_3$ solution until a permanent reddish-brown color appears.
4. In order to standardize the $AgNO_3$ solution used in the determination of Cl^-:

- Titrate 10 mL 0.01N NaCl solution against 0.01 *N* $AgNO_3$ after adding 4 drops potassium chromate solution until a permanent reddish-brown color appears.
- Take the reading, and calculate $AgNO_3$ normality:

$$N\,AgN0_3 = \frac{10 \times N\,NaCl}{V AgN0_3}$$

Where:

$NAgNO_3$= Normality of $AgNO_3$ solution
$VAgNO_3$= Volume of $AgNO_3$ solution used (mL) NNaCl= Normality of NaCl solution

$$Cl\ (me\ L) = \frac{V_1 \times N \times 1000}{V}$$

Where:

V1 = Volume of 0.01 N $AgNO_3$ titrated for the sample (mL)
N = Normality of $AgNO_3$ solution V = Volume of water sample used for measurement (mL)

Practical suggestions

1. Natural waters are often low in Cl^-, and 10 mL is a suitable aliquot in most cases.
2. Saline waters may be high in $Cl^-$and 5 mL (or even less than 5 mL) may then be more appropriate aliquots.

Estimation of Carbonate and Bicarbonate in irrigation water

Carbonate and bicarbonate in a sample can be determined by titrating the sample against standard acid using phenolphthalein and methyl orange or methyl red respectively as indicators. When the colour of phenolphthalein changes from pink to colorless, it indicates half the neutralization of carbonates. Now methyl red indicator is added and the titration continued. When the colour changes from yellow to rose red, end point reaches which show the complete neutralization of bicarbonates.

Principle

Carbonates

When the pH value of a sample of natural water is above 8.4, the CO_3^{--} is present, normally as sodium carbonate. The carbonate ion is converted to HCO_3. The amount of acid used a measure of the carbonate present.

$$C0_3^{2-} + H^+ = HCO_3^-;$$

Bicarbonate

Bicarbonate ions react with mineral acid and release carbon dioxide (CO_2) into the solution.

$$HCO_3^- + H^+ = H_2O + CO_2$$

Apparatus

Pipettes, Burette, Erlenmeyer flasks, Graduated pipette, Magnetic stirrer

Reagents

- Standard Sulphuric acid (0.01 N): Prepare 1 *N* acid from concentrated H_2SO_4 (36 N) (Dissolve approx 27 mL L^{-1} in distilled water) and standardized it with 0.01 *N* sodium carbonate by taking methyl red as indicator.
- Methyl red indicator (0.5%): Dissolve 0.5 gram dry methyl orange powder in 100 mL. of 95% ethanol.
- Phenolphthalein indicator (0.25%): Dissolve 0.25 gram of pure phenolphthalein powder in 100 mL. of 60% ethanol.

Procedure

1. Pipette 5 mL. of water sample in a 125 mL conical flask and add 2-3 drops of phenolphthalein. If

pink color appears it indicates the presence of carbonates.

2. Then titrate it with 0.01 *N* sulphuric acid with the help of a burette till the solution
3. becomes colorless. Record this reading and designate this reading as Y.

Now add 3-4 drops of methyl red indicator and titrate again, till the colour changes from yellow to rose red and record this reading also and designate it Z.

Calculations

If N_1 and V_1 are normality (CO_3^{2-} conc.) and volume of aliquot and N_2 V_2 are the normality and volume of H_2SO_4 used respectively, then

$N_1 V_1 = N_2 V_2$

$$N_1 = \frac{N_2 V_2}{V_1} = \frac{\text{Vol. of } H_2SO_4 \times \text{Normality of } H_2SO_4}{\text{ml of aliquot taken}}$$

Here N_1 = Normality = equivalents of CO_3^{2-} present in one Litre of aliquot.

Hence, milliequivalents of CO_3^{2-} per Litre:

$$\text{Carbonates (me L}^{-1}) = \frac{2y \times \text{Normality of } H_2SO_4 \times 1000}{V}$$

$$CO_3 \text{ (me L}^{-1}) = \frac{2y \times N \times 1000}{V}$$

$$HCO_3 \text{ (me L}^{-1}) = \frac{(Z - 2y) \times N \times 1000}{V}$$

Where:
2 = Valance of carbonate
Y= Volume of titrant against phenolphthalein indicator (mL)
Z = Volume of titrant against methyl red indicator (mL)
V= Volume of water sample used for measurement (mL)
N = Normality of H_2SO_4 solution

Practical suggestions

Standard HCl is used because H_2SO_4 may give rise to turbidity from calcium sulfate with Ca-rich samples.

Estimation of Sulphate in irrigation water

Sulphate ions usually occur in natural waters. Many sulphate compounds are readily soluble in water. Most of them originate from the oxidation of sulphate ores, the solution of gypsum and anhydrite, the presence of shales, particularly those rich in organic compounds, and the existence of industrial wastes.The SO_4^{--} in water is determined normally by barium sulfate ($BaSO_4$) precipitation.

Turbidimetric method

Principle

This method is used for the determination of sulphate ions. Sulphate ion (SO_4^{2-}) is precipitated in an acetic acid medium with Barium chloride ($BaCl_2$) so as to form Barium sulphate ($BaSO_4$) crystals of uniform size. The reaction involved is given below:

$Ba^{2+} + SO_4^{2-} = BaSO_4$ (White suspension)

Light absorbance of the $BaSO_4$ suspension is measured by a photometer or the scattering of light by Nephelometer.

Apparatus

Magnetic stirrer, Colorimeter for use at 420 mm or turbidimeter/nephelometer, Stopwatch, Nessler tubes, 100 mL, Measuring spoon (0.2 - 0.3 mL)

Reagents and standards:

- Buffer solution A: dissolve 30 g Magnesium chloride, $MgCl_2.6H_2O$, 5 g Sodium acetate
- $CH_3COONa.3H_2O$, 1 g Potassium nitrate, KNO_3 and 20 mL acetic acid, CH_3COOH (99%) in500 mL distilled water and make up to 1000mL.
- Buffer solution B: (required when the sample sulphate (SO_4^-) is less than 10mg/L). Dissolve 30 g Magnesium chloride, $MgCl_2.6H_2O$, 5 g sodium acetate, $CH_3COONa.3H_2O$, 1.0 g of
- Potassium nitrate, KNO_3: 0.111 g of sodium sulphate, Na_2SO_4 and 20 mL acetic acid (99%) in500 mL distilled water and make up to 1000 mL.
- Barium chloride: crystals, 20-30 mesh.
- Standard sulphate solution: dissolve 0.5434 g of oven dry AR grade K_2SO_4in distilled water and

dilute to 1 L. this contains 100 mg L^{-1} sulphate.

Calibration

Prepare standard curve by carrying standard sulphate solution through entire procedure. Space standards at 5mg L^{-1} increment in the 0 to 40 mg L^{-1}range. Read mg SO_4^{2-} present in the sample from the standard curve.

Procedure

1. Take suitable volume of sample and dilute to 100 mL into a 250 mL Erlenmeyer flask
2. Add 20 mL buffer solution, mix well
3. Keep the flask constantly stirred with the help of stirrer. Add 1spatula $BaCl_2$ crystals with stirring. Continue stirring for 1 minute after addition of $BaCl_2$
4. Pour suspension into an absorption cell of photometer an measure turbidity at 5 ± 0.5 min
5. To correct for sample colour and turbidity, run a blank to which $BaCl_2$ is not added.

Calculation

mg $SO_4^{2-}L^{-1}$ = (mg (SO_4^{2-}) form standard curve x 1000} / (mL of sample)

Estimation of Boron in irrigation water

In most natural waters boron (B) is rarely found in concentrations greater than 1 mg L^{-1}, but even this low concentration can have deleterious the effects on certain agricultural products. Water having B concentrations in excess of 2 mg L^{-1} can adversely affect many common crops. However, where levels are greater than 5 mg L^{-1}, toxicity may occur. Groundwater may have a greater B concentration, particularly in areas where the water comes in content with igneous rocks or other B-containing strata.

The hot-water procedure is still the most popular method for measuring B, and it was introduced by Berger and Truog (1939), and was modified by later researchers. The B is measured calorimetrically using Azomethine-H (Bingham, 1982).

Apparatus

Spectrophotometer, Erlenmeyer flasks, Polypropylene test tubes

Reagents

- Buffer Solution: Dissolve 250 g ammonium acetate(NH_4OAc), and 15 g EDTA disodium (ethylenediamine-tetraacetic acid, disodium salt)in 400 mL distilled water. Slowly add 125 mL glacial acetic acid(CH_3COOH), and mix well.
- Azomethine-H Solution ($C_{17}H_{12}NNa0_8S_2$):Dissolve1 g L-ascorbic acidin 100 mL distilled water, and then add 0.45 g Azomethine-H, and mix well. Fresh reagent should be prepared weekly and stored in a refrigerator.
- Standard Stock Solution: Dissolve 0.114 g boric acid(H_3BO_3) in distilled water, and bring to 1-L volume. This solution contains 20 ppm B (Stock Solution). Prepare a series of working Standard Solutions from the Stock Solution by diluting 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mL Stock Solution to 100 mL numbered flasks by adding distilled water, and then bring to volume. These solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm, respectively.

Procedure

1. Pipette 2 mL aliquot of the natural water sample in to a 10 mL polypropylene tube.
2. Add 4 mL buffer solution.
3. Add 4 mL Azomethine-H solution, and mix well.

4. Prepare a standard curve by pipetting 2 mL of each standard (0.5 - 3.0 ppm), and proceed as for the samples. Also make a blank with 2 mL distilled water, and proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 30 minutes on the Spectrophotometer at 420-nm wavelength.
6. Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations.
7. Read B concentration in the unknown samples from the calibration curve.

Calculation

B (mg L-1) = (S-A)
Where S =B (mg L-1) from standard curve in sample
A = B (mg L-1) from standard curve in blank.

Estimation of Sodium in irrigation water

The main cations present in irrigation samples are those of calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K). In effluents, swage and waste waters heavy metals are also found.

Sodium (Na)is a common element, the sixth most abundant, and present to some extent in most natural waters. Sodium is present in a number of minerals, the principal one being rock salt (sodium chloride). Sewage, industrial effluents, sea water intrusion in coastal area, and the use of Na compounds for corrosion control and water-softening processes all contribute to Na concentration in water because of the high solubility of sodium salts and minerals. Sodium levels in groundwater vary widely but normally range between 6 and 130 mg L^{-1}.

Reagents

A. Standard stock solution (100 me Na L^{-1})

- Dissolve 5.845g of AR grade dried NaCl in distilled water and make the volume to 1 L

B. Working standard solution

- Dilute 5, 10, 15, 20, 30, 40and 50 mL portion of the stock solution (containing 100 me Na L^{-1}) to 100 mL in volumetric flask to get working standards of 5, 10, 15, 20, 30, 40, and 50 me Na L^{-1} concentrations.

Procedure

1. Filter a portion of water sample through Whatman filter paper No. 42.
2. Calibrate Flame Photometer with a series of suitable Na standards with distilled water as blank sample.
3. Measure the water samples, take the emission reading on the Flame Photometer at 589-nm wavelength, and record the readings.
4. Draw a calibration curve.

Calculations

Na (me L^{-1}) = Na(ppm)from calibration curve/23
Where:
23 = Atomic weight of Na

Estimation of Calcium and Magnesium in irrigation water

Calcium (Ca) is dissolved easily out of almost all rocks and is, consequently, detected in most waters. Magnesium (Mg) are relatively abundant in the earth's crust and hence a common constituent of natural water. Waters associated with granite or siliceous sand usually contain less than 10 mg of calcium per litre and less than 5 mg magnesium per litre. Many waters from limestone areas may contain 30-100 Ca per litre, and those associated with gypsiferous shale may contain several hundred milligrams per litre. But for the water in contact with dolomite or Mg-rich limestone may content 10-50 mgL^{-1} and several hundred milligrams per liter may be present in water that has been in contact with deposits containing sulfates and chlorides of magnesium.

Calcium and Mg contribute to the hardness of water,it should be noted that the difference between total hardness and the Ca concentration can be used to calculate the magnesium concentration. However, some $CaCO_3$ is desirable for domestic waters because it provide a coating in the pipes which protects them against corrosion.

Principle

EDTA-disodium salt solution is used to chelate Ca^{2+} + Mg^{2+}. Calcium is separately estimated by the versenate method using ammonium purpuate (Murexide) indicator, when the pH is made sufficiently high, the Mg is largely precipitated as hydroxide and an indicator is used that combines with Ca only. Thus, Mg can be obtained by deduction of Ca from Ca+Mg content. Both cations can also estimated by atomic absorption spectrophotometer.

Apparatus

Burette (25 or 50 mL), Pipette, Stirring rods, Graduated cylinder, Beakers

Reagents

- Buffer Solution (NH_4Cl-NH_4OH): Dissolve 67.5 g NH_4Cl in 570 mL concentrated NH_4OH, and transfer the solution to a 1 L flask, let it cool, and bring to volume.
- Eriochrome Black T Indicator: Dissolve 0.5 g eriochrome black and 4.5 g hydroxylamine hydrochloride in 100 mL 95 % ethyl alcohol. Prepare a fresh batch every month.
- Ethylene Diaminetetraacetic Acid Solution (EDTA), 0.01N: Dissolve 2 g EDTA, and 0.05 g

magnesium chloride ($MgCl_2$) in distilled water, and bring to 1 L volume.

- Sodium Hydroxide Solution (NaOH), 2 N: Dissolve 80 g NaOH in about 800 mL distilled water, transfer the solution to a 1 L flask, cool, and bring to volume.
- Ammonium Purpurate Indicator ($C_8H_8N_6O_6$): Mix 0.5 g ammonium purpurate (Murexide) with 100 g potassium sulfate (K_2SO_4).
- **Standard Stock Calcium Chloride Solution ($CaCl_2.2H_2O$), 0.01.:**Dry about 3 g $CaCO_3$in an oven at 100 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle. Dissolve 0.5 g dried $CaCO_3$in 10 mL 3 N hydrochloric acid (HCL)and bring to1 L volume with distilled water. Standard stock can also be prepared by dissolving 0.735 g calcium chloride dihydrate($CaCl_2.2H_2O$) in 1 L volume with distilled water.

Procedure

A. Calcium

1. Pipette a suitable aliquot of water sample (10 mL natural water sample) into a 250 mL Erlenmeyer flask.
2. Dilute to 20 - 30 mL with distilled water, add 2 - 3 mL 2 *N* NaOH solution, and about 50 mg ammonium purpurate indicator.
3. Titrate with 0.01 *N* EDTA. The color change is from red to lavender or purple. Near the end point, EDTA should be added one drop every 10 seconds since the color change is not instantaneous.

B. Calcium plus Magnesium

1. Pipette a suitable aliquot of water sample (10 mL natural water sample), dilute to 20-30 mL with distilled water. Then add 3-5 mL buffer solution. And a few drops eriochrome black indicator.
2. Titrate with 0.01 *N* EDTA until the color changes from red to blue.
3. In order to standardize the EDTA solution used in the determination of Ca and Mg:
   - Pipette 10 mL 0.01 *N* calcium chloridesolution, and treat it as in determining Ca and Ca+Mg procedure, respectively.
   - Take the reading, and calculate EDTA normality:

$$\textbf{NaEDTA}\ (\text{me L}^{-1}) = \frac{10 \times N\ CaCl_2}{V\ EDTA}$$

Where:

N EDTA = Normality of EDTA solution
V EDTA = Volume of EDTA solution used (mL)
N CaCl2= Normality of $CaCl_2$ solution

Calculation

$$\textbf{Ca or Ca + Mg}\ (\text{me L}^{-1}) = \frac{V_1 \times N \times 1000}{V}$$

$$\text{Mg}\left(\text{me L}^{-1}\right) = \text{Ca + Mg}\left(\text{me L}^{-1}\right) - \text{Ca}\ (\text{me L}^{-1})$$

Where:

V1 = Volume of EDTA titrated for the sample (mL)
N = Normality of EDTA solution
V = Volume of water sample used for measurement (mL)

Practical suggestions

- In most water nearly all of the hardness is due to Ca and Mg, which react with soap to form precipitates .This in increases soap consumption, and react with certain constituents to form scale. As a general rule, a value less than 60 is considered soft, and values above 200 are considered very hard.
- If an Atomic Absorption Spectrophotometer is used, a small aliquot of the water sample is sufficient to determine Ca and Mg.
- Orthophosphate precipitates Ca at pH of the test. Strontium (Sr) and barium (Ba) interfere with the Ca determination, and alkalinity in excess of 300 mg L^{-1} may cause an indistinct end-point with hard waters. Under the conditions of the test, normal concentration of the following ions causes no interference with the Ca determination: Cu, Fe, Mn, Zn, Al, Pb, Cu, and Sn.

Estimation of Water quality indices and suitability

After determination of water samples for different parameters like total salts (EC), cations and anions, it is imperative to calculate some indices in order to asses water quality and its subsequent effect on soil as well as plant growth.

Sodium Adsorption Ratio (SAR):

It is calculate to indicate the sodicity or alkalinity hazards of irrigation water.

SAR= $$\frac{Na}{\frac{\sqrt{Ca+Mg}}{2}}$$

Residual sodium carbonate(RSC):

The RSC may be calculated simply by subtracting the quantity of Ca + Mg from the total of the carbonates and bicarbonates determined in different samples and expressed in me L^{-1}

RSC = $(CO_3^{-2} + HCO_3^{-}) - (Ca^{++} + Mg^{++})$

Business idea: <u>Development of Soil Salinity management centre</u>

Determination of soil texture by feel method and by Bouyoucos hydrometer method.

Determination of texture by feeling of the soil with the fingers requires considerable practices and experience. However, one can do fairly well if the following steps are followed carefully.

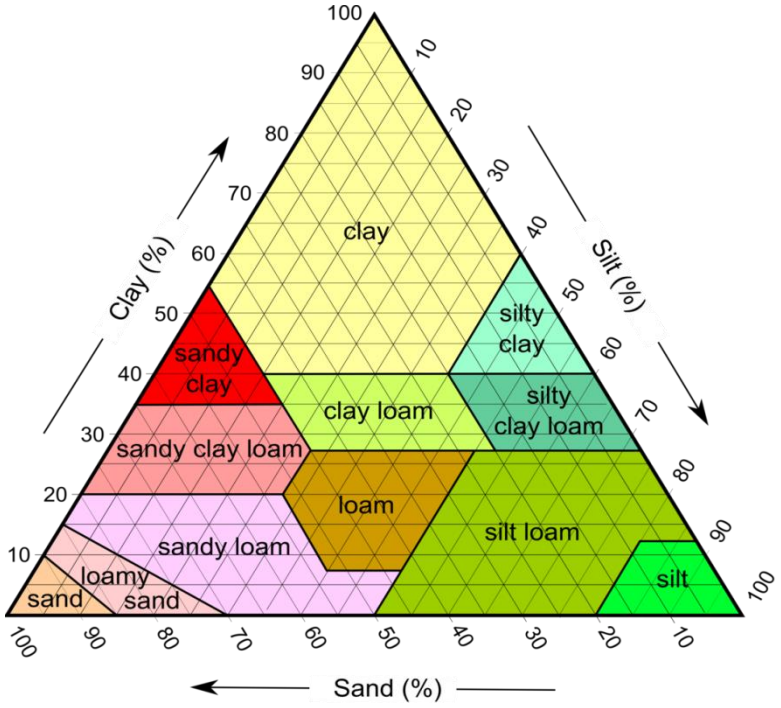


Fig: Soil Textural triangle

Procedure:

1. Determination whether the soil fits into a clay, clay loam and loam class based upon ribbon formation as good, medium or poor ribbon. To do this, place about ½ teaspoonful of soil in pour hand, Add water very slowly drop by drop, from a water bottle or the tap. Knead the soil all the while and bring it to the consistency of a moist, workable condition. When the soil is at the proper consistency try to press it into a ribbon between your thumb and fore-finger.
2. Once it has been determined whether the ribbon is good, medium or poor, the soil may be classified as a clay, clay loam or loam, respectively, as indicated in above fig.
3. When it has been determined that the given sample of soil is clay, clay loam or loam, re-examine the sample. If it has a gritty feeling indicating the presence of sand. Call it sandy clay, a sandy clay loam or sandy loam, depending upon a tube of ribbon it makes. If grittiness

can be detected by the teeth but not the fingers, call it a silty clay, loam or silt loam. If it is neither sandy nor silty but has exceptionally smooth feeling, call it clay or a clay loam depending the tube of ribbon it produces.

4. Soils that are loose and single-grained when dry and form a fragile cast when moist are called sand.
5. Determine texture of the sample provided and record the results on the data sheet.

Observations:

Note the textural class of the soil as per the visual observation and feel and mentioned textural class as per above triangle.

Result :

The textural class of the soil is

Questions:

1 .Determine the textural class of the soils as per visual observation.

2. Differentiate between textural class given by textural triangle, ISSS and feel method

Reference URL

- https://www.youtube.com/watch?v=GWZwbVJCNec

Determination of soil texture by Bouyoucos hydrometer method.

The process of determining the amount of individual soil separates below 2 mm in diameter i.e. sand, silt and clay is called particle size analysis. It consists of determination of the percentage of various sized particles. (Sand, fine sand, silt and clay) as they exist in the soil. An essential first step is to separate the particles so that they function as individuals. This is called dispersion. The completely dispersed individual primary particles are usually referred to as textural separates.

Method of particle size Analysis. :

Principle :

The hydrometer method is based on the principle that the density of the suspension at the given depth decreases as an initially homogeneous dispersed suspension settles. The rate of decreases in density at the given depth is related to the settling velocities of the particles, which in turn, are related to their sizes. The time required by the particles of a given size to settle can be calculated by using strokes Law.

Stroke's Law :

Strokes (1851) stated that the velocity of a falling particle is proportional to the radius square and not to its surface. Equationally.

$$V = \frac{2}{9}\left(\frac{d_p - d}{\eta}\right) g r^2$$

Where

V = Velocity of settling particle
dp = density of particle
d = density of liquid
g = acceleration due to gravity
r = radius of particle
η = Viscosity of liquid

Apparatus and Equipments

1) Dispersing machine - Mechanical stirrer

2) 1000 ml cylinders without spout with rubber stoppers/spout
3) Thermometer (Fahrenheit scale)
4) Wash bottle.
5) 600 ml beaker with cover
6) Hot plate or water bath.
7) Bouyoucos hydrometer.
8) Watch glass.
9) Chemical balance.
10) Oven

Reagents :

1) 5 per cent sodium hexametaphosphate (dispersing agent)
2) 30 per cent Hydrogen peroxide
3) Amyl alcohol.

Procedure :

1) Weight 40 g sample of air-dry soil passed through 2 mm sieve in duplicate. Dry one of the samples in the oven at 105^0 C to determine the moisture content of soil. Meanwhile transfer the other sample to a 600 ml beaker and add to it 200 ml of distilled water.
2) Add 4 to 5 ml of 30 per cent H_2O_2 cover the beaker with watch glass and place it on water bath until most of the organic matter is destroyed, then remove the beaker from water bath and allow it to cool.
3) Repeat the process until the colour of the suspension ceases to become lighter or until frothing stops. 15 ml of H_2O_2 will usually be sufficient for a 40 g sample but more may be needed for soils high in organic matter. After the last addition of $H_2 O_2$ the beaker with soil is placed on the water bath for two hours to remove the excess $H_2 O_2$.
4) While waiting for the oxidation of the organic matter, put 10 ml of sodium hexametaphosphate solution in 1000 ml cylinder and fill the cylinder with distilled water to make exactly one litre. Mix the suspension thoroughly and bring it to room temperature. Insert the hydrometer into the solution carefully and determine the scale reading. Record this reading as RL, the calibration correction.
5) Transfer the organic matter-free soil sample to the dispersing cup, fill it with distilled water to the level of 4 cm from the top and add 10 ml sodium hexametaphosphate solution. Allow the soil

to soak it for at least 15 minute. Then stir the soil suspension in the cup with the help of high-speed electrical stirrer for 10 minutes.

6) Pour and wash the contents of the dispersing cup into the special one litre cylinder with distilled water up to one-liter mark. Place a rubber stopper over the mouth of cylinder and shake it mechanically end-over-end for one minute.

7) Place the cylinder on the table and note the time immediately. Take the first hydrometer reading after 4 minute when the particles larger than 0.02 mm in diameter have settled (start inserting the hydrometer 10 seconds in advances of reading time). If the surface of the suspension is frothy, add one drop of amyl alcohol.

8) Remove the hydrometer carefully and wash it with distilled water. Measure and record the temperature of the suspension. The hydrometer is calibrated at 67^0F at any other temperature a correction must be applied if the working temp is above 67^0F the correction is added, if below 67^0F the correction is subtracted. The correction is equal to the difference between working temperature and 67^0 F multiplied by 0.2

9) Allow the suspension to remain undisturbed and re-insert the hydrometer at the end of two hours after the initial shaking was stopped. Now the particles larger than 0.002 mm. I.e. sand + silt will have settled. Record the hydrometer reading.

10) Calculate the percentage of sand, silt and clay in soil sample.

Observations

1.	Hydrometer reading at minutes	-	R_4
2.	Hydrometer reading at 2 hours	-	R_{120}
3.	Oven dry weight of soil sample	-	W
4.	Temperature correction	-	r
5.	Calibration correction	-	RL.

Calculations:

$$\boldsymbol{P_4 = \frac{R_4 - R_2 + r}{W} 100}$$

$$\boldsymbol{P_{120} = \frac{R_{120} - RL + r}{W} 100}$$

Calculate P_4 from the 4 minute reading and P_{120} from the 2 hours since P_{120} = % Clay and P_4 = % silt + % clay, then we may say that.

% Sand = 100- P_4

% Silt = P_4 - P_{120}

% Clay = P_{120}

Results :

Textural class of the soil is

Questions :

1. Define particle size of analysis..
2. Explain Stokes Law and how is it applied in soil particle analysis?
3. Explain the role of H_2O_2 in the particle size analysis.
4. Why organic matter is destroyed?
5. What is the role of Sodium hexametaphosphate?
6. Mention the size of different soil particles.

Reference:

1) Khanna, S.S. and Yadav, D.V. 1979. Practical Manual for Introductory courses in soils. pp. 33-43.
2) Piper C.S. Soil and Plant analysis 1966.

Reference URL

- https://www.youtube.com/watch?v=GfDW3Fw9cL0

Determination of Soil Suction by Tensiometer

Tensiometer is widely used for years together to measure soil tension/suction which can be used as the guide for scheduling irrigation. It is relatively cheaper, simple and easy to install. It works over a range of soil moisture tension of 0 to 0.8 bars. Tensiometer consists of a fine porous ceramic cup of 7.5 cm long connected with a manometer or vacuum gauge through a plastic tube.

Tensiometer

The manometer or vacuum gauge indicates vacuum pressure created relative to atmospheric pressure. Tensiometer is filled with water and is made air tight. Now, if the porous cup is placed in relatively dry. Soil water will come out through the pores of the ceramic cup to attain equilibrium between the suction on manometer or gauge and the soil (matric potential). This leads to a drop of hydrostatic pressure which is measured by manometer or vacuum gauge.

Characteristics of a Standard Porous Cup

The material of the ceramic cup should he such that it will pass through the following standards:

(i) Air Entry Value : Air entry value is the minimum air pressure difference required to cause air leak through the pores of saturated cup. In the laboratory it can be tested by connecting (ho cup with a compressor fitted with a gauge through a plastic lube. Water saturated cup is then dipped in a beaker filled with water. By releasing pressure slowly from the compressor observe the point at which air bubbles start to appear. This is the air entry value which must be in the range of 0.8 to

0.9 bars.

(ii) Cup Conductivity : It is the volume of water passing through the cup wall per unit time per unit hydraulic pressure head difference. Its value should be 3.95 ml/ sec/ atm.

Testing of Tensiometer

Before installation each tensiometer should be tested whether it passes through standards otherwise reject it.

Procedure

- Immerse the porous cup of the tensiometer into water for 2 to 3 days to get it saturated.
- For tensiometer having manometer add some mercury to the manometer and then fill the tensiometer and manometer with water. In case of tensiometer with vacuum gauge fill the tensiometer with water only.
- Close the opening of the tensiometer with the cap to make it air tight.
- Dry the porous cup in air so that about 0.7 bar suction develops on the tensiometer.
- Oil dipping the cup in water for good tensiometer the suction goes down to zero within 5 minutes.

Installation

- Make a hole in the field to slightly deeper than the desired depth with the help of screw type / tube auger having almost same outer diameter as that of porous cup.
- Drop some amount of loose excavated soil of the same depth and some amount of water into the hole.
- Insert the tensiometer ensuring close contact of the cup with the field soil.
- Compact the soil around the tube and make a small heap with the soil to avoid water stagnation.
- Fill the tensiometer with deaerated water; tensiometer will show zero reading.

- With time as water from the cup will come out to the soil to attain equilibrium record the tensiometer readings.
- Correlate the tensiometer readings with soil moisture content.

Calculation

Say,

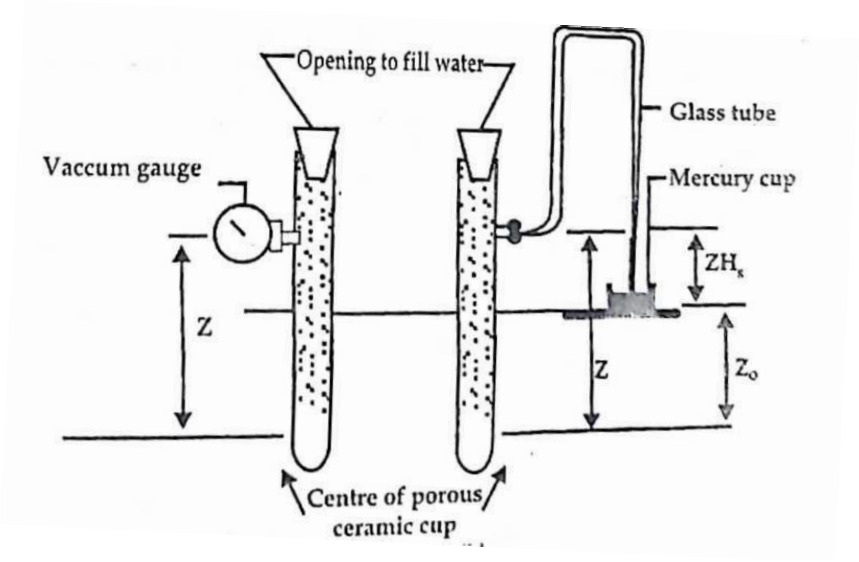
In case of tensiometer with vacuum gauge:

Matric potential (Φ_m) = - (F_g * R_g) + Z

Where, F_g = Gauge factor (suction value for each unit of gauge reading)

R_g = Gauge reading

Z = Vertical distance from the centre of the cup to the centre of dial



Business idea: Development of seed testing laboratory

Development of Seed Testing Laboratories

Seed Testing

Seed testing is determining the standards of a seed lot viz., physical purity, moisture, germination and ODV and thereby enabling the farming community to get quality seeds. The Seed Testing Laboratory is the hub of seed quality control. Seed testing services are required from time to time to gain information regarding planting value of seed lots. Seed testing is possible for all those who produce, sell and use seeds.

Objective & Importance of Seed Testing

Seed testing is required to achieve the following objectives for minimising the risks of planting low quality seeds.

1. To identify the quality problem and their probable cause
2. To determine their quality, that is, their suitability for planting
3. To determine the need for drying and processing and specific procedures that should be used
4. To determine if seed meets established quality standards or labelling specifications.
5. To establish quality and provide a basis for price and consumer discrimination among lots in the market. The primary aim of the seed testing is to obtain accurate and reproducible results regarding the quality status of the seed samples submitted to the Seed Testing Laboratories. Importance The importance of seed testing was realized more than 100 years ago for assured planting values. The adulteration of vegetable seeds by stone dust which was packed in some parts of the world particularly in Europe. Seed testing has been developed to aid agriculture to avoid some of the hazards of crop production by furnishing the needed information about different quality attributes viz., purity, moisture, germination, vigour and health. Quality control of seed depends on the different seed testing protocols which determine the genuineness of the cultivar. Testing of seed to evaluate the planting value and the authenticity of the certified lot. Seed testing is required to assess the seed quality attributes of the seed lots which have to be offered for sale. These quality attributes are seed moisture content, germination and vigour, physical and genetic purity, freedom from seed borne diseases and insect infestation. In India, seed testing is done mainly for moisture, germination and physical purity of seeds. Standard seed testing procedures for the evaluation of the seeds were developed by ISTA. It is obligatory on the part of the seed analyst to follow rules prescribed by ISTA (ISTA, 1985) if the seed is moving to

the International trade. The seed testing procedures which are described below are based mostly on the international rules because most of our rules (Chalam et al.. 1967) are based on, ISTA, 1996. Economic yield of a crop depends on the quality of seeds which can be evaluated by seed testing (1STA, 1996). The testing of seed quality is carried out on seed samples drawn from seed lot to be used for cultivation. The quantity of seed sample taken for testing in laboratory is minute compared to that of seed lot it represents.

Role of Seed Testing Laboratories

Sampling in Seed Testing Laboratory The seed samples received in the laboratory (submitted sample) are required to be reduced to obtain working samples for carrying out various tests. A number of methods are available for obtaining working samples. Mixing and dividing of seeds The main objective of mixing and dividing of seeds is to obtain the representative homogenous seed sample for analysis by reducing the submitted sample to the desired size of working sample.

Testing of Seed Viability (Kittock and Law, 1968)

Principle: Tetrazolium (TZ) testing is a rapid method (can be finished within less than two days) for the evaluation of seed viability. This method has been widely used by seed scientists to assess germination potentials, to determine the extent of seed damage, and to evaluate seed vigor and/or other seed lot problems.

The principle of TZ testing is based on the presence of dehydrogenase activity in viable seed tissues during the respiration process. Dehydrogenase can catalyze the colorless 2,3,5 triphenyl tetrazolium chloride solution into a red dye formazan. Therefore, living tissues of seeds that imbibe tetrazolium chloride will be stained red, while dead tissues will retain their natural color.

Apparatus: Spectrophotometer

Reagent:

1. Sprouted embryo of cereal, proteinaceous and fatty seed
2. Distilled Water
3. 2,3,5 triphenyl tetrazolium chloride
4. 2-methoxy ethanol

Procedure: Seed viability test of seed was estimated following the method of Kittock and Law (1968) with minor modifications. Ten seeds of each category seeds were placed for germination on the petridish at 20±1°C. After 24 h of germination, twenty uniformly sprouted embryos for each treatment was taken in a 10 ml capacity glass vial and then incubated with 2 ml of 0.2% solution of tetrazolium chloride for 3 h at 30°C in dark. After incubation, the tetrazolium chloride solution was decanted off and the embryos were thoroughly washed with distilled water. Five milliliter of methyl cellosolve (2-methoxy ethanol) were added on the vial containing embryos and kept for 8 h at room temperature (28±1°C) for red colour development (formazan

Observations: The red colour intensity of solution was measured in the Spectrophotometer at 470 nm.

Result: The red colour or development of formazan due to activity of dehydrogenase activity shos only in viable seeds.

Activity of viable seeds are measured in…….. OD value.

Seed Germination Test

Principle: The purpose of laboratory testing of seed germination is to assess seed quality or viability and to predict performance of the seed and seedling in the field. A NOTIFIED laboratory under SEEDS ACT or qualified laboratory of ISTA for testing seeds must test seed processed for sale. The ultimate aim of testing the germination in seed testing laboratory is to obtain information about the planting value of the seed sample and by inference the quality of the seed lot. In addition, the laboratory germination results are also required for comparing the performance potential or superiority of the different seed lots. In general, the farmers, seeds men and public agencies use the germination results for the following purposes:

1. Sowing purposes, with a view to decide the seed rate to achieve desired field establishment.
2. Labeling purposes.
3. Seed certification purposes.
4. Seed Act and Law Enforcement purposes.

Procedure: Seeds are placed on one or more layers of moist filter paper or blotter paper in petriplates. These petriplates are covered with lid and placed inside the germination cabinet. This is suitable for those seeds which require light.

Precautions: before germination seeds may be treated with fungicide for fungal occurrence.

Observation

Types of seed	Germination %	Root length (mm)	Shoot length (mm)	Total Seedling Length (mm)

Result: Germination of seed with seedling length measured by ……..mm.

Seed Physical Purity Test

Principle: The purity analysis of a seed sample in the seed testing laboratory refers to the determination of the different components of the purity viz., pure seeds, other crop seeds, weed seeds and inert matter. The objective of the purity analysis is to determine whether the submitted sample conforms to the prescribed physical quality standards with regard to physical components.

Procedure:

Sample preparation: The purity analysis is done on the working sample of prescribed weight drawn from submitted sample. The analysis may be made on one working sample of the prescribed weight or on two sub-samples of atleast half of this weight, each independently drawn.

Weighing the working sample: The number of decimal places to which the working sample and the componenets of the working sample should be weighed is given below.

Purity separation: The working sample after weighing is separated into its components viz., pure seed, other seed crop, weed seed and inert matter.

Pure seed: The seeds of kind / species stated by the sender. It includes all botanical varieties of that kind / species. Immature, undersized, shrivelled, diseased or germinated seeds are also pure seeds. It also includes broken seeds, if the size is >1/2 of the original size except in leguminacea, and cruciferae where the seed coat entirely removed are regarded as inert matter.

Other crop seed: It refers to the seeds of crops other than the kind being examined. Weed Seed It includes seeds of those species normally recognized as weeds or specified under Seed Act as a noxious weed.

Inert matter: It includes seed like structures, stem pieces, leaves, sand particles, stone particles, empty glumes, lemmas, paleas, chaff, awns, stalks longer than florets and spikelets.

Method of purity separation

Place the sample on the purity work board after sieving / blowing operations and separate into other crop seeds and inert matter. After separation, identify each kind of weed seeds, other crop seeds as to genus and species. The names and number of each are recorded. The type of inert matter present should also be noted.

Calculation

All the four components must be weighed to the required number of decimal places. The

percentages of the components are determined as follows.

$$\% \text{ of components} = \frac{\text{Weight of individual component}}{\text{Total weight of all components}} \times 100$$

Electrical conductance of seed leachate (Anderson *et al*., 1964**):**

Principle: The conductivity test is a measurement of electrolytes leaking from seeds. Changes in the organization of cell membranes occur during the development of seeds prior to physiological maturity, seed desiccation before harvest, and during imbibition prior to germination (ISTA 1995).

Apparatus: Conductivity Bridge (cell constant 0.756).

Reagent: Distilled water

Procedure: Electrical conductance of different category seeds were measured under ambient conditions. The electrical conductivity of seed leachate was done following the method of Anderson *et al*., (1964). Twenty wheat seeds were soaked in 30 ml distilled water for 30 min and then electrical conductance was measured in Conductivity Bridge (cell constant 0.756).

Observation:

Types of seed	Measure of electrical Conductance(dsm^{-1})

Result: Electrical conductance of different category seeds measured as ……..dsm^{-1}

Determination of test weight

Principle: The objective is to determine the weight of the 1000 seed from the submitted sample to determine the quality of the product. The working sample shall be the entire pure seed fraction of purity analysis. Either the whole working sample of replicates from it may be used.

Procedure: Count sample pure seeds and multiply with 1000 seed.

Calculation:

$$\text{Test weight} = \frac{\text{Weight of pure seed sample}}{\text{Total weight of sample}} \times 100$$

Development of GIS and RS based soil mapping

Introduction to GIS and RS software: downloading ,installation, plugins

Objective: Understand the basic concept of GIS, raster, vector,projections,geo-processing and analysis.Use a GIS for basic skills in: thematic mapping, importing tabular data and GIS interpretation, basic vector data analysis, finding and using open access data, styling and map design.

Software: Quantum GIS 3.6.3

System/Hardware Requirements:

- Processor-CPU : core i7 64 bit Processor
- Memory RAM : 8 GB or more
- Hard Disk : SSD de 128 Gb or 500 Gb SATA
- Graphic card : 2 Gb RAM
- Operating System : Windows 10

Web Browser : Google Chorme

Quantum GIS is an Open Source Geographic Information System. The QGIS software package currently runs on most versions of Unix, Windows and Mac OS X and supports a number of common raster and vector data formats. QGIS is available under the GNU General Public License (GPL).

QGIS aims to be a user-friendly GIS,providing common functions and features.The initial goal of the software was to provide a GIS data viewer. QGIS is available at no cost to users and provides an alternative to costly GIS software.

Methodology:

Downloading QGIS Software:

Steps followed

Step1. To install the software QGIS must choose the web browser of your choice on your computer (for demonstration we are using Google Chrome).In your preferred web browser navigate to the QGIS download page: http://www.qgis.org/en/site/forusers/download.html

Step2. Based on your operating system (Mac OSX, Windows, Unix) locate the “latest release” and download the “QGIS Standalone Installer.” The examples below outline the Download for

Windows and Mac OSX (Version3.6.3)

*If you are a Windows user, be sure to check your machine and download the correct file based on system type (**32 bit vs. 64 bit**). This can be done by right clicking on "Computer" in the start menu and selecting "Properties"

Installing QGIS Software:

1. After the file download has completed (about 2-5 minutes depending on connection), run the '.exe' program. The file name will be similar to "QGIS-OSGeo4W-3.6.3-1-Setupx86_64.exe" and can be located in your default downloads folder.

2. After running the file and accepting the terms of agreement, select where you would like the program saved under "Destination Folder" (default is recommended).

3. Select the components to install.

4. Click "Install."

5. After the installation, shortcuts will be placed to the desktop and the start menu. To open QGIS, click on the "QGIS Desktop 3.6.3" or QGIS Desktop 3.6.3 with GRASS 7.6.1" icon.

QGIS Plugins:

Plugins in QGIS add useful features to the software.Plugins are written by Qgis developers and other independent users who want to extend the core functionality of the software.These plugins are made available in QGIS for all the users.

Plugins are optional tools that you can download and import into your QGIS for additional functionality.

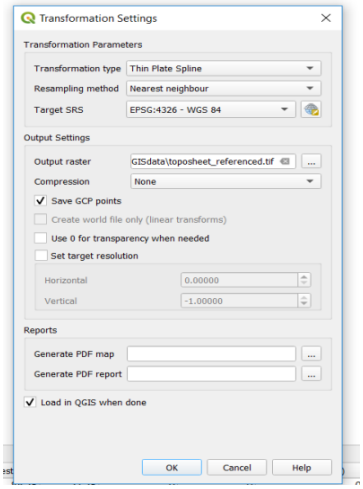
A useful plugin for QGIS is 'Georeferencing GDAL'. This is core plugin-meaning it is already part of your QGIS installation. You just need to enable it.

1. After opening the QGIS Desktop application, Go to the Plugins > Manage and Install Plugins and enable the Georeferencer GDAL plugin in the installed tab.

2.The plugin isninstalled in the Raster menu. Click on Raster> Georeferencer to open the plugin.

Conclusion:

Geo-referencing of a toposheet in GIS software



Objective: Geo-referencing a toposheet by using graticule intersections in a known coordinate system and datum.

Software: Quantum GIS 3.6.3

Georeferencing is a process of establishing a mathematical relationship between the image coordinate system and the real world spatial coordinate system. This mathematical relationship can be assigned by any one of the transformation settings, viz. Polynomial order 1, 2 or 3, Linear, Projective and Thin Plate Spline etc. Polynomial order 2 is the most widely used transformation in Georeferencing. Recently Thin plate Spline gaining popularity due its ability of incorporating the local deformations in the data, this is very useful when we are dealing with low resolution data. However in this practical we are using traditional polynomial order 2 transormation or Thin plate Spline to perform georeferencing/rectification of the Toposheet.

Workflow:

1. Open QGIS software
2. Add the topo sheet
3. Open georeferencer and add the import the topo sheet
4. Add GCP points or add points from map canvas
5. Transformation settings
6. Start georeferencing process

Methodology:

1. Registering a raster images using GCP's (Ground Control Point)

1. We start by opening the georeferenced image in QGIS by clicking on the **'Add Raster layer'** button or via the Menu bar **(Layer > Add Layer >Add Raster layer)**
2. Click on **Raster >Georeferencer** to open the Georeferencer GDL plugin.

The plugin window is divided into 2 sections. The top section where raster will be displayed and bottom section where table showing your GCPs will appear.

3. Go to **File > Open Raster**.Browse to the folder,select image (Toposheet.tif) and click open.

4. Image will be loaded in the top section.Need to assign coordinates to some points on this map.

To georeference an image we use Ground Control Points (GCPs). GCP is a location on the earth's surface with known coordinates on both earth and Toposheet.Zoom to area and click on **Add Point** Tool and click on the image location.

.

5. To start adding GCPs to our map, we first zoom to a corner of the map where we can easily identify the intersection of the latitude and longitude. Use the scroll wheel of the mouse to zoom in and out of the map. Use the 'Pan' Button when needed.
6. In the pop-up window,entercoordinates.Remember that **X=longitude and Y=latitude.**Click ok.

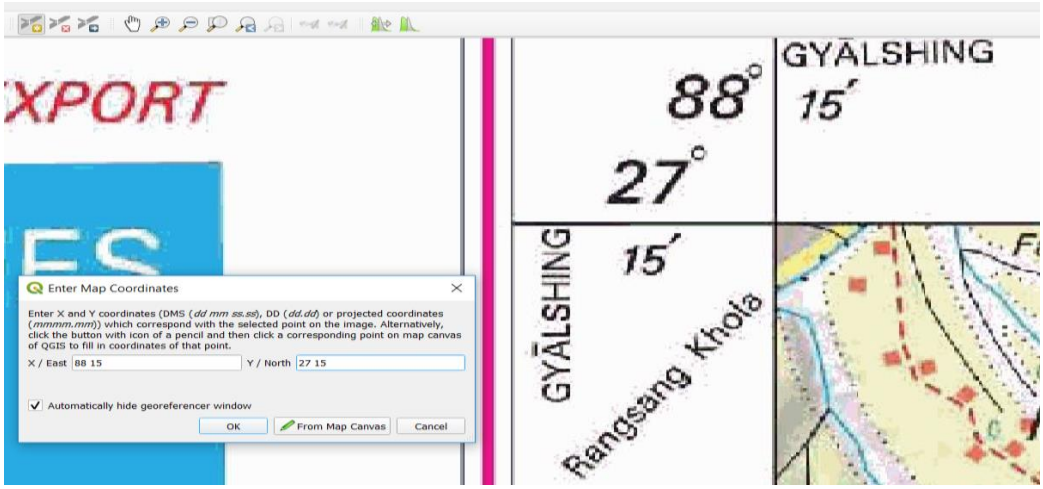
   we will require minimum 6+1(for check) i.e., 7 GCPs or more GCPs on the map. Therefore, we need to mark atleast 7 GCPs. The GCPs locations should be spread out as much as possible and they should not be co-linear at the same time they should enclose our whole area. Use the above procedure to mark six more control points.
7. Go to **Settings >TranformationSettings**.In the Transformation Setting Dialog ,choose The **Transformation Type** as Thin Plate Spline.Resampling method >Nearsrst neighbor Name Your output raster as toposheet_referenced.tif.Choose**EPSG:4326**(EPSG: European Petroleum Survey Group) as the target SRS so the resulting image is in a widely compatible datum.Make sure the Load **in QGIS** when done option is checked. Click **ok**.
8. Back to Georeferencerwindow,Go to **File > 'Start Georeferencing'**

Button. The processing will take at about 2 minutes.Once the process finishes,you will see the referenced raster layer (toposheet_referenced.tif)loaded in QGIS.

2. Registering a raster images using Map canvas

In case, the GCPs are not available to the users, georeferencing process can be done by using a map which is already georeferenced. In the step no 4 (Registering a raster images using GCP's (Ground Control Point)), Go to **Add point**Tool and click on the image location. Then select **From map canvas option.** The display window of QGIS will open where the georeferenced map is already loaded. Click exactly on the same position over the georeferenced map. It will take the coordinates of that point. After doing the same process for at least 7 points follow the

similar steps described in the above section.



Conclusion:

Digitization and area calculation using GIS software

Objective: To delineate different types of land use features on the toposheet.

Digitization is the process of converting analog data into digital data sets. In GIS context digitization refers to creating vector datasets viz., point, line or polygon from raster datasets. With the help of digitization we can create different set of layers Viz. Rivers, roads, schools, ward boundaries and building blocks from a single map; this process is known as **Vectorization**. Vector data is easy to edit, update and is more accurate as compared to raster data. Vector data is more efficient for GIS analysis.

Vector data is mainly of three types –

- Point: It consists of single points having (X, Y) coordinates, for example lamp posts, bus stops and postbox positions etc.
- Line: It consists a series of (X, Y) coordinates in a sequence (from start node to end node with a number of vertices joining these two nodes). For example roads, power lines, ward boundaries and contours etc.
- Polygon: It is a series of (X, Y) coordinates in a sequence closing a figure where first and last points are the same. For example lakes, building blocks, village blocks, ward areas and forests etc

Workflow:

1. Open QGIS software
2. Import the raster layer
3. Create a new shapefile layer
4. Choose the type of features (point, line or polygon)
5. Add the field names and add those to the list
6. Save the file with projection system
7. Select the file in the layer panel and activate the Toggle editing option
8. Digitize the features using Add feature tool
9. Calculate the area and length of polygon and line features respectively using the field calculator

Detailed methodology:

Open the raster layer of georeferenced toposheet in the map canvas of Quantum GIS via, **'Main**

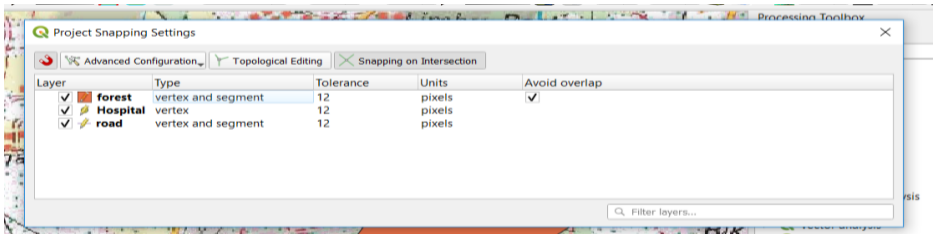
menu bar >Layer >Add Raster Layer' >browse and select the **georeferenced raster layer>Click** on 'Open' in the popup window.

Set snapping options:

1.Go to view menu > Toolbars>Snapping Toolbar

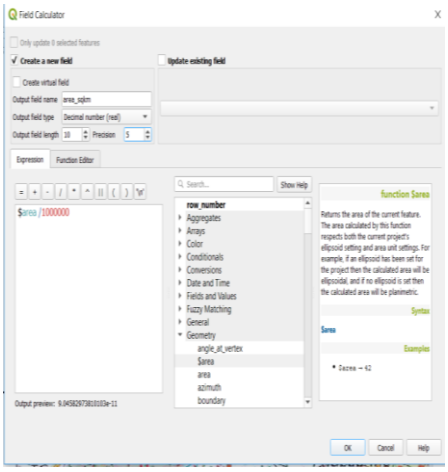
2. In the Snapping toolbar,First click the Advanced Configuration option,then Open Snapping Options;then open snapping options;as shown in the below figure

3. Now set the following things as shown given below



Rater to vector conversion(also called screen digitization)

1. To start digitization of polygon boundary,click Toggle editing tool on the Digitizing toolbar.
2. Click Add Polygon Feature Tool.to begin Digitizing.
3. To place last point right click .this will finalize the feature and show attribute dialog > click ok > click Save layer editing tool.



Geographic area calculation:

1. Select the Polygon/Line layerfrom layer panel.
2. Click the Open Field Calculator tool present in the Attribute toolbar.
3. Select the crate a new field checkbox to save the calculation into a new field.
4. Add Area/Length as Output Field name and Decimal number(real) as Output field type and define Output field length to be 10 and Precision 5.
5. Now double click on function $area for area and $length for length in the Geometry Group to add it into the Field calculator expression box.

Conclusion:

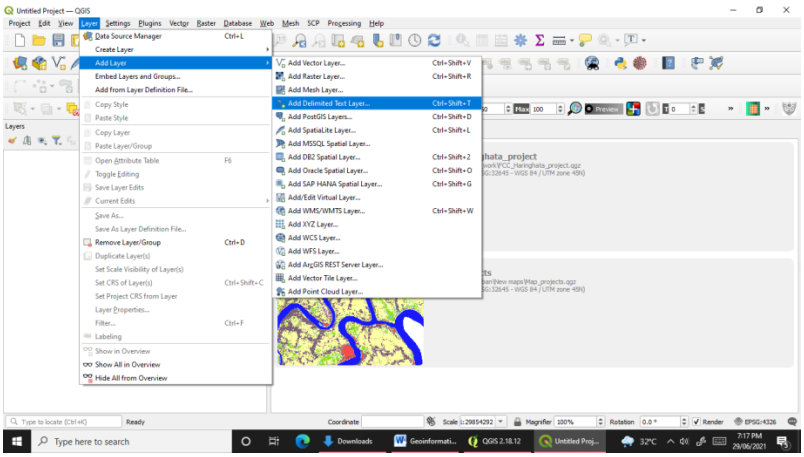
Field data collection using GPS device and data import to QGIS

Objective: To collect the field data (location) and import data to GIS system

Instrument: GPS Garmin e trex 10

Methodology:

1. Visit to the field. Collect the location (longitude and latitude) data using GPS device.
2. Note the location data. Save the data in a ".csv" (comma separated value) file
3. Import the ".csv" file in the QGIS through: Layer → Add layer → Add delimited text layer
4. Data is imported as point file in the GIS system



5. Export the file as a ".shp" file (vector file)
6. Open the attribute table of the vector file
7. Activate the "toggle editing" and add a new filed in the attribute table.
8. Fill the newly added fields with the information collected during field visit corresponding to a particular location

Conclusion:

Collection of open source satellite data and DEM ,Rainfall data, and Soil Map

Objective: To know and collect the open source satellite images.

Web Browser: Google Chrome

Data: For estimate the average soil loss due to erosion , we collect data from different sources. The following data is used to complete the task.

Download LANDSAT Satellite Data

Landsat :represents the world's longest continuously acquired collection of spacebased moderate resolution land remotesensing .All Landsat data are available to the public at no cost from U.S. Geological Survey (USGS) websites.

The USGS Earth Explorer interface uses Google Maps. You can zoom in and out with the mouse wheel as if you are in Google Maps. Google street view is also enabled, where you can drop a marker and get a real view of the location.

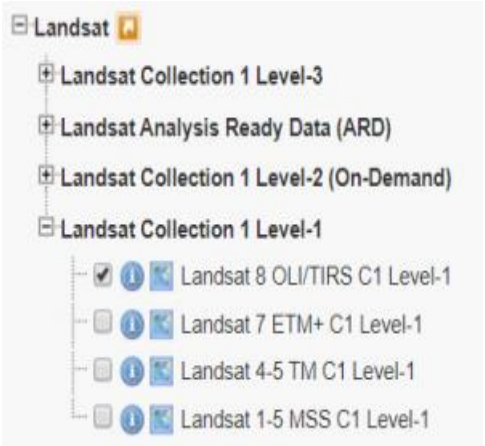
Download Landsat Satellite Data

First, you'll have to create an account with USGS. In the top-right corner, click the **Register** button. As it's a pretty painless process, you'll receive instructions to activate your account.

STEP 1. Register as a user of USGS data and create an account. To be able to download data from Earth Explorer sites, you must first register and create an account. To register and create an account, go here: https://earthexplorer.usgs.gov/register/?return TIP: Be sure to prior to starting your search for Landsat scenes on USGS as the system will not let you download any data unless you are logged in.

STEP 2. Log in (after registering)

1. Search criteria > Enter search criteria > Select a Geocoding Method > Path/Row > polygon > enter Path and Row number > show or at first study area find out > click Use map
2. Data Range > Search from and to > Search months
3. Cloud cover range 0 to 10%
4. Data sets > Landsat > Landsat Collection 2 Level -1 > Landsat 8 OLI/TIRS C2 L1 **or** Landsat 4-5 Tm C2 L1
5. Results > Click on the green download arrow



Download DEM ,Rainfall data, and Soil Map

1. SRTM Digital elevation model (USGS Earth explorer). The spatial resolution of DEM is about 1 arc second.

2. Annual average rainfall data is required to calculate R factor. Month wise rainfall data of Darjeeling district (2016) is collected from the climateknowledgeportal.worldbank.org website.

3. The soil map is collected from the EUROPEAN SOIL DATA CENTRE (ESDAC).

4. K factor values and the C factor values for different objects are collected from USDA (1972).

SL No.	**Parameters**	**Data Used**	**Source of Data Type**	**Techniques**
1	Slope	SRTM DEM 30 m × 30m	U.S Geological Survey	30 m × 30 m surface slope model
2	Elevation	SRTM DEM 30 m × 30m	U.S Geological Survey	30 m × 30 m digital elevation model
3	Rainfall	Rainfall data of Different Stations In 2016	Climateknowedgeportal.worldbank.org	IDW
4	Soil	Reference district soil map 1: 500,000	USDA	Digitization Process
5	Landcover	Landsat8 OLI/TIRS 30 m × 30m In 2018	U.S Geological Survey	Maximum likelihood Classification

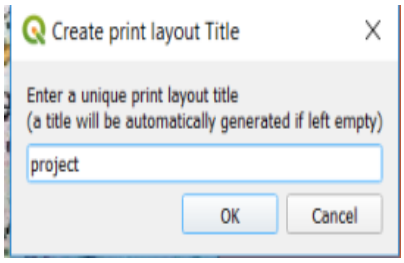
Conclusion:

Creation of different types of map in GIS Software

Objective: to use the QGIS Print layout to create a basic map.

Map Layout is the assembling of the varies elements of a map into a single whole,including the map itself,itslegend,title,scale bars and other elements.

1. Open the map layers via the menu **Layer → Add Vector Layer**. the layer type to **'ESRI Shapefiles**'. Select all the layers by clicking on one and pressing **CTRL+A**. Click 'Open'
   (Now rearrange the layers in such a way, all point features are on top to line features and line features over the polygon features)
2. Let's start the map preparation by setting the colours for the base polygon layers.Right click polygon.shapefile> properties>symbology> symbol layer type > simple fill> fill color choose > apply > ok



3. Go to **project > New print layout.**
4. You will be prompted to enter a title for the layout.So write down any project name and Click ok.In the print layout window,go to **Add Item > Add Map**
5. Once the Add Map button is active,hold the left mouse button and drag a rectangle where you want to insert the map.
6. Click on Item properties tab and enter value for scale
7. We will add grid and zebra border to the main map. In the item properties tab ,scroll down to the **Grids Section > Click the Add a new grid button**.
8. By default,the grid lines use the same units and projections as the currently selected.it is more common and useful to display grid lines in degrees.click on Modify grid…button and click change…. Button next to CRS.
9. In the coordinate Reference system selector dialog,enter 4326 in the Filter box.From the results,select the **WGS84 EPSG:4326** as the CRS.Click ok.
10. Select the interval values **as 0.03000 degrees** in both X and Y direction.
11. Choose the grid type as **Cross**.
12. Scroll down to the Frame Section and select a frame style that suits your taste.
13. Also check the Draw Coordinate section.Display left and right coordinates in Vertical ascending order.
14. In the draw coordinate section > format >**degree,minute and suffix**> font change

15. Now add a north arrow to the map.Click Add Item > Add picture.
16. Click on the item properties tab > Search directories section and select the North Arrow image .
17. Click on Add Item > Add Scale bar.
18. Click on Add Item > Add legend.
19. Click on Add Item > Add Lebel.
20. Export it as Image,PDF or SVG.Click Layout > Export as image
21. Save the project.

Conclusion:

Soil Erosion Risk mapping using RUSLE MODEL

Objective :

1. To briefly describe the soil erodibility in the area.
2. To estimate percentage soil loss per Landcover area
3. To estimate area of percentage soil loss in different slope range
4. To estimate soil loss area in different elevation
5. To estimate annual average soil loss
6. To estimate which area slope and elevation range is high.

Softwares:

Two softwares are used to done this work. For Image processing and classification purpose the ERDAS imagine 2018 is used. Also the ArcMap is used to calculate the R, LS, K, C factor by the raster calculator.

Furthermore, it is noteworthy that for the fulfillment of the study, the entire image processing jobs, GIS analysis and statistical testing has been performed using:

1. ERDAS IMAGINE (v 2018)
2. ArcGIS Desktop (v 10.5)
3. Excel (v 2007)
4. Word(v 2007)

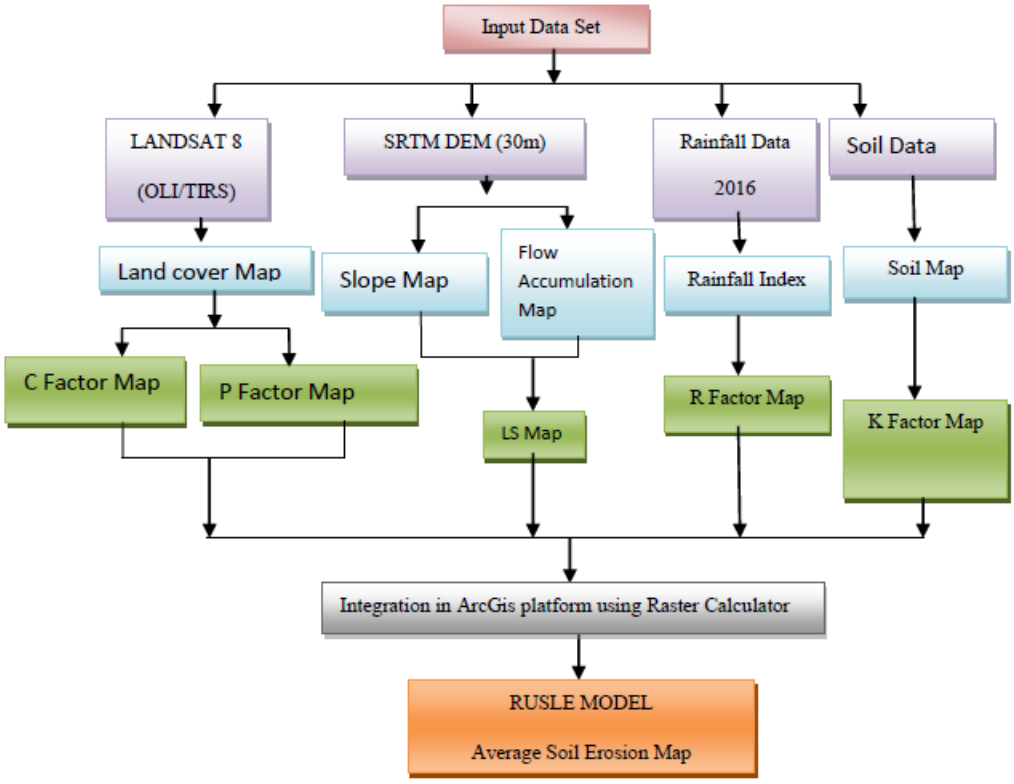
METHODOLOGY:

To survey the various territories of soil erosion through several models, different techniques are used. In this study, with help from the Revised Universal Soil Loss Equation (RUSLE) model, the estimation

of soil erosion is made . Due to the improved nature, the RUSLE model has been utilized and that replica is the modified shape of the Universal Soil loss equation (USLE) and MUSEL. The structure of RUSLE has greater adaptability in displaying disintegration in new conditions and generates more productive results than USLE. The RUSLE model has some additional flexibility in modeling erosion in new conditions and has further economic edge over the USLE model. There is an advantage of using the RUSLE technique because it can be a combination of empirical and process-based styles, and use optimum information as compared to the Universal Soil Loss Equation model. RUSLE factors enable additional adaptability in scheming soil loss by figuring exactly into the sub-factors. This permits the gauge by separating the substance into more substances, from the original substance, through the transportation of deposit . The RUSLE model has been utilized to identify the soil erosion in this study, which is based on GIS. The RUSLE model equation (Equation (1)) is used to estimate the average annual soil loss.

$$\mathbf{A = R \times K \times LS \times C \times P}$$

where, A = yearly mean soil loss (t ha−1 every year), R = precipitation erosivity factor (MJ ha−1 every year), K = soil erodibility factor (t ha h MJ−1 mm−1), C = cover management factor (dimensionless), LS = slope length and slope steepness factor (dimensionless), and P = support practices factor (dimensionless). The raster calculator tool was used to multiply the parameters



given above for the estimation of annual average soil loss of the region. The multiplication of isolated factors generated a combined map of soil loss. Quantity of eroded soil was taken from that final in terms of tons/ha/year. The outputs maps have a 90m resolution, which is acceptable for a regional study. The information elements of the exploration strategy came from different origins. The R-factor , K-factor , and P-factor were recovered from the investigations including the high goals of (100 meter). Through a correlation of the ongoing, the topographic factor (LS)-factor has been determined by comparing the Digital Elevation Model (DEM) data, as well as data that are analyzed from this current investigation. The information that is obtained from the present study has been compared to the information that was obtained through the Advanced Elevation Mode l (DEM).

Rainfall erosivity factor (R) :

R factor is the rainfall erosivity factor that is calculated by different formula which depends on the rainfall erosivity index. In this study the Modified Fournier's index (IMF) is taken to compute the rainfall erosivity index. The rainfall erosivity is depends on the amount of annual average rainfall.

ARNOLDUS (1978) proposed a modified Fournier Index,

$$IMF = \sum_{i=1}^{12} \frac{p_i^2}{P_t}$$

Where, *pi* is the precipitation of each month [mm] and *Pt* is the mean annual precipitation [mm]. RODRIGUEZ ET AL.(2004)who used the generic ICONA equation to estimate R .We consider this formula to calculate R for study area.

$$R = 2{,}56 \cdot IMF^{1{,}065}.$$

Soil erodility factor (K) :

The K factor is the soil erodibility factor of any soil. Which indicates the suspectibility of soil erosion and the rate of run off as measured under the standard unit plot condition. Suspectibilty is depends on the texture of the soil. Also the permeability, presence of organic matter have an impact on the soil erosion. The soil erodibility factor is estimated by using the soil map and the K values. Collection of soil data and assigning the value of K values from field data is too expensive also time consuming. The soil map has been derived with the help of the European Soil Data Centre (ESDAC).

Slope length and steepness factor (LS) :

Topography plays a significant role in erosion. LS factor represent as the multiplication of slope length and the slope steepness that's concern about the topographic condition of surface. Also the length and steepness of slope control the surface runoff speed. The slope value has been derived from the digital elevation model through the spatial analyst tool. Slope length is defined as the horizontal distance from the point of origin of overland flow to the point where either rthe

slope gradient decreases enough that deposition begins, or runoff is concentrated in a defined channel (Renard et al.1997; Wischmeier and Smith 1978) . Slope steepness reflects the influence of slope gradient on erosion. For compute the LS factor we consider the following formula. Moore and Burch (1986)

LS = (Flow accumulation * (cell size)/22.13) ^ (0.4) · [(sin slope/0.0896) ^ 1.3

Cover management factor (C) :

The C factor play significant role in the RUSLE model. The cover management is associated with land use and land cover of the given area. It is the ratio of soil loss from a cropped land in a particular condition to the soil loss in a continuous fallow on the same soil and slope. The C factor values are assigned to the different land use and land cover classes.

Support practices factor (P) :

The P factor is the ration of soil loss with a particular support practices to the corresponding loss of up and down slope cultivation. It is calculated as the relationship between terracing and slope in the paddy field area also the estimation is done according to the relation between contouring and slope in the crop field area. Here we take the value of P as 1, because we did not have any field data.

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Conclusion: