

B.Tech. (Environmental Engineering)

Laboratory Manual

Prepared by



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Laboratory Manuals for UG

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Department of Environmental Science & Engineering

B. Tech. Environmental Engineering

Water Pollution Practical (ESC 251)

Water Chemistry Laboratory (Room No: 307)

INDEX OF EXPERIMENTS

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12.	Determination of sodium and potassium by flame photometer

Do's and Don'ts for the Laboratory safety guidelines

Do's

- *Students will have to wear shoes and Laboratory apron while coming to the laboratory.*
- *Always wear full sleeves clothes. Wear safety glasses and gloves when recommended.*
- *Place all chemicals and supplies at the proper designated locations after use.*
- *It is necessary to take chemicals from reagent bottles and pour slightly more than the amount of chemical needed into a clean beaker.*
- *Always label the samples and reagents.*
- *At the end of each experiment, all used glassware must be cleaned and dried.*
- *Know the locations and operating Experimental Procedures of all safety equipment including: first aid kit(s), and fire extinguisher.*
- *Follow all written and verbal instructions carefully. If you do not understand a direction or part of an Experimental Procedure, ask your supervisor before proceeding with the activity.*

Don'ts

- *Never use broken or chipped glassware. Place broken glassware in specially marked containers.*
- *Never return unused chemicals to the original container.*
- *Always avoid mouth pipetting.*
- *Do not add water to concentrated acid. A concentrated acid should be added carefully to water.*
- *Never leave experiments while in progress.*
- *Do not block the laboratory sink and drains with debris.*
- *Do not work with chemicals until you are sure of their safe handling.*
- *Do not use your mobile phone while working with an experiment.*
- *Do not eat or drink inside the laboratory.*

Experiment No. 1

Aim

Calibration of pH meter, TDS and conductivity meter and determination of pH, TDS and conductivity of a given water sample.

The Experiment No: 1 has been divided into three distinct sections based on their measurable parameters. The Sections are: Experiment No: 1A, 1B & 1C.

Experiment No. 1A

Aim

To ensure calibration of pH meter and measurement of pH of given water sample.

Learning Outcomes

The student will learn the operating Experimental Procedure required to calibrate a pH meter and will gain the experience for measuring pH of unknown water samples.

Brief Theory

Measurement of pH is one of the most important and frequently used tests in water chemistry. The pH of a solution is measured as negative logarithm of hydrogen ion concentration (Eq.1).

$$\text{pH} = -\log [\text{H}^+] \quad \text{Eq. (1)}$$

Where $\{\text{H}^+\}$ is the concentration (or activity) of hydrogen ion (or proton) in moles per liter (M). At a given temperature, the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion concentration. pH values from 0 to 7 are diminishing acidic, 7 to 14 increasingly alkaline and 7 is neutral. The basic Brief Theory of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. Majority of modern pH electrodes are combination pH electrodes rather than a set of two separate electrodes. The combination pH electrode is virtually a tube within a tube arrangement. The inner tube housing the pH indicator electrode (pH sensing membrane, Ag/AgCl reference electrode, and HCl) and the outer one housing the reference electrode (Ag/AgCl) and its salt bridge. The pH sensing component of the indicator electrode is a glass bulb. When a pH probe is immersed in a sample solution, an electrical potential is developed inside and another electrical potential is developed outside, the difference in the potential is measured and is given as the pH of the sample.

Calibration of pH meter

To be certain of accurate and reliable measurements, you need to perform pH meter calibration. This is generally done by measuring different buffer solutions with standardized, well-defined values, and then adjusting the pH meter based on any deviations from the buffer's known pH value. Buffers are solutions that resist changes in pH when small amounts of a strong acid or a base are added. Because they maintain a constant pH, buffers are good reference solutions. Calibration should be performed periodically to maintain the pH data quality.

Apparatus

pH meter, Glassware (Beaker)

Reagents

Buffer Solutions

- pH 4: It is prepared by dissolving buffer tablet of pH 4 in a 100 mL volumetric flask and diluting it up to the mark with deionized water. Alternatively, it may be prepared by dissolving 1.021 g of potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$) in fresh 100 mL deionized water.
- pH 7: Dissolve a buffer tablet of pH 7 in deionized water in a 100 mL volumetric flask and make up to the mark. Alternatively, dissolve 0.340 g of potassium hydrogen phosphate (KH_2PO_4) and 0.355 g disodium hydrogen phosphate (Na_2HPO_4) in distilled water in a 100 mL volumetric flask and make up the solution to the mark.

Experimental Procedure

Calibration of pH meter

1. Plug in the meter and let it warm up for five to ten minutes.
2. Put the selector knob to pH mode.
3. Rinse the electrode with de-ionized water and blot dry using a piece of tissue.
4. Allow all of the buffers to reach the same temperature, since pH readings are temperature dependent. If the buffers are not at 25 °C, temperature compensation is recommended. Measure the temperature of the buffers using thermometer and manually adjust the “temperature compensation knob” to this temperature. Where, ATC probe is available the system automatically transmits the temperature of the buffers to the meter.
5. Place the electrode in the solution of pH 7 buffer. It should be ascertained that the glass electrode membrane is completely immersed in the solution. Electrode should not touch the sides or the bottom of the beaker. Allow the pH value to stabilize and adjust 'set buffer knob' in a manner that the reading matches with buffer pH. Remove the electrode from the buffer.
6. Rinse the electrode with de-ionized water and blot dry using a piece of tissue.
7. Place the electrode in the solution of next buffer i.e. pH 4. Follow the step no. 4 until pH value reaches pH 4. Remove the electrode from the buffer. Similarly, other pH standard such as 10.1 can be used, depending on the expected sample pH range. Generally, for groundwater sample pH 7.0 and 10.0 should be used for calibration purpose.
8. Rinse the electrode with de-ionized water and blot dry using a piece of tissue.
9. Now, the instrument is ready to measure pH of unknown sample.

pH Measurement of samples

1. Make sure that the meter is set to the pH mode.
2. Place the electrode in the sample (30-50 mL) to be tested.
3. The pH of the solution appears in the display. Allow the display to stabilize before taking your reading.
4. Note down the pH and temperature reading.
5. Rinse the pH electrode and place it back in the storage solution.

Precautions

1. Make sure to store a pH electrode in its wetting cap containing electrode fill solution (3 M KCl, purchased or prepared by dissolving 22.37 g KCl into 100 mL DI water). Do not store electrode in DI water.
2. Never touch the membrane of the glass electrode with anything else except soft tissue paper since it is fragile and is easily ruined if scratched or bumped.
3. Always bring the standards and the sample to the same temperature before measurement (preferably room temperature).

Observation Table:

Sample No.	pH of the sample	Temperature of the sample

Results:

Home Work

- Q1. Write down the environmental significance of pH measurement of water samples.
- Q2. One solution has a pH of 4.0 and another has pH of 6.0. What is (a) the hydrogen ion activity and (b) the hydroxide ion activity in each of the solutions?
- Q3. A 50 % decrease in hydrogen ion activity represents how much of an increase in pH units?

Experiment No. 1B

Aim

To calibrate conductivity meter and measure conductivity of given water sample

Learning Outcome

The students will learn the working Brief Theory of conductivity meter & how to calibrate and measure the conductivity of water samples.

Brief Theory

Conductivity, k , is a measure of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, valence and on the temperature of measurement. Since the charge on ions in solution facilitates the conductance of electric current, the conductivity of a solution is proportional to its ion concentration. Conductivity cells of different cell constants are chosen mainly to ensure that the actual resistance between the plates, when the cell is immersed in the solution, is within practical measurable range. It is customarily reported in micromhos per centimeter ($\mu\text{mho/cm}$). Freshly distilled water has a conductivity of 0.5-2 $\mu\text{mho/cm}$.

Apparatus

Conductivity meter with electrode, beaker

Reagents

- Conductivity water: Pass distilled water through a mixed bed deionizer and discard first 1000 mL of water. Conductivity of this water should be less than 1 $\mu\text{mho/cm}$.
- Standard potassium chloride solution, KCl, 0.0100M: Dissolve 745.6 mg anhydrous KCl in conductivity water and dilute to 1000 mL at 25°C. This is the standard reference solution, which at 25°C has a conductivity of 1412 $\mu\text{mhos/cm}$.

Experimental Procedure

Calibration and measurement of sample

1. Rinse the electrode thoroughly and then blot and dry.
2. Immerse the electrode in 0.01M KCl solution and the conductivity should be 1413 micro mhos at 25°C Temperature. Adjust the knob to get the accurate value.
3. Now the instrument is calibrated.
4. Remove the cell from KCl solution and wash it thoroughly with distilled water.
5. Immerse the electrode/cell in the unknown sample whose conductivity is to be determined.
6. Note down the instrument reading.

Observation Table:

Sample No.	Conductivity	Temperature

Results:**Home Work**

- Q1. Write down the environmental significance of measuring the conductivity water samples.
- Q2. How does conductivity represent ions in solution?
- Q3. How does temperature affect conductivity readings?

Experiment No. 1C

Aim

To calibrate TDS meter and measure TDS of given water sample

Learning Outcome

Students will learn to calibrate the TDS meters and will be able to measure the TDS of water samples.

Brief Theory

Although the basis for testing ppm of total dissolved solids is the conductivity of the solution, it is not correct to assume that this measurement is absolute. It is always necessary to calibrate all total dissolved solids meters with parts per million (ppm) total dissolved solids standard calibration solution that contains the same type of salts or mixtures of salts as the solution to be tested. Failure to do this will result in serious errors in the measurement of total dissolved solids.

Reagents

Deionised water, NaCl stock solution

Experimental Procedure

- Take certain amount (3 g) of NaCl in watch glass and dry it in oven at 120° C for 45 minutes.
- Cool it, and make 20 and 342 ppm solution of NaCl solution (Commercially available standard solution can also be used).
- Rinse the electrode with deionised water.
- Dip the electrode in 20 ppm solution and wait for the reading to stabilize.
- Next, use 342 ppm solution and adjust the knob to the correct value.
- Rinse the electrode with deionised water and wipe with tissue paper.
- Instrument is calibrated now and ready to be used.
- Now check the TDS of given sample and record the value.

*Alternatively, TDS content can be approximated by multiplying the specific conductance in micro-Siemens per cm ($\mu\text{S}/\text{cm}$) by an empirical factor varying from 0.55 to 0.90 depending on the chemical composition of the TDS.

Observation Table:

Sample No.	TDS	Temperature

Results:**Home Work**

- Q1. Write down the environmental significance of measuring the TDS of water samples.
- Q2. What value of TDS do you expect in portable water and drinking water?
- Q3. Write down the relation between TDS and Conductivity?

Experiment No: 2

Aim

To determine the different components of NOM by TOC analyzer and spectrophotometer

Learning Outcome

The students will learn about the different components of natural organic matter (NOM) and the working principle of TOC analyzer as well as of spectrophotometer.

Brief Theory

Natural organic matter (NOM) refers to a group of carbon-based compounds that are found in surface water and groundwater supplies. In practice, NOM is usually characterized by the measurement of its Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), absorbance at 254 nm (UV₂₅₄), and specific UV absorbance (SUVA). TOC represents the sum of the particulate and DOC when existing inorganic carbon is removed by acidification; while DOC is the organic carbon in a water sample filtered through a 0.45µm filter. SUVA is defined as the UV absorbance of a given sample at 254 nm divided by the DOC concentration of the sample. This ratio describes the hydrophobicity and hydrophilicity of NOM in water; a SUVA >4 indicates mainly hydrophobic and especially aromatic material, whereas a SUVA <3 illustrates mainly hydrophilic material. These different components of NOM can be analyzed using TOC analyzer and spectrophotometer.

TOC analyzer

Total Organic Carbon (TOC) analyzer utilizes a catalytic oxidation combustion technique at high temperature (the temperature raises up to 720 °C) to convert organic carbon into CO₂. The CO₂ generated by oxidation is then measured using any of the three methods:

- Non-dispersive infrared (NDIR)
- Direct Conductometric (Non-selective Conductometric)
- Membrane Conductometric Detection (Selective Conductometric)

NDIR TOC detectors measure CO₂ in the gaseous phase, while conductometric TOC detectors measure CO₂ in the liquid phase.

Spectrophotometer

Ultraviolet and Visible absorption spectroscopy is a semi-quantitative method to determine humic substances (NOM) in natural waters. It is based on the measurement of the attenuation of a beam of light after it passes through a sample or after its reflection from a sample surface. The concentration of an analyte in a solution can be determined by measuring the absorbance at a certain wavelength, applying the Beer–Lambert law. Absorption measurements can be taken at a single wavelength or over an extended spectral range. The UV absorbance spectra of NOM decrease monotonically with increasing wavelength; hence any wavelength from 220 to 280nm is appropriate for NOM measurements. However, UV₂₅₄ has been identified as a potential surrogate measure for DOC despite its tendency to represent only the aromatic character.

Apparatus

TOC analyzer and spectrophotometer

Reagents

Potassium hydrogen phthalate (KHP) (Required as TC standard solution): Accurately weigh 2.125 g of KHP that was previously dried at 105-120° C for about 1 h and cooled in dessicator. Then, transfer it to a 1L volumetric flask and dissolve in zero water (water absolutely free from total carbon and total nitrogen). Add zero water to 1 L mark and stir the solution.

HCl: Prepare 2M HCl by diluting 1 part commercially available conc. HCl (12M) with 5 parts of pure water.

Sodium hydrogen carbonate and sodium carbonate (Required as IC standard solution): Accurately weigh 3.50 g of reagent grade sodium hydrogen carbonate previously dried for 2h in a silica gel dessicator, and 4.41g of sodium carbonate previously dried for 1 h at 280-290° C and cooled in dessicator. Add 1L zero water to the mixture and stir the solution.

Experimental Procedure

Calibration and measurement of sample

The measurements of TOC and DOC are based on calibration with potassium hydrogen phthalate (KHP).

1. The TOC and DOC procedures require that all inorganic carbon (IC) be removed from the sample before the sample is analyzed for organic carbon content. If the IC is not completely removed, significant error will occur. IC is analyzed in liquid samples by acidifying with an inorganic acid (2M HCl) to pH 3 or lower, and then sparging with a stream of inert gas. The acidification converts carbonates and bicarbonates to carbon dioxide, which is then removed along with dissolved CO₂ by the gas stream, and measured to provide an IC value.
2. The sample, which is then free from IC interference, is injected into a TOC instrument system (TOC analyzer).
3. The organic carbon is oxidized to CO₂, which is released from the sample, detected, and reported as mg/L or ppm TOC or DOC.
4. The DOC procedure requires that the sample be passed through a 0.45-μm filter prior to analysis to remove particulate organic carbon (POC) from the sample.
5. The UVA procedure requires that the sample be passed through a 0.45-μm filter and transferred to a quartz cell. It is then placed in a spectrophotometer to measure the UV absorbance at 254 nm and reported in cm⁻¹.
6. The SUVA calculation requires both the DOC and UVA measurement. The SUVA is calculated by dividing the UV absorbance of the sample (in cm⁻¹) by the DOC of the sample (in mg/L) and then multiplying by 100 cm/M. SUVA is reported in units of L/mg-M.

Observation Table:

Sample No.	Total carbon (TC)	Total inorganic carbon (TIC)	Dissolved organic carbon (DOC)	Non-dissolved organic carbon (NDOC)	SUVA (UV ₂₅₄ /DOC)

Calculation:

$$\text{TOC} = \text{TC} - \text{TIC}$$

$$\text{TOC} = \text{DOC} + \text{NDOC}$$

Result:**Home work:**

1. What is the environmental significance of measuring different components of NOM?
2. Why is it necessary to acidify and spurge the sample during TOC analysis?

Experiment No. 3

Aim

To determine the acidity and alkalinity of a given water sample.

Learning Outcome

Students will learn the titration method to measure the acidity and alkalinity of water samples.

Brief Theory

Acidity

All waters having pH lower than approximately 8.3 should contain acidity. Acidity is the capacity of water to neutralize a base (OH). Sources of acidity in natural water include CO_2 (air, bacterial degradation), H_2PO_4 , H_2S , protein, fatty acids, and salt of trivalent metals such as hydrated Fe^{3+} and Al^{3+} . Acids contribute to corrosiveness and influence chemical reaction rates, chemical speciation and biological processes. Acidity of water is its quantitative capacity to react with a strong base to a designated pH. In polluted water, acidity may be caused by free mineral acid (H_2SO_4 , HCl) from metallurgical industry, acid mine drainage, acid rain, and organic acid waste. To measure acidity, an alkaline solution (NaOH) and two indicators are used. The measured value may vary significantly with the end point pH used in the determination. The first indicator (methyl orange) changes colour from red to yellow at approximately pH

3.7. This corresponds to mineral acidity or methyl orange acidity. If NaOH is continued to titrate, the second indicator phenolphthalein changes colour from colourless to pink at pH 8.3. Titration to the phenolphthalein end point of pH

8.3 measures acidity from both mineral acids and weak acids. This total acidity is also termed phenolphthalein acidity.

To calculate mineral acidity or total acidity from the titration data, the amount of NaOH must be converted to the equivalent amount of CaCO_3 (in mg/L) in water. Since the molecular weight (MW) of CaCO_3 is 100 g/mol or 1×10^5 mg/mol, its equivalent weight is $\frac{1}{2}$ MW or 5×10^4 . This is used as the conversion factor so that the acidity can be reported as mg CaCO_3 /L.

Note: As far as water analysis is concerned, acidity test does not bear significant importance because methyl orange acidity invariably remains absent in the raw water and even phenolphthalein acidity (that too principally due to the excessive-prevalence of dissolved carbon dioxide and carbonic acids) normally does not exist to a significant extent in the raw water.

Apparatus

Titration Stand with Burette, Conical Flask, Beaker, Measuring Cylinder, Pipette, Funnel, Wash Bottle etc.

Reagents

- 0.02 N NaOH Solution: Dissolve 0.8 grams of NaOH in 1 L of deionized water.

- Phenolphthalein Indicator: Dissolve 0.5 gm of Phenolphthalein Indicator in 500ml 95% of ethyl alcohol. Add 500 mL distilled water.
- Methyl orange Indicator: Dissolve 0.5 g of Methyl orange Indicator & dilute to 1000 ml with distilled water.

Experimental Procedure

Methyl orange acidity

- Take suitable volume of sample (25, 50 or 100 mL) in a conical flask (V)
- Add 2 drops of methyl orange and see the colour. If colour turns yellow, methyl orange acidity is absent. If colour turns orange, titrate with 0.02 N NaOH till colour changes to faint orange. Characteristic of pH 4.3-4.4. Note the volume of NaOH required (A).

Phenolphthalein Acidity

- Take suitable volume of sample (25, 50 or 100 mL) in a conical flask (V).
- Add 2-3 drops of phenolphthalein indicator. If the sample turns pink colour it means that, phenolphthalein acidity is absent and stop the experiment.
- If there is no colour change, titrate with 0.02N Standard NaOH solution till faint pink colour appears indicating pH 8.3. The end point is colourless to faint pink colour. Note additional volume of NaOH required (B).

Indicators: Phenolphthalein and Methyl Orange Indicator

End Point: a) Yellow to Faint Orange

b) Colourless to Faint Pink Colour

Calculations:

Mineral Acidity as CaCO_3 in mg/L = $[\text{Volume of NaOH (A)} \times \text{N} \times 50 \times 1000] / \text{Volume of sample}$

Phenolphthalein Acidity as CaCO_3 in mg/L = $[(\text{Volume of NaOH (B)} \times \text{N} \times 50 \times 1000) / \text{Volume of sample}]$

Total Acidity as CaCO_3 in mg/L = $[(\text{Volume of NaOH (A+B)} \times \text{N} \times 50 \times 1000) / \text{Volume of sample}]$

Alkalinity

In natural water, alkalinity is due to H_2CO_3 (CO_2 dissolved in water). Alkalinity from other materials is insignificant and may be ignored. In polluted waters, minor amounts of NH_3 and salts of weak acids such as borate, silicate, and phosphate (i.e., the conjugate bases of HBO_3 , H_4SiO_4 , H_3PO_4), and organic acids will contribute to alkalinity. Alkalinity of water is its quantitative capacity to neutralize a strong acid to a designated pH. In other words, its ability to maintain a relatively constant pH. The possibility to maintain constant pH is due to

the hydroxyl, carbonate and bicarbonate ions present in water. The ability of natural water to act as a buffer is controlled in part by the amount of calcium and carbonate ions in solution. Carbonate and calcium ion come from calcium carbonate or limestone. So, water that comes in contact with limestone will contain high levels of both Ca^{++} and CO_3^{2-} ions and have elevated hardness and alkalinity. Alkalinity is significant in many uses and in treatment of natural acid and wastewater. It is significant in determining suitability of water for drinking as well as irrigation purpose. The titration Experimental Procedure can distinguish two types of alkalinity, that is, phenolphthalein alkalinity and total alkalinity (methyl orange alkalinity). The phenolphthalein alkalinity is the acid-neutralizing power of hydroxide and carbonate ions (OH^- and CO_3^{2-}) present in the water sample, while total alkalinity represents all the bases in it (OH^- , CO_3^{2-} and HCO_3^-). The titration Experimental Procedure for alkalinity is exactly the opposite of the Experimental Procedure for acidity. Two same indicators are used but in the reverse order. The Experimental Procedure utilizes the first indicator, phenolphthalein, to signal the end points (pH 8.3) in titrating OH^- and CO_3^{2-} . Further titration with H_2SO_4 reaches the end point of the second indicator (methyl orange) at around pH 4.5, which measures the total alkalinity. Note that the pH for the colour change is not at a fixed value, but rather a range, that is, pH 3.1–4.4 and pH 8.0–9.6 for methyl orange and phenolphthalein, respectively.

Reagents:

- 0.02N H_2SO_4 : 3 mL. Conc. H_2SO_4 in 1 L deionized water to get 0.01 N H_2SO_4 . Phenolphthalein Indicator: Dissolve 0.5 gm of Phenolphthalein Indicator in 500 ml 95% of ethyl alcohol. Add 500 mL distilled water.
- Methyl orange Indicator: Dissolve 0.5 g of Methyl orange Indicator & dilute to 1000 ml with distilled water.

Experimental Procedure

Phenolphthalein Alkalinity

- Take suitable volume of sample (25, 50 or 100 mL) in a conical flask.
- Add 2-3 drops of phenolphthalein indicator.
- If the sample turns pink in colour, then titrate with 0.02 N H_2SO_4 , till the pink colour disappears. Note the volume of acid required as (A).
- If the pink colour does not exist it indicates that, phenolphthalein Alkalinity is absent. Then continue the titration with Methyl Orange indicator.

Methyl Orange Alkalinity

- Add 2-3 drops of methyl orange to the same flask; continue titration till colour becomes orange. Note down volume of H_2SO_4 consumed as 'B'.

Calculations:

Phenolphthalein Alkalinity as CaCO_3 in mg/L = $\frac{[(\text{Volume of NaOH (A)} \times N \times 50 \times 1000)]}{\text{Volume of sample}}$

Methyl Orange Alkalinity as CaCO_3 in mg/L = $\frac{[(\text{Volume of NaOH (B)} \times N \times 50 \times 1000)]}{\text{Volume of sample}}$

Total Alkalinity as CaCO_3 in mg/L = $[(\text{Volume of NaOH (A+B)} \times N \times 50 \times 1000) / \text{Volume of sample}]$

End Point: a) Pink to Colourless

b) Yellow to Faint Orange Colour.

Observation Table:

Measurement of Acidity				
Sample No.	Volume of Sample	Burette Reading		Volume NaOH
		Initial	Final	
Measurement of Alkalinity				
Sample No.	Volume of Sample	Burette Reading		Volume of H_2SO_4
		Initial	Final	

Results:

Home Work

Q1. Write down the environmental significance of measuring Acidity and Alkalinity of water samples.

Q2. How does pH play a role in affecting alkalinity and acidity of a given water sample?

Q3. A sample of water collected in the field had a pH of 6.8. By the time the water sample reached the laboratory for analysis, the pH had increased to 7.5. Give a possible explanation for this change.

Experiment No. 4

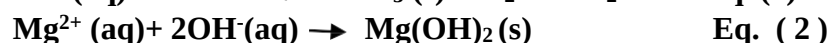
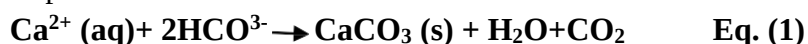
Aim: To determine the hardness of a water sample by measuring the amount of calcium present.

Learning Outcomes

Students will learn to measure the hardness of the water samples.

Brief Theory

All-natural waters have salts dissolved in them. These problems are caused mainly by the presence of calcium and magnesium ions in the water. Water hardness is caused by multivalent metallic cations, primarily the divalent Ca^{2+} and Mg^{2+} . Anions and monovalent cations such as Na^+ and K^+ do not contribute to hardness. Hard water adversely affects the suitability of water for uses in domestic and industrial purposes. You may have experienced this problem if you have taken a bath with water that contained too many calcium and magnesium salts. Eq. 1 and 2 present the respective hardness.



Although hardness is caused by cation, it may also be discussed in terms of carbonate (temporary) and non-carbonate (permanent) hardness. Carbonate hardness refers to the amount of carbonates and bicarbonates in solution that can be removed or precipitated by boiling. This type of hardness is responsible for the deposition of scale in hot water pipes and kettles. When total hardness is numerically greater than that of total alkalinity expressed as CaCO_3 , the amount of hardness equivalent to alkalinity is called carbonate hardness¹. When the hardness is numerically equal to less than total alkalinity, all hardness is carbonate hardness. The amount of hardness in excess of total alkalinity expressed as CaCO_3 is non- carbonate hardness. Non-carbonate hardness is caused by the association of the hardness- causing cation with sulphate, chloride or nitrate and is referred to as “permanent hardness”. This type of hardness cannot be removed by boiling. Hardness is determined by the EDTA method in alkaline condition; EDTA and its sodium salts form a soluble chelated complex with certain metal ions as shown in following reaction;



The reaction between calcium ions and EDTA only occurs at a high pH; therefore, a solution that has a constant pH of 10 (called a buffer) will be added to the water sample. Eriochrome black T, a metal ion indicator, will be used to visualize the endpoint. Eriochrome Black T (EBT) (blue colour solution) serves as an excellent indicator to show when all hardness ions have been consumed. When small amount of EBT is added to hard water with $\text{pH} > 10$, it combines with Ca^{2+} and Mg^{2+} ions to form weak complex ions (wine-red colour solution). A small amount of indicator is added to the solution containing the Ca^{2+} forming a wine-red complex.



The end point will be indicated when the original red solution turns to blue indicating that the EDTA has reacted with all the calcium ions in the water sample. In this experiment you will measure calcium carbonate (CaCO_3) in ppm. You will be able determine the hardness of your water sample by measuring the volume of your water sample and the volume (in mL) of the EDTA solution used to react with all of the calcium in the water sample.

Apparatus

Burette, Pipette, Spatula, Dropper

Reagents

- Ethylenediaminetetraacetic acid (EDTA).
- Buffer solution of pH 10.
- Eriochrome black T

Experimental Procedure

- Take 50 mL sample in a conical flask
- Using a dropper add few drops of the pH 10 buffer solution to the conical flask.
- Using spatula, add a pinch of indicator Eriochrome black T (in the form of a powder) to the flask. The colour of the solution will be red.
- Clean and fill burette with the EDTA solution and record the initial volume.
- Titrate the solution with EDTA till the colour of solution will changed from red to blue, record the final volume of burette and put in the given formula for calculating the hardness.

Calculations

$$\text{Hardness as per } \text{CaCO}_3 \text{ (mg/L)} = \frac{\text{mL of EDTA used in titration}}{\text{mL of Water sample}} \times 1000 \text{ mg/L}$$

Observation Table

Sample No	Volume of Sample	Burette Reading		Volume of EDTA	Hardness Concentration
		Initial	Final		

Results:**Home Work**

- Q1. Write down the environmental significance of measuring hardness of water samples.
- Q2. Among finished drinking water, raw wastewater and de-ionized water, which water is expected to have the highest carbonate hardness and why?
- Q3. A sample has 50mg/L Ca^{2+} , 150mg/L Mg^{2+} , 50 mg/L Na^+ , 20 mg/L Cl^- and 100 mg/L glucose. Calculate its total hardness, carbonate and non-carbonate hardness?

Experiment No. 5

Aim: To determine the concentration of nitrate for a given water sample.

Learning Outcomes

Students will learn to use UV Spectrophotometer for measuring Nitrate concentration in water samples.

Brief Theory

The Nitrate ion absorbs ultraviolet (UV) radiation at 220 nm, while it doesn't absorb the UV radiation of 275 nm. On the other hand, dissolved organic matter absorb both 220nm and 275 nm UV radiation Using this Brief Theory one can find out the Nitrate iron concentration of water sample. This can be accomplished by making a measurement of the water sample's absorbance at 275 nm, a wavelength that the organic matter can absorb UV radiation but in which the Nitrate cannot. Once known, an empirical correction factor can be applied to zero out the organic matter present at the water by measuring the water samples absorbance at 275 nm and then to the 220 nm to distinguish the nitrate from the organic matter.

Apparatus

UV Spectrophotometer, cuvette

Reagents

- Redistilled water: use redistilled water for the preparation of all solutions and dilutions.
- Stock nitrate solution: dissolve 721.8mg anhydrous KNO_3 (dried in an oven at 105 °C for 24 hours) and dilute to 1000ml with distilled water. To determine exact concentration the mass of KNO_3 need to be known that you actually used to the nearest milligram and calculate Nitrate concentration from this ($1\text{mL} = 100 \mu\text{g N} = 443\mu\text{g NO}_3$).
- Standard nitrate solution: dilute 100mL stock nitrate solution to 1000mL with distilled water. $1\text{mL} = 10\mu\text{g NO}_3 \text{ N} = 44.3\mu\text{g NO}_3$.
- 1N HCl solution.
- Aluminium hydroxide suspension: dissolve 125g potash alum in 1000mL distilled water. Warm to 60°C, add 55-60mL NH_4OH and allow to stand for 1h. Decant the supernatant and wash the precipitate a number of times till it is free from Cl, NO_2 and NO_3 . Finally, after setting, decant off as much clean liquid as possible, leaving only the concentrated suspension.

Calibration

Prepare nitrate calibration standards in the range 0 to 350µg N by diluting 1, 2, 4, 7 to 35mL of the standard nitrate solution per 50mL (Use 50.0 mL volumetric flasks and pipettes to create these solutions). Treat the nitrate standards in the same manner as the samples.

Measure the absorbance of known concentration of Nitrate solutions and plot it in a graph to prepare calibration curve.

Experimental Procedure

Read the absorbance or transmittance against redistilled water set at zero absorbance or 100% Transmittance. Use a wavelength of 220 nm to obtain the nitrate reading and, if necessary, a wavelength of 275nm to obtain interference due to dissolved organic matter. Set up a calibration curve by using at least five different standard solutions.

An Example of Calibration curve is given below:

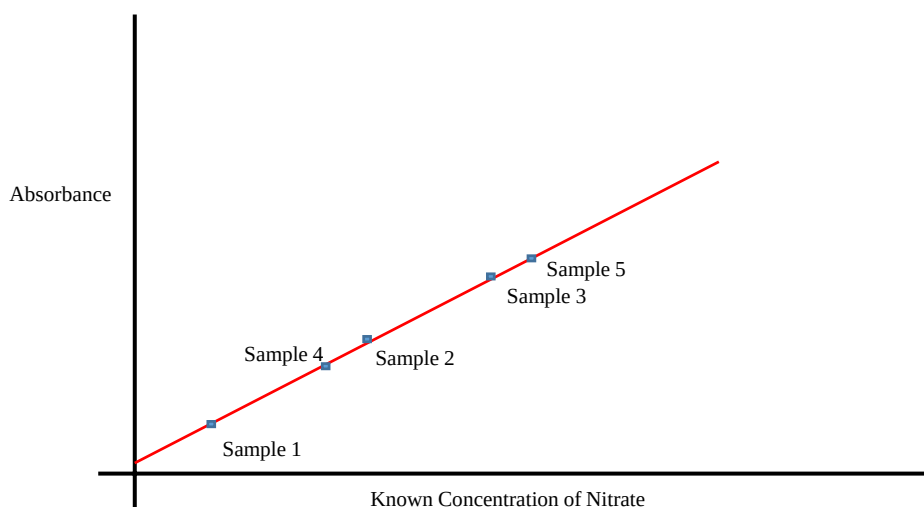


Fig. 1. Sample calibration curve

Calculations

For correction for dissolved organic matter, subtract 2 times the reading at 275nm from the reading at 220nm to obtain the correct absorbance due to nitrate. Convert this absorbance value into equivalent nitrate by reading the nitrate value from a standard calibration curve.

$$\text{Nitrate N, mg/L} = \text{mg nitrate-N} / \text{mL of sample}$$

$$\text{NO}_3, \text{ mg/L} = \text{Nitrate N mg/L} \times 4.43$$

Observation Table

Sample No.	Absorbance Value	Concentration of Nitrate

Results:

Home Work

Q1. Write down the environmental significance for measuring the Nitrate concentration of water samples.

Q2. What are the interferences that can produce erroneous results in nitrate measurement?

Q3. What are the other methods of measuring nitrates in water and wastewater?

Experiment No. 6

Aim: To determine the concentration of sulphate of a given water sample.

Learning Outcomes

Students will learn the working Brief Theory of spectrophotometer and will know the basis for measuring the sulphate concentration in water samples.

Brief Theory

Sulphate ions occur in natural waters. Sulphate compounds are readily soluble in water. Most of the Sulphate compounds 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. Sulphate ions can produce hydrogen sulfides as per following equations:

$\text{SO}_4^{2-} + \text{Organic matter} \Rightarrow \text{S}^{2-} + \text{H}_2\text{O} + \text{CO}_2$ (in the presence of anaerobic bacteria) **Eq. (1)**



The sulfate data is very useful to determine the applicability of different water types for their public and industrial applications. Concentration of sulphate indirectly indicates extent of problems that can arise due to reduction of sulfates to hydrogen sulfides. In addition, sulfate content of organic matter fed to anaerobic digester is important information as it gives idea of generation of hydrogen sulfides, which needs to be removed.

Apparatus

Whatman No. 1 filter paper; Spectrophotometer; Magnetic stirrer

Reagents

- Buffer solution A: dissolve 30g Magnesium chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5g Sodium acetate $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, 1g Potassium nitrate, KNO_3 and 20mL acetic acid, CH_3COOH (99%) in 500mL distilled water and make up to 1000mL.
- Buffer solution B: (required when the sample sulphate ($\text{SO}_4^{2-} < 10\text{mg/L}$). Dissolve 30g magnesium chloride, 5 g sodium acetate, 0.111 g sodium sulfate, and 20 mL acetic acid (99%) in 500 mL distilled water and make up to 1000 mL.
- Dry Barium Chloride (BaCl_2) crystals.
- Standard Sulfate Solution: Dissolve 0.1479 g of anhydrous sodium sulfate in distilled water to make the volume 1L. This solution contains 100 mg sulfate/L (i.e., 1 mL=100 μg SO_4^{2-}). Prepare standards of various strengths (preferably from 0.0 to 40.0mg/L at the intervals of 5 mg/L by diluting this stock solution). Above 40 mg/L accuracy decreases and BaSO_4 suspensions lose stability.

Calibration

- Standard Sulfate Solution: Dissolve 0.1479 g of anhydrous sodium sulfate in distilled water to make the volume 1L. This solution contains 100 mg sulfate/L (i.e., 1 mL=100µg SO_4^{2-}). Prepare standards of various strengths (preferably from 0.0 to 40.0mg/L at the intervals of 5 mg/L by diluting this stock solution). Above 40 mg/L accuracy decreases and BaSO_4 suspensions lose stability.
- Measure the absorbance against a distilled water blank (DO NOT ADD BARIUM CHLORIDE TO IT.) at 420 nm using spectrophotometer. Absorbance for the blank sample is taken to correct for sample colour and turbidity.
- Process the standard solution of different strengths in similar way and record the absorbance for each solution. Plot a standard sulfate calibration curve on a graph paper from these absorbance values putting strengths (mg/L) on X-axis and absorbance @ 420 nm on Y-axis. Fit a best-fit linear model to the data.

Experimental Procedure

- Filter the sample through filter paper (Whatman No. 1) and take 50 mL of filtrate in an Erlenmeyer flask.
- Add 20 mL buffer solution and mix in stirring apparatus. While stirring, add 0.15 g of barium chloride to the sample and stir the sample with the help of magnetic stirrer for about an hour.
- Measure the absorbance of the sample at 420 nm in spectrophotometer.

Calculations

Using the standard sulfate calibration curve (a linear-model; Equation 3), find out sulfate concentration in the given unknown sample in mg/L.

$$\text{Sulfate concentration (mg SO}_4^{2-}\text{/L)} = (1000 \times \text{mg SO}_4^{2-}) / (\text{mL sample})$$

Observation Table:

Sample No.	Absorbance Value	Concentration of Sulphate

Results:

Home Work:

- Q1. Write down the environmental significance of measuring sulphate in a water sample.
- Q2. Explain relevance of preparing standard sulphate solutions in determining sulphate concentration for unknown samples.
- Q3. Why must exactly the same Experimental Procedure be followed each time an analysis is made for sulphate concentration by turbidimetric system?

Experiment No. 7

Aim: To determine chloride ion concentration in a water sample.

Learning Outcomes

Students will learn to measure the chloride concentration of water samples.

Brief Theory

Chlorides are present in water usually as NaCl, MgCl₂ and CaCl₂. Although chlorides are not harmful as such, their concentrations over 250 ppm impart a peculiar taste to the water thus rendering the water unacceptable for drinking purposes. By Mohr's method, chloride ions in a water sample (neutral or slightly alkaline) can be determined by titrating it against standard silver nitrate (AgNO₃) solution using potassium chromate (K₂CrO₄) as an indicator. The pH should be in between 7-8. At higher pH, silver ions are precipitated as silver hydroxide. At lower pH, potassium chromate indicator is converted to potassium dichromate (K₂Cr₂O₇).

Mohr's method is based on the precipitation titration in which silver nitrate solution is released from the burette to the water sample which contains chloride ions and indicator. The silver ions (from silver nitrate solution) react with chloride ions (from water sample) and chromate ions (from indicator) to form white precipitate of silver chloride and red precipitate of silver chromate. The following reactions depict the detection of chlorine:



Apparatus

Burette, conical flask, pipette, measuring cylinder.

Reagents

- Standard silver nitrate solution ($\frac{N}{50}$), Indicator potassium chromate solution.

Calibration

- Transfer 25 ml of the distilled water in a conical flask and add 3-4 drops of indicator potassium chromate solution. Slowly add standard silver nitrate solution from the burette and shake the solution well. At the end point, light yellow colour starts changing to red colour. The titration is repeated until a concordant volume is obtained. The blank correction for the indicator should be subtracted from the volume of the titrant obtained after titrating the with the unknown water sample solution. Let us consider the titrated volume as (V₁)

Experimental Procedure

- Take 50 ml sample in a conical flask. Measure sample pH (to check for acidity or basicity of the sample, this step is optional).

- Add 1.0 ml of freshly prepared indicator solution of $K_2Cr_2O_4$.
- Titrate with standard silver nitrate solution until the brick red precipitate appears. This stage marks the end point of titration and note down volume of titrant used. Note the difference of volume as (V_2). Find the actual volume (V) of titrate used using: $V = V_2 - V_1$ equation.

Calculations

Calculate chloride ion concentration using the following equation:

$$\text{Chloride Ion Concentration (mg/L)} = (A \times N \times 35.45) \times 1000 / V_{\text{sample}} \quad \text{Eq. (3)}$$

Where, A = volume of titrant used, N is normality of silver nitrate (here we used N/50 or 0.02 N), and V_{sample} is volume of sample used (mL) (here we used 50 ml).

Observation Table

Sl.No	Volume of given water sample in ml	Burette Readings in ml		Volume of $AgNO_3$ solution in ml
		Initial	Final	
Blank Sample	50ml	X_1	Y_1	$Y_1 - X_1 = V_1$
Example 1	50 ml	X_2	Y_2	$V_2 = (Y_2 - X_2)$ $V = V_2 - V_1$

Results:

Home Work:

- Q1. Write down the environmental significance for measuring Chloride ion concentration of water samples.
- Q2. Why pH range is important in chloride determination?
- Q3. Would the analytical results by the Mohr method for chlorides be higher, lower or the same as the true colour value if any excess of indicator were accidentally added to the sample?

Experiment No. 8

Aim

To determine chlorine demand, residual chlorine and breakpoint of a given sample by iodometric method.

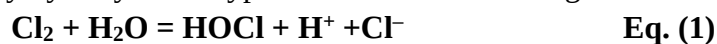
Learning Outcomes

Students will learn the iodometric method Brief Theory and know the concept of chlorine breakpoint and chlorine demand in water samples.

Brief Theory

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing micro-organisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, Sulphide and some organic substances. Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid and hypochlorite ion. Disinfectant capabilities of chlorine depend on its chemical form in water, which in turn is dependent on pH, temperature, organic content of water, and other water quality factors. Chlorine is used in the form of free chlorine [e.g., chlorine gas] or as hypochlorites [e.g., NaOCl and Ca(OC1)₂].

Chlorine gas rapidly hydrolyzes to hypochlorous acid according to following reaction:



The two chemical species formed by chlorine in water, hypochlorous acid (HOCl) and hypochlorite ion (OCl⁻), are commonly referred to as “free” or “available” chlorine. The difference between the amount of chlorine added to the water and the amount of residual chlorine (i.e., free and combined available chlorine remaining) at the end of a specified contact period is termed as “*chlorine demand*”. Free chlorine (hypochlorite ions and hypochlorous acid) and chloramines stoichiometrically liberate iodine from potassium iodide at pH 4 or less. Residual chlorine is determined by the starch-iodine method as described below:

The starch-iodine method is based on the oxidizing power of free and combined chlorine residuals to convert iodide ion into free iodine at pH 8 or less, as shown below.



The liberated iodine is titrated with a standard solution of sodium thiosulphate (Na₂S₂O₃) with starch as the indicator. The liberated iodine is directly proportional to the concentration of chlorine present in sample. Titrate at pH 3 to 4 because the reaction is not stoichiometric at neutral pH due to partial oxidation of thiosulphate to sulphate. In the starch-iodine method, the quantity of chlorine residuals is determined by measuring the quantity of iodine by titration with a reducing agent sodium thiosulfate (Na₂S₂O₃). The end point of titration is

indicated by the disappearance of blue colour, produced by the reaction between iodine and starch (which is added as indicator during the titration).

Reagents

- Bleaching powder solution, Potassium iodide crystal (KI), Glacial acetic acid, Starch indicator, Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$)

Experimental Procedure

(A) Determination of strength of bleaching powder

1. Take 25mL of bleaching powder solution. Add 5mL of acetic acid and mix well. Note down the pH value, it should be 3 to 4.
2. Add 1 g of KI crystals and mix well.
3. Add 1mL of starch indicator, the solution colour changes to dark blue colour.
4. Titrate immediately with sodium thiosulphate solution (0.025 N) till the blue colour just disappears. Note down the volume of titrant used (X).

Calculations:

Strength of bleaching powder solution;

$$\text{Cl (mg/L)} = (\text{X} \times \text{N} \times 35450) / \text{mL of sample used} \quad \text{Eq.(3)}$$

Where X is the volume of sample and N denotes the strength of sodium thiosulphate

(B) Determination of residual chlorine

- Take 200 mL identical samples in beaker.
- Add different amount of diluted bleaching powder solution to the samples (As per instruction).
- Now repeat the steps 2-4, as discussed in above section “A”.

(C) Break point chlorination

If chlorine is added to water containing reducing agents and ammonia (either naturally present or added to water to produce combined chlorine), a hump-shaped breakpoint curve is produced as shown in following figure. The different segment of the curve is described as follows:

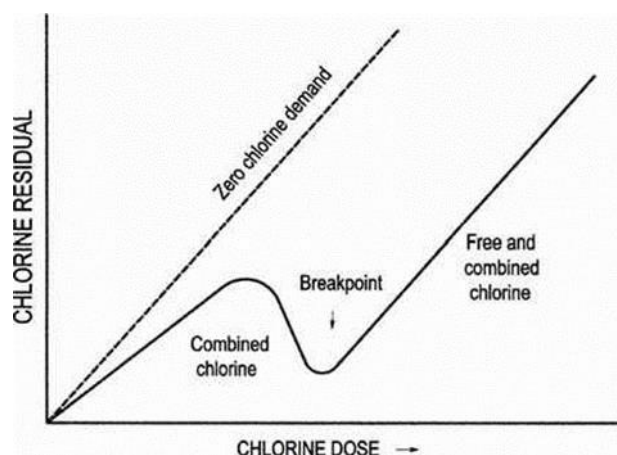


Fig. 2. Break point chlorination

- If the water is free of ammonia and other compounds that may react with chlorine, the application of chlorine will yield free available chlorine residual of same concentration. This is denoted by the 'no demand line' or the "zero demand line"
- Chlorine first reacts with reducing agents such as H_2S , Fe^{2+} , Mn^{2+} and develops no measurable residual as shown by the portion of the curve from Origin up to point A.
- Addition of chlorine beyond point C would produce free chlorine residuals and is referred to as "breakpoint chlorination". In other words, chlorination of water to the extent that all ammonia is converted to N_2 or higher oxidation state is referred to as "breakpoint chlorination".
- Plot the graph between chlorine dosage and residual chlorine to find the break point chlorination.

Observation Table

Sample No.	Chlorine dose (mg/L)	Volume of $\text{Na}_2\text{S}_2\text{O}_3$ solution (mL)	Volume of Bleaching powder solution (mL)	Residual Chlorine (mg/L)	Chlorine Demand (mg/L)

Results:

Home Work:

- Q1. Write down the environmental significance of measuring residual chlorine, chlorine demand and break-point chlorination of water samples.
- Q2. You would like to perform chlorination to a water sample with pH 7.5. At this pH, what would be the relative proportions of HOCl and OCl⁻? What kind of change in pH would you propose in order to increase the relative proportion of HOCl, which is a better disinfectant?
- Q3. What are the major disadvantages of chlorination? Name some of the alternate disinfectants.

Experiment No. 9

Aim

To calibrate and standardize Nephelometer and to determine the turbidity of a given water sample.

Learning Outcome

Students will learn the use and working Brief Theory of Nephelometer and will learn how to calculate turbidity of water samples.

Brief Theory

The Nephelometry method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher is the turbidity. Readings of the Nephelometer are given in Nephelometric Turbidity Unit (NTU). A primary standard suspension is used to calibrate the instrument. A secondary standard suspension is used as a daily calibration check and is monitored periodically for deterioration using one of the primary standards.

Apparatus

Nephelometer

Reagents

- Dissolve 1.000 g hydrazine sulfate $[(\text{NH}_2)_2 \cdot \text{H}_2\text{SO}_4]$ in filtered water and dilute to 100 mL in a volumetric flask.
- Dissolve 10.00 g hexamethylenetetramine $[(\text{CH}_2)_6\text{N}_4]$ in filtered water and dilute to 100 mL in a volumetric flask.
- Mix 5.0 mL of hydrazine sulfate and 5.0 mL of hexamethylenetetramine solutions in a 100-mL volumetric flask and let stand 24 hours at $25 \pm 3^\circ\text{C}$; dilute to the mark and mix. To prepare 500 mL of 400 NTU standard, mix 25 mL of the reagent solutions in a 500-mL flask, dilute to the mark, and mix.
- For a 40 NTU standard, dilute 10.00 mL of the 400 NTU stock suspension to 100 mL with turbidity-free water (sample or deionized water passed through a filter media of $\leq 0.2 \mu\text{m}$).

Experimental Procedure

Calibration of Nephelometer

The calibration of Nephelometer can be done using the following steps:

- Switch the turbidimeter on and allow it to warm up.
- Check instrument focus: insert template in the cell holder. The lamp image should just fill the inside circle.
- Draw out the sample cell from the Nephelometer and hold it by the rim not beneath the lip.

- Pour the deionized water into the sample cell and wipe out the exterior with a soft dry tissue paper.
- Using Set Zero dial calibrate the instrument to Zero.
- Next, pour the standard solution into the sample cell and wipe out the exterior with a soft dry tissue paper. Place the cell in its holder with correct orientation.
- Select the desired NTU range dial of the Nephelometer and rotate the calibrate dial of the Nephelometer to match the digital reading exactly with the preset range.
- The instrument is now calibrated.
- The Sample cell can now be filled with unknown sample to measure the NTU.

Calculation

The NTU of Diluted sample can be calculated using the following formula:

$$NTU = A \times (B+C) / C$$

Where,

A = NTU found in diluted sample,

B = volume of dilution water, in milliliters, and

C = sample volume taken for dilution, in milliliters.

Observation Table

Sample Number	NTU Reading

Results:

Home Work

- Q1. Write down the environmental significance of measuring turbidity of water samples.
- Q2. Why turbidity is important in “filtration” and “disinfection” processes?
- Q3. Discuss why turbidity in general cannot be correlated with weight concentration of suspended matter in water samples?

Experiment No. 10

Aim: To determine optimum coagulant dose using jar test apparatus.

Learning Outcomes

Students will learn to use the Jar test apparatus for measuring the optimum coagulant dosage for suspended colloidal particle removal from the water samples.

Brief Theory

Coagulants are used in water treatment plants

- For the removal of natural suspended and colloidal matter,
- For the removal of material which do not settle in plain sedimentation, and
- To assist in filtration.

Alum [$\text{Al}_2\text{S}(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$] is the most widely used coagulant. When alum solution is added to water, the molecules dissociate to yield SO_4^{2-} and Al^{3+} . The +ve species combine with negatively charged colloidal to neutralize part of the charge on the colloidal particle. Thus, agglomeration takes place. Coagulation is a quite complex phenomenon and the coagulant should be distributed uniformly throughout the solution. A flash mix accomplishes this.

Jar test device is a simple device used to determine this optimum coagulant dose required. The jar test, device consists of a number of stirrers (4 to 6) provided with paddles. The paddles can be rotated with varying speed with the help of a motor and regulator. Samples will be taken in jars or beakers and varying dose of coagulant will be added simultaneously to all the jars, and the paddles will be rotated at varying speed. The dose, which gives the least turbidity, is taken as the optimum coagulant dose

Apparatus

Jar test apparatus, Glass beakers, Pipette, Nephelometer, pH meter

Reagents

- Alum solution (1mL containing 10 mg of alum), Lime, Acid/alkali.

Experimental Procedure

- Take 1-litre beakers and fill them with sample up to the mark.
- Keep each beaker below each paddle and lower the paddles, such that each one is about 1cm above the bottom.
- Find the pH of the sample and adjust it to 6 to 8.5.
- Pipette 1, 2, 3, 4, 5, 6 mL of the alum solution into the test samples.
- Immediately run the paddles at 100 rpm for 1 minute.
- Reduce the speed to 30-40 rpm and run at this rate for 30 minutes.
- Stop the machine, lift out the paddles and allow to settle for 30 minutes.
- Find the residual turbidity of the supernatant using nephelometer.

- Plot a graph with alum dosage along x-axis and turbidity along y-axis.
- The dosage of alum, which represents least turbidity, gives Optimum Coagulant Dosage (O.C.D.).

Calculations

The optimum alum dose is the one, which gives least value of Turbidity

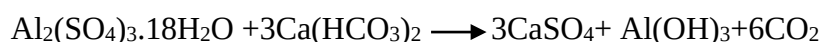
Observation Table

Sl. No	Alum Dosage in mg/L	Turbidity

Results:

Home Work:

- Q1. Write down the environmental significance of measuring OCD of water samples.
- Q2. Alum reacts with alkalinity as per following equation. For removing 90% of 300 mg/L suspended solids, say 50 mg/L alum (for pH 8 solution) is required. If solution also has bicarbonate alkalinity (250 mg/L as CaCO₃), how much alum would be required for getting 90% removal of suspended solids? How much solid waste (in terms of aluminium hydroxide precipitate and settled suspended solids) would be produced?



Q3. As per following information, identify electrolytes useful for coagulating (A) negatively charged particles and (B) charge neutral particles?

Electrolyte	Relative power of Coagulation	
	Positive colloids	Negative colloids
NaCl	1	1
AlCl ₃	1	1000
FeCl ₃	1	1000
Fe ₂ (SO ₄) ₃	30	>1000

Experiment No. 11

Aim

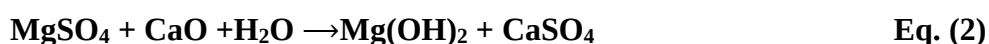
Determination of optimum lime soda dose for hardness removal.

Learning Outcomes

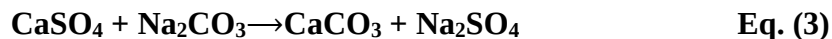
Students will learn the usefulness of lime soda method for hardness removal from water samples.

Brief Theory

Chemical precipitation is one of the more common methods used to soften water. Different species of hardness have different solubility. The least soluble forms are calcium carbonate and magnesium hydroxide. Chemical precipitation is accomplished by converting calcium hardness to calcium carbonate and magnesium hardness to magnesium hydroxide. The process uses lime (CaO), and soda ash (Na₂CO₃), to precipitate hardness from solution.



As seen in Eq. (2), removal of magnesium non carbonate hardness results in the formation of calcium non carbonate hardness. This calcium non carbonate hardness, as well as any initially present in the water, can be removed by addition of soda ash (Na₂CO₃):



Lime is used to remove carbonate hardness, and both lime and soda ash are used to remove non-carbonate hardness. Optimum chemical dosages can be evaluated with a jar test. Theoretically, for each molecule of calcium bicarbonate hardness removed, one molecule of lime is used. For each molecule of magnesium bicarbonate hardness removed, two molecules of lime are used. For each molecule of non-carbonate calcium hardness removed, one molecule of soda ash is used. For each molecule of non-carbonate magnesium hardness removed one molecule of lime plus one molecule of soda ash is used. After adding lime and/or soda ash, treated water will generally have a pH greater than 10. It is necessary to lower the pH to stabilize the water and prevent deposition of carbonate scale on filter sand and distribution piping. Re-carbonation is the most common process used to reduce pH. This Experimental Procedure adds carbon dioxide to water after softening. Generally, enough carbon dioxide is added to reduce the pH of the water to less than 8.7 promoting better precipitation of calcium carbonate and magnesium hydroxide.

Apparatus

Beakers, Pipette, Glass rod and pH meter

Reagents

Prepare 2% (or as instructed) of Lime [Ca(OH)₂] and soda [Na₂CO₃] solution

Experimental Procedure

- Take 50 or 100 mL of identical samples in a beaker.
- Measure the hardness of sample by EDTA method as discussed in experiment no. 4.
- Determine the pH of the raw water sample.
- Add various volume of lime and soda solution in increasing order (1, 1.5, 2, 2.5, 3, 3.5, 4 mL) to the sample.
- Stir the sample with glass rod for 3-5 minutes.
- Determine the pH of each treated water sample.
- Measure the hardness of the samples and record your observation.
- Plot a graph between volume of lime soda used and final hardness.
- The volume that gave the least hardness after treatment is the optimum dose of lime and soda.

Observation Table

Sample No.	Measured Hardness in raw water sample	Lime and soda ash volume	Hardness Measured After Lime soda Treatments

Results:

Home Work:

- Q1. Write down the environmental significance of removal of hardness of water samples.
- Q2. Calculate daily lime-soda ash requirement during softening of water (100 mg/L carbonate hardness (as CaCO_3) and 100 mg/L MgSO_4) (Given: Daily water production: 1000 m^3/day ; lime is 90% pure and soda ash is 80% pure). Assume all carbonate hardness is due to calcium bicarbonate.
- Q3. Water of IIT(ISM) has bicarbonate hardness equal to 100 mg/L as CaCO_3 . Calculate amounts of lime and soda ash required during the lime-soda ash process (Given: Daily flow rate: 10,000 m^3/day).

Experiment No. 12

Aim: To determine the concentration of sodium and potassium by flame photometer.

Learning Outcomes

Students will learn the working Brief Theory of flame photometer and how it can be used to measure the Na concentration and K concentration in water samples.

Detection of Sodium Concentration

Brief Theory: Amount of sodium can be determined by flame emission photometry at the wavelength of 589 nm. The sample is sprayed into a gas flame and excitation is carried out under carefully controlled and reproducible conditions. Desired wavelength of light is achieved by controlling the slit within the apparatus. The intensity of light is measured by a phototube potentiometer or other appropriate circuit. The intensity of light at 589 nm is approximately proportional to the concentration of the element. Minimum detectable concentration of the sodium in flame photometer depends on the instrument and techniques. The better flame photometers can be used to determine sodium levels approximating 100 μ g/L. With proper modifications in techniques the lowest range of sodium measurement can be lowered down to ~10 μ g/L.

Apparatus

Flame photometer

Reagents:

- Deionized distilled water: Use deionized distilled water to prepare all reagents and calibration standards and as dilution water.
- Stock sodium solution: Dissolve 2.542 g NaCl dried at 140°C and dilute to 1000mL with water, 1 mL = 1 mg Na.
- Intermediate sodium solution: Dilute 10 mL stock sodium solution with water to 100mL; 1 mL = 100 μ g Na. Use this intermediate solution to prepare calibration curve in sodium range of 1 to 10 mg/L.
- Standard sodium solution: Dilute 10 mL intermediate sodium solutions with water to 100 mL; 1.00 mL = 10 μ g Na. Use this solution to prepare calibration curve in sodium range of 0.1 to 1 mg/L.

Experimental Procedure

As the standard operating Experimental Procedure varies a lot with the type of instrument so it can't be generalized. Modern day instrument provides direct reading of Na. Here the Experimental Procedure for calculating Na form Calibration curve is given for detailed knowledge.

The Steps are following:

- Run the Flame photometer using the known standard solutions of Na at 589 nm.
- Use the reading of the known standards to construct a calibration curve.
- Run the Unknown water samples through flame photometer.
- Measure the emission intensity reading of the unknown sample from the flame photometer.
- Plot the value in calibration curve and find the concentration of the Na.

Detection of Potassium Concentration

Brief Theory:

Potassium ranks seventh among the elements in order of abundance, yet its concentration in most drinking water seldom reaches 100mg/L. Potassium is an essential element in both plant and human nutrition and occurs in groundwater as a result of mineral dissolution.

Trace amounts of potassium can be determined in either a direct-reading of internal standard type of flame photometer at a wavelength of 766.5 nm. Because much of the information pertaining to sodium applies equally to the potassium determination, carefully study the entire discussion dealing with the flame photometric determination of sodium before making a potassium determination.

Apparatus

Flame photometer

Reagents:

- Reagent water deionized distilled water: Use this water for preparing all reagents and calibration standards and as dilution water.
- Stock potassium solution: Dissolve 1.907g KCl dried at 110°C and dilute to 1000mL with water; 1mL = 1mg K.
- Intermediate potassium solution: Dilute 10mL stock potassium solution with water to 100mL; 1 mL = 0.1 mg K. Use this solution to prepare calibration curve in potassium of 1 to 10 mg/L.
- Standard potassium solution: Dilute 10mL intermediate potassium solution with water to 100mL; 1mL = 0.01 mg K. Use this solution to prepare calibration curve in potassium range of 0.1 to 1 mg/L.

Experimental Procedure:

The Experimental Procedure for detection of Potassium is similar to Na. The desired wavelength of Potassium detection is 766.5 nm.

Observation Table:

Determination of Na concentration	
Sample No.	Value of Na
Determination of K concentration	
Sample No.	Value of K

Results:

Home Work:

- Q1. Write down the environmental significance of measuring Na and K concentrations of water samples.
- Q2. How does flame photometry differ from atomic absorption spectroscopy?
- Q3. List out the differences between flame emission and flame absorption.

Web URLs for video tutorials

Exp. No.	Name of the Experiments	
2. (A) (B) (C)	Calibration of pH meter and determination of pH of given water sample	https://www.youtube.com/watch?v=vwY-xWMam7o&t=227s
	Calibration of conductivity meter and determination of conductivity of given water sample	https://www.youtube.com/watch?v=WhyMOVvAu3s
	Calibration of TDS meter and determination of TDS of given water sample	https://www.youtube.com/watch?v=yhACI_8asEU
2.	Determination of different component of NOM by TOC analyzer and spectrophotometer	https://www.youtube.com/watch?v=BAZZ9U_-Uhl ; https://www.youtube.com/watch?v=4mnqBLelDU
3.	Determination of acidity and alkalinity of given water sample	https://www.youtube.com/watch?v=zXvEmlFqicw ; https://www.youtube.com/watch?v=UL56Lr_TKTI
4.	Determination of hardness of given water sample	https://www.youtube.com/watch?v=Sa0WfA9UGG0
5.	Determination of nitrate of given water sample	https://www.youtube.com/watch?v=46oIaB4Niuo
6.	Determination of sulphate of given water sample	https://www.youtube.com/watch?v=1dsaz71BB8o&t=34s
7.	Determination of chloride of given water sample	https://www.youtube.com/watch?v=Set3XdRshGo
8.	Determination of chlorine demand, residual chlorine and breakpoint	https://www.youtube.com/watch?v=zelRO_lmDdc ;
9.	Calibration and standardization of Nephelometer and determination of turbidity of a water sample	https://www.youtube.com/watch?v=LeKqhMqEoKQ
10.	Determination of optimum coagulant dose using jar test	https://www.youtube.com/watch?v=Y8faha-CEtM
11.	Determination of optimum lime soda dose for hardness removal	Not Available
12.	Determination of sodium and potassium by flame photometer	https://www.youtube.com/watch?v=8vmrDTGWesE

Department of Environmental Science & Engineering

B. Tech. Environmental Engineering

Air and Noise Pollution Practical (ESC 252)

Air, Meteorological and Noise Laboratory (Room No: 308-A)

INDEX OF EXPERIMENTS

Exp. No.	Name of the Experiments
1.	Sampling of PM ₁₀ in ambient air and the determination of its concentration
2.	Sampling of PM _{2.5} in ambient air and the determination of its concentration
3.	Measurement of Sulphur dioxide (SO ₂) concentration in the ambient air
4.	Measurement of Nitrogen dioxide (NO _x) concentration in the ambient air
5.	To calculate emission rates (ER) for different exhaust gases emitting from vehicular tailpipe
6.	To calculate the air change rate (ACH) of indoor environment air and deposition rate of particulate concentration of different sizes
7.	Estimation of respiratory deposition doses (RDD) to the human beings under different conditions
8.	To plot Wind Rose Diagram
9.	To estimate the atmospheric stability within the atmospheric boundary layer of the study area
10.	a. Ambient noise monitoring b. Traffic noise monitoring
11.	Frequency spectrum analysis of machine noise
12.	Audiometric survey for assessing hearing accuracy

Air Quality Monitoring

Air quality monitoring is the systematic, long-term assessment of pollutant levels by measuring the quantity and types of certain pollutants in the surrounding, outdoor air.

Overview

Air quality monitoring is an integral part of an effective air quality management system. Reasons to collect such data include to:

- assess the extent of pollution;
- provide air pollution data to the general public in a timely manner;
- support implementation of air quality goals or standards;
- evaluate the effectiveness of emissions control strategies;
- provide information on air quality trends;
- provide data for the evaluation of air quality models; and
- support research (e.g., long-term studies of the health effects of air pollution).

There are different methods to measure any given pollutant. A developer of a monitoring strategy should examine the options to determine which methods are most appropriate, taking into account the main uses of the data, initial investment costs for equipment, operating costs, reliability of systems, and ease of operation.

The locations for monitoring stations depend on the purpose of the monitoring. Most air quality monitoring networks are designed to support human health objectives, and monitoring stations are established in population centers. They may be near busy roads, in city centers, or at locations of particular concern (e.g., a school, hospital, particular emissions sources). Monitoring stations also may be established to determine background pollution levels, away from urban areas and emissions sources.

Systems are needed to ensure that data are of acceptable quality, to record and store the data, and to analyze the data and present results.

Ambient air noise monitoring

The increasing ambient noise levels in public places from various sources, inter-alia, industrial activity, construction activity, fire crackers, sound producing instruments, generator sets, loud speakers, public address systems, music systems, vehicular horns and other mechanical devices have deleterious effects on human health and the psychological wellbeing of the people. Therefore, it is considered necessary to regulate and

control noise producing and generating sources with the objective of maintaining the ambient air quality standards in respect of noise. For that purpose, continuous monitoring of the noise level in the ambient is necessary in order to maintain the ambient standards with respect to noise level in respective area/zone.

Ambient air quality standards in respect of noise for different areas/zones

- (1) The ambient air quality standards in respect of noise for different areas / zones shall be such as specified in the appendices annexed to this manual (Appendix 5).
- (2) The State Government shall categorize the areas into industrial, commercial, residential or silence areas / zones for the purpose of implementation of noise standards for different areas.
- (3) The State Government shall take measures for abatement of noise including noise emanating from vehicular movements, blowing of horns, bursting of sound emitting firecrackers, use of loud speakers or public address system and sound producing instruments and ensure that the existing noise levels do not exceed the ambient air quality standards specified under these rules.
- (4) All development authorities, local bodies and other concerned authorities while planning developmental activity or carrying out functions relating to town and country planning shall take into consideration all aspects of noise pollution as a parameter of quality of life to avoid noise menace and to achieve the objective of maintaining the ambient air quality standards in respect of noise.
- (5) An area comprising not less than 100 meters around hospitals, educational institutions and courts may be declared as silence area / zone for the purpose of these rules.

Responsibility as to enforcement of noise pollution control measures

- (1) The noise levels in any area / zone shall not exceed the ambient air quality standards in respect of noise as specified in the schedule.
- (2) The authority shall be responsible for the enforcement of noise pollution control measures and the due compliance of the ambient air quality standards in respect of noise.
- (3) The respective State Pollution Control Boards or Pollution Control Committees in consultation with the Central Pollution Control Board shall collect, compile and publish technical and statistical data relating to noise pollution and measures devised for its effective prevention, control and abatement.

EXPERIMENT NO. 1

AIM: Sampling of PM₁₀ in ambient air and the determination of its concentration

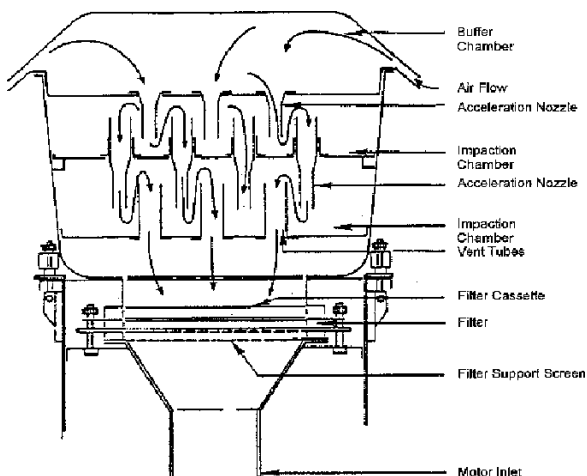
Reference: IS 5182 (Part 23): 2006/CPCB Guidelines Volume-I

Method: Gravimetric Method

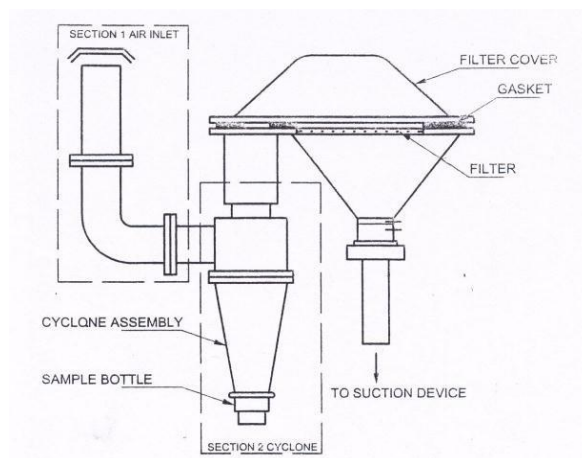
Principle: Air with dust enters the sampler through circular inlet and passed through the impactor where all particles size having aerodynamic diameter more than 10 microns are removed. Particles equal or less than 10 micron passed through impactor. Design of impactor has been done for a cut off at 10 micron at flow rate $2.3 \text{ m}^3/\text{hr}$. Particles greater than 10 microns hit on greased metallic surface and remains on the surface while dust less than 10 microns follow trajectory and reach to pre weighted filter paper where it accumulated. Accumulated dust on filter paper is obtained by weight difference while total volume of air sampled is given by difference of final and initial DG reading. PM₁₀ dust concentration calculated simply by dividing weight of dust by volume of air passed through filters.

Instrument/Equipment

1. Micro Analytical balance – 1 microgram
2. Desiccator – pre-conditioning of the filter paper
3. Sampler: A PM₁₀ sampler with size selective inlet for PM₁₀ and automatic volumetric flow control
4. Filter Jacket
5. Calibrated flow-measuring device to control the airflow at 16.67 l/min



PM₁₀ sampler (Impactation inlet)



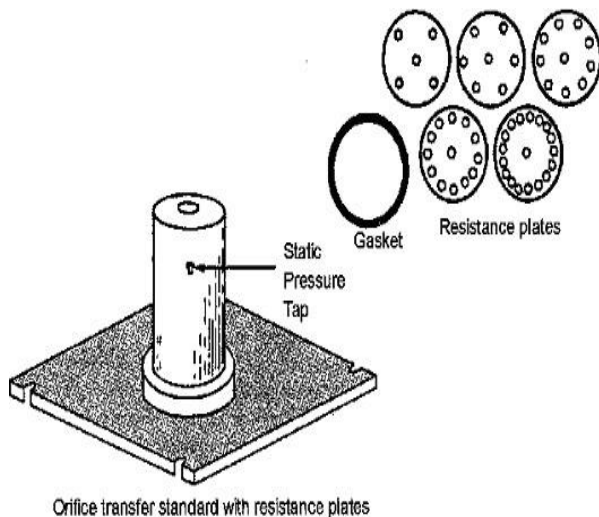
PM₁₀ sampler (Cyclonic inlet)

Filter Media: A Glass fiber 20.3 × 25.4 cm (8 × 10 in) filter of 47 mm size.

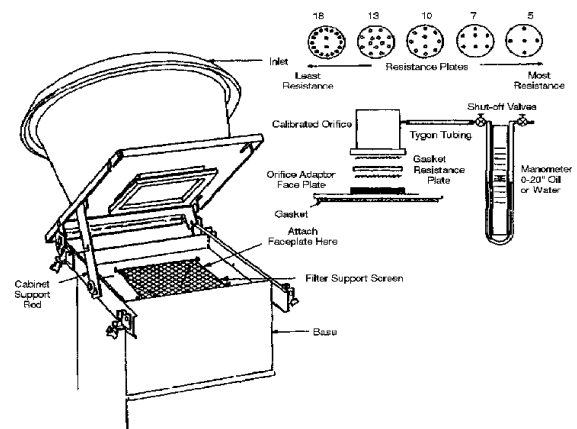
Calibration

Periodical calibration of the sampler is being done by Orifice Transfer Standard. The PM10 sampler calibration orifice consists of a 3.175 cm (1.25 in) diameter hole in the end cap of 7.62 cm (3 in) diameter by 20.3 cm (8 in) long hollow metal cylinder.

1. This orifice is mounted tightly to the filter support in place of the inlet during calibration. A small tap on the side of the cylinder is provided to measure the pressure drop across the orifice.
2. The relationship between pressure difference and flow rate is established via a calibration curve derived from measurements against a primary standard such as a Roots meter at standard temperature and pressure.
3. Flow resistances that simulate filter resistances are introduced at the end of the calibrator opposite the orifice by a set of perforated circular disks.



Top loading Orifice kit

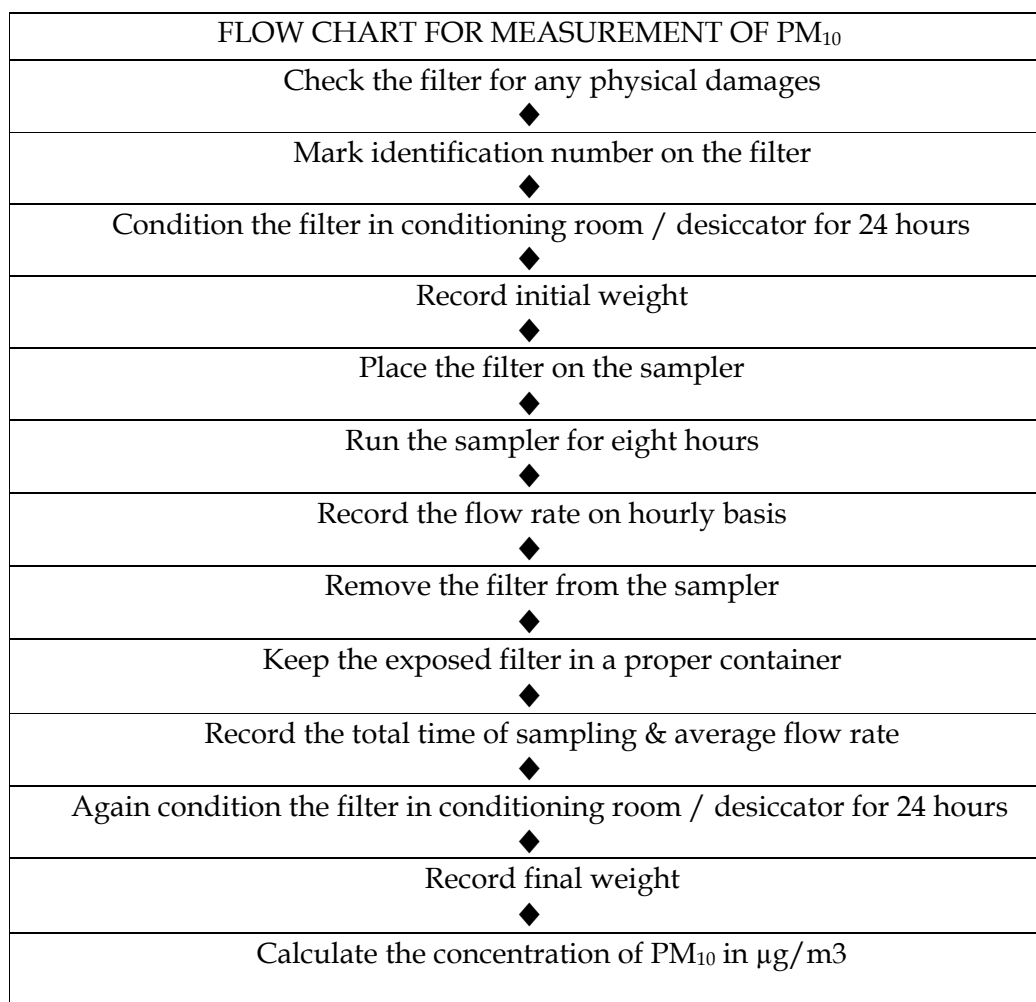


Calibration set up

Procedures

1. Filter inspection: Inspect the filter for pin holes using a light table.
2. Loose particles should be removed with a soft brush. Apply the filter identification number or a code to the filter if it is not numbered.
3. Condition the filter in conditioning room maintained within 20-30° C and 40-50% relative humidity or in an airtight desiccator for 24hours.

4. Take initial weight of the filter paper (W_i) before sampling.
5. Place the filter paper in the sampling system securely and tighten the screws of the bracket.
6. Note down the initial dry gas meter reading and time.
7. Start the sampler and adjust flow rate as per guidelines.
8. At the end stop the pump, note down final dry gas meter reading and time.
9. Remove filter cassette from the filter holder and store in filter carrier immediately and transfer it to desiccators as early as possible.
10. Condition the filter after sampling in conditioning room maintained within 20-30° C and 40-50% relative humidity or in an airtight desiccator for 24 hours.
11. Take final weight of the filter paper (W_f)



Calculation

$$PM_{10} = \frac{(W_f - W_i) \times 10^6}{V}$$

Where,

W_i : Initial mass of the conditioned filter before sample collection (gm)

W_f : Final mass of the conditioned filter after sample collection (gm)

10^6 : Unit conversion factor for grams (gm) to micrograms (μg)

V : Volume of air sampled (m^3)

(V = Final Dry Gas Meter Reading – Initial Dry Gas Meter Reading)

Conclusion/Interpretation: Judge the quality of air analyzed for your hometown city. (refer to CPCB)

Counter measures to improve the ambient air quality: List the contributing sources to the ambient PM.

EXPERIMENT NO. 2

AIM: Sampling of PM_{2.5} in ambient air and the determination of its concentration

Reference: CPCB Guidelines Volume-I

Method: - Gravimetric Method

Principle: Air with dust enters in the sampler through circular Omni directional inlet reaches to impactor where all particles having size more than 10 microns are removed and retained. Now air having particles less than 10 microns further proceed and pass through WINS impactor which is well shaped. A filter dipped in impaction oil is kept in the well where particles hit at a specific velocity (maintained by top critical hole of WINS impactor). This results in separation of particles above 2.5 micron to 10 micron. Only particles having size 2.5 micron and below proceed further and accumulated on PTFE membrane filter. Accumulated dust on filter is obtained by weight difference while total volume of air sampled is given by the difference of final and initial DG readings. PM_{2.5} dust concentration can be calculated simply by dividing weight of dust by volume of air sampled. The lower detection limit of PM_{2.5} dust is about $2\mu\text{g}/\text{m}^3$ which depends upon the accuracy of the digital balance. A sensitive digital balance with minimum detection of 0.00001 g is basic need for measurement of accumulated dust. If 6 digit balance (recommended by USEPA) is available one can measure weight more accurately and thus PM_{2.5} values through filters.

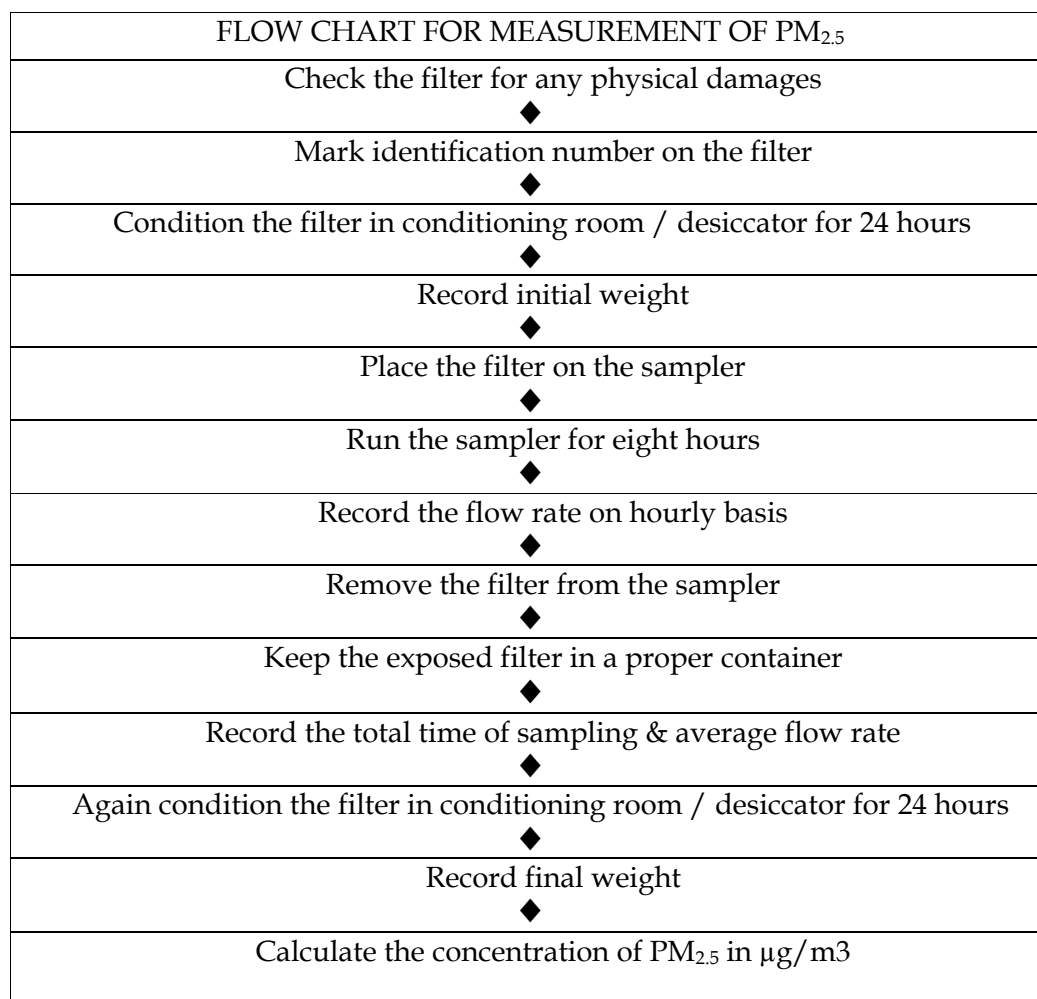
Instrument/Equipment

1. Analytical balance
2. Sampler : A PM_{2.5} Sampler with size selective inlet for PM_{2.5} and automatic volumetric flow control
3. Filter Jacket
4. Injector oil/grease
5. Calibrated flow-measuring device to control the airflow at 16.67 l/min

Filter Media: A polytetrafluoroethylene (PTFE) filter of 47 mm size.

Procedures

1. Filter inspection: Inspect the filter for pin holes using a light table.
2. Loose particles should be removed with a soft brush. Apply the filter identification number or a code to the filter if it is not numbered.
3. Condition the filter in conditioning room maintained within 20-30° C and 40-50% relative humidity or in an airtight desiccator for 24hours.
4. Take initial weight of the filter paper (W_i) before sampling.
5. Place the filter paper in the sampling system securely and tighten the screws of the bracket.
6. Note down the initial dry gas meter reading and time.
7. Start the sampler and adjust flow rate as per guidelines.
8. At the end stop the pump, note down final dry gas meter reading and time.
9. Remove filter cassette from the filter holder and store in filter carrier immediately and transfer it to desiccators as early as possible.
10. Condition the filter after sampling in conditioning room maintained within 20 30° C and 40-50% relative humidity or in an airtight desiccator for 24 hours.
11. Take final weight of the filter paper (W_f)



Calculation

Concentration of PM_{2.5} in µg/m³

$$PM_{2.5} = \frac{(W_f - W_i) \times 10^6}{V}$$

Where,

W_i : Initial mass of the conditioned filter before sample collection (gm)

W_f : Final mass of the conditioned filter after sample collection (gm)

10^6 : Unit conversion factor for grams (gm) to micrograms (µg)

V : Volume of air sampled (m³)

(V = Final Dry Gas Meter Reading – Initial Dry Gas Meter Reading)

EXPERIMENT NO. 3

AIM: Measurement of Sulphur dioxide (SO₂) concentration in the ambient air

Reference: IS 5182 (Part 2): 2001/CPCB Guidelines Volume-I

Method: - Improved West and Gaeke method

Principle: Sulphur dioxide is absorbed from air in a solution of potassiumtetrachloromercurate (TCM). A dichlorosulphitomercurate complex which resists oxidation by the oxygen in the air is formed. This complex is stable to strong oxidants such as ozone and oxides of nitrogen and therefore the absorber solution may be stored for some time prior to analysis. The complex is made to react with pararosaniline and methylsulphonic acid. The absorbance of the solution is measured by means of a suitable spectrophotometer.

APPARATUS & GLASSWARES

- Gaseous Pollutant Sampler
- Glass Impingers
- Spectrophotometer
- Volumetric flasks
- Pipettes
- Burette

REAGENTS

- **Distilled Water**
- **Absorbing Reagent (Potassium Tetrachloromercurate–TCM-0.04M):** Dissolve 10.86 gm mercuric chloride, 0.066 gm EDTA, and 6.0 gm potassium chloride in distilled water and bring to the mark in a 1 liter volumetric flask. The pH of this reagent shall be approximately 4.0 but it has been shown that there is no appreciable difference in collection efficiency over the range of pH 5 to 3. The absorbing reagent is normally stable for 6 months. If a precipitate forms, discard the reagent after recovering the mercury.

CAUTION: Highly poisonous if spilled on Skin. Flush off with water immediately

- **Sulphamic Acid (0.6%):** Dissolve 0.6 gm of Sulphamic acid in 100 mL of distilled water. Prepare fresh, when needed.
- **Formaldehyde Solution (0.6%):** Dilute 5 mL of formaldehyde solution (36 to 38 %) to 1 liter with Distilled water. Prepare fresh, when needed.
- **Stock Iodine Solution (0.1 N):** Take 12.7 gm of iodine in a 250 mL beaker; add 40 gm of potassium iodide and 25 mL of water. Stir to dissolve completely, then dilute to 1 liter with distilled water.
- **Iodine Solution (0.01 N):** Dilute 50 mL of stock solution to 500 mL with distilled water.
- **Starch Indicator Solution:** Triturate 0.4 gm of soluble starch and 0.002 gm of mercuric iodide preservative with a little water and add the paste slowly to 200 mL boiling water. Continue boiling until the solution clear, cool and transfer to a glass stoppered bottle.
- **Stock Sodium Thiosulphate Solution (0.1 N):** Take 25 gm of sodium thiosulphate pentahydrate in a beaker, add 0.1 gm of sodium carbonate, and dissolve using boiled distilled water making the solution up to final volume of 1 liter. Allow the solution to stand one day before standardizing

To standardize accurately weigh, to the nearest 0.1 mg, 1.5 gm of primary standard potassium iodate dried at 180°C, dissolve and dilute to 500 mL in a volumetric flask. Take 50 mL of iodate solution by pipette into a 500 mL iodine flask, add 2 g of potassium iodide and 10 mL (1:10) hydrochloric acid and stopper the flask. After 5 min titrate with stock thiosulphate solution to a pale yellow color. Add 5 mL starch indicator solution and continue the titration until the blue color disappears.

Calculate the normality of the stock solution

The normality of sodium thiosulphate solution, N is calculated as follows:

$$N = \frac{M \times 2.80}{V}$$

Where,

V : Volume in mL of Sodium thiosulphate solution used

M : Mass in gm of Potassium iodate

- **Sodium Thiosulphate Solution (0.01 N):** Dilute 100 mL of the stock thiosulphate solution to 1 liter with freshly boiled distilled water.

- **Standardized sulphite Solution for preparation of working sulphate-TCM Solution:** Dissolve 0.30 g of sodium Meta bisulphite (NaHSO_3) or 0.40 gm of sodium sulphite (Na_2SO_3) in 500 mL of freshly boiled and cooled distilled water. Sulphite solution is unstable, it is therefore important to use water of the highest purity to minimize this instability. This solution contains the equivalent of 320-400 $\mu\text{g/mL}$ of SO_2 .

The actual concentration of the sulphite solution is determined by adding excess iodine and back titrating with standard sodium thiosulphate. To standardize pipette out 50 mL of the 0.01 N iodine solution into each of two 500 mL iodine flask A (blank) and B (sample). To flask A add 25 mL of distilled water and into flask B measure 25 mL of sulphite solution. Stopper the flask and allow to react for 5 min. Titrate with standardized 0.01 N thiosulphate, to a pale yellow color. Then add 5 mL of starch solution and continue the titration until the blue color disappears.

- **Working sulphite- TCM Solution:** Take 2 mL of the standard sulphite solution into 100 mL volumetric flask to dilute with 0.04 MTCM.
- **Preparation of stock Para Rosaniline Solution:** Dissolve 0.5 gm of para rosaniline chloride in 100 mL distilled water. Keep it for 2 days and filter the solution. The filtrate solution is stable for 3 months, if stored in refrigerator.
- **Working Para Rosaniline Solution:** Add 15 mL concentrated hydrochloric acid to 10 mL stock para rosaniline solution and dilute to 250 mL with distilled water in a 250 mL volumetric flask. It may be stored at room temperature in an amber colored bottle for one to two weeks, if stored in a refrigerator.

PROCEDURES

Sampling

- Assemble the sampling apparatus at the sampling site. Procedures are described for short-term (30 minutes, 1 hour, 4 hours long-term (24 hours) sampling.
- **30 minutes, 1 hour, 4 hours sampling:** insert a midjet impinger into the sampling system. Add 10 ml of TCM solution to the impinger (30 ml TCM solution for 4 hours sampling)
- Collect sample at the rate of 1 l/min for 30 min, or 0.5 l/min for 1 hour and 4 hours using either a rotameter.
- **24 hours sampling:** Place 50 ml of TCM solution in a larger absorber and collect the sample at 0.2 l/min for 24 hour from midnight to midnight.

- Shield the absorbing reagent from direct sunlight during and after sampling by covering the impinger with aluminium foil to prevent deterioration.
- Start sampling only after obtaining a flow rate. Ensure packing of ice around bubbler for low temperature to improve absorption efficiency.
- Record the exact sampling time by recording initial time (t_i) and final time (t_f) of sampler.
- Measure and record the flow rate (f_i) before the sampling and flow rate (f_f) after the sampling.
- Record the atmospheric pressure and temperature.
- Remove the stopper the impinger. If the sample has to be stored for more than a day before analysis, keep it at 5° C in a refrigerator; during hot weather, sampling is not recommended unless it is possible to refrigerate the samples.
- After sampling measure the volume of sample and transfer to a sample storage bottle.
- Mark identification number on sample storage bottle and transport to the laboratory for analysis.

Sample Preparation

30-Minutes and 1-Hour Sample: Transfer the sample quantitatively to a 25 ml volumetric flask using about 5 ml of distilled water for rinsing. Delay the analysis for 20 min to allow any ozone to decompose.

24-Hour Samples: Dilute the entire sample to 50 ml with absorbing solution. Measure 5 ml of the sample into a 25 ml volumetric flask by pipette for chemical analysis. Bring volume to 10 ml with absorbing reagent. Delay analysis for 20 min to allow any ozone to decompose.

Analysis

- For each set of determinations prepare a reagent blank by adding 10 ml of unexposed TCM solution to a 25 ml volumetric flask.
- Prepare a control solution by measuring 2 ml of working sulphite-TCM solution into a 25 ml volumetric flask by pipette.
- To each flask containing sample or control solution or reagent blank, add 1 ml of 0.6% sulphamic acid and allow to react for 10 min to destroy the nitrite resulting from oxides of nitrogen.
- Add 2 ml of 0.2% formaldehyde solution and 5 ml of para rosaniline solution.
- Start a laboratory timer that has set for 30min.

- Determine the absorbance of the sample, reagent blank, and control solution at 560 nm using cells with a 1 cm path length.

Calibration

Preparation of Calibration Curve

- Measure 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml and 4.0 ml of working sulphite TCM solution in 25 ml volumetric flask.
- Add sufficient TCM solution to each flask to bring the volume to approximately 10 ml.
- Then add the remaining reagents as described in the procedure for analysis.
- A reagent blank with 10 ml absorbing solution is also prepared.
- Read the absorbance of each standard and reagent blank.

Standard Curve

- Plot a curve absorbance (Y axis) versus concentration (X axis). Draw a line of best fit and determine the slope.
- The reciprocal of slope gives the calibration factor (CF).

IS 5182 Part 2 Method of Measurement of Air Pollution: Sulphur Dioxide

FLOW CHART FOR MEASUREMENT OF SULPHUR DIOXIDE	
Place 30 ml of absorbing media in an impinger	◆
Connect it to the gas-sampling manifold of gas sampling device (RDS/HVS).	◆
Draw air at a sampling rate of 1 lpm for four hours	◆
Check the volume of sample at the end of sampling and record it	◆
Transfer the exposed samples in storage bottle and preserve	◆
Prepare calibration graph as recommended in method	◆
Take 10/20 ml. aliquot of sample in 25 ml. Vol. Flask	◆
Take 10/20 ml. of unexposed sample in 25 ml. Vol. Flask (blank)	◆

Add 1 ml Sulphamic acid. Keep it 10 minutes
◆
Add 2 ml formaldehyde
◆
Add 2 ml working PRA
◆
Make up to mark (25 ml.) with distilled water.
◆
Keep it 30 minutes for reaction
◆
Set Zero of spectrophotometer with Distilled water
◆
Measure absorbance at 560 nm
◆
Calculate concentration using calibration graph
◆
Calculate concentration of Sulphur Dioxide in $\mu\text{g}/\text{m}^3$

CALCULATION

Volume of Air Sampled: Convert the volume of air sampled to the volume in liter at the reference conditions of 25° C and 760 mm Hg:

$$V_n = V \times \frac{P}{760} \times \frac{298}{t + 273}$$

Where:

V_n : Volume of air at 25 °C and 760 mmHg

V : Volume of air sample (m^3)

P : Barometric Pressure, (mmHg)

T : Temperature of air sampled, (°C)

Concentration of Sulphite Solution:

$$C = \frac{(V_1 - V_2) \times N \times 32000}{V}$$

Where,

C : SO_2 concentration in $\mu\text{g}/\text{ml}$

V_1 : Volume of thiosulphate for blank (ml)

V_2 : Volume of thiosulphate for sample (ml)

V : Volume of standard sulphite solution (25ml)

SO₂ concentration in Air Sample: Calculate SO₂ as µg per cubic meter of air as follows:

$$SO_2 \left(\frac{\mu g}{m^3} \right) = \frac{(A - A_0) \times 10^3 \times B}{V_r} * D$$

Where,

A : Sample absorbance, (µg)

A_0 : Reagent blank absorbance, (µg)

10^3 : Conversion of factor to cubic meters

V_r : The sample corrected to 25 C and 760 mm Hg

B : Calibration factor, /absorbance unit

D : Dilution factor: for 30 min and 1 hr samples, $D=1$; for 24-hr samples, $D=10$

Conversion of µg/m³ into ppm

$$SO_2 \text{ (ppm)} = (SO_2 \mu g/m^3) \times 3.82 \times 10^4$$

EXPERIMENT NO. 4

AIM: Measurement of Nitrogen dioxide (NO_x) concentration in the ambient air

Reference: IS 5182 (Part 6): 2006/CPCB Guidelines Volume-I

Method: Modified Jacob and Hochheiser Method

Principle: Ambient nitrogen dioxides are collected by bubbling air through a solution of Sodium hydroxide and sodium arsenite. The concentration of nitrite ion (NO₂) produced during sampling is determined colorimetrically by reacting the nitrite ion with phosphoric acid, sulphanilamide and N-(1-naphtyl)-ethylenediamine di-hydrochloride (NEDA) and measuring the absorbance of the highly colored azo-dye at 540 nm.

APPARATUS & GLASSWARES

- Gaseous Pollutant Sampler
- Glass Impingers
- Spectrophotometer
- Volumetric flasks
- Pipettes
- Burette

REAGENTS

- **Distilled Water**
- **Absorbing Reagents:** Dissolve 4.0 g of sodium hydroxide in distilled water, add 1.0 g of sodium Arsenite, and dilute to 1000 ml with distilled water.
- **Hydrogen Peroxide**, 30percent
- **Hydrogen Peroxide Solution:** Dilute 0.2 ml of 30% hydrogen peroxide to 250 ml with distilled water. This solution may be used for one month, if, refrigerated and protected from light.
- **N-(1-Naphthyl)-ethylenediamine Di-hydrochloride (NEDA):** A 1%aqueous solution should have only one absorption peak at 320 nm over the range of 260-400

nm. NEDA showing more than one absorption peak over this range is impure and should not be used

- **NEDA Solution:** Dissolve 0.5 g of NEDA in 500 ml of distilled water. This solution is stable for one month, if refrigerated and protected from light.
- **Phosphoric Acid, 85percent**
- **Sodium Arsenite**
- **Sodium Hydroxide**
- **Sodium Nitrate:** Assay of 97 percent NaNO_2 or greater.
- **Sodium Nitrate Stock Solution (1000 $\mu\text{g NO}_2/\text{ml}$):** Dissolve 1.5 gm of desiccated sodium nitrate in distilled water and dilute to 1000 ml such that a solution containing 1000 $\mu\text{g NO}_2/\text{ml}$ is obtained. The amount of NaNO_2 to be used if the assay percent is less than 100 percent , is calculated as follows:

$$G = \frac{1.500}{A}$$

Where,

G: Amount of NaNO_2 in gm

1.500: Gravimetric Conversion factor

A: Assay percent (should be 97 or greater)

Note: This stock solution can be stored for six weeks, if refrigerated.

- **Sodium Nitrate Solution (10 $\mu\text{g NO}_2/\text{ml}$):** Pipette 5 ml of the stock solution into a 500 ml volumetric flask and dilute to volume with distilled water.
- **Sodium Nitrate Solution (1 $\mu\text{g NO}_2/\text{ml}$):** Pipette 25 ml of the solution into a 250 ml volumetric flask and dilute with absorbing reagent solution, prepare fresh daily.
- **Sulphanilamide:** Melting point 165 to 167°C
- **Sulphanilamide Solution:** Dissolve 20.0 gm of sulphanilamide in 700 ml of distilled water. Add with mixing 50 ml of 85 percent phosphoric acid and dilute to 1000ml.

(Note: This solution is stable for one month, if refrigerated.)

PROCEDURES

Sampling

- Assemble the sampling apparatus at the sampling site.
- Place 30 ml of absorbing solution in an impinger and sample for four hour at the flow rate of 0.2 to 1L/min.
- Connect the calibrated flow meter, set the flow rate as per method, measure the flow rate before sampling.
- Start sampling only after obtaining an initial flow rate of 1 liter per minute. Ensure packing of ice around bubbler for low temperature to improve absorption efficiency.
- Record the exact sampling time by recording initial time (t_i) and final time (t_f) of sampler.
- Measure and record the flow rate (f_i) before the sampling and flow rate (f_f) after the sampling.
- Seal the collected samples after making it up to 30 ml using distilled water.
- Mark identification number on sample collection bottle and transport to the laboratory for analysis.

Analysis

- Pipette out 10 ml of the collected sample into 50 ml volumetric flask.
Now. Add 1 ml of hydrogen peroxide solution, 10 ml sulphanilamide solution, and 1.4 ml of NEDA solution using pipette, with thorough mixing after the addition of each reagent and make up to 50 ml with distilled water.
- Prepare a blank in the same manner using 10 ml of un-exposed absorbing reagent.
- After a 10 min colour development interval, measure and record the absorbance at 540 nm against the prepared reagent blank.
- Use distilled water; not the reagent blank, as the optical reference.
- Determine NO_2 from the calibration curve.

Calibration

Preparation of Standard

- Prepare calibration curve using 1 $\mu\text{g/ml}$ working standards in accordance with the

above analytical procedure.

- Pipette 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 ml of working standard solution (sodium Nitrate Solution, 1 $\mu\text{g NO}_2/\text{ml}$) in 50 ml volumetric flask.
- Pipette out 10 ml absorbing solution in one volumetric flask as a blank and pipette out 10 ml absorbing solution to all other volumetric flask.
- Now add 1 ml of hydrogen peroxide solution, 10 ml sulphanilamide solution, and 1.4 ml of NEDA solution using pipette, with thorough mixing after the addition of each reagent and make up to 50 ml with distilled water.
- Take optical density at lambda 540 nm on spectrophotometer.

Standard Curve

- Plot a curve absorbance (Y axis) versus concentration (X axis). Draw a line of best fit and determine the slope.
- The reciprocal of slope gives the calibration factor (CF).

IS 5182 Part 6 Methods for Measurement of Air Pollution: Oxides of Nitrogen

FLOW CHART FOR MEASUREMENT OF NITROGEN DIOXIDE
Place 30 ml of absorbing media in an impinger
◆
Connect it to the gas sampling manifold of gas sampling device (RDS/HVS).
◆
Draw air at a sampling rate of 1 lpm for four hours
◆
Check the volume of sample at the end of sampling and record it
◆
Transfer the exposed samples in storage bottle and preserve
◆
Prepare calibration graph as recommended in method
◆
Take 10 ml. aliquot of sample in 50 ml. Vol. Flask
◆
Take 10 ml. of unexposed sample in 50 ml. Vol. Flask (blank)
◆
Add 1 ml hydrogen peroxide,
◆

Add 10 ml sulphanilamide
◆
Add 1.4 ml NEDA
◆
Make up to mark (50 ml.) with distilled water.
◆
Keep it 10 minutes for reaction
◆
Set Zero of spectrophotometer with Distilled water
◆
Measure absorbance at 540 nm
◆
Calculate concentration using calibration graph
◆
Calculate concentration of Nitrogen Dioxide in $\mu\text{g}/\text{m}^3$

CALCULATION

Air Volume: Calculate the volume of air drawn for sample as follows:

$$V_a = \frac{f_i + f_f}{2} \times (t_f - t_i) \times 60 \times 10^{-3}$$

Where,

V_a : Volume of air sample, (m^3)

f_i : Air flow rate before sampling, (LPM) s

f_f : Air flow rate after sampling, (LPM)

t_i : Initial time, (hr)

t_f : Final time, (hr)

10^{-3} : Conversion of litre to m^3

60 : Conversion of hours to min

NO_2 Concentration in Analyzed Sample: Determine $\mu\text{g NO}_2/\text{ml}$ graphically from the calibration curve or compute from the slope and intercept values.

NO_2 Concentration in Air Sample: Calculate NO_2 as μg per cubic meter of air as follows:

$$\text{NO}_2 \left(\frac{\mu\text{g}}{\text{m}^3} \right) = \frac{(A_s - A_b) \times CF \times D \times V_s}{V \times 0.82 \times V_t}$$

Where,

A_s: Absorbance of sample, (μg)

A_b: Absorbance of reagent blank, (μg)

CF: Calibration factor

D: Dilution factor (D:1 for no dilution, D:2 for 1:1 dilution)

V_a: Volume of air sample (m³)

V_s: Volume of sample (ml)

V_t: Volume of aliquot taken for analysis (ml)

0.82: Sampling efficiency

Conversion of μg/m³ into ppm

$$\text{NO}_2 (\text{ppm}) = (\text{NO}_2 \mu\text{g}/\text{m}^3) \times 5.32 \times 10^{-4}$$

EXPERIMENT NO. 5

AIM: To calculate emission rates (ER) for different exhaust gases emitting from vehicular tailpipe

Principle: The analyzer uses the principle of Non- Dispersive Infra-Red (NDIR) for measurement carbon monoxide (CO), carbon dioxide (CO₂), hydrocarbon (C_xH_y) and electrochemical sensors for Oxygen (O₂), Sulphur dioxide (SO₂), nitrogen dioxide (NO₂) and nitric oxide (NO) and hydrogen sulphide (H₂S).

Instruments/Equipment

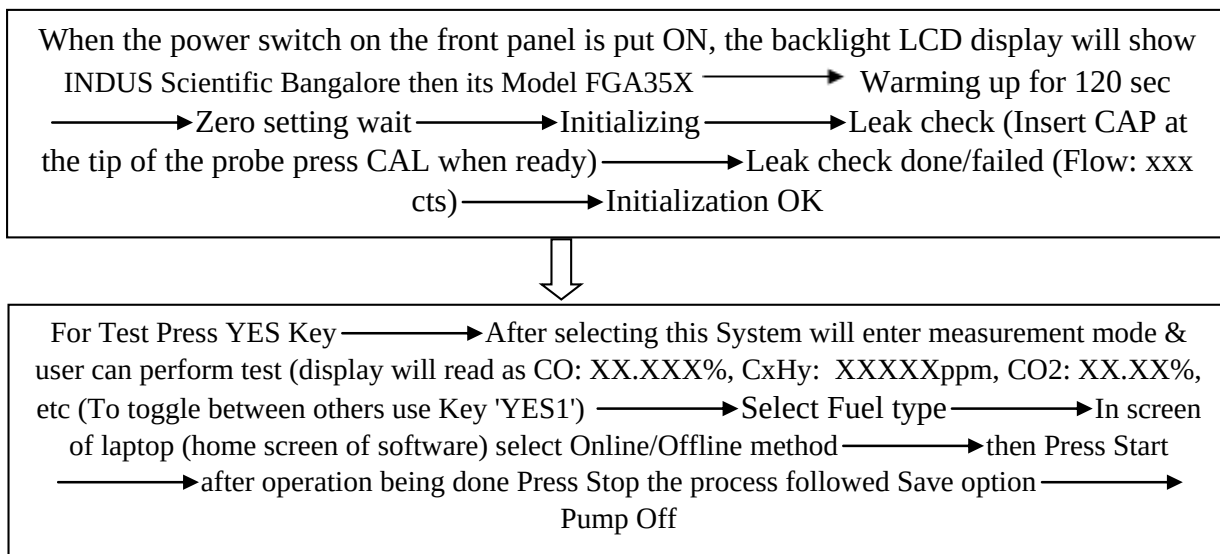
1. Flue gas analyzer: INDUS Model FGA53X is gas analyzer designed and manufactured for testing the emission from various types of exhaust. Sampling system consists of stainless steel probe, Primary particulate filter (attached), base instrument, flow meter- Acrylic.
2. Differential pressure measuring Instrument (Testo 510i) with Pitot Tube for measurement of exhaust velocity

Procedure

Connect well all the connecting tubes, place the equipment in the safe place connect RS232 (computer to equipment). Open the INDUS Software and follow the below described operation procedure.

Operation

From Initialization (power on) to Standby mode then to final operational step



For offline process additionally keys to remember: Select Fuel type; M1: Saving...; M3: Transmitting to PC (Data transmission).

Calculation Method

Step I: Convert the measured concentration values of different pollutant gases from PPM or % into mg/m³

Formula to be used

$$\text{Concentration}_i (\text{mg/m}^3) = \frac{\text{Concentration}_i (\text{ppm}) \times \text{Mw}_i}{V_{i2}}$$

Where,

i = pollutant type,

Mw_i = molecular weight

V_{i2} = volume of exhaust which can be calculated using gas law:

$$V_{i2}(\text{m}^3) = \frac{T_{i2}^{\circ\text{C}} + 273}{T_1^{\circ\text{C}} + 273} V_1(\text{m}^3)$$

Where,

T_1 = Standard temperature, T_{i2} = Exhaust temperature of individual vehicle

V_1 = unit molar volume of air at standard temperature.

Step II: Calculate the area of the exhaust pipe or tailpipe

Formula to be used:

$$A_i = \pi d_i^2$$

Where,

A_i = area of exhaust pipe or tailpipe of particular vehicle

d_i = diameter of the outlet of exhaust pipe or tailpipe.

Step III: Calculate the velocity of exhaust

Calculated from the Differential pressure measuring Instrument (Testo 510i) with Pitot tube for measurement of exhaust velocity.

Step IV: Calculate Emission rate (ER)

Formula to be used:

$$ER_i(\frac{mg}{s}) = Concentration_i(\frac{mg}{m^3}) \times A_i(m^2) \times V_{i2}(\frac{m}{s})$$

Where,

ER_i = required emission rate of the particular pollutant in exhaust.

Problem: Observed gaseous concentration values using flue gas analyzer for ten trials in a test vehicle, the diameter of exhaust pipe, measured data to be used in velocity calculation and exhaust temperatures are provided. What is the average ER (emission rate) of different gases for the exhaust of the tested vehicle?

Trial	CO(%)	CO ₂ (%)	CxHy(ppm)	NO(ppm)	NO ₂ (ppm)	SO ₂ (ppm)	H ₂ S(ppm)	Exhaust Temp(°C)
1	0.708	365	11.08	240	12	580	42	175
2	0.708	365	11.08	241	12	576	41	175
3	0.661	388	11.00	237	11	565	40	183
4	0.468	678	8.62	314	15	402	21	151
5	0.678	392	11.08	238	11	572	39	184
6	0.661	388	11.00	237	11	565	40	183
7	0.642	896	10.07	316	15	535	46	93
8	0.672	576	11.04	314	15	557	37	217
9	0.672	576	9.04	317	15	574	37	218
10	0.610	370	10.90	237	11	531	37	192

Diameter of tailpipe of test vehicle= 2.3 cm and exhaust velocity = 10 m/s.

Take M wt. (in g/mol) for CO-28, CO₂-44, CxHy-86 (Hexane equivalent) NO - 30, NO₂ - 46, SO₂ - 64 and H₂S - 34.

EXPERIMENT NO. 6

AIM: To calculate the air change rate (ACH) of indoor environment air and deposition rate of particulate concentration of different sizes

Instruments: Carbon monoxide meter (CO), carbon dioxide meter (CO₂) and aerosol monitor.

Principle

NDIR (Non-Dispersive Infra-Red): NDIR sensors are spectroscopic sensors that detect carbon dioxide in a gaseous environment by its characteristic absorption. It includes an infrared detector, an interference filter, a light tube and an infrared source. The gas is either pumped or diffused into the light tube, and the electronics measure the absorption of the wavelength of light. The carbon dioxide gas sensor measures gaseous carbon dioxide levels by detecting the quantity of IR radiation absorbed by carbon dioxide molecules. The sensor employs a hot metal filament that acts as an IR source to generate IR radiation. The IR source is located at one end of a sensor tube, and another end is provided with an IR sensor. The IR sensor measures the amount of radiation that passes through the sample without being absorbed by the carbon dioxide molecules.

Light scattering principle: The dust particles are measured by the physical principle of orthogonal light scattering. Here particles are illuminated by a laser light in an angle of 90 degree. All units of environmental dust monitor use light-scattering technology for single-particle counts. The scattered signal from the particle passing through the laser beam is collected at approximately 90° by a mirror and transferred to a recipient-diode. The signal of the diode is feed, after a corresponding reinforcement, to a multi-channel size classifier. This pulse height analyzer then classifies the signal high of each channel. These counts of each channel are converted each minute in a mass distribution from which the different PM values derive.

Experiment design

1. Air exchange rate estimation (AERs)

Air change rate is an important parameter that defines the time taken by exchange of air inside from outdoor. Whether it will reduce or increase the pollutants concentration in the indoor environments, depends on the relative concentration of pollutants indoor and outdoor. Many parameters like building size, building age and building characteristics are said to affect air change rates.

Procedure

- a) Assemble the inside room CO₂ measuring instruments at the height of 3 m from the ground level or at the breathing height.
- b) Keep the instruments away from the disturbance of direct obstacles.
- c) Set the time constant depending upon the required average period (1-minute average).

Calculation method

1. Convert the measured CO₂ concentration into per-minute average time resolution.
2. Calculate the area of indoor space and volume of the indoor room.
3. Calculate the air exchange rate using the following equation:

$$ACH = \frac{1}{t} \ln \frac{(C_t - C_a)}{(C_o - C_a)} \quad (1)$$

Where,

t is time, and C_t and C₀ are concentrations of the gas at times t and 0, respectively and C_a is the ambient concentration of CO₂ (assume ambient CO₂ concentration of 390 ppm).

2. Deposition rates (DRs)

Since people spend most of their time indoors and indoor concentrations of PM often exceeds the corresponding outdoor levels, indoor exposures are major contributors to total personal exposures. This necessitates the understanding of the mechanism of particle dynamics in an indoor environment. Deposition of particles on the surface is one of the significant mechanisms for particle dynamics in indoor.

Procedure

- a) Assemble the inside PM measuring instruments at the height of 3 m from the ground level or at the breathing height.
- b) Keep the instruments away from the disturbance of direct obstacles.
- c) Set the time constant depending upon the required average period (1-minute average).

Calculation method

1. Convert the measured PM concentrations into per-minute average time resolution.

2. Count the number of openings and its status in respect to open or closed.
3. With the assumption of well-mixed indoors, PM concentration indoor can be given by

$$\frac{dC_{in}}{dt} = p\alpha C_{out} + \frac{Q_s}{V} - (\alpha + k)C_{in} \quad (2)$$

Where, C_{in} and C_{out} are the indoor and outdoor particle concentrations, respectively; P is the penetration efficiency; α is the air exchange rate; k is the deposition rate; Q_s is the indoor particle generation rate; t is time, and V is the efficient volume of the room.

In absence of any indoor sources,

$$\frac{dC_{in}}{dt} = p\alpha C_{out} - (\alpha + k)C_{in} \quad (3)$$

In order to estimate the particle deposition rates (k), Eq (2) is simplified by assuming that α and k are constants and P equals one. It was considered that the uncertainty resulting from the assumption of P being equal to one in this study was negligible. This is because indoor concentrations were significantly higher (more than 10 times) than background levels ($C_{in0} \gg C_{out}$), thus the contribution of the outdoor source was comparatively small and could be ignored. Based on the above, the time-dependent solution to Eq (3) becomes:

$$\ln\left(\frac{C_{in}}{C_{in0}}\right) = -(\alpha + k)t \quad (4)$$

Where, C_{in0} is the peak indoor particle concentration. Based on this equation, the value of k is determined by fitting a line to a plot of C_{in}/C_{in0} versus time and subtracting α from the slope and k is particle deposition rate.

Interpretation

Table 1: Indoor environment air CO₂ (PPM) concentrations

Time (min.)	Inside CO ₂ concentration (ppm)
1	595.69
2	617.69
3	625.62
4	641.23
5	663.23
6	680.08
7	694.00
8	705.92

9	706.23
10	705.77
11	709.92
12	717.38
13	729.62
14	736.23
15	749.38
16	748.85
17	796.38
18	762.54
19	618.08
20	897.38
21	722.00
22	679.15
23	643.54
24	712.69
25	809.46
26	766.08
27	731.00
28	737.69
29	731.38
30	588.15
31	394.54
32	443.31
33	481.69
34	520.14

Table 2: Average inside PM ($\mu\text{g m}^{-3}$) concentration

Time (min.)	PM10	PM2.5	PM1
1	248.83	241.81	225.12
2	247.2	237.73	222.31
3	242.19	236.1	219.94
4	241.87	234.29	219.07
5	243.42	236.46	219.36
6	241.23	235.21	218.85
7	250.14	242.78	227.46
8	248.3	243.53	228.3
9	244.24	238.78	224.12
10	247.48	241.95	226.52
11	243.81	239.51	224.15
12	239.43	235.02	221.45
13	242.15	237.18	223.21
14	243.3	237.95	223.69
15	254.66	249.54	232.64
16	262.17	252.72	233.74
17	263.82	247.65	228.38
18	320.92	302.5	255.22

19	234.66	223.14	206.96
20	209.33	202.26	184.37
21	221.38	211.53	195.35
22	208.92	200.33	184.31
23	209.82	202.21	186.52
24	209.45	199.38	184.27
25	207.2	195.89	180.51
26	204.08	193.2	177.8
27	199.12	189.15	173.96
28	198.58	187.55	171.52
29	193.86	183.33	168.57
30	197.83	175.88	159.28
31	180.44	168.94	150.1
32	177.46	166.88	151.39
33	172.58	164.13	148.06
34	173.82	165.19	149.31

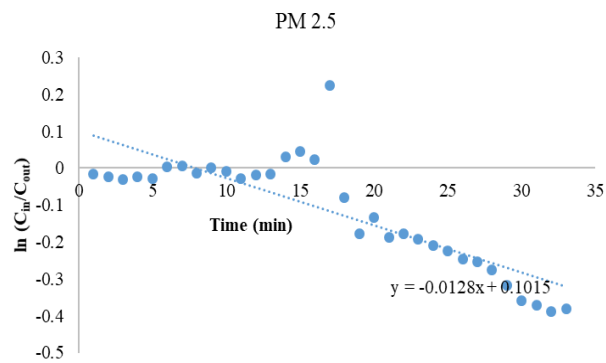
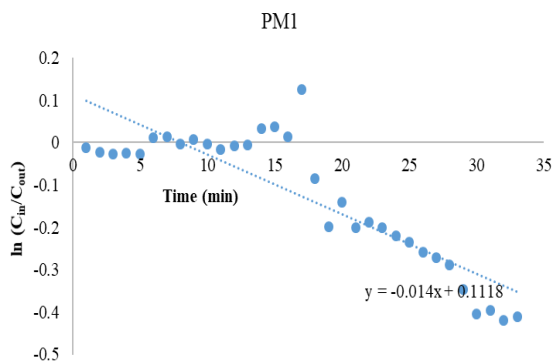
Experiment outcomes

Air exchange rate are calculated with the help of equation 1 and ambient concentration of CO₂ is taken as 390 ppm

Air exchange rate = 10.79 h⁻¹

Deposition rates

Based on this equation, the value of k is determined by fitting a line to a plot of C_{in}/C_{in0} versus time and subtracting α from the slope.



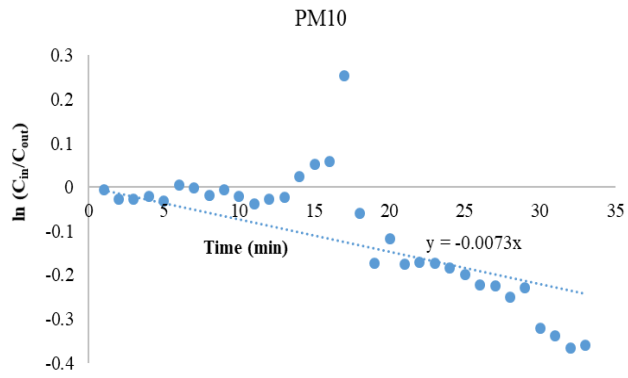


Fig. 2. A typical plot of $\ln(C_{in}/C_{in0})$ versus time for PM size fractions ranging from 0.225 to 0.375 μm

Table 3: Deposition rate for indoor environment for various size fractions of PM

PM size ($\mu\text{g m}^{-3}$)	Deposition rate (h^{-1})
10 μm	11.23
2.5 μm	11.56
1 μm	11.63

EXPERIMENT No. 7

Aim: Estimation of respiratory deposition doses (RDD) to the human beings under different conditions.

Instrument/Model used: Portable aerosol spectrometer GRIMM 1.109, ICRP Model Equation

Theory:

Information about deposition of inhaled aerosols in the respiratory tract is important for risk assessment of occupational and environmental air pollution, drug delivery via inhalers, and diagnosis of lung diseases. Accurate knowledge of the lung deposited fraction of inhaled particles is the key to relate the more readily measured aerosol exposure concentration levels (particle number, surface area, or mass per volume of inhaled air) to the tissue-delivered dose, which is one of the main determinants of the particle-induced biological response.

The semi-empirical ICRP model uses a theoretical prediction of diffusional particle deposition to compensate for the limited amount of data for particles below 100 nm. However, lung deposition is governed by a complex set of parameters comprising the breathing pattern, particle characteristics, flow dynamics, and morphological structure of the lung. In addition, these parameters are subject-dependent and influenced by age, sex, and state of health. Because of the complexity of these issues, numerical calculations of the lung deposition of particles necessarily involve simplifications and need to be validated by experiments.

From the standpoint of respiratory deposition, the respiratory system can be divided into three regions, each covering several anatomical units. These regions differ markedly in structure, airflow patterns, function, retention time, and sensitivity to deposited particles. The first is the head airways region, which includes the nose, mouth, pharynx, and larynx. It is also called the extrathoracic or nasopharyngeal region. Inhaled air is warmed and humidified in this region. The second region is the lung airways or tracheobronchial region, which includes the airways from the trachea to the terminal bronchioles. This region resembles an inverted tree, with a single trunk, the trachea, subdividing into smaller and smaller branches. Finally, beyond the terminal bronchioles is the pulmonary or alveolar region, where gas exchange takes place.

Total deposition: the combined deposition of particles in all regions of the respiratory system-is usually determined experimentally by measuring the concentration of inhaled and exhaled monodisperse test aerosols under controlled conditions.

Procedure:

The procedure for the estimation of respiratory deposition doses (RDD) using the ICRP model is discussed below.

Site selection:

Different working sites were selected for the measurement of particulate matters of different size range. One site was indoor site within the lab and another was outdoor site. Indoor and outdoor particle concentration was measured using the portable aerosol spectrometer, GRIMM 1.109 instrument.

Steps involved in measurement of particulates using GRIMM:

- The measuring principle of the GRIMM model 1.109 is the light scattering of single particles with a semiconductor laser as light source. Inside the measuring cell the scattering light is being led directly and via a mirror with a wide opening angle onto the detector.
- The detector is positioned in the right angle to the incident laser beam. This setup of the detector is denominated as 90° scattering light detection.
- If a particle crosses the laser beam, it creates a light pulse. The signal of the detector diode will be classified into different size channels after accordant amplification.
- The particle size is proportional to the intensity of the detected scattering light signal whereas the scattering light intensity is also being influenced by the particles refractive index, particle shape, and orientation of the particle within the measuring volume.
- GRIMM model 1.109 provides 31 size channels. By detection of the particle concentration and particle size, the size distribution of the aerosol particles can be determined which in turn is the basis of the evaluation of the particle mass.
- Within measuring mode “particle mass” an additional size channel is arithmetically adjoined below the smallest size channel. So, model 1.109 outputs within mass mode analogical 32 size channels.
- The entire sampling volume of 1.2 liter/minute is analysed for a precise and reproducible particle counting and particle sizing.
- GRIMM model 1.109 is started by pressing the ON/OFF key.
- The sample air is sucked through the measuring cell and a gravimetric filter by means of an internal volume flow-controlled pump.

- This filter serves as a dust collector and can be used for gravimetric control of the optical gained measurement results.
- The pump also conveys the rinsing air, which is gained out of the pump's exhaust air via a zero filter and being held constant by a rinsing air control.
- The rinsing air protects the laser optics just as other components of the optical measuring cell from pollution and serves during the self-test as particle-free reference air.
- At the beginning of every measurement the device makes a self-test. Here all optical, pneumatical, and electronical components are being checked.
- The self-test lasts about 30 seconds.
- Afterwards the actual measurement starts and the LCD-display shows continuously every six seconds data.
- This enables real-time measurements of the dust concentration.
- Measurement data was corrected according to the correction factor (C-factor). The particle passed through the measurement cell are collected onto the 47mm PTFE filter, thus enabling for gravimetrical verification of the dust particles mass reported by the optical analysis.
- $C - factor = \frac{\text{gravimetric dust weight on filter (loaded filter - empty filter)}}{\text{calculated dust weight (shown by instrument)}}$
- Verification of the calculated daily concentrations has been performed by gravimetric determination of the particle mass collected on the PTFE filter and correction factor was included (C-factor = 0.95). All the filters were equilibrated before initial and final weighing for a minimum of 24h in controlled chamber. The filters were weighed thrice before and after the sampling by using a microbalance. In addition, the dust monitor was calibrated by the manufacturer prior to starting these experiments. The real-time concentration measurement data are averaged every 6-sec to a data storage card which was aggregated into 1-min average data.
- At the same time all measuring results will be transmitted in certain, adjustable storage intervals to the storage card if one was inserted into the device.
- Via the built-in RS-232 interface data can be transmitted to an external PC (via **Grimm Windows software** with connected PC) or printer. Data output can happen in intervals from 6 seconds up to 60 minutes. With online PC connection even intervals of 3, 2, or 1 seconds are possible.

- GRIMM 1.109 measures the different size range (0.25 µm to 32 µm) of particle numbers in 31 size channels and further particle mass concentration is estimated in 32 size channels.
- Moreover, the standardized dust mass fraction in terms of **occupational health** (respirable, thoracic and alveolic) and **immission** (PM₁₀, PM_{2.5} and PM₁) is also measured by the GRIMM 1.109.
- Further the measured mass concentration of the particulates is averaged for 1-minute interval.
- Thereafter the respiratory deposition doses (RDD) are estimated using the equations mentioned below.
- These equations are for spheres of standard density, but can be applied to other particles by using the aerodynamic diameter for particles larger than 0.5 µm and the physical diameter or equivalent volume diameter for particles less than 0.5 µm.
- Deposition in the respiratory regions depend on particle size (d_p), breathing frequency (f), and tidal volume (V_T). The values for these respiratory parameters are tabulated in Table 1.

Respiratory deposition doses (RDD) estimation:

RDD is estimated using the equation given below, which is adapted from International Commission on Radiological Protection (ICRP; 1994).

$$RDD = (V_T \times f) \times DF_i \times PM_i \times t \quad \mu\text{g per person}$$

Where, V_T is the tidal volume (m³ per breath), f is the typical breathing frequency (breath per minute), DF_i is a deposition fraction of a size fraction i in respective regions, and PM_i is the mass concentration in different size ranges and t is the total monitoring time (minute). The V_T values and f depend on the person's gender and physical activity.

The deposition fraction (DF) in the head airways region DF_{HD} is estimated by using the following expression

$$DF_{HD} = IF \left(\frac{1}{1 + \exp(6.84 + 1.183 \ln d_p)} + \frac{1}{1 + \exp(0.924 - 1.885 \ln d_p)} \right)$$

The deposition fraction in the tracheobronchial region DF_{TB} is estimated by using the following expression

$$DF_{TB} = \left(\frac{0.00352}{d_p} \right) \left[\exp \left(-0.234 (\ln d_p + 3.40)^2 \right) + 63.9 \exp \left(-0.819 (\ln d_p - 1.61)^2 \right) \right]$$

The deposition fraction in the alveolar region DF_{AL} is estimated by using the following expression

$$DF_{AL} = \left(\frac{0.0155}{d_p} \right) \left[\exp \left(-0.416 (\ln d_p + 2.84)^2 \right) + 19.11 \exp \left(-0.482 (\ln d_p - 1.362)^2 \right) \right]$$

Inhalable fraction (IF) is estimated using the equation mentioned below (Eq. 2) and used for the estimation of deposition fraction in different regions inside the body.

$$IF = 1 - 0.5 \left(1 - \frac{1}{1 + 0.00076 d_p^{2.8}} \right)$$

Above equations are for spheres of standard density, but can be applied to other particles by using the aerodynamic diameter for particles larger than 0.5 μm and the physical diameter or equivalent volume diameter for particles less than 0.5 μm .

Table 1. Respiratory parameters used in the ICRP model

	Functional reserve capacity, FRC (L)	Breathing rate ($\text{m}^3 \text{h}^{-1}$)	Breathing frequency (breaths per minute)	Tidal volume (L)
<u>Female</u>				
Sitting	2.68	0.39	14	0.46
Light exercise	2.68	1.25	21	0.99
Heavy exercise	2.68	2.7	33	1.36
<u>Male</u>				
Sitting	3.30	0.54	12	0.75
Light exercise	3.30	1.5	20	1.25
Heavy exercise	3.30	3	26	1.92

Calculation: Calculation was performed in Excel using the ICRP model equations.

Result/Inference:

Gender: Male / Female	RDD ($\mu\text{g minute}^{-1}$)			RDD ($\mu\text{g per person}$)		
	DF _{HA}	DF _{TB}	DF _{AL}	DF _{HA}	DF _{TB}	DF _{AL}
Sitting						
Light exercise						
Heavy exercise						

The average respiratory deposition dose in head airways (DF_{HA}), tracheobronchial (DF_{TB}), and alveolar (DF_{AL}) regions were _____, _____ and _____ $\mu\text{g min}^{-1}$ respectively.

Environmental Significance:

The hazard caused by inhaled particles depends on their chemical composition and on the site at which they deposit within the respiratory system. Respiratory deposition dose study helps in assessment of the particulate exposure to the human beings under different conditions. It helps in understanding the deposition of different size fraction of particles in different regions of human lungs to evaluate properly the health hazards of particulates.

EXPERIMENT No. 8

OBJECTIVE: To plot Wind Rose Diagram

Instruments/software required

Portable weather tracker (Anemometer), Atmospheric weather station (AWS), WRPLOT View

Theory

A wind rose is a graphic tool used by meteorologists to give a succinct view of how wind speed and direction are typically distributed at a particular location. Historically, wind roses were predecessors of the compass rose (found on charts), as there was no differentiation between a cardinal direction and the wind which blew from such a direction. Using a polar coordinate system of gridding, the frequency of winds over a time period is plotted by wind direction, with colour bands showing wind speed ranges. The direction of the longest spoke shows the wind direction with the greatest frequency.

Presented in a circular format, the modern wind rose shows the frequency of winds blowing from particular directions over a specified period. The length of each "spoke" around the circle is related to the frequency that the wind blows from a particular direction per unit time. Each concentric circle represents a different frequency, emanating from zero at the center to increasing frequencies at the outer circles. A wind rose plot may contain additional information, in that each spoke is broken down into color-coded bands that show wind speed ranges. Wind roses typically use 16 cardinal directions, such as north (N), NNE, NE, etc., although they may be subdivided into as many as 32 directions. In terms of angle measurement in degrees, North corresponds to $0^{\circ}/360^{\circ}$, East to 90° , South to 180° and West to 270° .

The graph which contains the readings of wind speed and direction for a particular time is taken from a mechanical wind monitoring equipment and the readings are tabulated for percentage at winds in specific direction.

Methods

Two methods are mostly adopted to plot the wind rose diagram

- a) Graphical Method, and
- b) With the help of Software

Procedure

- Obtain the hourly averaged meteorological data (Time of measurement, wind speed and wind direction).
- In the observation table write down the number of times the wind flows in the range of speed in their respective directions.
- Find the frequency of wind flow by segregating the wind speed in specified range in the 16 different direction.
- Plot the value on a graph with direction indicated and concentric circle (where concentric circle indicates the frequency of the wind).
- Use different shades for different speed ranges to differentiate it.

Observation Table

	Direction → Range ↓	N	NNE	NE	ENE	E	ESE	SE	SSE	S	SSW	SW	WSW	W	WNW	NW	NNW
	0-1 m/s																
	1-5 m/s																
	5-10 m/s																
	>10 m/s																

Percentage is calculated by counting the number of observations recorded in each direction divided by total number of observation.

Application of software for the wind rose plot

Surface meteorological data from the weather monitoring stations or any other sources are used for plotting the windrose diagrams through the WRPLOT View software. WRPLOT View is a fully operational wind rose program for your meteorological data. It provides visual wind rose plots, frequency analysis, and plots for several meteorological data formats.

A wind rose depicts the frequency of occurrence of winds in each of the specified wind direction sectors and wind speed classes for a given location and time period.

The web link for downloading the WRPLOT View is

<https://www.weblakes.com/products/wrplot/index.html>

Installation steps for WRPLOT View software

- Download** the self-extracting installation file to a temporary folder in your computer.

2. **Submit your Registration** to obtain an activation code. An activation code will be e-mailed to you within 24 hours.
3. **Install WRPLOT View** onto your computer.
4. Once you have received your activation code, start-up WRPLOT View. When prompted to register, click on **Yes**, and the Registration dialog box will be displayed.
5. **Enter your activation code** (product registration key) you received by e-mail and press OK.
6. You can now **start using WRPLOT View**.

Meteorological input to the WRPLOT View is required in SCRAM file format. Hourly surface meteorological data are required to create a 28-character record also known as the SCRAM format. The SCRAM format can be processed directly by WRPLOT View.

Columns	Element
1-5	Surface Station Number
6-7	Year*
8-9	Month*
10-11	Day*
12-13	Hour*
14-16	Ceiling Height (Hundreds of feet)
17-18	Wind Direction (Tens of Degrees)*
19-21	Wind Speed (Knots)*
22-24	Dry Bulb Temperature (Degrees Fahrenheit)
25-26	Total Cloud Cover
27-28	Opaque Cloud Cover

* Only these variables are used by WRPLOT View to generate the wind rose statistics

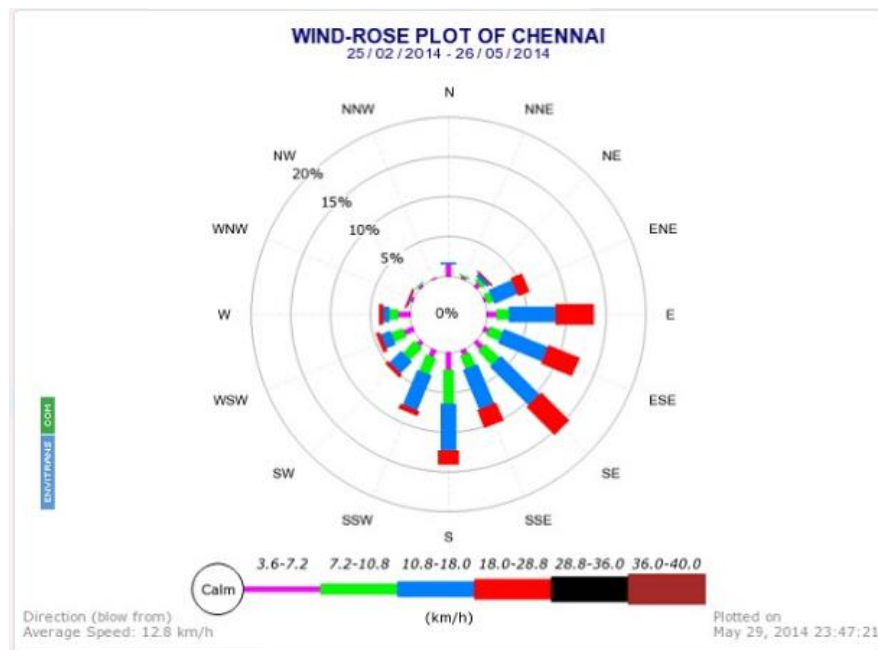
The variables used by WRPLOT View from the SCRAM record include the following:

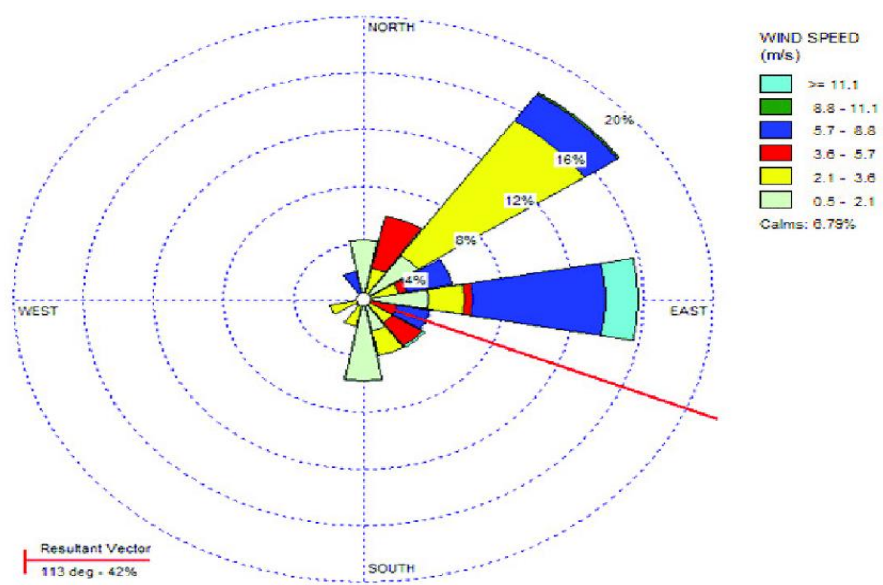
- **Surface Station Number:** The WBAN number identifying the NWS surface observation station.
- **Year, Month and Day of Record:** Identifies the year, month and day during which the meteorological data were observed. Only the last two digits of the year are reported.

- **Hour:** Identifies the hour of the meteorological data observation. Hour is based on the 24-hour clock and is recorded as 00 through 23.
- **Ceiling Height:** The height of the cloud base above local terrain which is coded in hundreds of feet.
- **Wind Direction:** The direction from which the wind is blowing, based on the 36 point compass, e.g. 09=East, 18=South, 27=West, 36=North, 00=Calm.
- **Wind Speed:** The wind speed measured in knots (00=Calm). · Dry Bulb Temperature: The ambient temperature measured in whole degrees Fahrenheit.
- **Cloud Cover:** There are two cloud cover parameters, opaque cloud cover and total cloud cover in the CD-144 and SCRAM meteorological data files. Both parameters identify the amount of cloud cover measured in tens of percent, e.g., 0 = clear or less than 10%, 4 = 40-49%, '-' = overcast or 100%

Results

- The prevailing wind direction in the downwind direction is
- Percentage calm period ($WS < 1 \text{ m/s}$) is





EXPERIMENT No. 9

OBJECTIVE: To estimate the atmospheric stability within the atmospheric boundary layer of the study area.

METHODS:

1. Stability class estimation using the surface wind speed and weather conditions (P-G method).
2. Estimation of Stability class using vertical temperature gradient (Lapse rate).
3. Estimation of Stability class using Richardson number.
4. Estimation of Stability class using Standard deviations of the horizontal wind direction (σ_θ).
5. Monin-Obukhov length for atmospheric stability classification.

INSTRUMENTS:

Atmospheric weather station (AWS), Portable weather tracker (Anemometer)

METEOROLOGICAL REQUIREMENT:

Wind speed, Wind direction, Ambient temperature, Measuring height, Cloud cover, Ceiling height

THEORY:

Atmospheric stability is defined as the ability of the atmosphere to enhance or to resist atmospheric motions. In other way it is the tendency of an air parcel to move upward or downward after it has been displaced vertically by a small amount. Stable atmospheres suppress the vertically upward movement of the air parcels and reduce the turbulence intensity. Different methodologies adopted to estimate the atmospheric stability class in this study are discussed in this practical.

The most commonly used method of categorizing the amount of atmospheric turbulence present was the method developed by Pasquill in 1961. The atmospheric turbulence was categorized into six **stability classes** as tabulated below (APPENDIX 9).

The atmospheric stability is influenced by different meteorological conditions such as solar radiation, cloudiness, wind speed etc. Solar radiation increases atmospheric instability through warming of the Earth's surface so that warm air is below cooler (and therefore denser) air

promoting vertical mixing. Clear nights push conditions toward stable as the ground cools faster establishing more stable conditions and inversions. Wind increases vertical mixing, breaking down any type of stratification and pushing the stability class towards neutral (D). Meteorological conditions that define the atmospheric condition of the study area based upon the Pasquill stability class is tabulated in APPENDIX 10.

A simple way to determine the atmospheric stability is to use dry adiabatic lapse rate (Γ_d). The lapse rate is the rate of change of temperature with height and is defined as, $\Gamma = -\Delta T / \Delta Z$. The value of Γ varies throughout the atmosphere. The conditions for different atmospheric lapse rates is shown in table in APPENDIX 11. The value of the dry adiabatic lapse rate in the table below is **1°C/100 m**. The environmental temperature gradient is positive in a strongly stable atmosphere while it is negative in an unstable atmosphere.

Richardson number (Ri) plays an important role in atmospheric turbulence and dispersion, as it characterizes the relative importance of heat convection and mechanical turbulence in the theory of atmospheric turbulence and dispersion (Tricio et al., 2012). Turbulence characteristic with various Ri number is shown in APPENDIX 12.

Ri of large magnitude indicate that convection predominates and, in such situation, the winds are weak, and there is strong vertical motion. As the mechanical turbulence increases, the Ri approaches zero, and the angular dispersion decreases. For the positive Ri, the stratification becomes stable and damps the mechanical turbulence. For Ri greater than 0.25 (strong inversions, weak winds), vertical mixing effectively disappears, and only weak horizontal eddies remain (Tricio et al., 2012).

Yamartino developed an estimator based on the assumption that the distribution of wind direction was isotropic, and selected an interpolation function based on its performance against Monte-Carlo simulations also based on this assumption. Yamartino benchmarked this method against the Ackerman-Verrall and Williams methods (which used more complicated estimators) and showed that the Yamartino method performed better for high values of sigma theta compared to the others.

CALCULATIONS:

METHOD – 1: Stability class estimation using the surface wind speed and weather conditions (P-G method).

Parameters required for Stability class estimation

- Surface wind speed (m/s).
- Day time insolation:
 - Strong: Clear summer day with sun higher than 60° above the horizon.
 - Moderate: Summer day with a few broken clouds of a clear day with sun 35-60° above the horizon.
 - Slight: A fall afternoon, or a cloudy summer day, or clear summer day with sun 15-35° above the horizon.
- Night time cloudiness: Cloudiness means the fraction of sky covered by clouds.
 - $\geq 4/8$ cloudiness or thinly overcast
 - $\leq 3/8$ cloudiness

The meteorological conditions mentioned above helps in determining the stability class using the table shown in APPENDIX 10.

METHOD – 2: Estimation of Stability class using vertical temperature gradient (Lapse rate)

Another way to define the stability class is by measuring the vertical temperature gradient ($\Delta T/\Delta z$) at various depth. By knowing the temperature at various depth, the ($\Delta T/\Delta z$) value is calculated and thus the stability class will be estimated using the APPENDIX 13.

METHOD – 3: Estimation of Stability class using Richardson number

By knowing the vertical temperature profile and the vertical wind profile at various depth, Ri number are evaluated using the equations mentioned below and thus, stability class is known by using the correlation between the Ri number and the Pasquill-Gifford stability class as shown in APPENDIX 13.

The Richardson number is calculated using the relation (Tricio et al., 2012):

$$Ri = \frac{\left(\frac{g}{\theta}\right)\left(\frac{d\theta}{dz}\right)}{\left(\frac{d\bar{u}}{dz}\right)^2} \quad \text{or} \quad Ri_g = \frac{N^2}{\left(\frac{d\bar{u}}{dz}\right)^2}$$

where, Ri is the traditional Richardson number, Ri_g is the gradient Richardson number, $\left(\frac{g}{\theta}\right)$ is the buoyancy parameter ($m/s^2/K$), $\left(\frac{d\theta}{dz}\right)$ is the potential temperature gradient (K/m), $\left(\frac{d\bar{u}}{dz}\right)$ is the mean

wind shear (s^{-1}), $N = \sqrt{\left(\frac{g}{\theta}\right)\left(\frac{d\theta}{dz}\right)}$ is the Brunt–Väisälä frequency or Buoyancy frequency (in s^{-1}),

g is the acceleration due to gravity, z is the geometric height, $\theta = T \left(\frac{P_0}{P}\right)^{0.286}$ is the potential temperature, P (mb) is the atmospheric pressure, P_0 (mb) is the Reference pressure, T (K) is the air temperature at reference height.

METHOD – 4: Estimation of Stability class using Standard deviations of the horizontal wind direction (σ_θ)

Yamartino method

The Yamartino method computes the average sine of wind direction and the average cosine of wind direction. From these two quantities it computes epsilon (the sine of sigma theta). Sigma theta is then computed as the arcsine of epsilon, multiplied by an interpolation factor to correct for large values of sigma theta. $S = \frac{1}{N} \sum_{i=1}^N \sin \theta_i$

$$C = \frac{1}{N} \sum_{i=1}^N \cos \theta_i$$

$$\varepsilon = \sqrt{1 - (S^2 + C^2)}$$

$$\sigma_\theta = \arcsine(\varepsilon) \left[1 + \left(\frac{2}{\sqrt{3}} - 1 \right) \varepsilon^3 \right]$$

For each hour of valid data, a 60-minute sigma theta was computed by finding the Root Sum Squared (RSS) average of the four 15-minute sigma theta values. Four 15-minute sigma theta (σ_θ) values should be combined to represent an hourly sampling period as follows:

$$\sigma_\theta = \sqrt{\frac{\sigma_{\theta_1} + \sigma_{\theta_2} + \sigma_{\theta_3} + \sigma_{\theta_4}}{4}}$$

METHOD – 5: Monin-Obukhov length for atmospheric stability classification

$$L = -\frac{\rho_{air} C_p T u_*^3}{kg H_v}$$

$$u_* = - \frac{ku}{\ln \frac{Z_{ref}}{z_0} - \psi_m \left(\frac{Z_{ref}}{L} \right) + \psi_m \left(\frac{z_0}{L} \right)}$$

Physical constant in the above formula are

- a) k - von Karman constant (0.4)
- b) ρ_{air} – density of ambient air (kg/m^3)
- c) C_p – specific heat capacity of air (J/kg/K)
- d) g – acceleration due to gravity (m/s^2)

u – wind speed at reference height (m/s)

Z_{ref} – wind reference height (10 m)

z_0 – surface roughness length (sector and season dependent)

T – the ambient temperature at the reference height in Kelvin

u^* – the friction velocity

and H_v – the vertical heat flux, which is difficult to obtain by measurement, H_v .

The correlation developed by Pasquill and Smith (1971) for flow over short grass, with $z_0 = 0.01$ m is given APPENDIX 13.

RESULTS:

The obtained value for hourly average meteorological data from different methods are correlated with different atmospheric stability class as per the APPENDIX 13. Thus by using above mentioned methods the atmospheric stability class is easily known for the hourly average meteorological data under various conditions.

The atmospheric stability class obtained using the different methods for specific area are tabulated below:

Method adopted	Stability class	Description

Plot the graph for different parameters obtained from the above methods showing their variation with the time.

Plot the graph for different parameters obtained from the above methods showing their variation with the altitude.

EXPERIMENT NO. 10 (a)

AIM: Ambient noise monitoring.

Theory

Noise level measurement procedure are processes which are followed while monitoring sound level or acoustic energy level in specified area. These days annoying noise levels have started to become a major threat to public health. Noise in simple terms can be defined as unwanted or unpleasant sound which disturbs the environment and has a significant impact on the quality of life. We all very well know how exposure to high noise level can result in hearing impairment, headache, sleep disturbance and impaired task performance.

Why Noise Monitoring is required?

- Compliance with noise regulation
- Hearing loss prevention
- Noise control
- Community Annoyance
- To find out the location or place or areas where noise is high than the TLV and problematic
- Workers or people are complaining about noise or are affected

Noise Measurement

Three techniques are used to measure noise in the work place and community environment

- (1) Personal sound exposure meter or also known as dosimeter
- (2) Sound Level Meter
- (3) Tape recorders, data recorders and level recorders

Types of Noise

- Steady or Continuous Noise: It is uninterrupted noise that varies less than 5 dB-A during the period of measurement eg Noise from house hold fan , boiler in a power house, lathes, diesel engine ,grinder etc
- Impulsive or Impact noise :When source causes vibration for a short time eg firing from the gun or hammer

- Intermittent/Fluctuating Noise: Large workshop number of machines are in operation, noise levels varies from time to time or dentists drilling

Selection of instrument, sampling duration and scale

- To measure noise level, the most extensively used instrument is a Sound Level Meter (SLM) which commonly is known as a noise meter.
- After selection of instrument, selection of sampling duration and scale is must. With these it is also necessary to consider the parameters which we need to measure like; Lmax, Lmin, Leq, etc.

Sound Level Meter

- Sound level meter SLM consist of a microphone, electric circuit and digital display. Microphone detects and reads minimal air pressure changes and convert them into electric signals.
- These signals are processed by electric circuits and converted into decibels. SLM can read noise level for one location at a time.
- While measuring noise levels it is held in an arm's length at the ear height. It does not matter whether microphone is point towards source.
- Sound level monitor must be calibrated before and after use.
- Every sound level meter has two modes and those are SLOW and FAST.
- It is the response rate which SLM averages before showing on screen.
- For workplace noise level monitoring it should be taken as SLOW.

Information required for noise survey/monitoring

- Location and Nature of work (Sketch of the measurement site, including size of the room, machine dimensions etc.)
- Nature of work process and task
- No of workers working in noisy area
- Current control including protection devices (e.g. Personal Protection devices like ear plugs or ear muffs)
- Community noise monitoring is required? If yes, Status of Community monitoring, whether higher than permissible level?
- Type of sound level meter

- Operating condition during the measurement and job description
- Results of the noise assessment
- Date of assessment or monitoring
- Name and signature of the person conducting the monitoring.

Noise Level measurement Procedure

- Must be check the battery full before goes onside monitoring.
- Switch ON of equipment and wait for one minute.
- SLM has two modes of operation selection via the menu key.
- Two mode:(1) In continuous Mode (2) In Recording Mode
- In continuous Mode: For only onsite observation.
- In Recording Mode: For recording the data in sound level meter.
- Select the appropriate mode of sound level.
- Set the slow or fast time as per site condition. Slow time is applicable for normal measurement. Fast time is applicable for specific measurement of noise like moving train, heavy traffic etc.
- Adequate Distance (1 to 3 meter) maintain between source and equipment.
- Take reading for at least one minute at one location. Number of reading can take for one minute intervals.
- Monitoring period should be decided in such a manner that one reading is available after one minute of monitoring at one location.
- When we change the location, off the machine and again Switch ON of equipment.

Place of the Instrument

Many factors need to be taken into account when measuring because sound levels vary at different heights above ground level. They will also vary depending on the distance between the measurement point and facades and obstacles. These are some important factors for doing monitoring:

- a. Away from facades
- b. Away from obstacles
- c. With the microphone 1.2 - 1.5 meter above ground level

- d. Monitoring inside Industrial facility distance from source shall be 1.5 meter

Monitoring Locations

The locations for monitoring to assess the ambient noise levels shall be mix up of all zones i.e. Residential, Commercial and Industrial to find the variations with different zones. The monitoring schedule carried out on working days and weekend to differentiate the noise levels between normal days to weekend days. The Sampling locations should be sufficient enough provide representative samples for the project.

Observation

Time	North				East				South				West			
	Max	Max	Min.	L _{eq}	Max	Max	Min.	L _{eq}	Max	Max	Min.	L _{eq}	Max	Max	Min.	L _{eq}
	P	L	L		P	L	L		P	L	L		P	L	L	

Results:

Comparison with Noise standards

Once we get the monitoring records then we can compare them with standards to understand if noise levels are exceeding the standards and if they are then by how much. In India, the standards extensively used are prescribed by Central Pollution Control Board (CPCB).

Noise level Measurement Standards

- Limits or noise level standards defined by pollution control board during Day time for Residential Noise < 55 dB and Limits during Night time for Residential Noise < 45 dB.
- In industry, there are of two types Sound Monitoring & testing or Noise Level Measurement: (A) Ambient Noise level Monitoring & (B) In-plant Noise level Monitoring.

A) Ambient Noise level standards

Ambient Noise level Monitoring or Noise pollution Measurement within industrial zone at ambient conditions. (e.g. Near Main Gate, Near Canteen, Near Manufacturing plant etc.) As per Central Pollution control Board (as per Factory

act 1948).

Limits or acceptable noise level during Day time for Ambient Noise < 75 dB and
Limits during Night time for Ambient Noise < 70 dB.

B) In-plant Noise level Standards

In-plant Noise level means Sound level measurement allocated in the plant. As per
Central Pollution control Board (as per Factory act 1948) Limits for In-plant Noise
level < 90 dB.

The below table shows the Noise level Monitoring standards prescribed by Central Pollution
Control Board (CPCB). Noise Pollution (Regulation & Control) Rules 2000 under the
provision of the Environment (Protection) Act, 1986. These rules provides standards in respect
of noise for different areas/zones of a city/town.

Area code	Category of area/Zone	Limits in db (A)	
		Day time	Night time
(A)	Industrial area	75	70
(B)	Commercial area	65	55
(C)	Residential area	55	45
(D)	Silence zone	50	40

Day time: 6:00 AM to 10:00 PM

Night time: 10:00 PM to 6:00 AM

EXPERIMENT NO. 10 (b)

AIM: Traffic noise monitoring

Theory

There are two types of road traffic noise:

- (a) From 'bulk' traffic flow (i.e. a reasonably continuous stream of vehicles), and
- (b) From individual noisy vehicles.

The first occurs on busier roads and manifests itself as a continuous hum or backdrop of noise, although it can have peaks and troughs according to the traffic flow. It is the aggregate noise of all the vehicles in the traffic stream.

The second is produced by single vehicles (typically a high-revving motorbike, a car with a modified exhaust system, or a commercial vehicle's exhaust brakes) and can occur anywhere and at any time. This may be loud enough to rise above bulk flow traffic noise and can be extremely disruptive, particularly at night in quiet residential areas where sleep can be disturbed.

Although measures can be taken to protect the community against both types, the second is by far the harder to deal with because of the random nature of occurrences, and can only be controlled by vehicle design requirements and in-service monitoring of these requirements. These Guidelines essentially deal with bulk flow traffic noise resulting from normal operation of the road system.

Factors affecting traffic noise generation

Individual vehicle noise is a combination of noises produced by:

- the engine;
- the transmission;
- the exhaust;
- the interaction of tyres and road pavement;
- air turbulence; and
- body and load rattles.

Noise Descriptors

There are two descriptors commonly used to describe road traffic noise which varies over time. These are L_{10} and L_{eq} .

The noise descriptor L_{10} refers to the noise level which is exceeded for 10% of the time in a given measurement period, and corresponds to the average of the upper noise levels. In Figure 2.5 below, which shows typical noise level variations over a one hour measurement period, L_{10} is the noise level which is exceeded for 6 minutes (10% of one hour).

The noise descriptor L_{eq} (the equivalent noise level) refers to the value of a steady noise that would have the same acoustic energy as the varying noise, over the same time period.

The descriptor L_{10} is used by some organisations as the descriptor for traffic noise. The descriptor L_{eq} has now replaced L_{10} as the commonly used descriptor. Generally, L_{10} is around 3 dB higher than L_{eq} , although this difference varies.

Noise Level measurement Procedure

- Must check the battery full before goes onsite monitoring.
- Switch ON of equipment and wait for one minute.
- SLM has two modes of operation selection via the menu key.
- Two mode:(1) In continuous Mode (2) In Recording Mode
- In continuous Mode: For only onsite observation.
- In Recording Mode: For recording the data in sound level meter.
- Select the appropriate mode of sound level.
- Set the slow or fast time as per site condition. Slow time is applicable for normal measurement. Fast time is applicable for specific measurement of noise like moving train, heavy traffic etc.
- Adequate Distance (1 to 3 meter) maintain between source and equipment.
- Take reading for at least one minute at one location. Number of reading can take for one minute intervals.
- Monitoring period should be decided in such a manner that one reading is available after one minute of monitoring at one location.
- When we change the location, off the machine and again Switch ON of equipment.

Observation

Time	North				East				South				West			
	Max	Max	Min.	Leq	Max	Max	Min.	Leq	Max	Max	Min.	Leq	Max	Max	Min.	Leq
	P	L	L		P	L	L		P	L	L		P	L	L	

Comparison with ambient air standards Appendix 5

Noise level Measurement Standards

The Noise level Monitoring standards prescribed by Central Pollution Control Board (CPCB) is presented in Appendix 5. Noise Pollution (Regulation & Control) Rules 2000 under the provision of the Environment (Protection) Act, 1986.

Results:

EXPERIMENT NO. 11

AIM: Frequency spectrum analysis of machine noise.

Theory

When more detailed information about a complex sound is needed, the frequency range of 20 Hz to 20 kHz can be split into sections or bands. This is done electronically within a sound level meter.

These bands usually have a bandwidth of one octave or one third octave. More advanced instruments may be able to give a narrow band analysis of the noise data. This may be an FFT (Fast Fourier Transform) or information in 1/12 octaves.

An octave band is a frequency band where the highest frequency is twice the lowest frequency. we are frequently interested in knowing how sound energy is distributed as a function of the sound frequency. As such the measure acoustic energy is electronically filtered into contiguous frequency bands. For each frequency band we measure the overall sound pressure level of the filtered frequency. The octave band is the common contiguous band used in the study of machine noise. In the octave band upper frequency limit is twice the lower frequency limit. For more detailed analysis of sound wave, finer set of contiguous band such as 1/3 octave band is used.

Initially frequency spectrum analysis is conducted with 1/1 octave band to identify the alarming range of frequencies, thereby detailed analysis is conducted with 1/3 octave band within the identified alarming range in 1/1 octave band to determine the dominant frequency i.e. frequencies with highest sound pressure levels.

The frequency spectrum analysis is very helpful in suggesting the effective control measure to combat the noise situation within permissible level. The common octave frequency bands in 1/1 octave bands are: — 31Hz, 63Hz, 125Hz, 250Hz, 500Hz, 1kHz, 2kHz, 4kHz, 8kHz and 16kHz – and their composition is made up of the Lower Band Limit, Centre Frequency and Upper Band Limit. The lower limit (F_l) and upper limit (F_u) of 1/1 octave band is given below:

1/1 octave band:

$$F_l = F_c / \sqrt{2} ; \quad F_u = 2F_l$$

1/3 octave band:

$$F_l = F_c / 2^{1/6} ; \quad F_u = 2^{1/3} F_l$$

Apparatus used: Modular precision sound level meter with 1/3 and 1/1 octave filter set

Procedure

1. Set the instrument on a stand, directing the microphone towards the source of the sound.
2. Set the frequency weighting to linear.
3. Set the frontal/random knob to frontal.
4. Set the full scale detection as per requirement.
5. Push the bottom of the filter out to in before taking readings.
6. The readings are taken for both 1/1 octave band and 1/3 octave band at central frequency.

Results:

EXPERIMENT NO. 12

AIM: Audiometric survey for assessing hearing accuracy

Theory

When we are exposed to loud sound, some or all the hair cell in the organ of Corti may get damaged temporarily or permanently. Temporary damages mean that our hearing threshold level has shifted temporarily due to loud sound of short exposure and by giving adequate time for recovery in quiet surroundings, our hearing threshold returns to its original threshold level.

Permanent damage means, due to prolonged exposure to loud sound causes permanent damage to hair cells with the result that hearing ability becomes impaired. At first, damage to first few hair cells is not noticeable but as more for the hair information. This may lead to permanent threshold shift.

Assessment of average hearing level

- As per American Academy of Ophthalmology and Otolaryngology (AAO)-
Average of better ear HTL of 500 Hz, 1KHz and 2KHz
- As per national institute of occupational safety and health(NIOSH)-
Average of HTL of 1KHz, 2KHz and 3KHz

Degree of hearing loss: degree of hearing loss regards to the severity of the loss. The table below shows one of the more commonly used classification system _

Degree of hearing loss	Hearing loss range (dB HL)
Normal	-10 to 15
Slight	16 to 25
Mild	26 to 40
Moderate	41 to 55
Moderately severe	56 to 70
Severe	71 to 90
Profound	91+

Instrument used: lafred peters plc- AP251 microcomputer audiometer

Procedure

1. The earphone was adjusted to to the ears of the subject.
2. The subject was made understand the process, i.e. to process the button whenever he or she listen the sounds and the button when sounds stopped.
3. The audiometer generates pure tone sound from 250Hz to 8000Hz in each frequency. Each ear is tested for eight times.
4. The test stars from left ear, complete all frequencies from 250Hz to 8KHz with 8 times testing for each frequency. Then goes to right ear, do the same thing and again comeback to left ear to retest at 1000Hz. The hearing level for left ear for original test and retest at 1000Hz should not vary more than 5Hz.
5. If it is more than 5Hz, the test result should be discarded and test must be repeated.

Observation: The test was run for the subject "--" and the self-generated data sheet of the audiometer is attached.

Calculations: 1. From the data sheet

(A) As per AAOO- Average of better ear HTL of 500Hz, 1000Hz and 2000Hz

(B) As per NIOSH – Average of HTL of 1KHz, 2KHz and 3KHz

2. Check for retest at 1 KHz

Results: for the subject the degree of hearing loss

(i) According to AAOO

(ii) According to NIOSH

APPENDICES

APPENDIX 1

NATIONAL AMBIENT AIR QUALITY STANDARDS (2009)

Pollutants	Time Weighted Average	Concentration in Ambient Air		Methods of Measurement
		Industrial, Residential, Rural and other Areas	Ecologically Sensitive Area (Notified by Central Government)	
Sulphur Dioxide (SO₂), µg/m³	Annual * 24 Hours **	50 80	20 80	-Improved West and Gaeke Method -Ultraviolet Fluorescence
Nitrogen Dioxide (NO₂), µg/m³	Annual * 24 Hours **	40 80	30 80	-Jacob & Hochheiser modified (NaOH-NaAsO ₂) Method -Gas Phase Chemiluminescence
Particulate Matter (Size less than 10µm) or PM₁₀, µg/m³	Annual * 24 Hours **	60 100	60 100	-Gravimetric -TEOM -Beta attenuation
Particulate Matter (Size less than 2.5µm) or PM_{2.5}, µg/m³	Annual * 24 Hours **	40 60	40 60	-Gravimetric -TEOM -Beta attenuation
Ozone (O₃), µg/m³	8 Hours * 1 Hour **	100 180	100 180	-UV Photometric -Chemiluminescence -Chemical Method
Lead (Pb), µg/m³	Annual * 24 Hours **	0.50 1.0	0.50 1.0	-AAS/ICP Method after sampling on EPM 2000 or equivalent filter paper -ED-XRF using Teflon filter
Carbon Monoxide(CO), mg/m³	8 Hours ** 1 Hour **	02 04	02 04	-Non dispersive Infrared (NDIR) Spectroscopy
Ammonia (NH₃), µg/m³	Annual * 24 Hours **	100 400	100 400	-Chemiluminescence -Indophenol blue method
Benzene (C₆H₆), µg/m³	Annual *	05	05	-Gas Chromatography (GC) based continuous analyzer -Adsorption and desorption followed by GC analysis
Benzo(a)Pyrene (BaP) Particulate phase only, ng/m³	Annual *	01	01	-Solvent extraction followed by HPLC/GC analysis
Arsenic (As), ng/m³	Annual *	06	06	-AAS/ICP Method after sampling on EPM 2000 or equivalent filter paper
Nickel (Ni), ng/m³	Annual *	20	20	-AAS/ICP Method after sampling on EPM 2000 or equivalent filter paper
* Annual Arithmetic mean of minimum 104 measurements in a year at a particular site taken twice a week 24 hourly at uniform intervals. ** 24 hourly or 8 hourly or 1 hourly monitored values, as applicable, shall be complied with 98% of the time in a year. 2% of the time, they may exceed the limits but not on two consecutive days of monitoring. NOTE: Whenever and wherever monitoring results on two consecutive days of monitoring exceed the limits specified above for the respective category, it shall be considered adequate reason to institute regular or continuous monitoring and further investigations.				

APPENDIX 2

Emission Standards for Thermal Power Plant (w.e.f. 7-12-2015)

Parameters	Standards		
	TPP installed before December 31, 2003	TPP installed after 2003 to December 31, 2016	TPP to be installed from January 2017
Particulate Matter	100 mg/Nm ³	50 mg/Nm ³	30 mg/Nm ³
SO₂	600 mg/Nm ³ (units smaller than 500MW capacity) 200 mg/Nm ³ (Units having capacity of 500MW and above)	600 mg/Nm ³ (units smaller than 500MW capacity) 200 mg/Nm ³ (Units having capacity of 500MW and above)	100 mg/Nm ³
NO_x	600 mg/Nm ³	300 mg/Nm ³	100 mg/Nm ³
Mercury	-	0.03 mg/Nm ³	0.03 mg/Nm ³

APPENDIX 3

Proposed BS VI standards for 2- and 3- wheel vehicles (g/km)

Date	Stage	type	CO	HC	NMHC	NO _x	PM
2-Wheel vehicles (new models only)							
2020	BS VI	SI	1.0	0.10	0.068	0.06	0.0045
		CI	0.50	0.10	0.068	0.09	0.0045
3-Wheel Vehicles (all models)							
2020	BS VI	SI	0.44	0.35	-	0.085	-
		CI	0.22	0.10	-	0.10	0.025

Emission standards for passenger car (g/km)

Stage	year	CO	HC	HC+NO_x	NO_x	PM	PN
Gasoline vehicles							
BS IV	2017	1.0	0.10	-	0.08	-	
BS VI	2020	1.0	0.10	-	0.06	0.0045	6*10 ¹¹ (b)
Diesel vehicles							
BS IV	2017	0.50	-	0.30	0.25	0.025	
BS VI	2020	0.50	-	0.17	0.080	0.0045	6*10 ¹¹

b- Applicable only to vehicles using DI engines

APPENDIX 4

Methods of measuring and analyzing air pollutants

Method	Variable Measured	Principle
Gravimetric	PM10, PM2.5	Particles are trapped or collected on filters, and the filters are weighed to determine the volume of the pollutant.
Atomic absorption spectrometry (AAS)	more than 60 metals or metalloid elements (e.g. Pb, Hg, Zn)	This technique operates by measuring energy changes in the atomic state of the analyte. Emitted radiation is a function of atoms present in the sample.
Spectrophotometry	SO ₂ , O ₃	Measure the amount of light that a sample absorbs. The amount of light absorbed indicates the amount of analyte present in the sample.
Chemiluminescence	SO ₂ , O ₃	Based upon the emission spectrum of an excited species that is formed in the course of a chemical reaction.
Gas chromatography (GC) - flame ionization detector (FID)	VOC	Responds in proportion to number of carbon atoms in gas sample.
Gas chromatography-mass spectrometry (GC-MS)	VOC	Mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized atoms or molecules to separate them from each other.
Fourier Transform Infrared Spectroscopy (FTIR)	CO, VOC, CH ₄	Sample absorbs infrared radiation and difference in absorption is measured.

APPENDIX 5

Ambient air quality standards in respect of noise

Area code	Category of area / zone	Limits in dB (A) L_{eq} *	
		Day time	Night time
(A)	Industrial area	75	70
(B)	Commercial area	65	55
(C)	Residential area	55	45
(D)	Silence zone	50	40

- Note:-
1. Day time shall mean from 6.00 a.m. to 10.00 p.m.
 2. Night time shall mean from 10.00 p.m. to 6.00 a.m.
 3. Silence zone is an area comprising not less than 100 meters around hospitals, educational institutions, courts, religious places or any other area which is declared as such by the competent authority
 4. Mixed categories of areas may be declared as one of the four above mentioned categories by the competent authority.

* dB(A) L_{eq} denotes the time weighted average of the level of sound in decibels on scale A which is relatable to human hearing.

A “decibel” is a unit in which noise is measured.

“A”, in dB (A) L_{eq} , denotes the frequency weighting in the measurement of noise and corresponds to frequency response characteristics of the human ear.

L_{eq} : It is an energy mean of the noise level over a specified period.

APPENDIX 6

Health impact caused due to exposure to different concentrations of ambient air pollutants

1. SO_x

Concentration (ppm)	Effects
0.2	Lowest concentration causing a human response
0.3	Threshold for taste recognition
0.5	Threshold for odour recognition
1.6	Threshold for inducing reversible broncho-constriction in healthy individuals
8-12	Immediate throat irritation
10	Eye irritation
20	Immediate coughing

2. NO₂

Effect	Concentration (ppm)	Exposure
Increase in acute respiratory disease	0.06 to 0.1	2 to 3 years
Increase in acute bronchitis in school children	Upto 0.1	6 months
Human olfactory threshold	0.12	<24 hours
Increase in airway resistance	5	10 minute
Pulmonary edema	90	30 minute

3. CO

Concentrations (ppm)	Effects
1-5	Blood flow to certain vital organs increases to compensate for reduction in oxygen carrying capacity of blood
5-9	Visual light threshold increased
16-20	Laboured respiration during exertion, visual evoked response abnormal
20-30	Headache, nausea
30-40	Severe headache, nausea and vomiting, dizziness
40-50	Slurring of speech; tendency to collapse
50-70	Convulsions; coma -Fatal coma if not treated

4. Ozone and photochemical oxidant

Effetcs	Concentrations (ppm)	Exposure
Ozone		
Increased airway resistance	0.1-1	1 hour
Extreme fatigue, lack of coordination	1-3	2 hour
Severe cough	2	2 hour
Pulmonary edema	9	Unknown
Total oxidants		
Eye irritation	0.1	Instantaneous
Aggravation of asthma	0.05-0.06	1 hour
Impaired performance of athletes	0.03-0.3	1 hour

APPENDIX 7

Various ambient air pollutants and their anthropogenic and natural sources

Pollutants	Man Made source	Natural source
SO_2	Burning of fossil fuels such as coal, oil and natural gas	Volcanic eruptions and hot springs
NO_2	Formed by reaction of atmospheric nitrogen and oxygen due to heat produced by the vehicle	Lightning
O_3	formed when pollutants emitted by cars, power plants, industrial boilers, refineries, chemical plants, and other sources react chemically in the presence of sunlight	Formed in upper atmosphere by reaction between the oxygen molecules in presence of Ultra Violet Radiations
CO	produced in the incomplete combustion of carbon-containing fuels, such as gasoline, natural gas, oil, coal, and wood	Formed <i>naturally</i> by certain types of vegetation and marine Organisms (siphonophores)
PM	from direct emissions from the burning of fuels or from wear of tyres or vehicle-generated air turbulence on roadways	Bushfires, dust storms, pollens and sea spray

APPENDIX 8

Surface data:

Surface data are meteorological data that are measured at the earth's surface (technically, somewhere between the ground level and 10m). This data contains physical parameters that are measured directly by instrumentation, such as temperature, dew point, wind direction, wind speed, cloud cover, cloud layer(s), ceiling height, visibility, current weather, and precipitation amount. Surface data are reported by the National Weather Service for each hour.

SCRAM (Support Center for Regulatory Atmospheric Modeling)

SAMSON (Solar and Meteorological Surface Observation Network)

Upper air data:

Upper air data are meteorological data that are measured in the vertical layers of the atmosphere. Upper air data are usually measured by twice daily radiosonde soundings, taken at 00 and 12Z (Greenwich Time). There are other collection methods for gathering upper air data, though radiosonde soundings are the method usually employed for local-scale dispersion modeling.

Potential temperature (θ):

The potential temperature θ is defined as the fictitious temperature that an air parcel would have if it were brought adiabatically to a reference pressure (usually chosen as ground-level pressure). The potential temperature, θ , can be used instead of the actual temperature, T , to better account for the normal decrease in temperature with height, where

$$\theta = T - \lambda (Z_2 - Z_1)$$

and ' λ ' is the adiabatic lapse rate, $\approx -0.01 \text{ K.m}^{-1}$ over the distances to the mixing height.

The advantage of the potential temperature is that it eliminates the pressure effect (*i.e.*, compressibility) when comparing different air parcels, exposing the thermal effect in isolation.

$$\theta = T \left(\frac{P_0}{P} \right)^{\frac{R}{c_p}}$$

The stratification frequency N is defined as: $N^2 = \frac{g}{T} \left(\frac{dT}{dz} + \frac{g}{c_p} \right)$ Note: $\alpha = 1/T$

With the inclusion of ' θ ' instead of actual temperature ' T ' in the definition, the definition of the stratification frequency N reduces to:

$$N^2 = \frac{g}{\theta} \left(\frac{d\theta}{dz} \right)$$

APPENDIX 9

Pasquill atmospheric stability class

Stability class	Definition
A	Very unstable
B	Unstable
C	Slightly unstable
D	Neutral
E	Slightly stable
F	Stable

APPENDIX 10

Meteorological conditions that define the Pasquill stability class

Surface wind (measured at 10m above ground) (m/s)	Daytime insolation			Night time cloudiness	
	Strong	Moderate	Slight	Cloudy Thinly overcast or $\geq 4/8$ cloudiness	Clear $\leq 3/8$ cloudiness
< 2	A	A–B	B	E	F
2-3	A–B	B	C	E	F
3-5	B	B–C	C	D	E
5-6	C	C–D	D	D	D
> 6	C	D	D	D	D

APPENDIX 11

Conditions for different atmospheric lapse rates

Lapse rate	Atmospheric stability
$\Gamma > \Gamma_d$	Unstable
$\Gamma = \Gamma_d$	Neutral
$\Gamma < \Gamma_d$	Stable

APPENDIX 12

Turbulence characteristics with various Richardson numbers

Richardson number (Ri)	Turbulence characteristics
$0.24 < Ri$	No vertical mixing
$0 < Ri < 0.25$	Mechanical turbulence, weakened by stratification
$Ri = 0$	Mechanical turbulence only
$-0.03 \leq Ri < 0$	Mechanical turbulence and convection but mixing mostly due to the former
$Ri < -0.04$	Convective mixing dominates mechanical mixing

(Source: Tricio et al., 2012)

APPENDIX 13

Atmospheric stability class correlated with Richardson number, Vertical Temperature Gradient ($\Delta T/\Delta z$), Standard Deviations of Horizontal Wind Direction (σ_θ) and Monin-Obukhov length

Pasquill Stability Class	Richardson number (Ri)	$\Delta T/\Delta z$ ($^{\circ}\text{K}/100 \text{ m}$)	σ_θ (degrees)	Monin-Obukhov length (L) (m)
A	$\text{Ri} < -0.86$	$\Delta T/\Delta z < -1.9$	$\sigma_\theta > 22.5$	-2 to -3
B	$-0.86 \leq \text{Ri} < -0.37$	$-1.9 \leq \Delta T/\Delta z < -1.7$	$22.5 \geq \sigma_\theta > 17.5$	-4 to -5
C	$-0.37 \leq \text{Ri} < -0.10$	$-1.7 \leq \Delta T/\Delta z < -1.5$	$17.5 \geq \sigma_\theta > 12.5$	-12 to -15
D	$-0.10 \leq \text{Ri} < 0.053$	$-1.5 \leq \Delta T/\Delta z < -0.5$	$12.5 \geq \sigma_\theta > 7.5$	∞
E	$0.053 \leq \text{Ri} < 0.134$	$-0.5 \leq \Delta T/\Delta z < 1.5$	$7.5 \geq \sigma_\theta > 3.75$	35 to 75
F	$0.134 \leq \text{Ri}$	$1.5 \leq \Delta T/\Delta z < 4.0$	$3.75 \geq \sigma_\theta > 2.0$	8 to 35
G	---	$4.0 \leq \Delta T/\Delta z$	$2.0 \geq \sigma_\theta$	---

(Source: Woodward, 2010; Essa et al., 2006)

Department of Environmental Science & Engineering

B. Tech. Environmental Engineering

Geology Practical (ESC 253)

Land Use and Hydrogeology Laboratory (Room No: 303-A)

ESC 253	Geology Practical (B.Tech Env. Engg.) IV Semester	2 Hr
Course Objective: ➤ The identification of different types of rocks and understanding their behaviour. ➤ To apply geologic concept and approaches on environmental engineering project. Learning Outcomes: Upon successful completion of this course, student will ➤ The student will be able to demonstrate the basic lab skills: identifying minerals and rocks; inferring rock origin from examination of specimens; reading, drawing and interpreting contour maps and profiles; using terrestrial coordinates.		

INDEX OF EXPERIMENTS

Exp. No.	Name of Experiment	No. of Practical Hours
1.	Toposheet Analysis, Their Number, Scale and Index. Determination of physiographical features with the help of toposheet.	2
2.	Preparation of Contour line map (choose one suitable sector of toposheet) and demarcation of higher and lower landmarks with direction.	2
3.	Preparation of Drainage map and drainage density of a given watershed area.	2
4.	Preparation of watershed map and analysis of slope of sub watershed area	2
5.	Laboratory observations of different folds and faults	
6.	To draw the profile/section of different beds in the given Geological map	2
7.	Drawing of strike line & determination of true dip & apparent dip	2
8.	Laboratory Study and Observations of Physical Properties of Minerals.	2
9.	Study of rock specimens and its physical properties (Igneous, Sedimentary and Metamorphic rock).	2
10.	Study through GPS-Latitude, Longitude, Elevation etc	2
11 & 12.	Study of Water table fluctuation through secondary data and Calculation of TARR value	4

Books and References

1. Textbook of Geology – G. B. Mahapatra, CBS.
2. Textbook of Geology – P. K. Mukherjee, World Press.
3. Practical Geology – Dr. Harish Kapasya, Himanshu Publication.

Geology Practical (ESC253)

General Instructions

Do's

1. Learn objective & significance of the practical.
2. Always perform the experiment or work as per the instruction of the Instructor.
3. Bring calculator, graph sheet and instrument box regularly
4. Before performing practical, read Lab Manual carefully.
5. Count all rocks and minerals before leaving the lab.

Dont's

1. Don't use mobile phone during lab hours.
2. Dont's try to repair any faulty instrument.
3. Don't touch rock & minerals without permission.

EXPERIMENT NO. - 1

Aim of the Experiment: Toposheet Analysis, their Number, Scale and Index, Determination of physiographical features with the help of toposheet.

1. Learning outcomes: The information regarding concepts of Toposheet analysis. The students will also acquire the information regarding physiographical features and tools of the Toposheet.

2. Brief Theory: Topographic maps are based on topographical surveys. Performed at large scales, these surveys are called topographical in the old sense of topography, showing a variety of elevations and landforms. This is in contrast to older cadastral surveys, which primarily show property and governmental boundaries. The first multi-sheet topographic map series of an entire country. Topographic map series became a national resource in modern nations in planning infrastructure and resource exploitation. A Toposheet is a shortened name for “Topographic Sheet”. They essentially contain information about an area like roads, railways, settlements, canals, rivers, electric poles, post offices etc. According to their usage, they may be available at different scales (e.g. 1:25000, 1:50000 etc. where the former is a larger scale as compared to the latter). They are made on a suitable projection for that area and contains Lat-Long information at the corners. Thus, any point on it can be identified with its corresponding Lat-Long, depending upon the scale (i.e. if the scale is large, more accurate Lat- Long).

Representative Fraction (R.F.): It is the ratio between the distances on the map to its corresponding distance on actual ground.

Scale: Scale is the ratio between the distance of any two points on the map and the actual distance of the same points on the ground. The scale of the given map extract is 2 cm. ,1 km or 1:50000

Contour: contours are imaginary lines drawn on maps, joining all places with the same height above sea level.

Contour Interval: The Interval between two consecutive contours is called contour interval.

Bench Marks: Height of a place actually marked on a stone pillar, rock or shown on building as a permanent reference. It is written as BM 200 m.

Stony Waste: A large area usually in arid/ semi-arid regions where the finer sand/ soil has blown away leaving a surface covered with boulders, stones and pebbles.

Mixed Forest: A forest with more than two varieties of trees growing in close proximities to each other.

Open Jungle: A forest where trees are widely scattered.

Open Scrub: Scrub is the vegetation found in region with less than 100 cm. of rainfall. Therefore it indicates a dry region.

Embankment: They are raised rock or soil filled constructions on which roads / railways tracks are built. Also made near tanks and rivers to prevent flooding.

3.Apparatus/Components Required: SOI Toposheet map, Scale, Graph Paper, Pencil, Practical Copy.

4. Experimental Procedure:

First given the different No. of Toposheets to every student for individual study.

a)Definition of Toposheet: Toposheet is a map representing basically topography of the regions in details further it also represents total land use and land cover in a given area.

b)Chronology of preparation of Toposheets: Survey of India is a very old GOI organization and it was established right back in 1767,for preparation of toposheets. Initially the map of total south east Asia was divided into small squares $4^{\circ} \times 4^{\circ}$ square grids and then each grid was surveyed and the details were shown on a map having scale $1:10^6$.

c)Numbering: Numbering assigned to each map was very important because it is a unique number. Each square grid ($4^{\circ} \times 4^{\circ}$)was numbered, starting from top left corner 1,2,3,, vertically downward and ending before a grid having no landmass(i.e. only water).The scheme of numbering started again from the top into the adjacent column of grids and continued in similar fashion. The scheme of numbering the toposheets have been shown overleaf. Total number of Toposheets thus prepared were around 90.

d)Enlargement of Scale: Subsequently it was observed that the topographic details could not be shown conveniently on the toposheets with existing scale. Therefore, each toposheet was subdivided into 16 squares with $1^{\circ} \times 1^{\circ}$ square grid and assigned number from 'A'to'P' prefixed by number of original map i.e. for example

73 A 73B,73C,73D.....73P

76A,73B,73C,73D.....76P and so and each was enlarged to size of the original sheet.

a) Reference

1. Toposheet Number- 73M/5 (for example)
2. Longitude - 87°15' E -87° 30' E
3. Latitude - 23° 45' N – 24° 0' N
4. Scale-1:50000

b) Location

1. State- Bihar, West Bengal
2. District- Santhal Pargana, Birbhum

c) Communication

- a. National Highway (NH)/Railway - No national Highway
 - i. Starting from SW part of map to - Eastern Railway
 - ii. NE part of the map [Andal-Sainthiya Section]
 - iii. Starting from SW part of map and Ends there- Eastern Railway
- b. Distance from prominent Location- Andol 26 km to Sainthiya 23 km
- Palasbari RS 22km to Bhimgara Jn.2km

d) Topographical Features

- a. Reduced level (R.L.) -Minimum 60 m & Maximum -140 m
- b. Topography - Plan area with some undulation
- c. General Slope Direction - Sloping towards North East, towards NE
 1. 60 m (R.L) & REST IN 140 m (RL)
- d. Drainage Direction -Mayurakshi following from North west to South

e) Water Regime

a) River -

- Hingla Nadi, Seasonal flowing from NE to SW direction at southern part of the map.
- Chandrabhaga Nadi, Seasonal flowing from NW to SW direction in Eastern part of the map.

b) Tributaries -

- Kushkarni Nadi, Seasonal tributary of Mayurakshi flowing from NW Direction to NE direction.
- Sil river seasonal tributary flowing from western part of the map to the southern part of the map.

c) Reservoir

Natural tanks/ponds
Manmade tanks

- **No reservoir found**
- 3 Nos.
- approx 90

f) Sector One Only

Name of the sector	A1
(5.4) Longitude	- 87° 15'E- 87°20'E
Latitude	-23° 55'N- 24°0' N
(5.5) Natural tanks /Ponds	-3Nos
Man made tanks	-90 Nos

g) Land Use (General)

-Spare vegetation is maximum, covering about 60% of the area

Total area for water bodies= 54 ha

h) Forest (measurement its area by planimeter/graph paper)

- i) 2.75 km²=275 ha
- ii) 0.7 km² = 70 ha
- iii) 4.025 km²=402.5 ha
- iv) 0.35 km²=35 ha
- v) 0.675 km²=67.5 ha
- vi) 0.375 km² = 37.5 ha
- vii) 1.1 km² =
- viii) 0.2 km² = ha

Total forest area = 1017.5 ha

i) Settlement (area measured by transparency graph paper)

- i) 27(0.5x0.5) = 6.75 cm²=1.6875 km²=168.75 ha.
- ii) 1x1 = 1 cm² = 0.25 km² = 25 ha.
- iii) 1.5x1.5 = 2.25 cm² = 0.5625 km²=56.25 ha.
- iv) 2.5x2.5 = 6.25 cm²=1.5625 km²=156.25 ha.
- v) 9.5x9.5 =90.25cm²=22.5625 km²=2256.25 ha
- vi) 1.2 x1.5 = 1.9 cm²=0.45 km²=45 ha
- vii) 0.5 x2 = 1.0cm²=0.25 km²=25 ha
- viii) 0.5x0.6 = 0.30 cm²=0.075 km²=7.5 ha
- ix) 0.5 x1.3 = 0.65 cm²=0.1625 km²=16.25 ha
- x) 0.5 x 1.5 = 0.75 cm²=0.1875 km²=18.75 ha

Total settlement area = 2775 ha.

1.5. Observation: After studying the Toposheet map one can easily identify and find out the human settlement (roads, cities, buildings etc), but may also include some thematic information such as vegetation or the boundaries of national parks. Identified different topographical features from the Toposheet in percentage.

Thus the scale of each toposheet became 1:2,50,000 again for showing more details each of these was divided into 16 equal parts and assigned serial numbers in the same system prefixed by the number of original map as follows:-

73A/1,73A/2.....73A/16

73B/1,73B/2.....73B/16

73P/1,73P/2.....73P/16, and enlarged to size of the original sheet. The scale thus became 1:63360. The sheets were further enlarged to 1:50,000. As at this point to time. System for measurement was changed from foot pound system (FPS) to metric system, further the area of toposheet was again sub-divided into 4 equal parts and the number assigned was follows:

73M/5

73M/5/NW,73M/5/NE

73M/5/SW, 73M/5/SE

NW	NE
SW	SE

Thus the scale finally settled to 1:25,000

Size: Toposheet are prepared on uniform size paper

e) Study of Toposheets: Toposheets contain following features

1. References No. or Index No. at the top right hand side .
2. Name of State in the middle.
3. Name of the districts covered on left hand side.
4. The year of publication, survey and edition.
5. Scale of the toposheet-presented by a bar scale & R.F.
6. Contours interval in m
7. Index of surrounding toposheets-
8. The longitude and latitude of the area in degree and minute.

9. Index showing various topographic features represented on the toposheet with suitable color and symbols detailed overleaf.

f) Importance of Study of Toposheet

I. It helps to study the local topography and geography of an area under consideration

II. Study of land use.

III. Study of land cover.

IV. It helps to preparation of contours map.

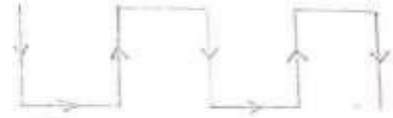
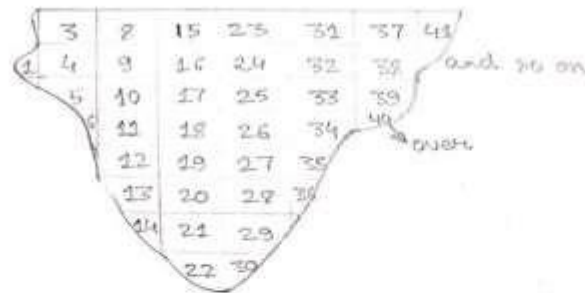
V. Point out of sampling area/location.

VI. Study of watershed characteristics.

Toposheets of certain sensitive areas are restricted i.e. they are not being made available by Survey of India without prior permission from ministry of defence.

Toposheets are destroyed after certain period of time, which new sets are prepared, leaving only some limited copies for the archives of survey of India.

SCHEME OF NUMBERING OF TOPOSHEET



Stage I :-

	60°E	64°E	68°E	72°E	76°E
36°N	1	5	9	13	
32°N	2	6	10	14	
28°N	3	7	11	15	
24°N	4	8	12	16	
20°N					

4° x 4° (square grid)
scale 1:10⁶

Stage II :-

		12		
A	E	I	M	
B	F	J	N	
C	G	K	O	
D	H	L	P	

1° x 1° square
scale → 1:2,50,000

Scheme of Numbering of Toposheet

Stage III

12 M

1	5	9	13
2	6	10	14
3	7	11	15
4	8	12	16

12 M/1, 12 M/2, 12 M/3, 12 M/4, 12 M/5, 12 M/6
scale 1:63,360

Stage IV

12 M

1	5	9	13
2	6	10	14
3	7	11	15
4	8	12	16

scale enlarged
1:50,000

System of measurement
was changed from

Stage V

12 M/6

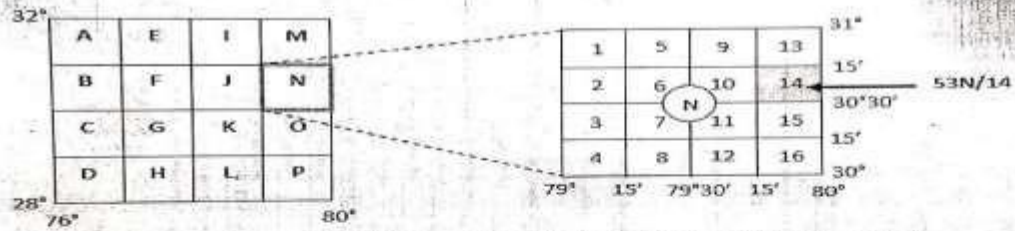
NW	NE
SW	SE

12 M/6/NW, 12 M/6/NE
12 M/6/SW, 12 M/6/SE

Scale: 1:25,000

Scheme of Numbering of Toposheet

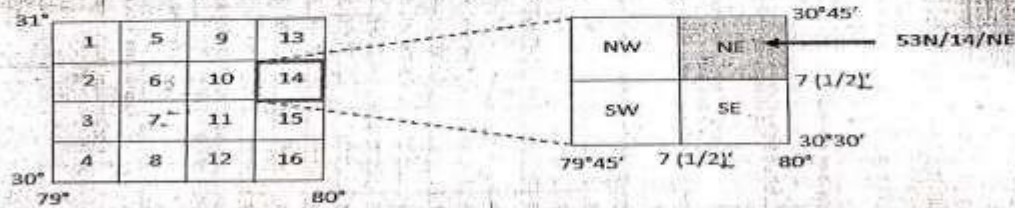
1° × 1° sheets can also be divided into 16 sections each of 15' latitude × 15' longitude and are numbered 1 to 16 in a columned manner.



A sheet of 4° × 4° (scale: 1: 1,000,000)

A sheet of 15' × 15' (scale: 1: 50,000)

A 15' × 15' sheet can be divided into 4 sheets, each of 7(1/2)' and are numbered as NW, NE, SW and SE.



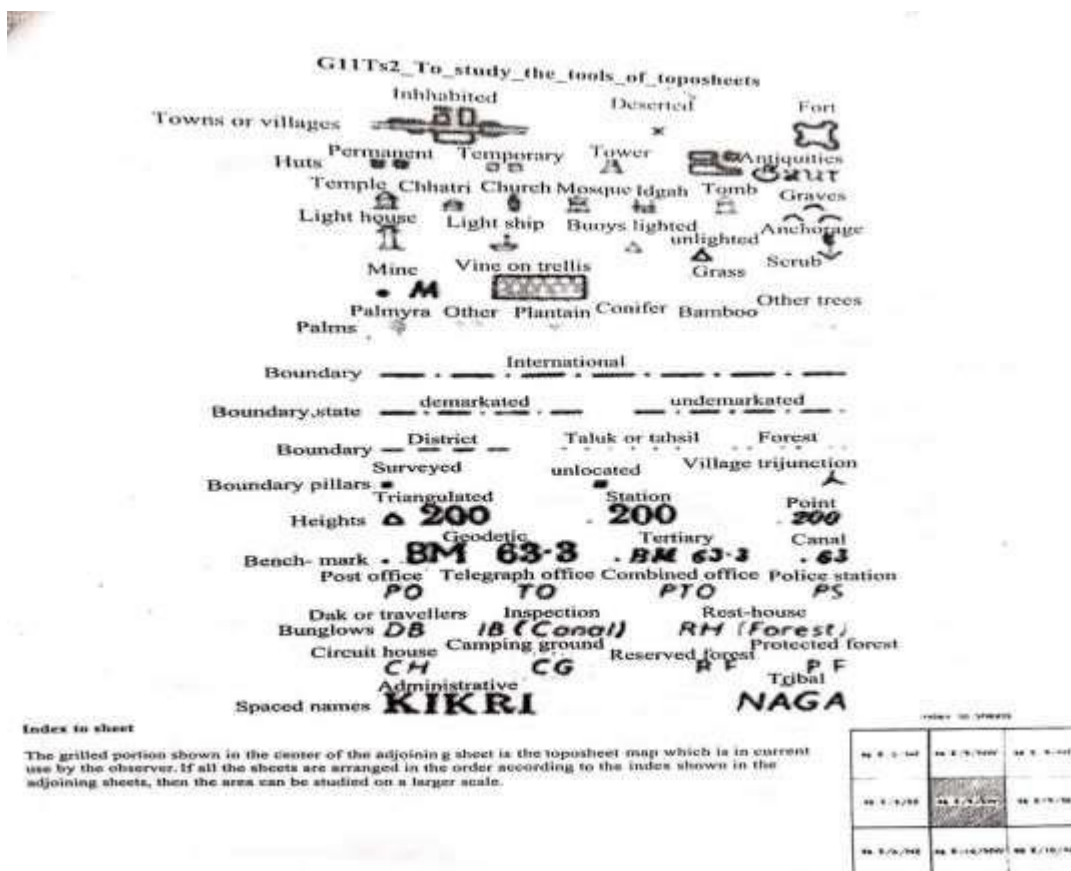
A sheet of 1° × 1° (scale: 1: 250, 000)

A sheet of 7(1/2)' × 7(1/2)' (Scale: 1: 25,000)

Classification of maps has already been discussed in unit 1, given below is a brief account of different types of maps.

Scheme of Numbering of Toposheet

Different tools of Toposheets



1.6 Observation table:

Tabel.1. Observation of different features of Toposheet map

S.No.	Observations
1.	Locating features with the help of a four figure or a six figure grid reference.
2.	Identification and definition of types of scale given on the map.
3.	Identity: Site of prominent villages and/or towns, types of land use / land cover and means of communication with the help of the index given at the bottom of the sheet.
4.	Identification of drainage (direction of flow and pattern) and settlement patterns.
5.	Identification of natural and man-made features.

Table.2. Observation of different types of topographical features

S.No.	Topographical features	Area in percentage
1.	Road	0.34%
2.	Water bodies	0.686% -0.69%
3.	Forest	12.94%
4.	Settlement	35.294- 35.30%
5.	Sparse vegetation	50.73%

1.7. Environmental Significance: Topographic maps are an important tool because they can represent the three-dimensional landscape in two dimensions. A person who can read a Toposheet map can find out the location of peaks, valleys, ridges and saddles, among other land features. Toposheet maps can also show you whether you will be traveling uphill or downhill on a particular road or trail . Topography describes the physical features of an area of land. These features typically include natural formations such as mountains, rivers, lakes, and valleys. Manmade features such as roads, dams, and cities may also be included.

8. Questions based on the Experiment:

Study the extract from the topographical sheet No. 63K/12, as shown in the Toposheet and answer the following questions —

1. Convert 1:50,000 into a statement of scale.
2. Name the major settlements of the area.
3. Name the villages/settlements where Post Office is located ?
4. Name the two types of vegetation shown on part of the topographical sheet.
5. Give the location of the area, its extent, the scale and the contour interval used?
6. Find the maximum and minimum elevations. How high above the sea is the general sea level?
7. What physiographic features is seen here? Give its elevation.

EXPERIMENT NO. – 2

Aim of the Experiment :Preparation of Contour line map (choose one suitable sector of toposheet) and demarcation of higher and lower landmarks with direction.

1. Learning outcome: The students will learn from Toposheet map regarding different contour map.

2. Brief Theory: The delineation of any property in map form by constructing lines of equal values of that property from available data points is the contour mapping. A topographic map, for example, reveals the relief of an area by means of contour lines that represent elevation values; each such line passes through points of the same elevation. The method is not wholly objective because two investigators may produce somewhat different maps whenever interpolation between data points is necessary for construction of the contours. In addition to topography, there are scores of geophysical, geochemical, meteorological, sociological, and other variables that are mapped routinely by the method.

3. Apparatus Required: SOI Toposheet with Contour map, Scale, Pencil, Practical Copy.

4. Experimental Procedure:

a) To draw the contour lines:

- Choose an appropriate contour interval by examining the elevation points on the map.
- Two common relief (the highest and lowest elevation) is taken depending on the contour interval.
- The most common intervals are 10 or 20 ft for less relief and 100 ft for greater relief.

b) Preparation of a contour map

- A contour map can be made by taking elevation reading in the field, plotting them on a map and drawing contour lines on the basis of those figures.
- The following map shows specific elevations above sea level, a stream with its tributaries and several contour lines (some partly drawn).
- Using a light or colored pencil, finish the map by adding lines at ten foot intervals.

5. Observation: Through this practical, we can see how Contour lines on a map show different lines of altitude from the ground level. These contour lines are an important way of showing the rise and fall of the land on a map. Contour lines show all the places that are the same height above sea level.

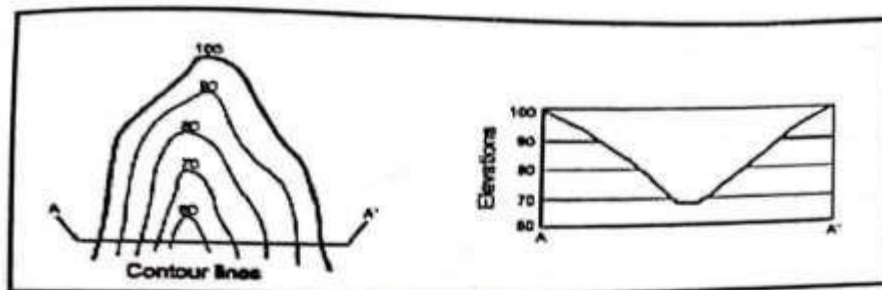
6. Observation table:

S.No.	Observations
1.	Each topographic contour represents a curve of equal elevation.
2.	The image is a graphical scale
3.	Topographic contour lines that are closer together than other contour lines indicate steeper slopes
4	contour lines that are uniformly spaced indicate a constant slope

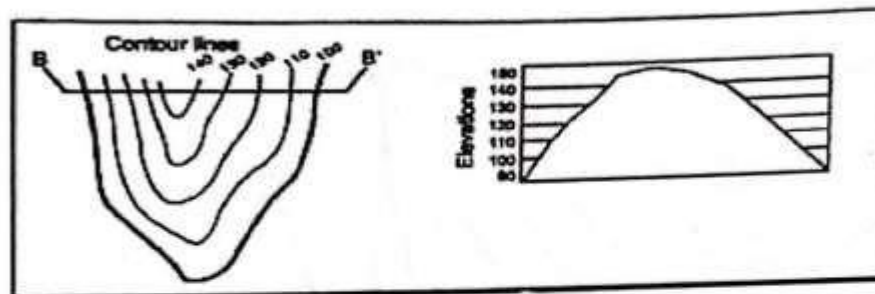
7. Environmental Significance: Contour lines are lines drawn on a map connecting points of equal elevation, meaning if you physically followed a contour line, elevation would remain constant. Contour lines show elevation and the shape of the terrain. They're useful because they illustrate the shape of the land surface and its topography on the map. These contour lines are an important way of showing the rise and fall of the land on a map. Contour lines show all the places that are the same height above sea level. They also tell us about the slope of the land. On a steep slope, the lines are close together.

8. Questions based on the Experiment:

1. How do you calculate contour interval?
2. How many methods of contouring are present?
3. What do close contour lines represent on a map?
4. What is the difference in elevation between Point X and Point Y?

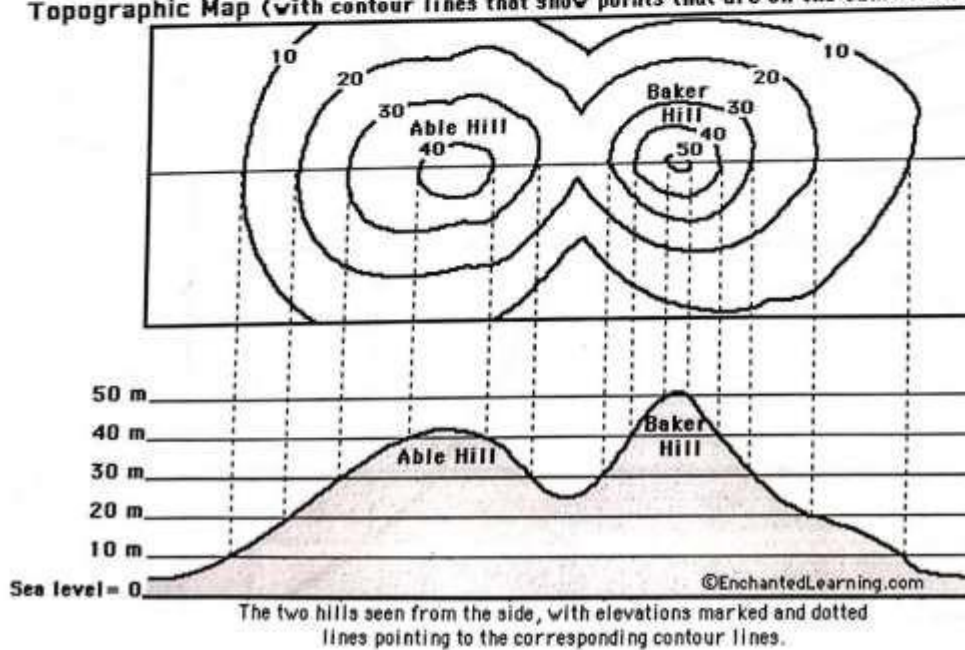


4a. A Depressed Area



4b. A Ridge

Topographic Map (with contour lines that show points that are on the same level)



Topographic map showing contour lines

EXPERIMENT NO. – 3

Aim of the Experiment: Preparation of Drainage map and drainage density of a given watershed area.

1. Learning outcome: The information regarding different types of drainage pattern in the given watershed area.

2. Brief Theory: A drainage basin is an area of land that drains or contributes runoff to the same water body whether it be a retention or detention pond, river, stream, lake or other body of water.

Drainage density:- It is defined as the ratio of the total channel length existing in watershed to the total drainage area.

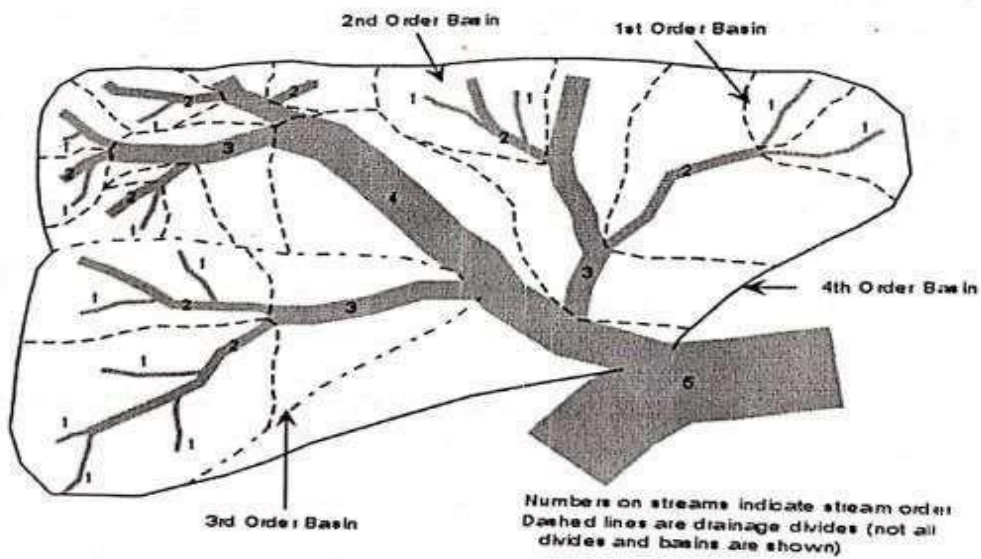
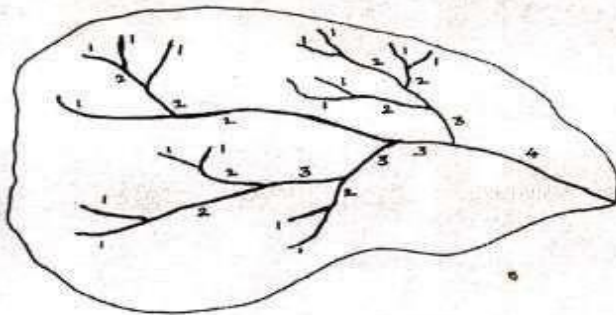
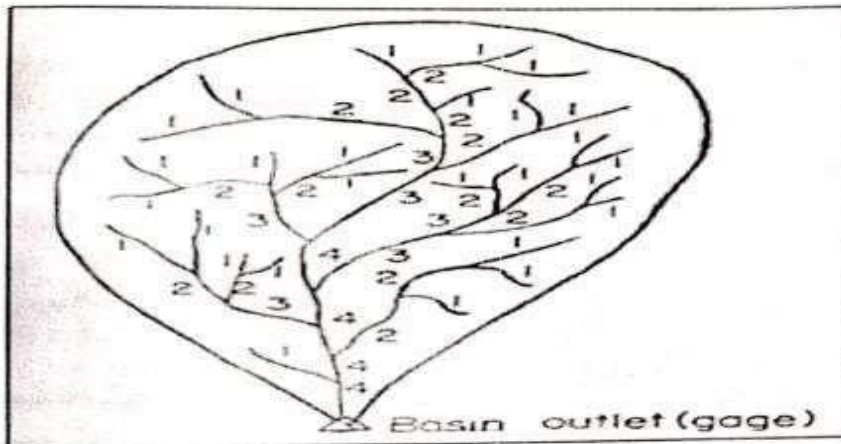
$$D.D. = \frac{\text{Total channel length}}{\text{Total drainage area}}$$

$$D_a = L/A$$

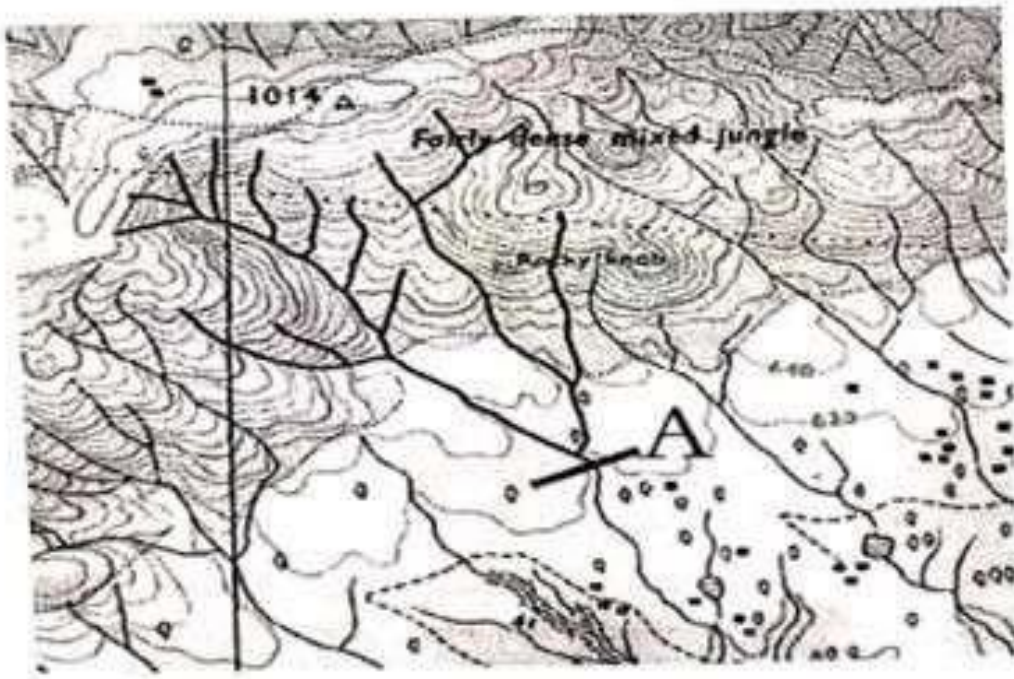
3. Apparatus Required: SOI Toposheet map, Planimeter, Rotameter, Scale, Pencil, Graph Paper

4. Numerical Procedure:

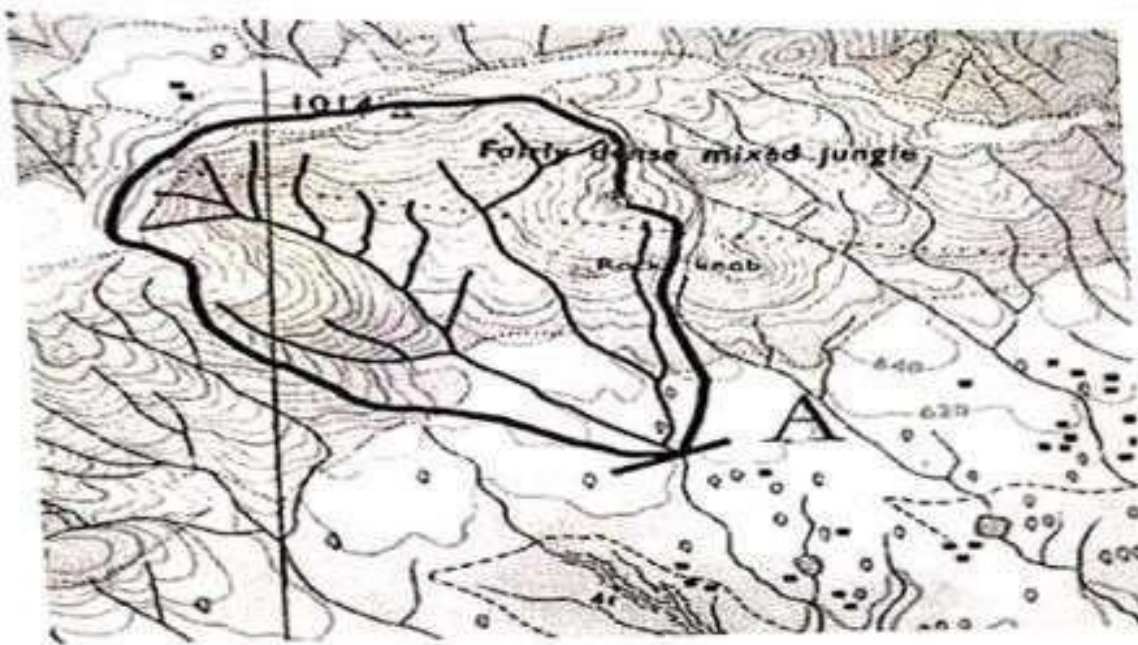
1. To draw a watershed boundary (Toposheet No)
2. To draw a watershed boundary the area of a Toposheet chosen for which mini watershed map is to be prepared.
3. The river system with all its tributaries from the starting was considered.
4. The highest points around the river and tributaries were joined together to get the watershed boundary enclosing the area from which runoff and overland flow will pass into the river system.
5. The drainage area under study enclosed is between longitude..... & Latitude.....
6. Within the watershed boundary all drainage lines were draw (order of the tributaries are drainage line are also marked on the map).
7. The scale, total area of drainage basin location of area are shown in map:
 - a) To calculate drainage density
 - b) With the help of Planimeter/graph paper the total area of watershed was measured.
 - c) With the help of Rotameter, the total channel length was measured.



Basin outline with drainage lines



Mark the Drainage Lines (Source: BACPE 2007)



Mark out the Watershed Boundary

5. Observation: Observed the different types of drainage basin pattern made by drainage line from the map to study the watershed boundary (like - Dendritic Pattern). And measured values will be as follow:

6. Observation table:

S.No.	Drainage Features	Measured values
	Area of Watershed =	-
1	Total length of drainage line=	-
2	As per map scale 2 cm =	1 Km
3	Drainage density (DD)=	-

7.Environmental Significance: Drainage pattern are the reflection of so many factors it is evident that they are of very real significance. They form one of the most immediate approach to understanding of geologic structure.

8. Questions based on Experiment:

1. What is drainage pattern explain?
2. What is drainage pattern name the factors that influence the drainage pattern?
3. What are the different drainage patterns and what do they tell us about the about the ground surface or the landscape over/through which the river is flowing?
4. Define the terms: drainage basin, interfluve, drainage divide, drainage pattern, stream order, and base level.

EXPERIMENT NO. – 4

Aim of the Experiment:- Preparation of watershed map and analysis of slope of sub watershed area.

1.Learning outcome: The knowledge about watershed map and its use in watershed management and planning.

2.Brief Theory: Watershed is a basic hydrologic unit based on the natural boundary. For all analytical purposes watershed is defined by all points enclosed within an area from which rain falling at these points will contribute water to the outlet (watershed outlet).

Watershed Slope: Watershed slope (S) reflects the rate of change of elevation with respect to distance along the principal flow path which is delineated as:

$$S = \Delta E/L$$

Where, ΔE = elevation difference between the end points of the principal flow path; and

L . = length of the flow path.

3.Apparatus Required: SOI Toposheet map, Transparency paper, Measuring thread, Scale and Planimeter to measure the length and area, Pencils of different desired colors.

4. Experimental Procedures:

Start by introducing the students to the concepts and the key vocabulary terms to help them begin to understand and define its boundary or watershed delineation.

1. Choose the point of the watershed outlet. This is generally our point of interest to design a structure or monitoring location.
2. Draw the perpendicular lines across the contour lines for land that drains to the point of interest. A watershed boundary always runs perpendicular to the contour lines. Contours (contour lines) that point upstream denote valleys and that point downstream denotes ridges. The boundary of a watershed will essentially be a ridgeline.
3. Measure the area of the watershed (A) delineated under step 2 with the help of planimeter (cm').
4. Measure the perimeter of the watershed on map (P) by putting the thread over the boundary of the watershed, mark it and measure the length of thread in cm with the help of scale.
5. Identify and draw the valley line (major channel) from outlet point and extend it to the basin divide keeping the path of travel of water in view. Measure the Watershed Length (L) with the help of thread and scale.
6. Note the elevation of elevation of outlet and the other end of the principal path of flow. Elevation of these areas may be calculated through interpolating between adjacent contours. The elevation difference of these point may denoted by till (m).

7. Similar to the length of watershed measure the following:

L_{ca} : The length to the center of area measured along the main channel from the watershed outlet to the point on the main channel opposite the center of area.

L_m : The maximum length of the basin parallel to the principal drainage lines.

(All the measurements taken above are in cm (linear) and cm^2 (areal) measured on map)

5. Observation: Studied and identification of stream headwaters, length, tributaries, final stream destination, and watershed boundaries are best determined through maps.

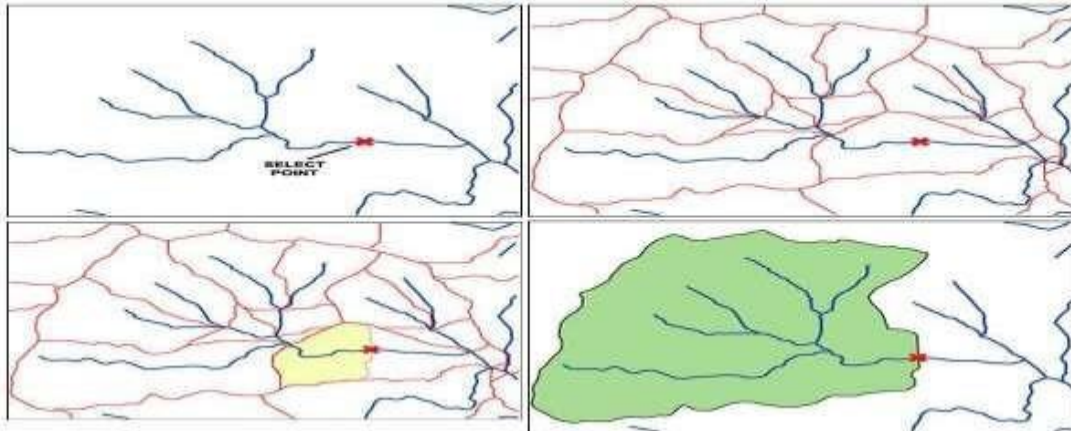
6. Observation table:

S.No.	Different measurements for watershed delineation	Measured values
1.	Length of the tributary	Cm (on map) = km
2.	Length of the main river	Cm (on map) = km
3.	Total length of channel	km
4	Watershed perimeter	Cm (on map) = km

7.Environmental Significance: Watersheds are important because the surface water features and storm water runoff within a watershed ultimately drain to other bodies of water. It is essential to consider these downstream impacts when developing and implementing water quality protection and restoration actions. Everything upstream ends up downstream.

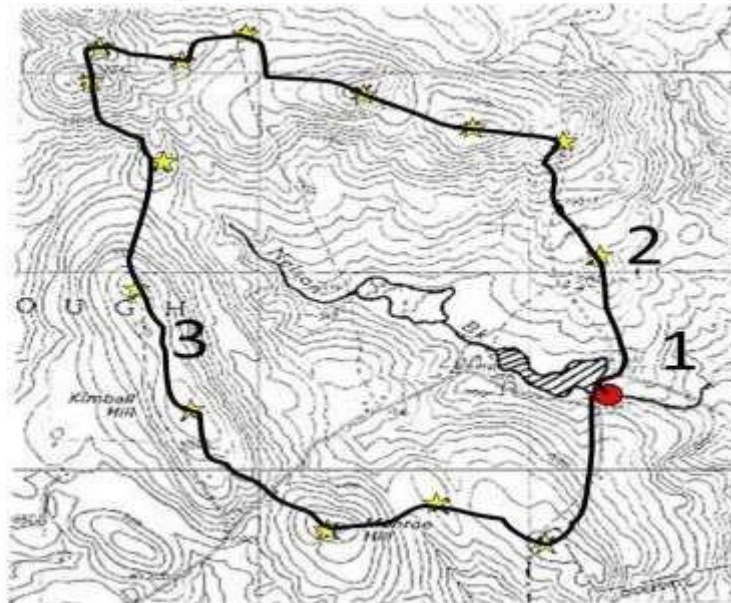
8. Questions based on Experiment:

1. What is delineation of watershed?
2. Why is watershed delineation important?
3. How do you calculate watershed?
4. How do you delineate a watershed?



Delineating Watersheds

1. Identify outlet point
2. Identify high points
3. Link high pts crossing contour lines at right-angles



Delineated Watershed with Boundary

EXPERIMENT No. – 5

Aim of the Experiment:- Laboratory observation of different fold and faults.

1. Learning outcome: To provide knowledge about different types of faults and folds.

2. Brief Theory: Geologic maps show various types of outcrop patterns. By studying these patterns the geologic structures such as folds, faults, unconformities and igneous intrusions can easily be recognized.

The term fold is used in geology when one or a stack of originally flat, level surfaces, such as sedimentary strata, are bent or curved as a result of pressure and high temperature. The basic cause is likely to be some aspect of plate tectonics.

A fault is a crack in the Earth's crust. Typically, faults are associated with, or form, the boundaries between Earth's tectonic plates. In an active fault, the pieces of the Earth's crust along a fault move over time. The moving rocks can cause earthquakes:

3. Apparatus Required: Models of different folds and faults, Practical Copy.

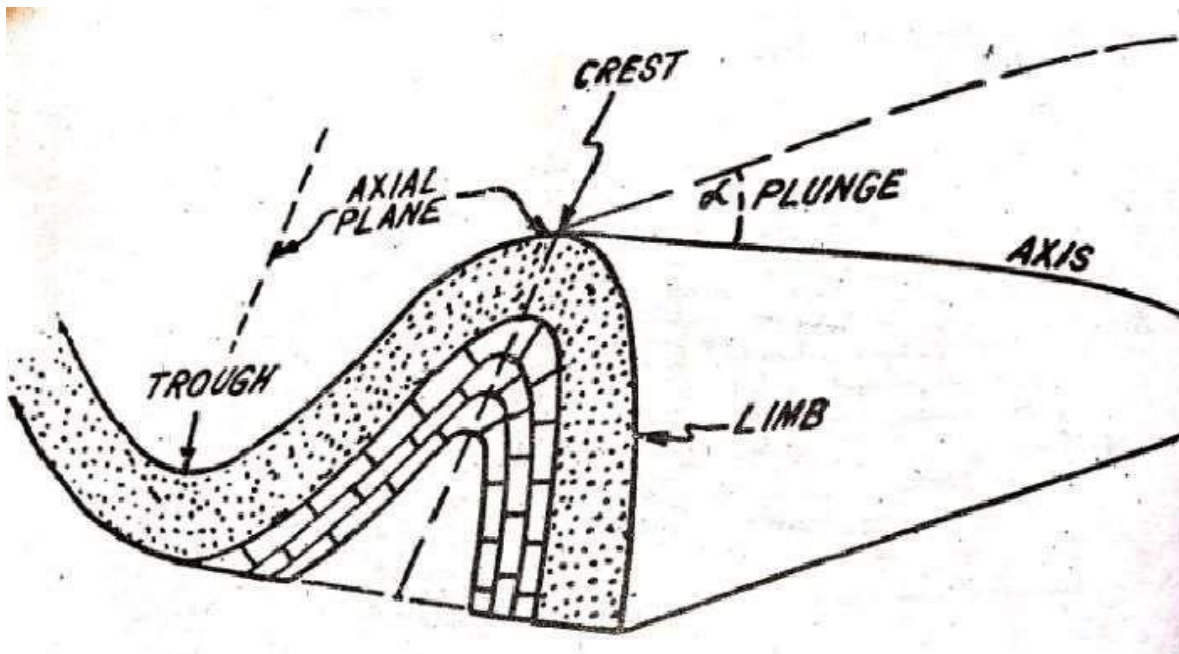
4. Experimental Procedures:

List of different types of folds & faults models:

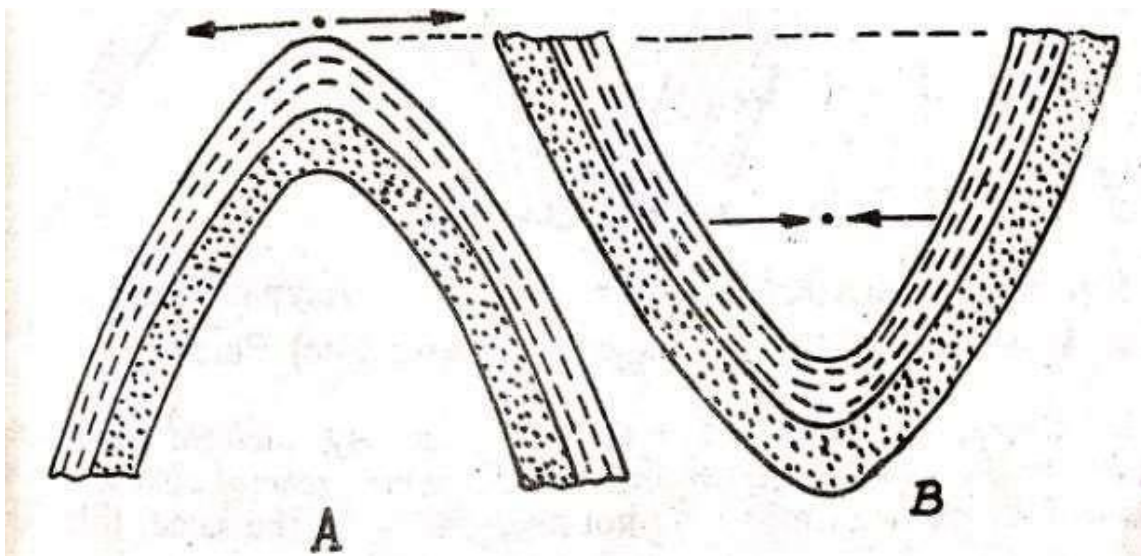
1. Unconformities Set of Fire (Lava)
2. Unconformities Set of Fire (Sandstone)
3. Isoclinal Fold Anticline, Syncline
4. Plunging Fold
5. Chevron Fold
6. Reverse Fault
7. Kit Fault
8. Symmetrical Fault
9. Hing Fault
10. Dome Fold
11. Monoclinic Fold
12. Step Fold
13. Vertical Dip Slip Strike Fault
14. Structural Terrace Fold
15. Tear Fault
16. Normal Fault
17. Thrusts Fault
18. Bending Fault
19. Drag Fault
20. Trace Slip Fault
21. Recumbent Fold

- 22. Rotational Fault
- 23. Overturned Fold

Folds and Faults

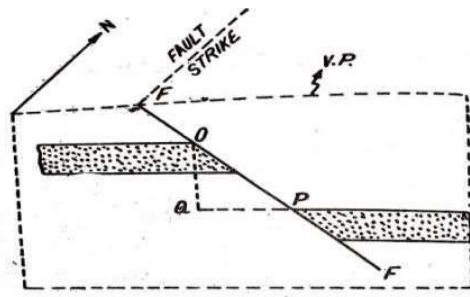


Parts of a Fold



Types of Folds: A (Anticline) and B (Syncline)

The arrow indicate direction of dip of limbs

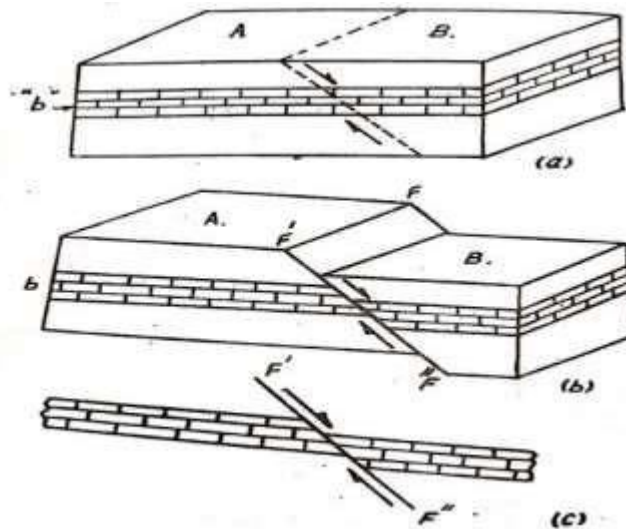


Fault

OP= net slip, PQ = horizontal slip

OQ = vertical slip

As PQ and OQ are measured in a vertical plane at right angles to the fault strike, they are termed here as Heave and Throw respectively



Normal Faulting

a = Fracture appear in a block of crust containing bed 'b' (along with many others)

b= Movement occurs along the fracture; one block is thrown down relative to the other

c = Normal fault in section.

5. Observation:

- To describe how geological features provided clues that allow us to decipher the geologic past.
- Folds are classified to discuss for knowing the geometry and composition of rocks.
- Folds are recognized in the field by plotting the attitude of the beds .i.e., their dip and strike on the map.
- Faults are well defined cracks along which the rock masses on either side have relative displacement.

6. Observation table:

S.No.	Observation
1	Description of Faults
2	Fault Displacements
3	Recognition of folds of different types

7.Environmental Significance: An essential importance of structural geology is to know areas that contain folds and faults because they can form traps in which the accumulation and concentration of fluids such as oil and natural gas and minerals occur. To understand the geologic history of a region, the analysis of folds, faults, and joints help in gaining knowledge about mountain building forces and to interpret them in the light of plate tectonics. It also helps to make geologic maps, which is used to infer underground structures where one can't see the rocks and also to understand the formation of geologic resources to locate and manage them.

8. Question based on Experiment:

1. The orientation of a plane in space is expressed by its attitude; a term consisting of two
2. components, name them?
3. There are two end-member varieties of faults:
4. The orientation of a plane in space is expressed by its attitude; a term consisting of two
5. components,
6. What is a fault?
7. When does a normal fault occur?

EXPERIMENT NO. - 6

Aim of the Experiment:- To draw the profile/section of different beds in the given Geological map.

1.Learning outcome: to understand and to identify regions of groundwater movement, evaluate potential sites for economic mineral deposits, and locate oil and gas reservoirs.

2.Brief Theory:-A given geological map represents all geological features of the earth that are below the surface of the earth including outcrop, folds, faults etc. The profile of given area's geological map can be prepared by drawing strike line and drawing corresponding using the dip angles.

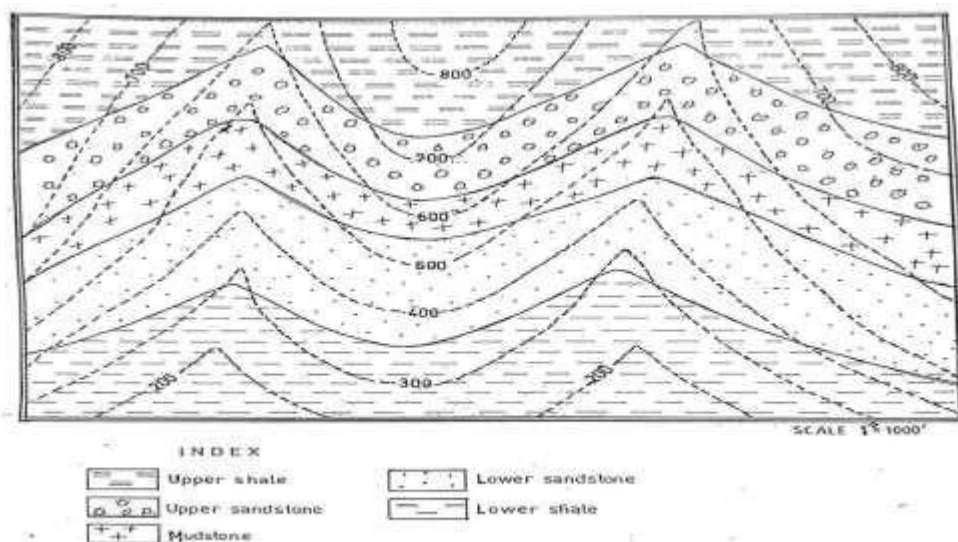
Topography:-In the given map the maximum topographic elevation of the area is more than 800 feet and the lowest is lower than 200 feet above MSL. The topography is a require sloping terrain having a common slope along south direction.

Lithology:-The area is represented by a single formation and constituted of five beds namely lower shale, lower Sand stone, Mud stone Upper sandstone and Upper shale. The contact of individual sequence is simple and continuous.

Strike:-The direction that is made by horizontal line which intersects the boundary of a bed at certain contact points in a plane perpendicular to it. The strike is a scalar quantity as it has only direction.

Dip:- The angle of inclination of the beds with the horizontal line. It is a vector as it has both magnitude and direction.

3. Apparatus Required: GSI Geological map, Scale, Graph Paper, Compass.



Geological map

4. **Experimental Procedure:**

1. **Drawing a Topographic Profile:** The map is first folded along the XY line on a drawing paper. A horizontal line equal to the XY line is drawn, which serves as a base line to the Geological sections to be made over this line.
2. **Determination of Dip and Strike:** Generally on Geological maps both the contour lines as well as boundaries of rock beds are shown. To determine the dip of a rock bed by graphical method such a contour is selected which cuts the particular rock boundary at two points.
3. **Construction of Geological Structures:** Now the map is folded along the XY line and is placed over the base line of the profile. The points of intersection of the boundary lines between different beds and the XY line are transferred. These points are then projected to the surface profile. From these points lines are drawn according to the Dip of the respective boundary surfaces. This is usually done by drawing angles of Dip first at the base line and then drawing parallel lines from the corresponding points at the surface profile.

5.Observation: Through this exercise one can able to understand that when rocks have been squeezed, they show plastic deformation features (folding), especially if deformation has occurred relatively rapidly, with high-confining pressures and elevated temperatures in Earth's upper crust. In contrast, rapid application of stress, low-confining pressure, and low temperatures are more likely to produce brittle deformation, known as faulting.

6. **Observation table:**

S. No.	Observation
1	Outcrop patterns on geological maps
2	Effects of dip of beds and width of outcrop
3	cross section drawn using structure contours.

7.Environmental Significance: Geologic cross sections are constructed to understand and to identify regions of groundwater movement, evaluate potential sites for economic mineral deposits, and locate oil and gas reservoirs. Also to understanding the orientation of flow-enhancing layers (aquifers) versus flow-preventing layers (aquicludes) is the key to evaluating the motion of groundwater. This also provides an application for determining where wells are best to be drilled. It also allows for analysis of aqueous pollutant movement and possible mitigation strategies.

8. **Questions based on Experiment:**

1. What features are shown on a geologic map?
2. How to draw a cross section?
3. How to measure strike and dip from structure contours?

EXPERIMENT NO. - 7

Aim of the Experiment: Drawing of strike line & determination of true dip & apparent dip.

1. Learning outcome: To understand the attitude of rock layers or other planar geologic features) in constructing of accurate geologic maps and geologic cross-sections.

2. Brief Theory: Dip and Strike on Geologic Maps refer to the orientation or attitude of a geologic feature.

Strike is the direction of the line formed by the intersection of a rock surface with a horizontal plane. On a geologic map, this is represented with a short straight line segment oriented parallel to the strike line. Strike (or strike angle) can be given as either a quadrant compass bearing of the strike line (N25°E for example) or in terms of east or west of true north or south, a single three digit number representing the azimuth, where the lower number is usually given (where the example of N25°E would simply be 025), or the azimuth number followed by the degree sign (example of N25°E would be 025°).

Dip:- Dip is the acute angle that a rock surface makes with a horizontal plane. One technique is to always take the strike so the dip is 90° to the right of the strike, in which case the redundant letter following the dip angle is omitted (right hand rule, or RHR). The map symbol is a short line attached and at right angles to the strike symbol pointing in the direction which the planar surface is dipping down. The angle of dip is generally included on a geologic map without the degree sign. Beds that are dipping vertically are shown with the dip symbol on both sides of the strike, and beds that are level are shown like the vertical beds, but with a circle around them. Both vertical and level beds do not have a number written with them.

Apparent dip:- is the name of any dip measured in a vertical plane that is not perpendicular to the strike line. True dip can be calculated from apparent dip using trigonometry if the strike is known. Geologic cross sections use apparent dip when they are drawn at some angle not perpendicular to strike.

First instruction is given to have graph with pencil to draw strike line and dip in the graph.

3. Apparatus Required: Graph Paper, Pencil, Practical Copy.

4. Experimental Procedures:

1. Determination of Strike & Dip.
2. Draw a line between the outcrop at the highest elevation and the outcrop at the lowest elevation. (i.e. between point A and B).
3. Measure the map distance of that line (i.e. line AB) with your ruler (about 1600 m).
4. Set up a triangle with the map distance (1600 m) between A & B and elevation difference (600-200=400 m) between A & B to calculate apparent dip.
5. The apparent dip tells you that the true dip has to be at least that value, it cannot be less.

6. The dip is perpendicular to the strike line. The dip direction will be in the general direction of the lower elevation.
7. With a high point on the left side, the bed would have to be dipping from the left to right.
8. To draw a dip line, take any point on strike line (CD) and draw a line perpendicular.
9. Find out the true dip angle, extend the strike line.
10. Connect that strike line to the point B so that the line is perpendicular to the strike line.

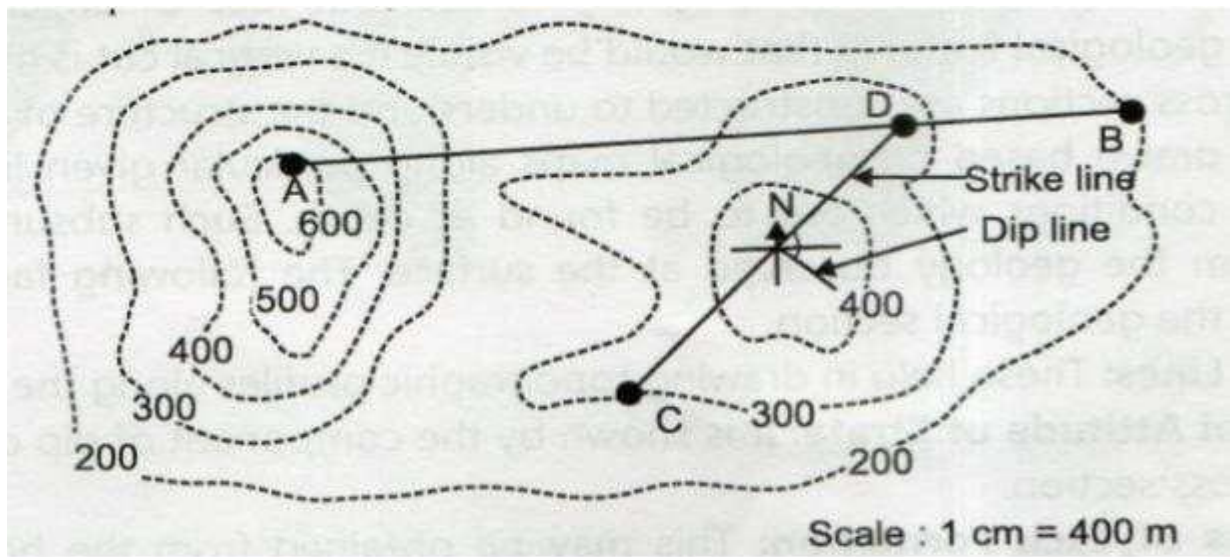
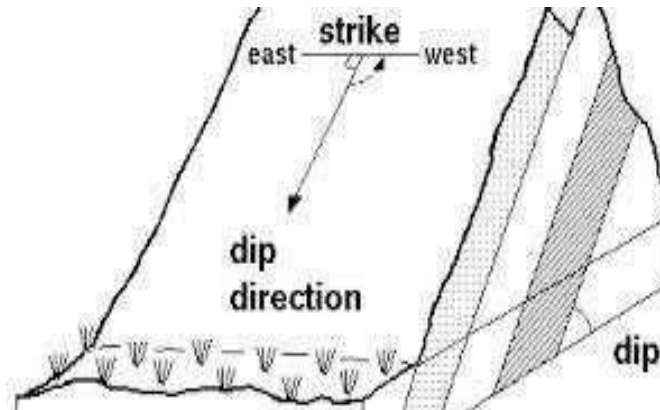


Figure showing Strike and dip line

5. **Observation:** Observation of the strike and dip on Geologic Maps.

6. **Observation table:**

S.No.	Observations
1.	Identified strike and dip in the geological map
2.	Trend of outcrop lines and true dip and strike of the bed are determined by the use of the stereographic projection.

7.Environmental Significance: Strike and dip are measurements of the orientation and slope of a rock. The strike and dip are important in determining the younger bed or formation. The younger beds will always be found in the direction of dip. It also helps in the classification and nomenclature of folds and faults. Measurement of strike and dip (i.e., the attitude of rock layers or other planar geologic features) helps geologists in constructing of accurate geologic maps and geologic cross-sections.

8. Questions based on Experiment:

1. How do you identify strike and dip?
2. What is the difference between strike and dip?
3. What are strike and dip measurements used for?
4. What is importance of strike and dip in geology?

EXPERIMENT NO. – 8

Aim of the Experiment : Laboratory Study and Observations of Physical Properties of Minerals.

1. **Learning outcome:** To understand the behavior and characteristics of minerals for remediation of contaminated soils and groundwater.
2. **Brief Theory:** Minerals are homogeneous and have specific chemical composition. Minerals are solid inorganic chemical substances .

Detail of the Physical Properties of the Minerals-

- **Color:** It is the usual body color of mineral.
- **Streak:** The color of the mineral powder is called the streak of a mineral. This is tested by rubbing the mineral on streak plate (An unglazed white porcelain plate).
- **Hardness:** Hardness may be defined as the resistance offered by the mineral to abrasion or scratching. It is determined with the help pH Moh's scale of hardness which consists of ten reference minerals arranged in increasing order of hardness and numbered accordingly.
- **Cleavage:** If a mineral breaks along a flat plane, it is said to possess a cleavage, if it breaks with an irregular surface, it is said to show a fracture. Thus, the "cleavage" may be defined as the tendency of a mineral to break more easily with smooth surfaces along planes of weak bonding. Hence cleavage is the property which is related to the atomic arrangement within the mineral. Because the cleavage always occurs parallel to a possible crystal face, it is designated in terms of the crystal face to which it lies parallel, such as cubic, octahedral, prismatic, basal and so on. For example, galena has cubic cleavage, fluorite has octahedral cleavage, mica has basal cleavage and calcite has rhombohedral cleavage. Depending on the ease with which a crystal cleaves and the perfection of the surface obtained, the cleavage is classified as "perfect", "good", "poor" and "indistinct". Examples of minerals which show perfect cleavage are mica, galena, calcite and fluorite. Quartz has no cleavage at all.
- **Crystalline structure:** A crystalline structure is any structure of ions, molecules, or atoms that are held together in an ordered, three-dimensional arrangement. Crystalline structure is one of two types of structural ordering of atoms, the other being the amorphous structure. types of crystals may appear as Triclinic, Monoclinic, Orthorhombic, Tetragonal, Trigonal, Hexagonal, and Cubic.
- **Lustre:** Lustre is the nature of shining on the surface of the mineral. Types of lustre are as follows:
 - i. **Metallic Lustre-** Minerals which have the appearance of metal, are said to have a metallic lustre'' eg-pyrite and galena.
 - ii. **Sub-metallic Lustre-** The feebly displayed metallic lustre is called the submetallic lustre'' eg- chromite and hematite
 - iii. **Adamantine Lustre-** A hard brilliant lustre like that of a diamond is called adamantine lustre''. It is due to the mineral's high index of refraction eg – transparent cerussite.

- iv. **Vitreous Lustre**- It is the lustre exhibited by the broken glass eg- quartz.
- v. **Pearly Lustre**- It is the lustre exhibited by the pearls eg-muscovite, talc and calcite.
- vi. **Silky Lustre**- It is the lustre exhibited by the silk fibers. Minerals which crystallize in fibrous habit commonly show silky lustre eg-asbestos and fibrous gypsum.
- vii. **Resinous Lustre**- It is the lustre exhibited by the resin eg- sphalerite
- **Specific gravity**: Specific gravity or Density of minerals depends on their chemical composition and atomic structure.
- **Fracture**: Fracture is the nature of the randomly broken surface of a mineral. Types of fracture:
 - **Conchoidal Fracture**- It is a curved fracture surface showing concentric lines like a shell. Quartz and glass show conchoidal fracture.
 - **Even fracture**- It is a fracture surface which is almost flat. Flint shows even fracture.
 - **Uneven fracture**- It is a fracture surface which is irregular and rough. A large number of minerals shows uneven fracture.
 - **Hackly Fracture**- It is a fracture surface which is rough with sharp and jagged points. Native metals show hackly fracture.
- **Odor**: Some minerals give a characteristic smell when rubbed upon or heated. The chief type of odors are as follows:-
 - **Arsenical**- The arsenical odor is like the odor of garlic. Orpiment and other arsenic minerals give arsenical odor.
 - **Sulfurous**- This odor is like the odor of burning sulphur. Pyrite gives sulphurous odor.
 - **Argillaceous**- This odor is like the odor of clay. Kaoline gives argillaceous odor.

Mineral Physical Property chart		
Physical Property	Definition	Testing Method
Cleavage	Breakage of a mineral along planes of weakness in the crystal structure	Examine the mineral for area where the mineral is broken. Look for area where the light reflects from planar surfaces. This can be easily confused with a crystal face and is the most difficult properties for student to master
Color	Visible light spectrum radiation reflected from a mineral.	look at the sample and determine its color white, green, black, clear etc.
Crystal forms	Geometric shape of a crystal or mineral	examine and describe the geometric shape of the mineral, cubic, hexagonal, etc. Not commonly seen in most lab samples
Fractures	Breakage of a mineral, not along planes of weakness in the crystal structure	Examine the mineral for area where the mineral is broken. Describe the breakage as either irregular or conchoidal (has the appearance of broken glass)
Hardness	Resistance to scratching or abrasion	Use mineral of known hardness from the Mohs hardness Kits. Scratch the unknown mineral with a known hardness to determine which is harder. Continue doing this with harder or softer minerals from the kit until the hardness is determined.
Lustre	Character of the light reflected by a mineral	Look at the samples to determine if the mineral is metallic in appearance or non metallic. Vitreous,

		like glass and earthy (like dirt, or other Powderly material)
Magnetism	Electromagnetic force generated by an object or electric field.	Use of magnet to determined in an introductory lab.
Specific gravity	Ratio of the mass of a mineral to the mass of an equal volume of water	Generally not determined in an introductory lab.
Streak	Color of the mineral when it is powdered	Grind a small amount of a mineral into a powder on a porcelain streak plate and determine the color of the powder
Transparent	Stages of transparency of mineral	A mineral is Transparent when Light is able to pass through it or partially let light pass through; or do not let any light through it.

Mohs's Scale of Hardness

Mohs hardness	Mineral	Chemical Formula
1	Talc	$\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$
2	Gypsum	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
3	Calcite	CaCO_3
4	Fluorite	CaF_2
5	Apatite	$\text{Ca}_5(\text{PO}_4)_3(\text{OH}^-, \text{Cl}^-, \text{F}^-)$
6	Feldspar	KAlSi_3O_8
7	Quartz	SiO_2
8	Topaz	$\text{Al}_2\text{SiO}_4(\text{OH}^-, \text{F}^-)_2$
9	Corundum	Al_2O_3
10	Diamond	C

List of Laboratory Minerals -

- Quartz
- Pyroxene
- Pyrite
- Chalcopyrite
- Sphalerite
- Psilomelane
- Haematite
- Magnetite
- Chromite
- Pyrolusite
- Graphite

1.3. Apparatus Required: Specimens of different minerals, Practical Copy

4.Experimental Procedure : Different rocks specimen are given to the student to identify it and write their physical properties in the practical notebook.

5. Observation: After observation of different minerals following properties has been identified .

6. Observation table:

S.No.	Physical Properties	Detail description
1	Form	
2	Color	
3	Streak	
4	Lustre	
5	Fracture	
6	Cleavage	
7	Hardness	
8	Specific Gravity	
9	Specific Gravity	
10	Degree of Transparency	
11.	Special Property	

7.Environmental Significance: The behavior and characteristics of minerals are an integral part of all remediation of contaminated soils and groundwater. In general, the influence of minerals in soil and groundwater clean-up can be grouped into their effects on fate and transport of contaminants, their use as amendments to facilitate contaminant destruction or stabilization, their indirect influence on remediation, and their role as sources of natural contaminants.

8. Questions based on Experiment:

1. What are the minerals?
2. What are the properties of minerals?
3. Which of the following physical properties is likely to be most useful in identifying a mineral?
4. Which of the following minerals is the hardest according to the Mohs' scale?

EXPERIMENT NO. – 9

Aim of the Experiment: Study of rock specimens and its physical properties (Igneous, Sedimentary and Metamorphic Rocks).

1.Learning outcome: The knowledge about the important rocks and give idea about what the Earth was like in the past.

2.Brief Theory: Rocks does not have homogeneous property and does not have specific chemical composition. Rocks are the mixture of one or two minerals and can also have organic traces. There are three different types of rock:

a) Igneous Rocks: All those rocks which have formed from cooling of magma while beneath the surface or after its eruption over the surface of the earth (as lava) have been defined as Igneous or Eruption rocks. They are distinguished as Intrusive igneous rocks when formed beneath the surface or Extrusive when their cooling take place after the magma has been erupted over the surface. Obviously the intrusive rocks would become exposed to the surface only when their covering rocks are removed and this is achieved in nature by destructive processes like erosion and weathering. Some of the laboratory specimen of Igneous rocks:

- Granite
- Rhyolite
- Pegmatite
- Syenite
- Diorite
- Gabbro
- Dolerite
- Basalt
- Peridotite

Recognition of Igneous Rocks				
Grain size	Usual color	Other	Composition	Rock type
Coarse	Green	Dense	Approximately 90 to 95% Olivine	Dunite
Fine	Dark	Contain Quartz	Low - silica lava	Basalt
Coarse	Light	Wide range of color and grain size	Large grains of quartz, feldspar, Olivine and pyroxene	Granite
Coarse	Medium to dark	Little or no Quartz	Plagioclase and dark mineral	Diorite
Coarse	light	Wide range of color and grain size but no Quartz	Feldspar with Pyroxene, amphibole and mica	Syenite
Coarse	Medium to	Quartz may have Olivine	Calcium Plagioclase and	Gabbro

	dark		dark minerals	
Fine	Medium	Between felsites and basal	Medium Silica Lava	Andesite
Fine	Light	Contain Quartz	High Silica Lava	Felsites

b) Sedimentary Rocks: Sedimentary Rocks are also called secondary rocks. This group includes a wide variety of rocks formed by accumulation and compaction and consolidation of sediments or particles or even organic remains. The accumulation and compaction of the solid matter of sedimentary rocks most commonly takes place under water or at least in the presence of water. A great bulk of all the sedimentary rocks is comprised of the sediments or grains broken from pre-existing rocks by the action of natural agencies such as wind, water and ice. Sedimentary rocks are known to cover as much as 75 percent surface area of the land, the rest being covered by igneous rocks. Sedimentary rocks are formed by simple or complex mechanical or chemical processes. Sedimentary rocks cover a great part of the earth's crust in its horizontal extension which according to CLARK, approaches to 75 percent of the total area of the earth. It is with these types of rocks that a civil engineer has to deal with in majority of cases. It is therefore desirable for a civil engineer to know as much as possible about important characters of sedimentary rocks. Some of the laboratory specimen of Sedimentary rocks :

- Sandstone
- Shale
- Limestone
- Conglomerate
- Breccia
- Laterite

Recognition of Sedimentary Rocks				
Hardness	Grain Size	Other	Composition	Rock type
Hard	Coarse	White to brown, Foliated	Clean Quartz	Sand stone
Hard	coarse	Quartz and Feldspar	Usually very coarse	Arkose
Hard to Soft	Mixed	Mixed rocks and sediments	Round rocks in finer sediment matrix	Conglomerate
Hard to soft	Mixed	Mixed rocks and sediments	Sharp and angular pieces of rocks in finer sediments matrix	Breccia
Soft	Fine	Fizzes with acid	Calcite	Limestone
Soft	Fine	Foliated	Clay minerals	Shale
Hard	Fine	Chalcedony	No fizzing with acid	Chert
Hard	Fine	Feel gritty on teeth	Very fine sand no clay	Silts tone

Metamorphic Rocks: Metamorphism rocks are defined as those rocks which have formed through the operation of various types of metamorphic processes on the pre-existing primary (igneous) or secondary (sedimentary) or other rocks involving either textural and structural changes or changes in the mineralogical composition or reconstitution in both directions. They are megascopically distinguished

from other types of rocks by the development of features like cleavage, foliation, schistosity and granulation and by the presence of such minerals which are known to be of metamorphic origin. All those changes in the body of a rock that are due to variations in the factor of pressure, temperature and chemical environment are known as the metamorphic changes and the process itself is termed metamorphism. Metamorphism is also termed local when the effects are limited to a part of the region and as regional when the metamorphic effects are of varied nature and widespread over an enormous extent of the area. Presence of water is almost essential in many cases to start and complete the chemical reactions in the rock mass undergoing metamorphism. Some of the laboratory specimen of Metamorphic rocks:

- Quartzite
- Slate
- marble

Recognition of Metamorphic Rocks					
Grain Size	Hardness	Foliation	Usual Color	Other	Rock Type
Coarse	Hard	Foliated	Mixed dark and light	Wrinkled foliation; often has large crystal	Schist
Coarse	Hard	Foliated	Mixed	Banded	Gneiss
Fine	Soft	Foliated	Dark	"tink" when struck	Slate
Coarse	Soft	Nonfoliated	light	Calcite or dolomite by the acid test	Marble
Coarse	Hard	Nonfoliated	light	Quartz (no fizzing with acid)	Quartzite
Fine	Soft	Foliated	Dark	Shiny, Crinkly foliation	Phyllite
Coarse	Hard	Foliated	Dark	Mostly hornblende	Amphibolite
Coarse	Hard	Foliated	Mixed	Distorted "metled" layer	Migmatite

3. Apparatus Required: Rock specimens, lenses, Colored pencils etc.

4. Experimental Procedures: To identify a rock following things must be considered:

Rock Origin: The first step to identify a rock is to try categorizing the rock into one of the three main types or group of rocks. These include igneous, sedimentary and metamorphic types. The only rocks which do not fall into one of these categories are meteorites. Igneous, sedimentary and metamorphic rock types are distinguished by the processes of their formation.

Rock Composition: The rock composition is determined by the identification of mineral make up the rock. By the identification, a rock is a solid mass or compound consisting of at least two minerals (although there are some exceptions when a rock may consist entirely of one mineral). The minerals comprising the rock can be identified using common field testing method for individual minerals, particularly where the texture is sufficiently coarse grained enough to distinguish the individual minerals

with the naked eye or a hand lens. Where the grain sizes of the minerals comprising the rock are too fine grained to recognize identification in many cases.

Rock Texture: The texture of a rock is defined by observing two criteria: 1) grain size, 2) grain shapes.

Grain Size: it is the average size of the mineral grains. The size scales used for sedimentary, igneous and metamorphic rock are different.

Grain Shape: It is the general shape of the mineral grain (crystal faces evident, or crystals are rounded)

5. **Observation:** After observation of different rocks following properties has to be identified.

6. **Observation table:**

S.No.	Physical Properties	Detail descriptions
1.	Color	
2.	Grain	
3.	Texture or Structure	
4.	Mineral Present	

7.**Environmental Significance:** Geologists study rocks because they contain clues about what the Earth was like in the past. Different rocks form under only certain conditions and even the dullest gray lump of a rock can tell us something important about the past.

8. **Questions based on Experiment:**

1. How will you define rocks
2. How are rocks distinguished from each other?
3. What are the different types of rock forming processes?

EXPERIMENT NO. – 10

Aim of the Experiment: Study through GPS-Latitude, Longitude, Elevation etc.

1. Learning outcome: The knowledge about the latitude and longitude of any location can easily be determined.

2. Brief Theory: The GPS receivers used for surveying are generally more complex and expensive than those used in everyday life. A GPS baseline uses two survey-quality GPS receivers with one at each end of the line to be measured. They collect data from the same GPS satellite at the same time. GPS Mapping is the digital mapping which can be found in a variety of computer applications the main use of these maps is with the global Positioning system, or GPS satellite network used in standard automotive navigation systems.

3. Apparatus Required: GPS (Global Positioning System), Notebook, Pen.

4. Experimental Procedures:

1. GPS is a satellite-based navigation system made up of a network of 24 satellite orbiting the earth at all times.
2. This system when the proper signals are received allows a user to tell where they are located.
3. GPS is a network of about 30 satellite orbiting the earth at an altitude of 20,000 km.
4. Once it has information on how far away at least three satellites are your GPS receiver can pinpoint your location using a process called trilateration.

5. Observation: Observation of latitude and longitude of the location of any area.

6. Observation table:

S.No.	Latitude	Longitude	Elevation
1	23.7957° N	86.4304° E	227 m

7. Environmental Significance: GPS stands for Global Positioning System. GPS units receive signals from United States Government satellites that they use to determine location. The remarkable thing about GPS is that the satellite navigational information is available to anyone, anywhere, free of charge. GPS and other GNSS and enhancements raise productivity; reduce and avoid costs; save time; enable improved and new production processes, products and markets; increase health and well-being; reduce injury and loss of life; improve the environment; and increase security.

8. Question based on Experiment:

1. How do you read GPS coordinates?
2. What is the use of coordinates system?
3. How GPS help to indicate latitude and longitude coordinates of any location?

EXPERIMENT NO. - 11 & 12

Aim of the Experiment : Study of Water table fluctuation through secondary data and Calculation of TARR value

1.Learning outcome: To estimate the groundwater recharge by analysis of water-level fluctuations from the observation wells. To understand the importance of total annual replenishable recharge and for assessment of groundwater utilization and its stage of groundwater development.

2.Brief Theory: The water-table fluctuation provides an estimate of groundwater recharge by analysis of water-level fluctuations in observation wells. The method is based on the assumption that a rise in water-table elevation measured in shallow wells is caused by the addition of recharge across the water table. This water table fluctuation is measured with the help of water level meter. A water level meter is a system that relays information back to a control panel to indicate whether a body of water has a high or low water level. Some water level indicators use a combination of probe sensors or float switches to sense water levels. The Water Level Indicator employs a simple mechanism to detect and indicate the water level in an overhead tank or any other water container. for accurately measuring the depth of water in wells, boreholes, standpipes, and tanks. They are sturdy, easy to use and read accurately to 1/100 ft.

The basic principle followed in this methodology is the estimation of annual groundwater recharge from rainfall and other sources, including irrigation, water bodies and artificial recharge, determination of present status of groundwater utilization and categorization of assessment units based on the level of groundwater utilization and long-term water level trend. These estimations are widely used in formulating various groundwater development and management plans. TARR (Total Annual Replenishable Recharge).

3.Apparatus Required: Water level Recorder, Tape, Scale, Notebook. TARR Formula, Calculator, Practical Copy.

4. Experimental/Numerical Procedure:

The working principle of a water level indicator is actually quite simple. Water level indicators work by using sensor probes to indicate water levels in a well. These probes sense water levels and indicate the level of water in the well or bore hole.

1. Measuring water levels in wells.
2. Single measurements.
3. Simple tape measures inaccurate.
4. Long-term monitoring.
5. Once the probe touches the level of water it sensor automatically indicate and measurement is taken on the tape.



Water Level Recorder

Calculation of TARR

- **Calculation of TARR (Total Annual Replenishable Recharge) by using these formula:**

- **TARR = Area × Avg. water table fluctuation × yield.**
- **Yield = $P \times RG / HW(P-RS)$**

Where:

P= Precipitation

RG= Groundwater runoff

HW= Average water table fluctuation

RS= Surface runoff

i. Computation of total annual replenishable recharge (TARR) (million m³/year)

a. By ground water table fluctuation method:

The average water fluctuation in study area

$$\text{Specific yield} = P \times R_g / H_w \times (P - R_s)$$

Where P is the Annual Rainfall, R_g is the annual groundwater runoff, R_s is the annual surface runoff and H_w is the water table fluctuation.

$$\text{TARR (million m}^3\text{/year)} = \text{Area} \times \text{average water table fluctuation} \times \text{specific yield}$$

a) By rainfall infiltration factor method (RIF) (million m³/year)

$$= \text{Area} \times \text{average rainfall} \times \text{infiltration}$$

$$\text{Average TARR} = \text{m}^3\text{/year}$$

ii). Annual draft excluding estimated draft through mine discharge (million m³/year)

The total population in the sub-watershed area is around 6044 so the domestic withdrawal of water has been computed by considering 65 L per day per head water consumption. The total annual domestic water withdrawal for the sub-watershed becomes:

$$= \text{Population} * \text{consumption} * \text{days in (million m}^3/\text{year)}.$$

iii) Estimated draft through mine discharge (million m³/year)

iv) Net ground water availability = TARR- (Estimated draft through mine discharge + Domestic draft) = (million m³/year).

v) Stage of ground water development (in %)= Net draft/TARR

vi) Radius of Influence R (t) -

According to Bear 1979 formula the radius of influence for a pumping well will be:

$$R(t) = 1.5 (T*t/S)^{1/2}$$

Where T is Transmissivity (m²/day), t is the time (day), S is the storativity of the discharging well.

Radius of Influence (m)

5. Observation:

Water level data will be recorded with help of Water Level Recorder and then Water level fluctuation will be calculated to observe the level of water of any location. Obtained the total annual replenishable recharge of any given watershed area.

6. Observation table:

Table.1. Observation of Water level Data

S.No.	Parapit level	Diameter of well	Total depth	Depth of Water	Water level	Water level fluctuation
1.						
2.						

Table.2. Observation of TARR Calculation

S.No.	Formula used	Result
1	TARR (Total Annual Replenishable Recharge)	(million m ³ /year)
2	Annual draft excluding estimated draft through mine discharge	(million m ³ /year)
3	Estimated draft through mine discharge	(million m ³ /year)
4	Net ground water availability	(million m ³ /year).
5	Stage of ground water development = Net draft/TARR	(in %)
6	Radius of Influence R (t)	m

7.Environmental Significance: The water-table fluctuation (WTF) method provides an estimate of groundwater recharge by analysis of water-level fluctuations in observation wells. The method is based on the assumption that a rise in water-table elevation measured in shallow wells is caused by the addition of recharge across the water table.

The sustainable development of groundwater requires a precise quantitative assessment based on reasonably valid scientific procedure. Assessing managing and planning of groundwater resources for sustainable use becomes an important issue for human life especially in the region where the rainfall is low, erratic and with very high groundwater depth. The groundwater resources are a replenishable resource but infinite resource. Precipitation is the main sources of groundwater recharge whereas the other sources which replenish the ground water are seepage from canal system, return flow from the applied irrigation, subsurface inflow from the adjoining region, recharge from the river system are also contribute significantly to the groundwater recharge. So, this can be estimated using TARR for assessment of groundwater utilization and its stage of groundwater development.

8. Question based on Experiment:

1. What is water level ?
2. Why does the depth of the water table vary from place to place and season to season?
3. Why to study water level?
4. What is groundwater fluctuation?
5. How can we maintain groundwater levels?
6. What is ground water draft?
7. What is artificial ground water recharge?
8. How do you replenish ground water?
9. What is groundwater How is it replenished?

Department of Environmental Science & Engineering

B. Tech. Environmental Engineering

Soil and Environmental Microbiology (ESC 254)

Environmental Microbiology Laboratory (Room No: 309)

B. Tech (Environmental Engg.) - IV Semester (W 2021-2022)
ESC 254: Soil & Environmental Microbiology

INDEX OF EXPERIMENTS

ESC 254	Soil and Microbiology Practical	L	T	P	C
		0	0	3	3
Course Objectives: ➤ Develop idea about culture media and staining techniques. ➤ Develop understanding about the microbiology of air, water and soil environment. Learning Outcomes: ➤ Plan and conduct an experiment for physico-chemical properties of soil ➤ Understand the preparation of culture media and staining techniques. ➤ Learn methods for enumeration of microbes from air, water and soil.					

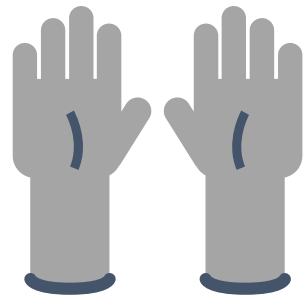
Exp. 1.	General Introduction	2 hrs
Exp. 2.	(a) Determination of Specific Gravity, Bulk Density and Moisture content of a given sample.	2 hrs
	(b) Determine the In-Situ (Bulk Density/ Wet Density), Dry Density of Soil by Core Cutter Method.	
	(c) Determination of soil Moisture Content	
Exp. 3.	Determination of the soil Organic Carbon (%) of the soil samples by Titrimetric Method (Walkley and Black, 1974)	2 hrs
Exp. 4.	Determination of Total Nitrogen in Soil Sample by Kjeldahl method Determination of Available Phosphorous by Dickman and Bray method.	
Exp. 5.	(a) Determination of K and Na by Flame Photometric Method Determination of Cation Exchange Capacity (CEC) of Soil	2 hrs
Exp. 6.	Determination of bio-available and total heavy metals in soil	2 hrs
Exp. 7.	Culture media preparation– Semi-synthetic and Synthetic media. Liquid, Solid and semisolid media, Nutrient agar, PDA media.	2 hrs
Exp. 8.	Gram staining techniques for detection of gram positive and gram-negative bacteria.	2 hrs
Exp. 9.	Study of fungi (medium – Rose Bengal agar).	2 hrs
Exp. 10.	Bacteriology of drinking water and domestic sewage -MPN techniques for total coliform, Faecal coliform and Faecal Streptococci (FS). IMViC test (Biochemical Identification)	2 hrs
Exp. 11.	Microbiology of Air: Enumeration of microbes by exposure plate method.	2 hrs
Exp. 12.	Microbiology of soil: Isolation and Identification of microbes by serial dilution methods and colony count by colony counter.	2 hrs

Practical PLAN

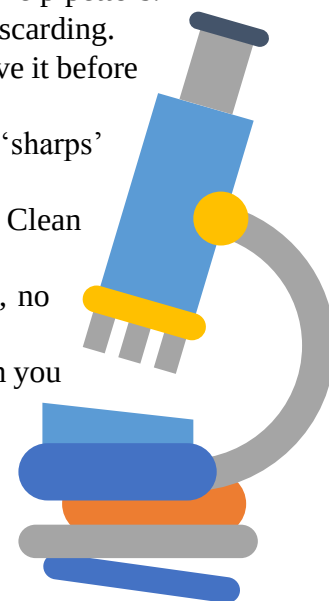
EXPERIMENT NO. 1

GOOD LABORATORY PRACTICES: Basic Practices, Safety Rules & Requirements

- Wash your hands with disinfectants soap when you arrive the lab and again before you leave.
- Absolutely no food or drinks should be consumed or opened in lab. Do not store food in areas where microorganisms are stored.
- Use lab coat and safety glasses, alternatively, a long sleeved shirt that buttons or snaps closed is an acceptable protective clothing. Avoid loose fitting items of clothing.
- Wear appropriate shoes (sandals are not allowed) in the laboratory.
- Keep your workspace free of all unnecessary materials. Backpacks, purses and coats should be placed away from work area.
- Disinfect work areas before and after use with 70% ethanol.
- Label everything clearly.
- Replace caps on reagents, solution bottles, and bacterial cultures. Do not open Petri dishes in the lab unless absolutely necessary.
- Inoculating loops and needles should be flame sterilized in Bunsen burners before you lay them down.
- Turn off Bunsen burner when not in use. Long hair must be restrained if burners are in use.
- Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of laboratory.
- Wear disposable gloves when working with potentially infectious microbes or samples.
- Sterilize equipment and materials.



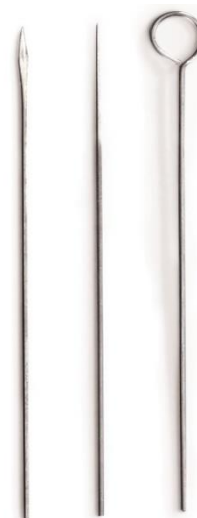
- Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors.
- Autoclave liquids and broth cultures to sterilize them before discarding.
- Dispose all solid waste material in a biohazard bag and autoclave it before discarding.
- Dispose blades, syringe needles, and sharp metal objects in the 'sharps' container.
- Report spills and accidents immediately to your instructor. Clean small spills with care, seek help for large spills.
- Report all injuries or accidents immediately to the instructors, no matter how small they seem.
- Always wear lab coat and gloves when you are in the lab. When you enter the lab, switch on the exhaust fan and make sure that all the chemicals and reagents required for the experiment are available. If they are not available, prepare the reagents using the components for reagent preparation.
- Clean all glassware with soap and distilled water. Once the experiment is completed, recap the reagent bottles. Switch off the light, exhaust fan, hot air oven and gas cylinder before leaving the lab.
- Discard the used gloves in a waste bin.



COMMONLY USED TOOLS AND APPARATUS

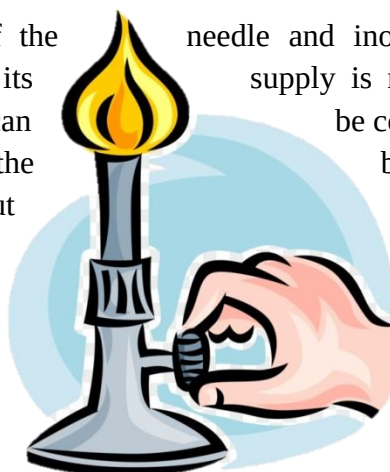
1) Inoculation Needle & Inoculation Loop

These are the most commonly used tools. Inoculation needle/loop is made up of a long platinum wire fixed into a metallic rod. A wire loop has a handle with steel screw shaft in which nichrome or platinum wire is to be fitted. The straight wire needle is used for transferring culture from solid medium. Even smaller amount of liquid culture can be manipulated by using straight needle. The loop and wire are also used for picking small quantities of solid materials from a microbial colony and can be used to inoculate either a liquid or a solid medium. Both the loop and straight wire must be flamed immediately after use to avoid contamination.



2) Bunsen Burner

It is used for the sterilization of the needle and inoculation loop. Gas enters the burner at the base, and its supply is regulated externally by the gas cock. The amount of air can be controlled by rotating a sleeve that fits over the holes in the body of the burner. To keep the flame from blowing out frequently used to fit over the top of the burner. The proper method of lighting the burner is to close off the air supply, turn on the gas, and light. The flame will be large and yellow. Gradually open the air intake until the flame takes



needle and inoculation loop. Gas supply is regulated externally by the gas cock. The amount of air can be controlled by rotating a sleeve that fits over the holes in the body of the burner. To keep the flame from blowing out frequently used to fit over the top of the burner. The proper method of lighting the burner is to close off the air supply, turn on the gas, and light. The flame will be large and yellow. Gradually open the air intake until the flame takes

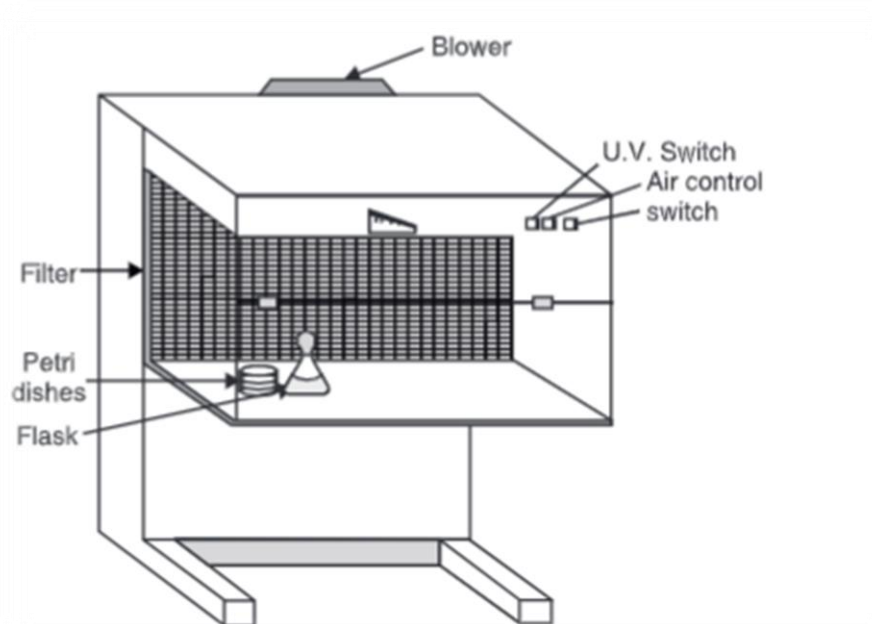
3) Water Bath

Water bath is an instrument that is used to provide constant temperature to a sample. It consists of an insulating box made up of steel fitted with electrode heating coil. The temperature is controlled through a thermostat. In some of the water baths, plate form rotates, then it is called water bath shaker. It is more useful to microbiologists because it provides a uniform heat to the sample material meant for incubation. The main use of water bath is the incubation of samples at a desired and constant temperature.



4) Laminar Air Flow Chamber

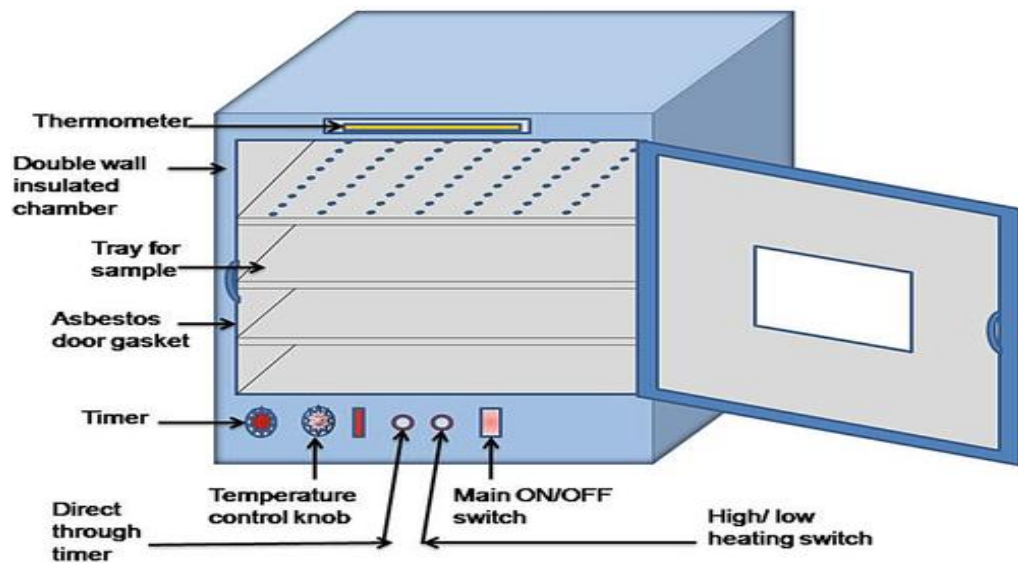
Laminar air flow is an apparatus consists of an air blower in the rear side of the chamber which can produce air flow with uniform velocity along parallel flow lines. There is a special filter system of high efficiency particulate air filter (HEPA) which can remove particles as small as 0.3 μ m. In front of the blower, there lies a mechanism through which air blown from the blower produces air velocity along parallel flow lines. The laminar air flow is based on flow of air current of uniform velocity along parallel flow lines which help in transferring microbial cultures in aseptic conditions. Air is passed through the filters into the enclosure and the filters do not allow any kind of microbe to enter in to the system. Inside the chamber one fluorescent tube and another UV tube are fitted. Two switches for these tubes and a separate switch for regulation of the air flow are fitted outside the LAF.



Initially, dust particles are removed from the surface of the laminar air flow with the help of smooth cloth containing alcohol. Switch on the UV light for a period of 30 minutes so as to kill the germs, if any present in the area of working space. The front cover sheet of the apparatus is opened to keep the desired material inside. The air blower is set at the desired degree so that the air inside the chamber is expelled because the air inside the chamber may be contaminated / bring contaminants. Sit properly in front of the chamber and wipe the working table with alcohol to reduce the contaminants. All the work related to pouring, plating, streaking etc. are to be carried out in the flame zone of the burner or spirit lamp. In microbiology laboratory, horizontal type of laminar air flow is used to supply the air through filter.

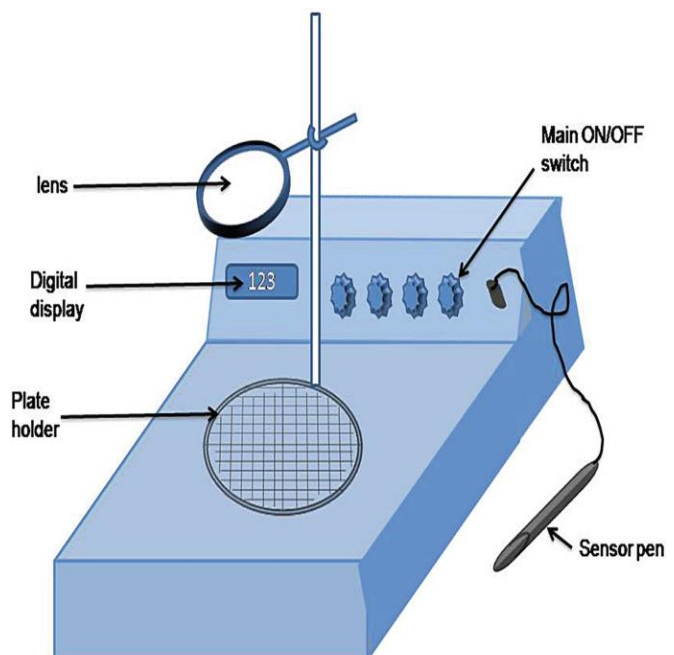
5) Incubator

An incubator is an instrument that consists of copper/steel chamber around which warm water or air is circulated by electric current or by means of small gas flame. The temperature of the incubator is kept constant due to its control by using thermostat. The incubator is made up of double walled chamber adjusted to a desired temperature. It is done by using an external knob controlling the thermostat system. The gap between two walls is insulated to check heat condition. A thermometer is inserted from the top for recording the temperature. Temperature greatly influences the microbial growth. Therefore, instrument is generally designed that can allow the desired microorganism to grow at a particular temperature. It is operated to allow the microbial growth on a suitable medium under proper temperature. In an incubator, the variation in temperature should not be more than one degree. Small square type incubators are better than large ones. If a lower temperature than the room is required, the water is circulated around the chamber to pass through an ice chest.



6) Colony counter

Colony counters are used to estimate a liquid culture's density of microorganisms by counting individual colonies on an agar plates, slide, mini gel, or Petri dish. Instruments accommodate various sizes and formats, including units that can scan plates upto 300 x 300 mm. The instrument consists of a illuminated base with a grid pattern on which the plates are laced. They are optimized for use with UV illumination, white light, fluorescent, and/or green fluorescent protein colonies. The counting can be accomplished manually, often with touch pressure and a digital counter, or can be semi or fully automatic.



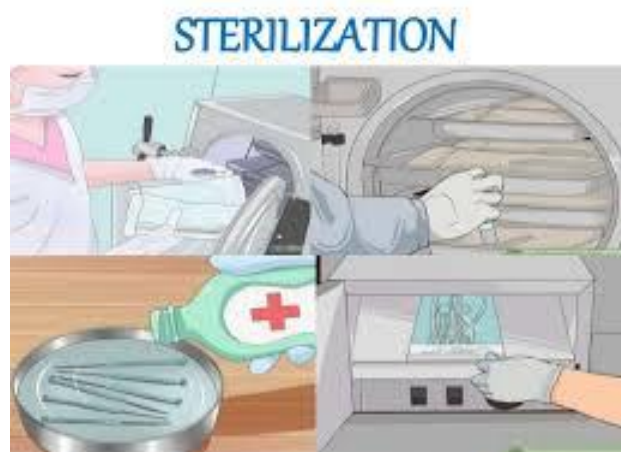
• Sterilization

Sterilization is the process of destroying or physically removing all forms of microbial life including vegetative cells, spores and viruses from a surface, a medium or an article. The principal reasons for controlling microorganisms are:

1. To prevent transmission of disease and infection.

2. To prevent contamination by undesirable microorganisms.
3. To prevent deterioration and spoilage of materials by microorganisms.

The methods of sterilization employed depend on the purpose for which sterilization is carried out, the material which has to be sterilized and the nature of the microorganisms that are to be removed or destroyed. The various agents used in sterilization can be grouped into physical and chemical agents



❖ Physical Agents (Physical Methods)

• Sunlight

Direct sunlight has an active germicidal effect due to the combined effect of the ultraviolet radiation and heat. This is a natural method of sterilization.

• Drying

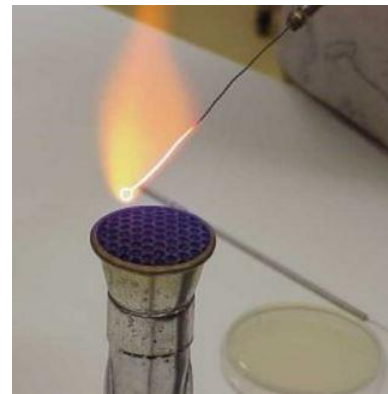
Moisture is essential for the growth of bacteria. Drying in air has therefore a deleterious effect on many bacteria. But spores are unaffected by drying. Hence this is very unreliable method.

• Heat

Heat has a killing effect on microorganisms and is one of the most popular reliable methods to destroy. Microorganisms has a minimum, optimum and maximum growth temperatures. Temperature below the minimum usually produces static (inhibition of metabolism).

➤ Dry heat

Many objects are best sterilized in the absence of water by dry heat sterilization; killing by dry heat is due to protein denaturation, oxidative damage and toxic effect of elevated levels of electrolyte.

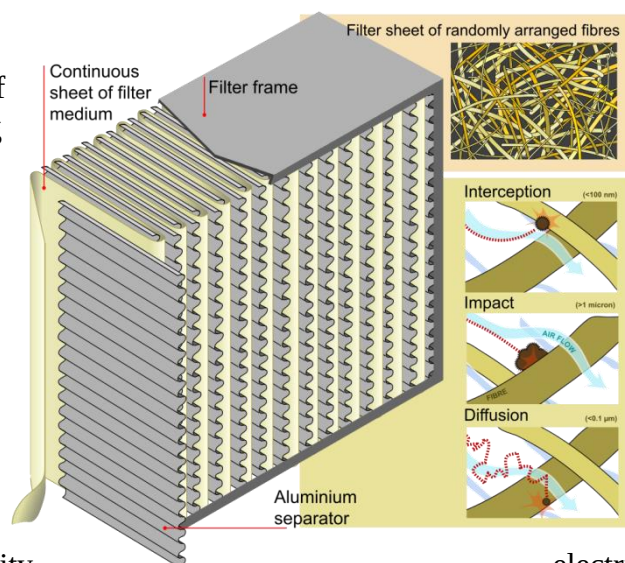


➤ Moist heat

Moist heat kills microorganisms by coagulating their proteins and is much more rapid and effective than dry heat because water molecules conduct heat better than air. Lower temperature and less time of exposure are therefore required than for dry heat. Moist heat readily kills viruses, bacteria, fungi etc.

• Filtration

Filtration is the process of removal of microorganisms from liquid or gases using a mechanical device known as filter. This is an excellent way to reduce the microbial population in solution of thermo labile materials such as sera, antibiotic solutions, intravenous solutions, carbohydrates solutions used in the preparation of culture media etc. As fluid passes through the filter, microorganisms are trapped in the pores of the filtering material. The solution that drips through the filter is collected in a previously sterilized container. Porosity,



electric

charges of the filter, electric charge carried by the organisms, nature of the fluid being filtered etc. can influence efficiency of filtration. Types of filters: Seltz Filter, Berkefeld Filter, Membrane Filter, High Efficiency Particulate Air (HEPA) filter

• Irradiation

The process of exposing organisms to any one of the radiation such as UV-rays, X-rays, gamma rays etc. is known as irradiation. Irradiation is an effective method of sterilization. Two types of radiations are used for sterilization.

a) Non ionizing radiation like UV radiation and Infrared radiation

b) Ionizing radiation like X-rays and Gamma rays

Disinfection

It is the destruction, inhibition or removal of microbes that may cause disease or other problems, e.g. spoilage. It is usually achieved by the use of chemicals. The list of chemicals commonly used are:

- Alcohol
- Chlorine and chlorine compounds
- Formaldehyde
- Glutaraldehyde
- Hydrogen peroxide
- Iodophors
- Ortho-phthalaldehyde(OPA)
- Peracetic acid
- Ultraviolet radiation
- Pasteurization



➤ QUESTIONS

1. Differentiate between Disinfection and sterilization.
2. A student disinfects a pair of tweezers with 99% pure ethanol and keeps it with other tools for some days. Can he use the tool for microbiological purpose later?
3. Why is pasteurization considered as a disinfection method?
4. Can UV rays be used for sterilization?

SOIL SAMPLING PROCEDURES

- **Soil Sampling**

The method and procedure for obtaining soil samples vary according to the purpose of sampling. Analysis of soil samples may be needed for engineering and agricultural purposes. Soil sampling for agricultural purpose is described which is done for soil fertility evaluation and fertilizer recommendations for crops.

The results of even very carefully conducted soil analysis are as good as the soil sample itself. Thus, the efficiency of soil testing service depends upon the care and skill with which soil samples are collected. Non-representative samples constitute the largest single source of error in a soil fertility programme. It is to be noted that the most important phase of soil analysis is accomplished not in a laboratory but in the field where soils are sampled.

Soils vary from place to place. In view of this, efforts should be made to take the samples in such a way that it is fully representative of the field. Normally one to ten gram of soil is used for each chemical determination and represents as accurately as possible the entire surface 0-22cm of soil, weighing about 2millionkg/ha.

- **Sampling tools and accessories**

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

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



Soil sampling Tubes, Augurs

- **Selection of a sampling unit**

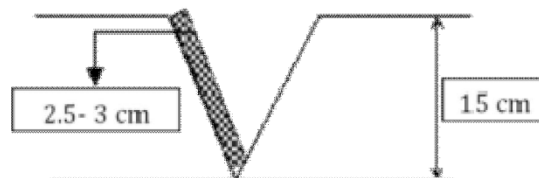
A visual survey of the field should proceed the actual sampling. Note the variation in slope, colour, texture, management and cropping pattern by traversing the field. Demarcate the field in to uniform portions, each of which must be sampled separately. If all these conditions are similar, one field can be treated as a single sampling unit. Such unit should not exceed 1 to 2 hectares, and it must be an area to which a farmer is willing to give separate attention. The unit of sampling is a compromise between the expenditure, labour and time on one hand and precision on the other. In view of limited soil testing facilities, it has been suggested to adopt an alternate approach where a sample may be collected from an area of 20-50 ha to be called as composite area soil sample and analyze the same for making a common recommendation for the whole area.

- **Sampling procedure**

Prepare a map of the area to be covered in a survey showing different sampling unit boundaries. A plan of the number of samples and manner of composite sampling is entered on the map, different fields being designated by letters A, B, C etc. Each area is traverse separately. A slice of the plough layer is cut at intervals of 15 to 20 steps or according to the area to be covered. Collect at least 10 to 15 samples from each sampling unit and place in a bucket or tray. Generally 10 to 20 spots must be taken for one composite sample depending on the size of the field.

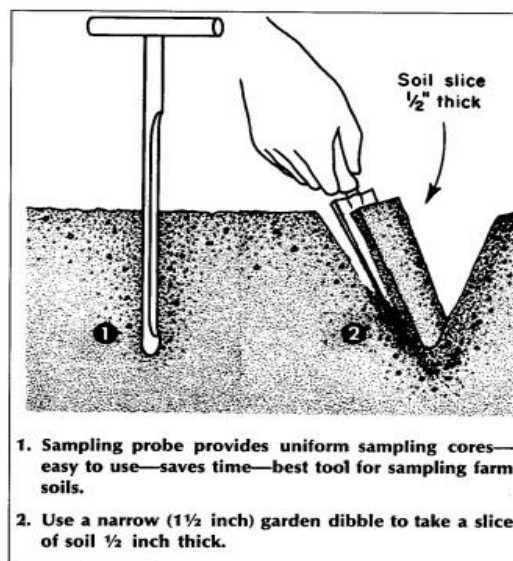
Scrap away surface liter; Obtain a uniform thick slice of soil from the surface to the plough depth from each place. A “V” shaped cut to a depth of 15 cm in the sampling spot using spade to remove 1 to 2 cm slice of soil. The sample may be collected on the blade of the spade and put in a clean bucket. In this way collect samples from all the spots marked for one sampling unit. In case of hard soil, samples are taken with the help of augur from the plough depth and collected in the bucket.

- **V shaped method of soil sampling**





Spade is stuck in soil

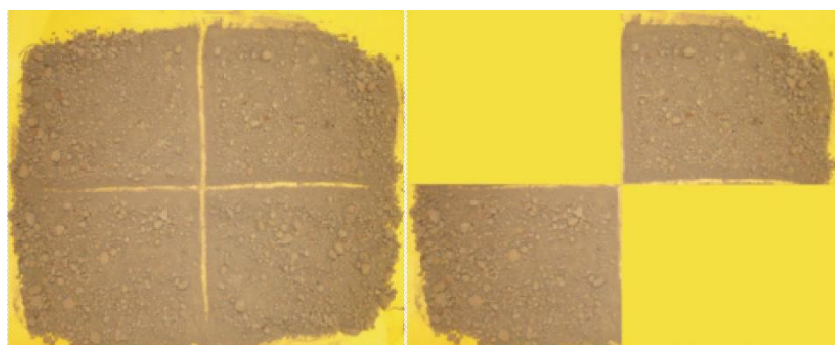


Sampling of Soil for fertilizer & lime recommendation

GUIDELINES FOR SAMPLING DEPTH

S. No.	Crop	Soil sampling depth	
		Inches	cm
1	Grasses and grasslands	2	5
2	Rice, finger millet, groundnut, pearl millet, small millets <i>etc.</i> (shallow Rooted crops)	6	15
3	Cotton, Redgram, sugarcane, banana, tapioca, vegetables <i>etc.</i> (deep rooted crops)	9	22
4	Perennial crops, plantations and orchard crops	Three soil samples at 12, 24 and 36 inches	Three soil samples at 30, 60 and 90 cm

Pour the soil from the bucket on a piece of clean paper or cloth and mix thoroughly. Spread the soil evenly and divide it into 4 quarters. Reject two opposite quarters and mix the rest of the soil again. Repeat the process till left with about half kg of soil, collect it and put in a clean cloth bag. Each bag should be properly marked to identify the sample.



Quarter method of soil sampling

The bag used for sampling must always be clean and free from any contamination. If the same bag is to be used for second time, turn it inside out and remove the soil particles. Write the details of the sample in the information sheet like name of the farmer, location of the farm, survey number, previous crop grown, present crop, crop to be grown in the next season, date of collection, name of the sampler etc. Put a copy of this information sheet in the bag. Tie the mouth of the bag carefully.

➤ **PRECAUTIONS**

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

EXPERIMENT NO. 2

(A) Determination of Specific Gravity, Bulk Density and Moisture Content of a given soil sample.

Specific Gravity

- I. AIM :** Specific Gravity Test of Soil (IS-2720-Part-3-1980)
- II. PRINCIPLE:** This test is done to determine the specific gravity of fine-grained soil by density bottle method as per IS: 2720 (Part III/Sec 1) – 1980. Specific gravity is the ratio of the weight in air of a given volume of a material at a standard temperature to the weight in air of an equal volume of distilled water at the same stated temperature.
- III. REQUIREMENTS:** Two density bottles of approximately 50 ml capacity along with stoppers, Constant temperature water bath ($27.0 \pm 0.2^{\circ}\text{C}$), Vacuum desiccator, Oven (capable of maintaining a temperature of 105 to 110°C), Weighing balance (with an accuracy of 0.001g), Spatula
- IV. PROCEDURE:**
 - **Preparation of sample:** The soil sample (50g) should if necessary be ground to pass through a 2mm IS Sieve. A 5 to 10g sub-sample should be obtained by riffing and oven-dried at a temperature of 105 to 110°C .
 - **Procedure to determine the Specific Gravity of Fine-grained Soil:**
 - i. The density bottle along with the stopper, should be dried at a temperature of 105 to 110°C , cooled in the desiccator and weighed to the nearest 0.001g (W_1).
 - ii. The sub-sample, which had been oven-dried should be transferred to the density bottle directly from the desiccator in which it was cooled. The bottles and contents together with the stopper should be weighed to the nearest 0.001g (W_2).
 - iii. Cover the soil with air-free distilled water from the glass wash bottle and leave for a period of 2 to 3hrs, for soaking. Add water to fill the bottle to about half.
 - iv. Entrapped air can be removed by heating the density bottle on a water bath or a sand bath.
 - v. Keep the bottle without the stopper in a vacuum desiccator for about 1 to 2hrs until there is no further loss of air.
 - vi. Gently stir the soil in the density bottle with a clean glass rod, carefully wash off the adhering particles from the rod with some drops of distilled water and see that no more soil particles are lost.
 - vii. Repeat the process till no more air bubbles are observed in the soil-water mixture.
 - viii. Observe the constant temperature in the bottle and record.
 - ix. Insert the stopper in the density bottle, wipe and weigh (W_3).

- x. Now empty the bottle, clean thoroughly and fill the density bottle with distilled water at the same temperature. Insert the stopper in the bottle, wipe dry from the outside and weigh (W_4).
- xi. Take at least two such observations for the same soil.

V. OBSERVATION & CALCULATION:

The specific gravity G of the soil = $(W_2 - W_1) / [(W_4 - W_1) - (W_3 - W_2)]$

The specific gravity should be calculated at a temperature of 27°C and reported to the nearest 0.01. If the room temperature is different from 27°C, the following correction should be done:-

$$G' = kG$$

where,

G' = Corrected specific gravity at 27°C

k = [Relative density of water at room temperature] / Relative density of water at 27°C.

A sample proforma for the record of the test results is given below. Relative density of water at various temperatures is taken from table here.

SPECIFIC GRAVITY OF SOIL

S. No.	Description	Determination No.		
		I	II	III
1	Temperature in °C	31	31	31
2	Weight of bottle (W_1) in g	18.57	18.50	18.62
3	Weight of bottle + Dry soil (W_2) in g	28.57	28.50	28.62
4	Weight of bottle + Soil + Water (W_3) in g	90.88	90.20	91.02
5	Weight of bottle + Water (W_4) in g	84.74	84.00	84.83
CALCULATION:				
1	Specific gravity $G = \frac{W_2 - W_1}{(W_4 - W_1) - (W_3 - W_2)}$	2.59	2.63	2.62
2	Average G (at 31°C)	2.61		
3	Corrected G (at 27°C), $G' = G \times \frac{\text{Relative density of water at room temperature}}{\text{Relative density of water at 27°C}}$ $= 2.61 \times \frac{0.995369}{0.996542} = 2.6069, \text{ say } 2.61$			

Note: The figures given in the above table are for illustration purpose only.

Void ratio (e) is the ratio of the volume of voids (V_v) to the volume of soil solids (V_s), and is expressed as a decimal. 2. Porosity (n) is the ratio of the volume of voids to the total volume of soil (V), and is expressed as a percentage.

(B) Determination of Bulk Density

- I. AIM:** Determine the In-Situ (Bulk Density/ Wet Density), Dry Density of Soil by Core Cutter Method
- II. PRINCIPLE:** Bulk density of a soil is required to be known for determining the degree of compactness. It also helps to determine the soil structure, to calculate the soil pore space etc. It is also an indicator of aeration status of soil and it provides information on the environment available to the many soil microorganisms, which live within them. This test is done to determine the in-situ dry density of soil by core cutter method as per IS: 2720 (Part XXIX) – 1975.
- III. REQUIREMENTS:** Cylindrical core cutter, Steel dolly, Steel rammer, Balance (with an accuracy of 1g), Straightedge, Square metal tray (300mm x 300mm x 40mm), Trowel
- IV. PROCEDURE:**
- The internal volume (V) of the core cutter in cc should be calculated from its dimensions which should be measured to the nearest 0.25mm.
 - The core cutter should be weighed to the nearest gram (W₁).
 - A small area, approximately 30cm square of the soil layer to be tested should be exposed and labeled. The steel dolly should be placed on top of the cutter and the latter should be rammed down vertically into the soil layer until only about 15mm of the dolly protrudes above the surface, care being taken not to rock the cutter. The cutter should then be dug out of the surrounding soil, care being taken to allow some soil to project from the lower end of the cutter. The ends of the soil core should then be trimmed flat in level with the ends of the cutter by means of the straightedge.
 - The cutter containing the soil core should be weighed to the nearest gram (W₂).
 - The soil core should be removed from the cutter and a representative sample should be placed in an air-tight container and its water content (w)



V. OBSERVATION & CALCULATION:

Bulk density was determined in the laboratory in repacked cubes as per the procedure of Chopra and Konwar [1986] and the values were given by

$$\text{Bulk density (g /cm}^3\text{)} = \frac{W_2 - W_1}{V}$$

Where,

W₁ = Weight of the empty core cutter.

W₂ = Weight of core cutter packed with Wet/ moist soil

V = Volume of core cutter, obtained by measuring the volume of water required to fill it completely.

$$\text{Dry Density (g /cm}^3\text{)} = \frac{W_3 - W_1}{V}$$

Where

W₁ = Weight of the empty Measuring cylinder.

W₃ = Weight of the cylinder packed with oven dry soil and

V = Measured volume of the core cutter,

(C) Determination of Soil Moisture or Water Content

I. AIM: Moisture or Water Content of Soil

II. PRINCIPLE: This test is performed to determine the water (moisture) content of soils. The water content is the ratio, expressed as a percentage, of the mass of “pore” or “free” water in a given mass of soil to the mass of the dry soil solids.

- **Standard Reference:** ASTM D 2216 - Standard Test Method for Laboratory Determination of Water (Moisture) Content of Soil, Rock, and Soil-Aggregate Mixtures.
- **SIGNIFICANCE:** For many soils, the water content may be an extremely important index used for establishing the relationship between the way a soil behaves and its properties. The consistency of a fine-grained soil largely depends on its water content. The water content is also used in expressing the phase relationships of air, water, and solids in a given volume of soil.

III. PROCEDURE:

- i. Record the moisture can and lid number. Determine and record the mass of an empty, clean, and dry moisture can with its lid (MC)
- ii. Place the moist soil in the moisture can and secure the lid. Determine and record the mass of the moisture can (now containing the moist soil) with the lid (MCMS).
- iii. Remove the lid and place the moisture can (containing the moist soil) in the drying oven that is set at 105 °C. Leave it in the oven overnight.
- iv. Remove the moisture can. Carefully but securely, replace the lid on the moisture can using gloves, and allow it to cool to room temperature. Determine and record the mass of the moisture can and lid (containing the dry soil) (MCDS).
- v. Empty the moisture can and clean the can and lid.

V. OBSERVATION & CALCULATION:

(1) Determine the mass of soil solids.

$$M_s = M_{CDS} - M_{SC}$$

(2) Determine the mass of pore water.

$$M_w = M_{CMS} - M_{CDS}$$

(3) Determine the water content W (%) =
$$\frac{M_w}{M_s} \times 100$$

EXPERIMENT NO. 3

Determination of Organic Carbon, NPK and CEC of a given soil sample

Organic Carbon

- I. **AIM:** To Determine the Organic Carbon (%) of the Soil Samples by Titrimetric Method (Walkley & Black, 1974)
- II. **PRINCIPLE:** The role of organic matter in soil in relation to soil fertility and physical conditions is widely recognized [Stevenson, 1986, Johnston, 1986], The organic matter is the source of plant nutrients, which are released in assimilable forms during microbial degradation.

A major proportion of nitrogen (95-99% of the total), phosphorous (33-67% of the total) and sulphur (75% of the total) in soil occur in organic combination, which mineralize them to release the nutrients in inorganic form to be used by plants. It serves as a reservoir of plant nutrients and promotes water storage as well as regulates microbial activity.

- III. **REQUIREMENTS:** 1 g of the sieved soil sample, 10 ml of 1 (N) potassium dichromate solution, 0.5 N ferrous ammonium sulphate, 20 ml of concentrated sulphuric acid (containing 1.25 % Ag_2SO_4), 10 ml of 85 % H_3PO_4 , 1 ml of diphenylamine indicator, Distilled Water, Volumetric flasks (100 ml, 250ml, 1000ml capacity), Conical flasks (100 ml, 250ml, 500 ml capacity), Measuring cylinder (10 ml, 50ml, 100 capacity), Pipette (1ml, 10ml capacity), Burette with Stand (50 ml Capacity)

IV. **PROCEDURE:**

➤ **Determination of Soil Organic Carbon**

- Take 1 g of soil in a 500 mL conical flask.
- Add 10 mL of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ solution and shake to mix it.
- Then add 20 mL Con. H_2SO_4 and swirl the flask 2 or 3 times.
- Allow the flask to stand for 30 minutes on an asbestos sheet for the reaction to complete.
- Pour 200 mL of water to the flask to dilute the suspension. Filter if it is expected that the end point of the titration is not to be clear.
- Add 10 mL of 85% H_3PO_4 and 1 mL of Diphenylamine indicator and back titrate the solution with 0.5 N Ferrous Ammonium Sulphate, till the colour flashes from violet through blue to bright green. H_3PO_4 gives sharper endpoint, by making the colour change, distinct through a flocculating effect.
- Note the volume of Ferrous Ammonium Sulphate.
- Carryout blank titration (without soil) in a similar manner.

V. OBSERVATION & CALCULATION:

% of Organic Carbon in Soil (R) is,

$$R = \frac{(V_1 - V_2) \times N \times 0.003 \times 100}{W} \times C$$

Where,

W - Weight of Sample

V₁ - Blank Titre value

V₂ - Titre value of the Sample

N - Normality of K₂Cr₂O₇ (Here it is 1N)

C - Correction Factor (1.334, 1.724)

Exp. 4 : Determination of Total Nitrogen in Soil Sample

- I. **AIM:** Determination of Total Nitrogen in Soil Sample by Kjeldahl Method.
- II. **PRINCIPLE:** Nitrogen determination has a long history in the area of analytical chemistry. Johan Kjeldahl first introduced the Kjeldahl nitrogen method in 1883 at a meeting of the Danish Chemical Society.
Since 1883, the Kjeldahl method has gained wide acceptance and is now used for a variety of applications. Kjeldahl nitrogen determinations are performed on food and beverages, meat, feed, grain, waste water, soil and many other samples.

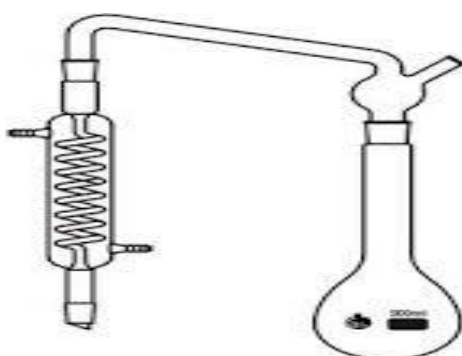
Of the total amount of nitrogen present in soil, nearly 95 - 99 % is in the organic form and 1 - 5% in the inorganic form as NH₄⁺ and nitrate (NO₃⁻) [Troeh and Thompson 1993]. Total nitrogen is merely an indicator of the soil potential for the element, but not the measure in which it becomes available to the plant. During growth and development an average of only 0.5 - 2.5 % (rarely 5 %) of the total nitrogen is converted into forms accessible to plants [Rao et al., 1997]. Total nitrogen content of soil is needed for the evaluation of C/N ratio of soil, which gives an indication of the process of transformation of organic N to available nitrogen like ammonical nitrite and nitrate N. The most common method for estimating nitrogen in soil is Kjeldahl method, which measures organic and ammonium forms excluding nitrate. In the present work, soil nitrogen was estimated by micro Kjeldahl method [Jackson, 1967], the brief procedure for which is as follows:

The Kjeldahl method is a means of determining the nitrogen content of organic and inorganic substances. Although the technique and apparatus have been altered considerably over the past 100 years, the basic principles introduced by Johan Kjeldahl endure today. The Kjeldahl method may be broken down into three main steps:

- **Digestion** - the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution.

- **Distillation** - adding excess base to the acid digestion mixture to convert NH_4^+ to NH_3 , followed by boiling and condensation of the NH_3 gas in a receiving solution.
- **Titration** - to quantify the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution.

III. REQUIREMENTS: Very early Kjeldahl digestions and distillations were performed using stone fume hoods and gas mantles as a heat source. In the 1920's these were replaced by what is now known as classical macro-Kjeldahl digestion and distillation apparatus. Macro setups use Kjeldahl flasks from 500 to 800 ml volume and handle sample sizes from 0.5 to about 5.0 g



Kjeldahl distillation Apparatus

- **Reagents:**
 1. Digestion catalyst mixture (20 g copper sulphate, 3 g mercuric oxide, 1 g of selenium powder were mixed together and 1 g of this was mixed with 20 g of sodium sulphate to obtain the catalyst mixture).
 2. Conc. Sulphuric Acid (H_2SO_4)
 3. 40 % NaOH solution
 4. Mixed indicator: Mixed indicator was prepared by mixing alcoholic solutions of bromocresol green (0.5 %) and methyl red (0.1 %) in 2:1 ratio.
 5. 4 % boric acid solution
 6. 0.1 N HCl

IV. PROCEDURE:

➤ **Digestion of Soil Sample:**

- 10 g of soil sample in a 300 ml kjeldahl flask was mixed with 25 ml of distilled water to make a suspension. 20 g of the digestion catalyst mixture (20 g copper sulphate, 3 g mercuric oxide, 1 g of selenium powder were mixed together and 1 g of this was mixed with 20g of sodium sulphate to obtain the catalyst mixture) and

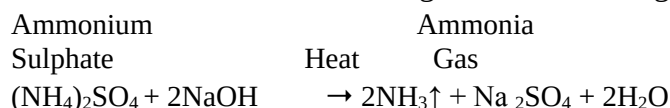
35 ml of concentrated H₂SO₄ were added to the suspension and were mixed by gentle swirling motion. The content was heated at low heat for about 10-30 minutes until the frothing stops. The heat was then raised and the flask was rotated at every five minutes interval for about two hours.

- Then the digested solution was cooled and the supernatant liquid was transferred to 100 ml volumetric flask. The residue was washed several times with distilled water and after each washing, the supernatant liquid was transferred to the flask.

Organic N + H₂SO₄ → (NH₄)₂SO₄ + H₂O + CO₂ + other sample matrix by-products

➤ **Distillation**

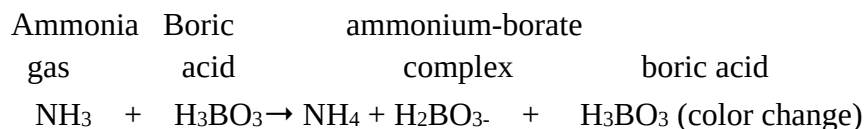
- 25 ml of the above solution was taken in a round bottom flask of the micro Kjeldahl Apparatus.
- 25 ml of 40 % NaOH solution and pieces of Zn were added to the flask and swirled gently.
- A 500 ml conical flask containing 25 ml of boric acid and mixed indicator (Mixed indicator was prepared by mixing alcoholic solutions of bromocresol green (0.5 %) and methyl red (0.1 %) in 2:1 ratio.
- 5 ml of this solution is added to 100 ml of 4 % boric acid solution) was placed below the condenser.
- Distillation is commenced and about 100 ml of distillate is collected. This was then titrated with 0.1 N HCl until the colour changes from blue to light pink.



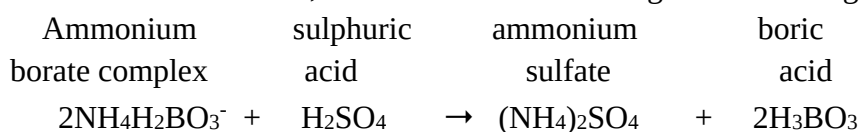
- A blank titration was run with distilled water using other chemicals in same quantities.

➤ **Titration**

- Nitrogen Determination by DIRECT TITRATION.
- If boric acid is used as the receiving solution instead of a standardized mineral acid, the chemical reaction is:



- The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the ammonia collects, the colour of the receiving solution changes.



V. OBSERVATION & CALCULATION: Total nitrogen was calculated from the formula

Let,

Mass of the organic compound = W g

Volume of the standard acid required for complete neutralization of evolved ammonia = v ml.

Normality of the standard solution of acid = N

From the law of equivalence (normality equation),

1000 ml of 1N acid = 1000 ml NH_3 = 17 g NH_3 = 14 g nitrogen

Then,

V ml of N acid = v ml of NH_3

NV milli equivalent of acid = NV milli equivalent of ammonia

Therefore,

$$\text{Mass of Nitrogen in the evolved Ammonia} = \frac{14 \times N \times V}{1000} \text{ g}$$

Then,

$$\text{Percentage of Nitrogen in the sample} = \frac{14 \text{ NV}}{1000} \times \frac{100}{W} = \frac{1.4 \text{ N V}}{W}$$

Or,

$$\% \text{ of Nitrogen in the sample} = \frac{1.4 \times \text{N of HCl used for complete neutralization of } \text{NH}_3}{\text{Mass of compound taken}}$$

Or,

$$\% \text{ nitrogen in the sample} = \frac{1.4 \text{ V N}}{W}$$

Where,

V=Acid used in titration (ml)

N= Normality of the standard acid (0.1)

W= Weight of the sample (g)

Exp. 4A: Determination of Available Phosphorous

I. AIM: To Determine Available Phosphorus by Dickman & Bray, 1940 method

II. PRINCIPLE: Both inorganic and organic forms of phosphorous occur in soils, both are important to plants as sources of this element, and the relative amounts in the two forms vary greatly from soil to soil (Zhang and Karatbanasis, 1997) The term available Phosphorous (P) refers to the inorganic form occurring in the soil solution, which is almost exclusively orthophosphate. This orthophosphate occurs in several forms and combination, and only a small fraction of the total amount present may be available to plants. The phosphate concentration in solution is governed by heterogeneous equilibrium in which it takes part. The situation can be represented as:

P adsorbed in solid phase <-----> P in soil solution<-----> P Precipitated,

The available P is considered to be a fairly good indicator of the P-supplying capacity of a soil. It exists in various forms in mineral soils, and is equally divided between that in soil organic matter and in various inorganic forms. The phosphorous present in organic form is not extracted easily and therefore is not included in the determination of available phosphorous. The inorganic phosphorous exists primarily as a mixture of phosphates of Al, Ca and Fe tightly bound to the soil particles. The Ca-P is most predominant in the neutral and alkaline soils while the percentages of Al and Fe phosphates is relatively higher in the acidic soils. Plants absorb P as orthophosphates - 4 (H_2PO_4^-) and 2-4 (HPO_4^{2-}) and only a small fraction of the total P present in the soil is available to the plants. The fraction of total phosphorus which can be absorbed by plants during their growth is known as **available phosphorous**.

○ **Extracting agents**

A number of extracting agents have been suggested for the determination of phosphorous. The amount extracted depends on the type of extractant used and also the soil pH. Of these two extracting agents viz., Bray's P-1 extractant (0.03 M NH_4F + 0.025 M HCl) and Olsen's reagent

(0.5 M NaHCO_3 , pH = 8.5) are commonly employed. The former is suitable for acidic soils while the latter works better with neutral and alkaline soils. Both these methods are explained here. You can use one of these methods depending on the nature of the soil. You have learnt to determine the pH (acidity/alkalinity) of the soil in the first experiment of this laboratory course.

○ **Objectives**

After studying and performing this experiment you should be able to:

- State the available form of phosphorous
- State the extractants used for phosphorous determination in soil
- Describe the principle involved and the methods used for determining the amount of available phosphorous in the given soil sample.
- Draw a standard curve for phosphorous using colorimetric method of analysis, and
- Calculate the amount of available phosphorus in the given soil sample.

○ **Bray's P-1 Method**

Principle

In Bray's method a mixture of ammonium fluoride and hydrochloric acid (NH_4F + HCl) act as the extracting agent. It works in two ways, firstly, the hydrogen ions from HCl have a solubilising effect, especially on Ca-P, which makes phosphate get extracted. Secondly, the fluoride ions (from ammonium fluoride) complex with the Al^{3+} and Fe^{3+} ions, thereby releasing the Al and Fe bound phosphorous into the extract. The liberated phosphorous (present as orthophosphates) in solution is treated with ammonium

molybdate under acidic conditions. It forms a complex called ammonium phosphomolybdate. The complex is then treated with a reducing agent like, stannous chloride or ascorbic acid to obtain intense blue colour compound called molybdenum blue. The intensity of colour of this complex is proportional to the concentration of phosphate and can be read with the help of a photoelectric colorimeter at a wavelength of 660 nm or 880 nm depending on the reducing agent used. A number of standard solutions containing known concentration of the phosphorus are prepared. The colour is developed under conditions used for the soil extract and their absorbance is measured at a suitable wavelength. A plot of absorbance as a function of concentration is drawn and the concentration of the phosphorus in the soil extract is found out using this standard curve. This method is suitable for acidic soils and does not give good results with clay soils and calcareous soils. These soils have high degree of base saturation and neutralize the acid in the extractant, thereby reducing its solubilizing effect.

- III. REQUIREMENTS:** Conical flask (100 cm³), Horizontal shaker, Funnel, Whatman No.1 filter paper, Pipette, Volumetric flask (25 cm³, 100 cm³, 1dm³), Photoelectric colorimeter (UV-VIS spectrophotometer), Ammonium fluoride, Hydrochloric acid, Stannous chloride, Ammonium molybdate, Monobasic potassium Phosphate

Solutions Provided

1. Bray's P-1 extractant: Mix 30 cm³ of 1M NH₄F solution (prepared by dissolving 3.7 g of solid ammonium fluoride in water and making up the volume to 100 cm³) and 50 cm³ of 0.5M HCl (prepared by diluting 2.2 cm³ of concentrated HCl to 50 cm³) in a 1 dm³ volumetric flask. Make up the volume with distilled water. Store the solution in a polyethylene bottle. The extractant is stable for about a year.

2. Dickman and Bray's reagent (1.5%): Dissolve 15 g of ammonium molybdate (in powdered form) in about 300 cm³ of distilled water taken in a 1dm³ volumetric flask and warm it to ~ 50°C. Cool and add 350 cm³ of 10 M HCl to it with constant stirring. Make up the volume with distilled water.

3. Stannous chloride (stock solution): Dissolve 10 g of SnCl₂·2H₂O in 25 cm³ of conc. HCl in a 100 cm³ beaker. Warm if necessary. Cool, add a piece of pure metallic tin (or Zn) to it so as to prevent oxidation and store the solution in a glass stoppered brown bottle. The solution is stable for short time.

4. Stannous chloride (working solution): Dilute 1 cm³ of the stock solution to 66.0 cm³ by distilled water just before use.

5. Phosphorous standard (100 mg P dm⁻³): Dissolve 0.4394 g of oven dried (~ 1 hr. at 100°C) monobasic potassium phosphate in some distilled water. Add 25 cm³ of ~ 3.5 M H₂SO₄ (prepared by diluting 5 cm³ of concentrated sulphuric acid to 25 cm³) and make up the volume.

6. Phosphorous standard (working solution, 2 mg P dm⁻³ or 2 ppm P): Pipette out 1 cm³ of 100 ppm standard solution of phosphorous in a 50 cm³ volumetric flask and make up the volume with distilled water.

IV. PROCEDURE

The procedure involves three steps and each step further involves a sequence of activities as per the following details.

➤ **Extraction**

1. Weigh 2 g of air dried soil sample and put into a 50 cm³ extraction vessel or flask.
2. Pipette 20 cm³ of the Bray's P-1 extraction reagent and add to the vessel.
3. In another flask (marked as blank) take 20 cm³ of the Bray's P-1 extraction reagent without the sample of the soil. This would act as a blank or reference. Treat this solution in exactly the same way as for the test solution.
4. Shake the mixture for 5 minutes on a mechanical shaker.
5. Quickly filter the extract through Whatman No. 1 filter paper. Ensure that the filtrate is collected within 10 minutes and save the extract.

➤ **Development of Colour**

1. Transfer 5 cm³ of the filtrate to a 25 cm³ volumetric flask.
2. Add 5 cm³ of ammonium molybdate solution.
3. Gently shake the flask for some time and dilute the mixture to about 20 cm³ with distilled water (add water along the sides of the flask so as to wash them.)
4. Add 1 cm³ of working solution of SnCl₂, shake the solution and make up the volume with distilled water.
5. Transfer an aliquot to a spectrophotometric tube or cuvette and measure the absorbance at 660 nm using a UV-VIS spectrophotometer. Use the 'blank' solution as the reference (for zero absorbance setting). Record the readings in the observation table given below.

➤ **Calibration or Standard Curve**

1. Take six volumetric flasks of 25 cm³ each and label them as 0, 1, 2, 3, 4 and 5 mg respectively.
2. Transfer 0, 0.5, 1.0, 1.5, 2.0 and 2.5 cm³ of the working standard solution of 2 mg dm⁻³ respectively to the flasks labeled above and add 5 cm³ of the extractant to each of them.
3. Develop the colour as with the soil extract. Follow steps 2 to 4 as given under development of colour.
4. Measure the absorbances of these solutions one by one using a spectrophotometer and record them in the table given below. Use blank as the reference.

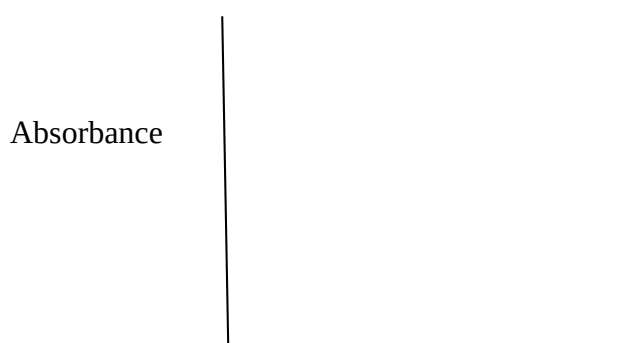
V. OBSERVATIONS AND CALCULATIONS

➤ Observation Table

SL. No	Concentration of phosphorous in standard solution (in mg)	Absorbance (at 660 nm)
1.	1.0	
2.	2.0	
3.	3.0	
4.	4.0	
5.	5.0	
6.	“Z” (soil sample)	

➤ Calculations

- Plot a graph (Calibration curve) between the concentrations of phosphorous (x-axis) in the standard solutions and the corresponding absorbance values (y-axis) as recorded in the observation table. (Column 2 and 3)



Concentration of P (in □g)

- Mark the absorbance reading for the soil sample on the calibration curve and read the corresponding value of concentration on the X- axis.
- The value of available phosphorous (in kg ha⁻¹) can be obtained from the following formula:

$$\text{Available phosphorous (in kg ha}^{-1}\text{)} = \frac{Z \times V \times 2.24}{A \times W}$$

Where

Z = the amount of P (in mg) as read from the graph

V = the volume (in cm³) of the extracting agent used

A = the volume (in cm³) of the aliquot used in colour development

W = the mass (in g) of the soil sample

Substituting the values, we get:

$$\text{Available P} = Z \times 4.48 \text{ kg/ ha}$$

➤ **PROCEDURE:**

- Phosphorus in soil is generally determined as available phosphorus, which can be extracted from soil with 0.002 N H₂SO₄.
- After extraction, the phosphorous is estimated spectrophotometrically by Dickman and Bray [1940] method.
- 10 g of air-dry soil sample was taken in a 500 ml conical flask and 200 ml of 0.002 N H₂SO₄ was added. The suspension was shaken for about half an hour and filtered through Whatman No. 50 filter paper to get a clear solution.
- 2 ml of ammonium molybdate solution and 5 drops of stannous chloride reagent were added to 50 ml of the extract and a blue colour developed. The intensity of the blue colour was measured by using UV-visible spectrophotometer (Peridn-Elmer Lambda EZ 201) at 690 nm.
- A standard curve was prepared with potassium hydrogen orthophosphate solutions in the range of 0.0 to 10 mg dm⁻³ following the same procedure.

➤ **CALCULATION:**

The available phosphorus is given by

$$\text{Pmg/kg} = \frac{\text{mg P/dm}^3 \text{ in soil extract} \times V}{S \ v}$$

Where V = total volume of the soil extract prepared (200 ml)

S = wt of soil taken in gram,

v = volume o f the aliquot taken for analysis (50ml)

Exp. 5(a) : Determination of Available Potassium by Flame Photometric method

- I. **AIM:** To Determine Potassium by Flame Photometric method
- II. **PRINCIPLE:** Ammonium acetate is known to extract the soil exchangeable potassium among other cations. This is rapid procedure for extraction of potassium separately from other exchangeable metallic cations. Potassium adsorbed on the soil particles is exchanged with ammonium of the acetate solution, maximally at the latter's strength.

Potassium can be determined quantitatively when they are atomized from solution, led to burner and excited to spectral emission in a flame. Since the intensity of the light emitted by each element depends primarily on the concentration of its atoms in the flame at any given instant, a measurement of the light intensity produced by a given element makes possible the quantitative determination of that element.

- III. **REQUIREMENTS:** Shaker, plastic bottle with caps, cylinder, beaker, Ammonium acetate 1 N (To 700 – 800 ml distilled water, add 57 ml. conc. acetic acid + 68 ml of conc. ammonium hydroxide. Dilute to a volume of 1 liter and adjust to pH 7.0 by the addition of more ammonium hydroxide or acetic acid, or, The same solution can be also made by dissolving 77 g ammonium acetate in 1 liter dist. water.)

Flame Photometer,

Potassium chloride 1000 ppm (Dissolve 1.9117 g dried KCl in distilled water and make to 1 L volume),

Sodium chloride 1000 ppm (Dissolve 2.5422 g dried NaCl in distilled water and make to 1 L volume),

Standard curve solutions (Prepare the following dilution 10, 20, 30,, 100 ppm from the standard 1000 ppm solution in solution of 1 N ammonium acetate pH 7.0.)

IV. **PROCEDURE:**

➤ **Extraction from Soil**

- i. Add 100 ml of ammonium acetate solution to 5 g air dried soil (passed through 1-2 mm sieve) in a plastic bottle. Place the bottles in the shaker.
- ii. Shake for 1 hour.
- iii. Filtrate through filter paper whatman 41 or S & S 512; M & N 280. Receive in a plastic container. Discard the first third of the filtrate.

➤ **Determination**

- i. Pipet an aliquot of the solution to be analyzed into a 50 ml volumetric flask, complete with 1 N ammonium-acetate solution (pH 7).
- ii. Determine the potassium concentration by use of the flame photometer and the appropriate calibration curve.
- iii. Terminating operations:
- iv. Introduce distilled water for approximately 3 minutes to clean the intake and the atomizer/burner assembly.
- v. Close the valve of supplying gas.
- vi. Push down button of gas selection and wait until the flame is extinguished.

- vii. Switch off main switch.
- viii. Place cover over the housing after allowing sufficient time for cooling off.

V. OBSERVATION & CALCULATION

- (1) Concentration of K or Na in sample-extract can be calculated by slope calculation.
For every concentration of the standard solution (10-100 ppm) the concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope.
- (2) Content of K and Na in soil sample: Content of K or Na as mg/100 g soil (at 105°C).

VI. QUESTIONS:

- 1. What are the sources of K in soil?
- 2. In what form do plants absorb K?
- 3. Name different forms of K present in soil.
- 4. How is plant-available K from the soil extracted?
- 5. How is non-exchangeable K in soil?
- 6. Give K rating of Indian soil.
- 7. Name few common K fertiliser.
- 8. A fertiliser is guaranteed to contain 30% K₂O. To what percentage of K does this correspond?

Exp. 5(b): Determination of Cation Exchange Capacity of Soil

- I. **AIM:** To determine the Cation Exchange Capacity (CEC) of the supplied soil
- II. **PRINCIPLE:** The Cation Exchange Capacity of a soil is a measurement of its ability to bind or hold exchangeable cations. In other words it is a measure of the number of negatively-charged binding sites in the soil.
- The cation exchange capacity (CEC) of a soil is a measure of the quantity of negatively charged sites on soil surfaces that can retain positively charged ions (cations) such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}), by electrostatic forces. Cations retained electrostatically are easily exchangeable with cations in the soil solution so a soil with a higher CEC has a greater capacity to maintain adequate quantities of Ca^{2+} , Mg^{2+} and K^{+} than a soil with a low CEC. A soil with a higher CEC may not necessarily be more fertile because a soil's CEC can also be occupied by acid cations such as hydrogen (H^{+}) and aluminum (Al^{3+}). However, when combined with other measures of soil fertility, CEC is a good indicator of soil quality and productivity.
- Cation exchange sites are found primarily on clay minerals and organic matter (OM) surfaces. Soil OM will develop a greater CEC at near-neutral pH than under acidic conditions (pH-dependent CEC). Thus, addition of an organic material will likely increase a soil's CEC over time. On the other hand, a soil's CEC can decrease with time as well, through natural or fertilizer-induced acidification and/or OM decomposition.
- Soil CEC is normally expressed in one of two numerically equivalent sets of units: meq/100 g (milliequivalents of charge per 100 g of dry soil) or cmolc/kg (centimoles of charge per kilogram of dry soil).
- III. **REQUIREMENTS:**
- i) Flame photometer,
 - ii) Mechanical shaker, reciprocating,
 - iii) Centrifuge (capable of 3000 rpm), Conical centrifuge tubes (50 mL),
 - iv) Sodium Acetate Solution :1 N (Dissolve 136 g sodium acetate trihydrate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) in about 950 mL DI water, mix well, and let the mixture cool. Adjust pH to 8.2 by adding more acetic acid or sodium hydroxide, and bring to 1-L volume with DI water.),
 - v) Ethanol 95% ($\text{C}_2\text{H}_5\text{OH}$),
 - vi) Ammonium Acetate Solution :1N (Add 57 mL concentrated acetic acid (CH_3COOH) to 800 mL DI water, then add 68 mL concentrated ammonium hydroxide (NH_4OH), mix well, and let the mixture cool. Adjust to pH 7.0 by adding more acetic acid or ammonium hydroxide, and bring to 1-L volume with DI water),
 - vii) Standard Stock Solution (Dry about 5 g sodium chloride (NaCl) in an oven at 105°C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle. Dissolve 2.5418 g dried sodium chloride in DI water, and bring to 1-L volume with DI water. This solution contains 1000 ppm Na (Stock Solution).

- viii) Prepare a series of Standard Solutions from the Stock Solution as follows: Dilute 2, 4, 6, 8, 10, 15, and 20 mL Stock Solutions to 100 mL final volume by adding 1 N ammonium acetate solution. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm Na.)

IV. PROCEDURE:

- i) Weigh 4 g (for medium to fine textured) or 6 g (for coarse textured) air-dry soil into a 40 mL centrifuge tube, and add 33 mL 1 N sodium acetate trihydrate solution, stopper tube, and shake for 5 minutes.
- ii) Remove stopper from tube and centrifuge at 3000 rpm until supernatant liquid is clear. Decant the supernatant as completely as possible and discard.
- iii) Repeat with 33-mL portions 1 N sodium acetate trihydrate solution, a total of four times, discarding the supernatant liquid each time. Then add 33-mL 95% ethanol to the stopper tube, and shake for 5 minutes, unstopper tube, and centrifuge until the supernatant is clear and decant.
- iv) Wash the sample with 33 mL portions 95% ethanol, a total of three times, discarding the supernatant liquid each time. The electrical conductivity (EC) of the supernatant liquid from the third washing should be less than 400 $\mu\text{S}/\text{cm}$.
- v) Replace the adsorbed sodium (Na^+) from the sample by extraction with three 33-mL portions 1 N ammonium acetate solution. Each time shake for 5 minutes, and centrifuge until supernatant liquid is clear.
- vi) Decant the three supernatant liquids as completely as possible into a 100-mL volumetric flask, bring to volume with 1 N ammonium acetate solution, and mix well.
- vii) Run a series of suitable Na standards, and draw a calibration curve.
- viii) Measure the samples (soil extract) and take the emission readings by a Flame Photometer.
- ix) Calculate sodium (Na) concentration according to the calibration curve.

V. OBSERVATION & CALCULATIONS:

For Cation Exchange Capacity in soil:

$$\text{CEC (meq/100 g)} = \text{meq/L Na (from calibration curve)} \times \frac{A}{Wt} \times \frac{100}{1000}$$

Where: A = Total volume of the extract (ml)

Wt = Weight of the air-dry soil (g)

Comments

1. CEC is an indicator of the nutrient storage capacity of soil.
2. In the reclamation of sodic (alkali) soil, the principle is to treat them with gypsum which is the sum of Ca^{2+} , so that a major portion of exchangeable Na is replaced with Ca^{2+} .
3. When potential pollutants, such as Pb (Pb^{++}) or cadmium (Cd^{++}), etc., are disposed on soil surface, instead of leaching easily, they are absorbed to the cation exchange sites.

VI. QUESTIONS:

1. What is CEC?
2. Is there any relationship between CEC and % base saturation?
3. Which soil has high CEC sandy soils or clay soils—why?
4. List the different soil cations.
5. CEC is an important indicator of storage capacity of soils

EXPERIMENT NO. 6

Determination of Bioavailable and Total Heavy Metals in soil.

- I. AIM:** To determine of Bioavailable Metals in soil.
- II. PRINCIPLE:** Of the seven essential micronutrients, the plant abundance/requirements are as follows: $\text{Fe} > \text{Mn} > \text{B} > \text{Zn} > \text{Cu} > \text{Mo} > \text{Cl}$. These elements if present, greater than the threshold limits, become toxic. The other metals, which are not essential for plant growth, also present in soil or are added by anthropogenic activities such as the following: arsenic (As), cadmium (Cd), Cobalt (Co), Chromium (Cr), Mercury (Hg), Nickel (Ni), Lead (Pb), Selenium (Se), etc. The DTPA (diethylenetriaminepentaacetic acid) micronutrient extraction method is a non-equilibrium extraction for estimating the potential soil availability of Zn, Cu, Mn, and Fe. It has been used for cadmium, nickel and lead in soils. The method has shown to be well correlated to crop response to fertilizer for zinc and copper. The amount of micronutrients and trace metals extracted are affected by solution pH, temperature, soil extraction ratio, shaking time, extraction time, and extractant concentration. Extracts are analyzed by AAS.
- III. REQUIREMENTS:**
- i) Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ -1.47 g/L),
 - ii) DTPA (Diethylene triamine pentaacetic acid -1.967 g/L),
 - iii) TEA (Triethanolamine - 14.92 g/L),
 - iv) Dilute HCl (AR HCl diluted with double distilled water 1:5)—use for pH adjustment,
 - v) DTPA (Diethylene triamine pentaacetic acid) extracting solution—0.005 M DTPA, 0.01 M CaCl_2 and 0.1 M TEA adjusted to pH 7.30 with HCl: take 1-L volumetric flask. Dissolve 1.967 g DTPA, 14.92 g TEA and 1.47 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in approximately 200 mL of double distilled water. Allow sufficient time to dissolve DTPA and add 800-mL double distilled water. The pH of the solution is adjusted to 7.3 with dilute HCl while stirring. This solution is stable for several months. (DTPA is acting as a chelating agent; the pH of 7.3 buffered with TEA is used to prevent excess dissolution of trace metals, which is highly pH dependent. In the presence of 0.01-M CaCl_2 , enable the extract to attain equilibrium with CaCl_2 , which minimizes the dissolution of CaCO_3 from calcareous soils.)

IV. PROCEDURE: Take 10 g of air-dried soil in a 100-mL conical flask, add 20-mL DTPA extracting solution and shake for 2 h. After exactly 2 h of shaking, filter the suspension by gravity through Whatman filter no. 42. The time of shaking is very important because extraction is not complete before 2 h. Determine Pb, Cu, Zn, Mn, Ni, Cr, Cd and Fe in ASS.

V. OBSERVATION & CALCULATION:

Concentration of DTPA-extractable elements are as follows: (in ppm)

$$= 20 \times \frac{(\text{ppm of elements in sample solution} - \text{ppm in blank solution})}{\text{wt of air-dried sample, g}}$$

VI. QUESTIONS:

1. List the essential micronutrients required for plant growth.
2. List the important toxic metals added to the soil by anthropogenic activities.
3. What is the mechanism of DTPA extraction of metals?

Exp. 6: Determination of Total Heavy Metals in Soil (contd...)

I. AIM: To determine of Total Heavy Metals in soil.

II. PRINCIPLE: Heavy metals are considered to be one of the main sources of pollution in the environment, because of their significant effect on the ecological quality. The main sources of heavy metal pollution in the environment are man-made effects, including combustion of fossil fuels, mining activities, wastewater discharges of manufacturing industries, and waste disposal. High levels of heavy metals in the sediments and soils may pass to the aquatic environment, groundwater, and plants through the transfer processes and reach to the animals and humans. Therefore, the use of simple and accurate methods for monitoring heavy metals has a great importance among the environmental studies.

III. REQUIREMENTS:

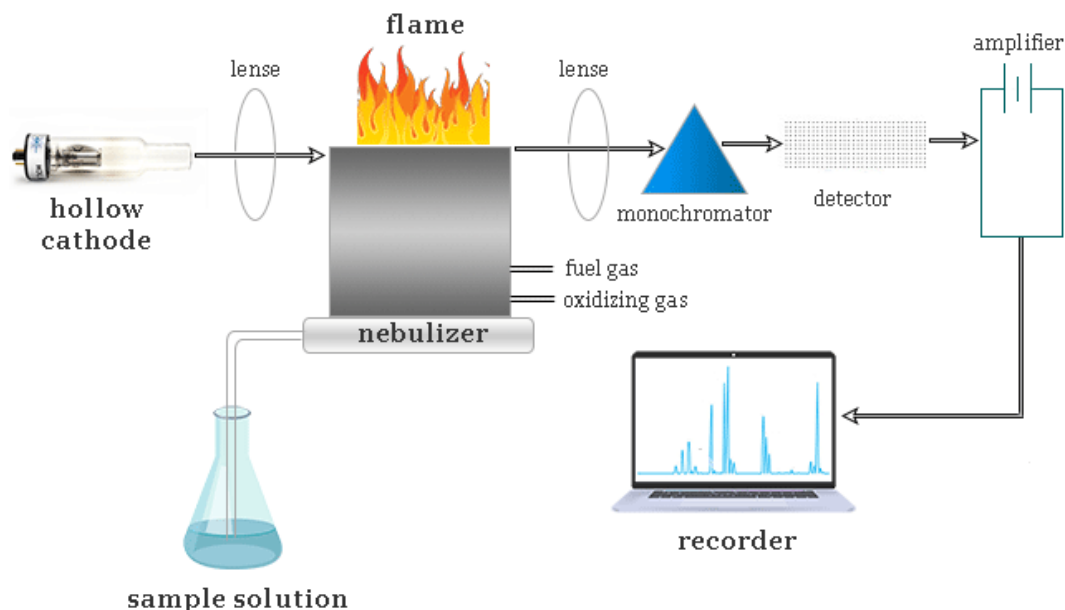
- i) Atomic Absorption Spectrophotometer (AAS),
- ii) Digestion Vessels – 250 mL,
- iii) Vapor recovery device (appropriate refluxing device),
- iv) Drying ovens (able to maintain 30°C + 4°C),
- v) Temperature measurement device (capable of measuring to at least 125°C with suitable precision and accuracy (e.g., thermometer, IR sensor, thermocouple, etc.)
- vi) Filter paper (Whatman No. 41 or equivalent),
- vii) Centrifuge and centrifuge tubes,

- viii) Analytical balance - capable of accurate weighing's to 0.01 g,
- ix) Heating source - Adjustable and able to maintain a temperature of 90-95°C. (e.g., hot plate, block digester, microwave, etc.),
- x) Funnel or equivalent,
- xi) Graduated cylinder or equivalent volume measuring device,
- xii) Volumetric Flasks – 50 mL and
- xiii) Nitric acid (69%),
- xiv) Hydrogen peroxide (30%)

IV. PROCEDURE:

- i. Mix the sample thoroughly to achieve homogeneity and sieve, if necessary. For each digestion procedure, weigh to the nearest 0.01 g and transfer a 1-2 g sample (wet weight) or 1 g sample (dry weight) to a digestion vessel. For samples with high liquid content, a larger sample size may be used as long as digestion is completed.
- ii. For the digestion of samples for analysis by AAS, add 10 mL of 1:1 HNO₃, mix the slurry, and cover with a watch glass or vapour recovery device. Heat the sample to 95°C ± 5°C and reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 mL of concentrated HNO₃, replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO₃, repeat this step (addition of 5 mL of conc. HNO₃) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO₃. Use a watch glass or vapour recovery system, either allow the solution to evaporate to approximately 5 mL without boiling or heat at 95°C ± 5°C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.
- iii. After the previous step has been completed and the sample has cooled, add 2 mL of water and 3 mL of 30% H₂O₂. Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the vessel.
- iv. Continue to add 30% H₂O₂ in 1 mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged.
- v. Cover the sample with a ribbed watch glass or vapour recovery device and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL or heat at 95°C ± 5°C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.
- vi. After cooling, dilute to 50 mL with water. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle. The sample is now ready for analysis by AAS.

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Filtration - Filter through Whatman No. 41 filter paper (or equivalent).

Centrifugation- Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

The diluted digestate solution contains approximately 5% (v/v) HNO_3 . For analysis, withdraw aliquots of appropriate volume and add any required reagent or matrix modifier.

The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

If percent solids is desired, a separate determination of percent solids must be performed on a homogeneous aliquot of the sample.

V. QUESTIONS:

1. Name the important sources of Cr and Ni in soil
2. 'Translocation of heavy metals to the plants is governed by soil pH'-why?
3. 'Increasing of organic matter in soil helps immobilization of Ni'- how?
4. Why is Cd considered to be a serious soil pollutant?

EXPERIMENT NO. 7

Culture media preparation– Semi-synthetic and Synthetic media. Liquid, Solid and semisolid media, Nutrient agar, PDA media.

- I. **AIM:** To study the different types of culture media for the growth of microorganisms.
- II. **PRINCIPLE:** The food materials on which the organism is grown is known as culture medium and the growth of organism is known as culture. Different microorganisms require different nutrient materials. Thus, culture media vary in form and composition, depending upon the species to be cultivated. It must contain all the ingredients required by the organism and in certain proportions. Basically there should be an energy source, various macro and micronutrients, vitamins etc. it must have a suitable pH. Moreover, it must be sterile so that the organism cultivated may form a pure culture. A culture is an in vitro technique of growing or cultivating microorganisms or only other cells in a suitable nutrients medium called culture medium in the laboratory. The primary aim of constructing a culture medium for any microorganism is to provide a balanced mixture of required nutrients, at concentrations that will permit good growth. Culture media give artificial environment simulating natural conditions necessary for growth.

Basic Requirements of culture medium are: Energy source, Carbon source, Nitrogen source, Salts, pH, Adequate oxidation, Growth factor.

➤ ***Classification on the basis of physical state media can be classified as:***

- Liquid Media
- Semi Solid Media
- Solid Media

a) Liquid Media or Broth

No solidifying agents (e.g.: agar) is added while preparing the medium. The most commonly used non-synthetic liquid media are nutrient broth, peptone solution, milk, blood, serum etc. broth is a clear transparent straw coloured fluid prepared from meat extract or peptone. Beef infusions are rich in minerals, organic micronutrients, protein, protein derivatives and carbohydrates. They are often supplemented with 1% peptone or yeast extract culture fluids made from beef infusion are commonly called infusion broth, where as those made from beef extract are called extract broth.

➤ ***Advantages of Liquid Media***

- For obtaining bacterial growth from blood or water when large volumes have to be tested.
- For preparing bulk cultures for preparation of antigens or vaccines.

- It's used to study growth rate and the sedimentation rate of bacterial cells.

➤ **Disadvantages of Liquid Media**

- It's difficult to isolate different types of bacteria from mixed population.
- It's difficult to study colony characteristics.



b) Semi-Solid Media

The semi-solid medium remains in the semi-solid condition. It is prepared by adding small amount of agar (0.5%) or gelatine. Agar is a complex carbohydrates prepared from algae like gelidium and gracillaria. Agar forms a colloidal solution in hot water and sets in the form of a jelly when cool. Gelatine is an animal extract prepared by hydrolysis of collagen with boiling water. When dissolved in water, it's in the form of a liquid when warm and sets in the form as it cools. Many bacteria, when grown on a gelatine medium, produce a digestive enzyme gelatinase, which liquefies gelatine. This feature is important in the identification and classification of bacteria. The semi-solid medium may be selective which promotes the growth of one organism and retards the growth of another organism. This type of medium can be used to study bacterial motility (semi solid media are useful for cultivation of microaerophilic bacteria).

c) Solid Media

The solid medium is solid in consistency. It is prepared by adding 2% or 1% gelatine; agar or silica gel or sometimes an inorganic solidifying agent for autotrophic bacteria. It's used for colony characterization, colony identification, etc.

➤ **Classification of culture media on the basis of composition:**

a) **Synthetic or chemically defined medium**

A chemically defined medium is one prepared from purified ingredients and therefore its exact composition is known.

b) **Non synthetic or chemically undefined medium**

Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts.

➤ ***Classification of bacterial culture media on the basis of purpose/functional use/application:***

a) **General purpose media/Basic media**

Basic media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar (NA) are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

b) **Enriched medium**

Addition of extra nutrients in the form of blood, serum, egg yolk etc., to basal medium makes enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc., are few of the enriched media.

c) **Selective and enrichment media**

They are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

Examples of selective media

- ***Mannitol Salt agar and Salt Milk Agar*** used to recover *S. aureus* contains 10% NaCl.
- ***Potassium tellurite medium*** used to recover *C. diphtheria* contains 0.04% potassium tellurite.
- ***MacConkey's Agar (Cetrimide Agar)*** used to recover *E. coli* contains cetrimide (antiseptic agent).
- ***Crystal Violet Blood Agar*** used to recover *S. pyogens* contains 0.0002% crystal violet.

Preparation of Liquid Medium (broth) for Routine Microbiological Work

REQUIREMENTS: Nutrient Broth Medium (Peptone: 5.0 g; Beef Extract : 3.0 g; Distilled Water: 1 L), pH Meter, Distilled Water, Autoclave, Culture Tubes, Glass Rod, Measuring Cylinder.

PROCEDURE:

- Accurately weigh the chemical ingredients of the nutrient broth and transfer them to a beaker containing 500 ml distilled water.
- Gently heat the contents with slight agitation to dissolve the ingredients.
- Add more distilled water to make the volume to 1 litre.
- Measure pH of the growth using a pH meter and adjust the pH to 7.0 adding drops of either HCL or NaOH solution.
- Dispense 10 ml broth to each culture tubes.
- Close the mouth of broth tubes with the help of cotton plug or autoclavable caps.
- Transfer the broth tubes inside the autoclave and sterilize at 121 degree Celsius for 30 minutes.
- When temperature cools down check out the broth tubes.
- Use the broth tubes when required or store at room temperature for the use.

AIM: Preparation of Solid Media for Routine Microbiological Work

REQUIREMENTS: Nutrient Agar, pH Meter, Distilled Water, Autoclave, Culture Tubes, Glass Rod, Measuring Cylinder

PROCEDURE:

- Carefully measure 28 grams of Nutrient Agar(s) media using spatula and weighing balance and transfer it into separate 1.5 L conical flask.
- Measure 1000ml of distilled water using measuring cylinder and transfer to the conical flask containing the culture media.
- Close the mouth of the conical flasks using cotton plug, cover with aluminium foil to prevent from getting wet during autoclaving and label them.
- Wrap petri dishes in aluminum foil and along with culture media transfer them to autoclave.
- Autoclave the content at 15 lbs. pressure (121°C) for 15 minutes.
- Allow the media to cool a bit and take the nutrient agar media and plates to laminar flow chamber.
- When temperature drops to around 40°C pour the nutrient agar media in sterilized petri dishes.
- Allow cooling with lid partially closed until the media solidifies.
- After cooling to room temperature media is ready to use.

OBSERVATION: Carefully observe the color and change in transparency of media when it cools and solidifies.

RESULT AND DISCUSSION: The solid nutrient agar media has been prepared for the bacterial culture. Discuss what kind of microbes can grow on the media.

QUESTIONS

- i. A student adds HgCl_2 in the nutrient broth media and cultures soil bacteria in it to obtain mercury resistant species. What class of media can this specific nutrient broth be classified as?
- ii. What are the advantages of using a solid media?
- iii. What kind of media is potato dextrose agar media? What is the use of such media?

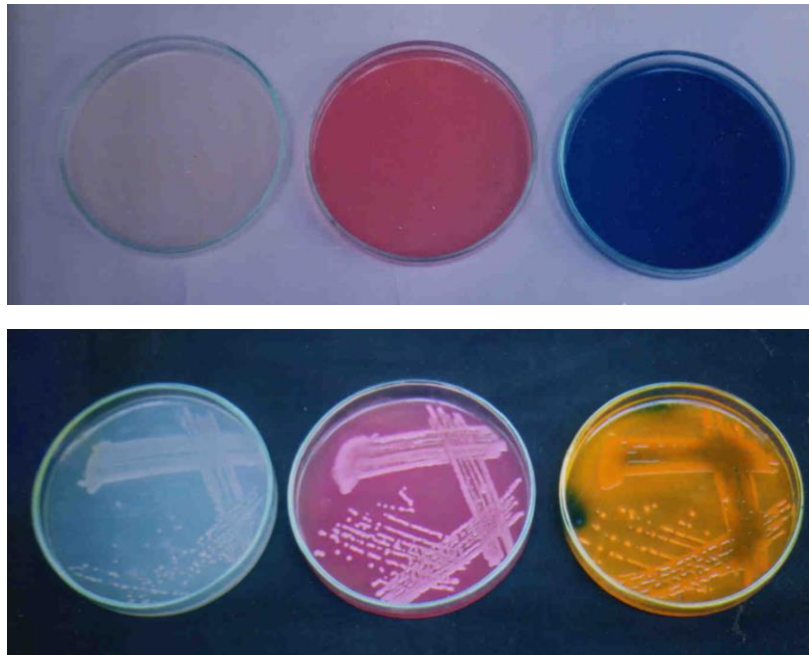


Fig. L-R; Nutrient agar, MacConkey's agar, Bromothymol blue agar

EXPERIMENT NO. 8

- I. **AIM:** Gram staining techniques for detection of gram positive and gram-negative bacteria.
- II. **REQUIREMENTS:** Glass slides, Bunsen burner, cotton, sample, microscope, crystal violet dye, iodine, alcohol (95% ethyl alcohol), and safranin dye.
- III. **PRINCIPLE:** Gram staining is a most widely used staining technique used in examination of microorganisms. It was discovered by Danish scientist and physician Hans Christian Joachim Gram in 1884. This technique differentiates bacteria in 2 groups' i.e., Gram positive and Gram negative bacteria. The procedure is based on the ability of microorganisms to retain colour of the stain during Gram reaction. Gram negative bacteria are decolorized by alcohol losing the colour of primary stain, purple. After the decolorization step, a counter stain is used to impart pink colour to the gram negative microorganism. Gram positive bacteria have a thick mesh like cell wall which is made up of peptidoglycan (50-90%) of cell wall which stain purple. Gram negative bacteria have a thinner layer of peptidoglycan (10% of cell wall) and lose the crystal violet iodine complex during decolourisation with alcohol rinse but retain the counter stain safranin thus appearing reddish or purple. The gram positive and negative bacteria are significantly different on basis of their cell wall structure, which is exploited for their differentiation.

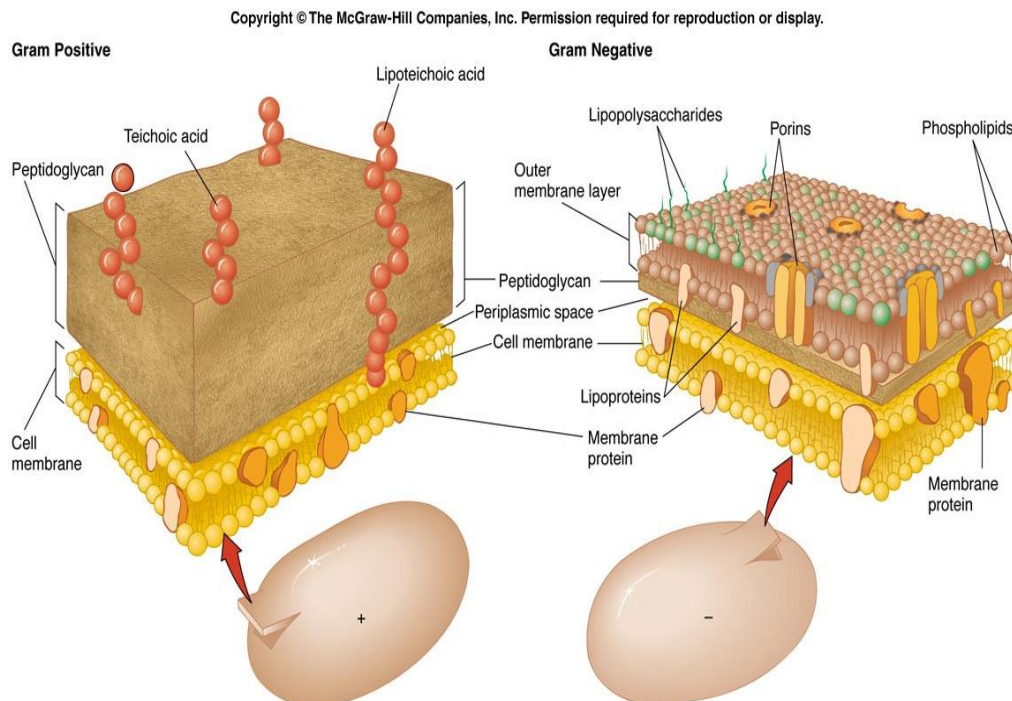


Fig. Bacterial cell wall composition

Stain Reactions:

1. Application of crystal violet(CV) to heat fixed smear:

CV dissociates in aqueous solution into CV⁺ and Cl⁻ ions. These two penetrate the cell wall and cell membrane of both gram positive and gram negative. CV⁺ interact with negative component of bacterial cell and stain it purple.

2. Addition of gram iodine:

Iodine acts as a mordant and a trapping agent. A mordant is a substance that increase the affinity of cell wall for a stain by binding to primary stain, thus forming an insoluble complex that get trapped in cell. During the reaction CV-I complex is formed and all the cells turn purple.

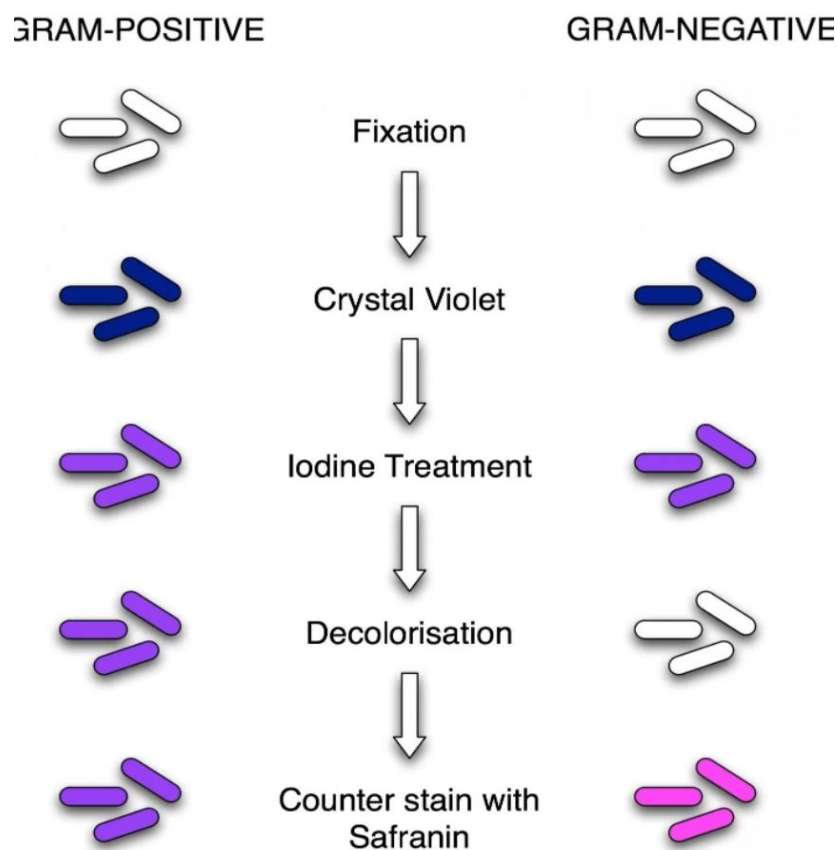


Fig: steps of gram staining
(Source: microbiologynote.com)

3. Decolourization with ethyl alcohol:

Alcohol dissolve the lipid outer membrane of gram negative bacteria, thus leaving the peptidoglycan layer exposed and increase the porosity of cell wall. The CV-I complex is then washed away from the peptidoglycan layer leaving gram negative bacteria colourless. In gram positive bacteria, alcohol has dehydrating effect on cell wall causing

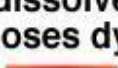
cell wall to shrink, then CV-I complex get tightly bound into multi layered leaving the cell with purple colour.

4. Counter stain with safranin dye:

The decolourized gram negative cell can be visible with a suitable counter stain which is usually positively charged safranin which is stained in pink.

IV. PROCEDURE:

- Prepare very thin smear of sample on glass slide and heat fix it.
- Flood the smeared slide with crystal violet dye. Avoid over flooding and keep it for 1 minute.
- Wash the slide under running tap water.
- Apply iodine solution gently all over the slide and keep for 1 min.
- Wash it under tap water.
- Apply 95% ethyl alcohol all over the slide dropwise and keep it for 10sec.
- Immediately rinse with water.
- Finally, flood the sample with safranin dye to counter stain and keep for 45mins.
- Wash the slide with running water.
- Observe it under microscope.

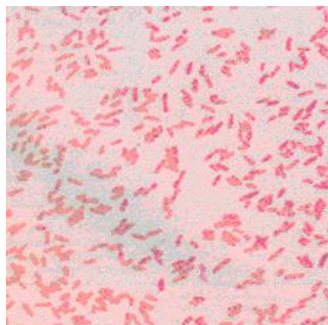
	Microscopic Appearance of Cell		Chemical Reaction in Cell Wall (very magnified view)	
Step	Gram (+)	Gram (-)	Gram (+)	Gram (-)
1. Crystal violet				
2. Gram's iodine				
3. Alcohol				
4. Safranin (red dye)				

V. OBSERVATION: Report your observation on the basis of the colour of the bacteria present. Also observe the shape of the microbes and their association.

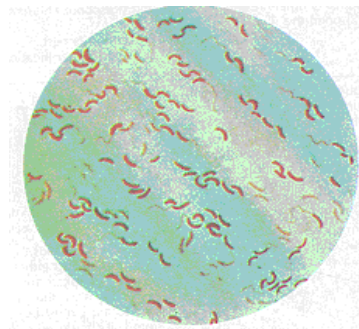
VI. RESULTS & DISCUSSION: Report the gram nature of the bacteria as Gram positive or negative along with observed shape. Discuss the reason for the role of test in identifying bacteria.



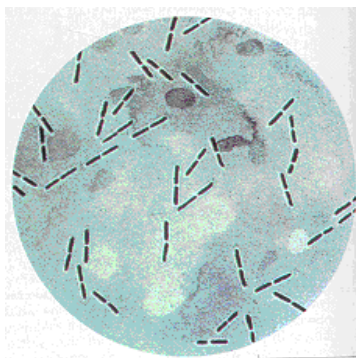
Gram + rod



Gram – rod



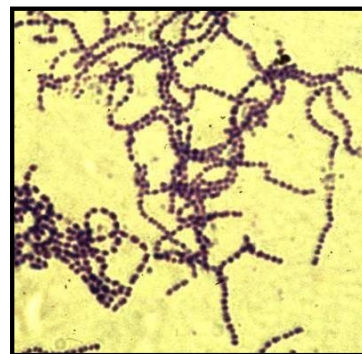
Vibrio



Diplo bacillus



Staphylococcus



Streptococcus

➤ **Precautions:**

- Do not overheat the smear for heat fixing the bacteria on the slide.
- Do not touch the surface of the slide after the preparation of the smear.
- Avoid over staining by reducing the time of exposure to stains if necessary.

VII. QUESTIONS:

- Differentiate between gram positive and gram negative bacteria.
- Can we skip the step where the bacterial smear is washed with alcohol? Why?
- Can we identify the figure as cell wall of gram positive and negative strain?

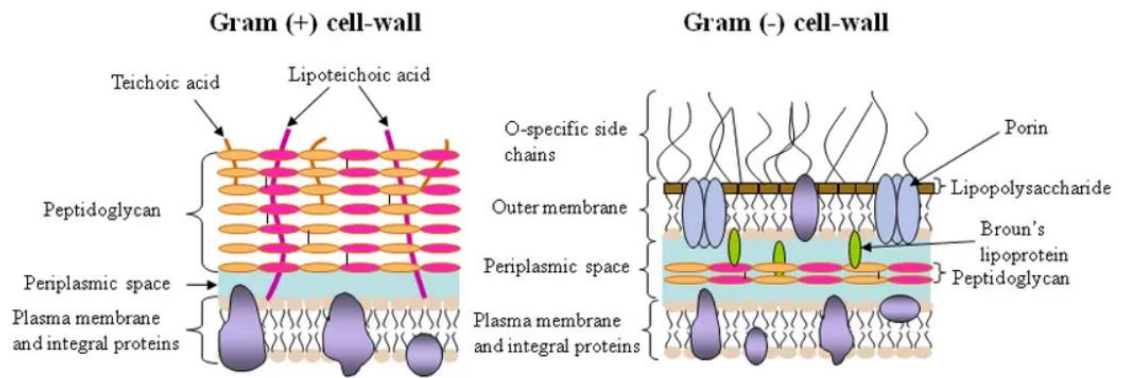


Fig: difference between the cell wall of gram positive and gram negative bacteria.
Source: microbiologynote.com

EXPERIMENT NO. 9

- I. AIM:** Study of fungi (medium – Rose Bengal agar), enumeration and identification of fungal mycelium and spores by Lactophenol cotton blue mounting method.
- II. Principle:** Fungi are eukaryotic organisms and are classified into two main groups that is yeast and molds. Its cell wall is made up of chitin. Fungal cells have both macroscopic as well as microscopic structure. This microorganism is useful as well as harmful to human being. It is useful because as it produces many antibiotics, natural products as well as used in industrial fermentation process. It is harmful in the sense as it causes human diseases, produce toxic substances as well as harm important crops. So it is very important to study fungal species. The branch of science that deals with study of fungal species is called as Mycology. Staining of these fungal cells is a very important step. Some examples of fungi are *Rhizopus*, *Aspergillus*, *Penicillium*, *Fusarium*, *Candida*, *Mucor* etc. Here we are going to stain fungal cells by using Lactophenol cotton blue method. This staining technique is also called as mounting of fungus.

The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components:

1. **Phenol:** kills any live organisms;
2. **Lactic acid :** It preserves fungal structures, and
3. **Cotton blue:** It stains the chitin in the fungal cell walls.

Lactophenol Cotton Blue Solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue.

- III. REQUIREMENTS:** Young fungal culture, Glass slide, Cover slip, Needle, Lactophenol cotton blue, Phenol, Cotton Glycerol, Lactic Acid, Deionized Water

IV. PROCEDURE:

➤ **Preparation of lactophenol cotton blue (LPCB) slide mounts**

1. Place a drop of seventy percent alcohol on a clean microscope slide.
2. Material from cultures of filamentous fungi should be removed using a stiff inoculating wire not the loop used for manipulations with bacteria or yeasts.
3. Flame the wire by holding it upright in the hottest part of the Bunsen flame, just above the blue cone, until the whole length of the wire glows red hot.
4. You must ensure that the inoculating wire has cooled before placing it in a fungal culture – it should have cooled sufficiently after approximately ten seconds.
5. Remove the cap from the tube but do not put it on the bench. Kill any contaminating microorganisms by flaming the neck of the tube.
6. Remove a small amount of the culture. For fungal cultures, it is often useful to take a little of the agar medium together with the fungus. In any case, the material should be disturbed as little as possible when being transferred to the slide.
7. Flame the neck of the tube once more and replace the cap.
8. Immerse the fungal material in the drop of seventy percent alcohol. This drives out the air trapped between the hyphae.

9. Tease out the material very gently with mounted needles.
10. Do not forget to sterilise the inoculating wire and the needles after use by heating to red heat in a Bunsen flame
11. Fungal structures are readily visualised after staining with a lactophenol cotton blue dye preparation.
12. Before the alcohol dries out add one or at most two drops of the stain. A common fault is to add too much to the preparation. Holding the coverslip between your index finger and thumb, touch one edge of the drop of stain with the edge of the coverslip.
13. Lower the coverslip gently onto the slide, trying to avoid air bubbles. Your preparation is now ready for examination.
14. Make the initial examination using a low power objective lens. The thinner parts of the preparation, generally around the edges of the mounted material, will yield the best images.
15. Switch to a higher power 40X objective for more detailed examination of spores and other structures.

V. OBSERVATION: After staining fungus cell we can observe different parts of a cell like conidia, hyphae as well as conidiophore etc.

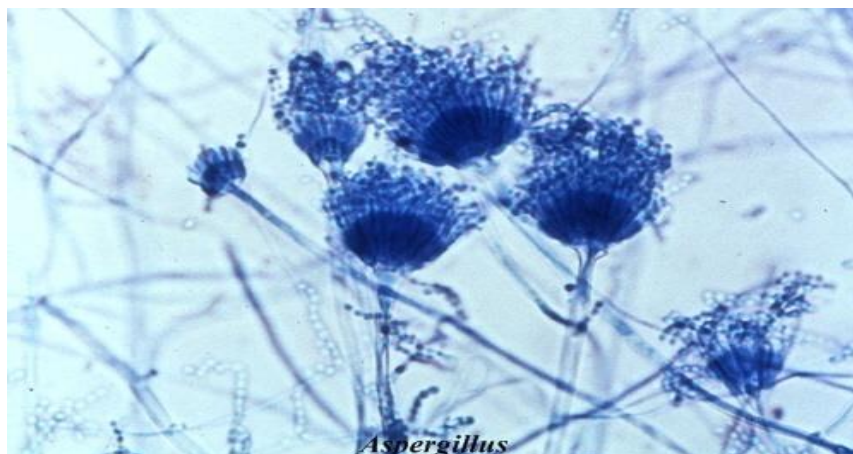


Fig: Blue stained fungi

VI. RESULTS AND DISCUSSION: Observed the slide(s) under low and high power objectives of a microscope and note the types of conidia, conidiospore, hyphae and their arrangement. Identify the fungi following the mycological literature.

VII. QUESTIONS:

- i. What is the difference between stain and dye?
- ii. Which stain is used for fungi? What is its chemical composition?

EXPERIMENT NO. 10

- I. AIM:** Bacteriology of drinking water and domestic sewage -MPN techniques for total coliform, Faecal coliform and Faecal Streptococci (FS), Membrane filtration techniques for faecal coliform and total coliform.
- II. THEORY:** The presence of bacteria and pathogenic (disease-causing) organisms is a concern when considering the safety of drinking water. Pathogenic organisms can cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera, and other illnesses.

Human and animal wastes are a primary source of bacteria in water. These sources of bacterial contamination include runoff from pastures and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities, and natural soil/plant bacteria. Bacteria from these sources can enter wells that are either open at the land surface or do not have water-tight casings or caps.

Coliform bacteria have been used to evaluate the general quality of water. Testing for coliform bacteria is faster and cheaper than testing for specific organisms and pathogens. U.S. Public Health Service established a standard in 1914. Coliforms include all aerobic and facultative anaerobic, gram-negative, non-spore forming bacilli that, when incubated at 35° C, can ferment lactose and produce gas (CO₂) within 48 hrs. Faecal coliforms are the coliform bacteria that originate specifically from the intestinal tract of warm-blooded animals (e.g., humans, beavers, racoons, etc.).

Most probable number (MPN) analysis is a statistical method based on the random dispersion of microorganisms per volume in a given sample. In this method, measured volumes of water is added to a series of tube containing a liquid indicator growth medium. The media receiving one or more indicator bacteria show growth and a characteristic color change. Color change is absent in those receiving an inoculum of water without indicator bacteria. From the number and distribution of positive and negative reactions, the MPN of indicator organisms in the sample may be estimated by reference to statistical tables

III. Materials Required:

- **Medium:** Lactose broth or Mac Conkey Broth or Lauryl tryptose (lactose) broth
- **Glass wares:** Test tubes of various capacities (20 ml, 10 ml, 5 ml), Durham tubes, Micropipette, Autoclave, Laminar Air Flow, Incubator

- IV. Principle:** Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilize the lactose present in the medium to produce acid and gas. The presence of acid is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction (*i.e. both color change and gas production*) and comparing the pattern of positive results (*the number of tubes showing growth at each dilution*) with standard statistical tables.

Multiple Tube Fermentation technique for Total Coliform & Faecal Coliform Test is performed in 3 steps

1. Presumptive test

The presumptive test, is a screening test to sample water for the presence of coliform organisms. If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe. If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction.

2. Confirmatory test

Some microorganisms other than coliforms also produce acid and gas from lactose fermentation. In order to confirm the presence of coliform, confirmatory test is done.

3. Completed test

V. METHODOLOGY:

1. Presumptive test:

Prepare medium (either mac Conkey broth or Lactose broth) in single and double strength concentration.

For untreated or polluted water:

Dispense the double strength medium in 10 tubes (10ml in each tube) and single strength medium in 5 tubes (10 ml in each tube) and add a Durham tube in inverted position.

For treated water:

Dispense the double strength medium in 5 tubes (10ml in each tube) and 50 ml single strength medium in 1 bottle and add a Durham tube in inverted position.

Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.

Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

➤ For untreated water

1. Take 5 tubes of double strength and 10 tubes of single strength for each water sample to be tested.
2. Using a sterile pipette add 10 ml of water to 5 tubes containing 10 ml double strength medium.
3. Similarly add 1 ml of water to 5 tubes containing 10 ml double strength medium and 0.1 ml water to remaining 5 tubes containing 10 ml double strength medium.
4. Incubate all the tubes at 37°C for 24 hrs. If no tubes appear positive re-incubate up to 48 hrs.

- Compare the number of tubes giving positive reaction to a standard chart and record the number of bacteria present in it.

For example: a water sample tested shows a result of 3–2–1 (3 × 10 ml positive, 2 × 1 ml positive, 1 × 0.1 ml positive) gives an MPN value of 17, i.e. the water sample contains an estimated 17 coliforms per 100 ml.

➤ **For treated water**

- Take 1 tube of single strength (50ml) and 5 tubes of double strength (10ml) for each water sample to be tested.
- Using a sterile pipette add 50 ml of water to the tubes containing 50 ml single strength medium.
- Similarly add 10 ml of water to 5 tubes containing 10 ml double strength medium. Incubate the tubes at 37°C for 24 hrs. If no tubes appear positive re-incubate up to 48 hrs.
- Compare the number of tube giving positive reaction to a standard chart and record the number of bacteria present in it.

For example: a water sample tested shows a result of 1-4 (1 × 50 ml positive, 4 × 10 ml positive) gives an MPN value of 16, i.e. the water sample contains an estimated 16 coliforms per 100 ml

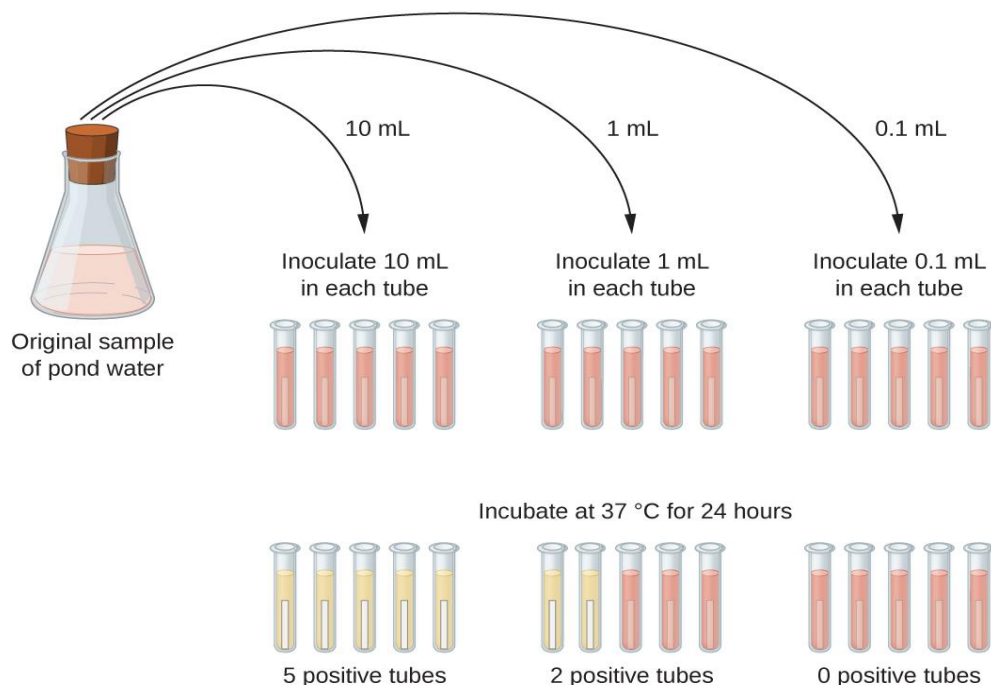
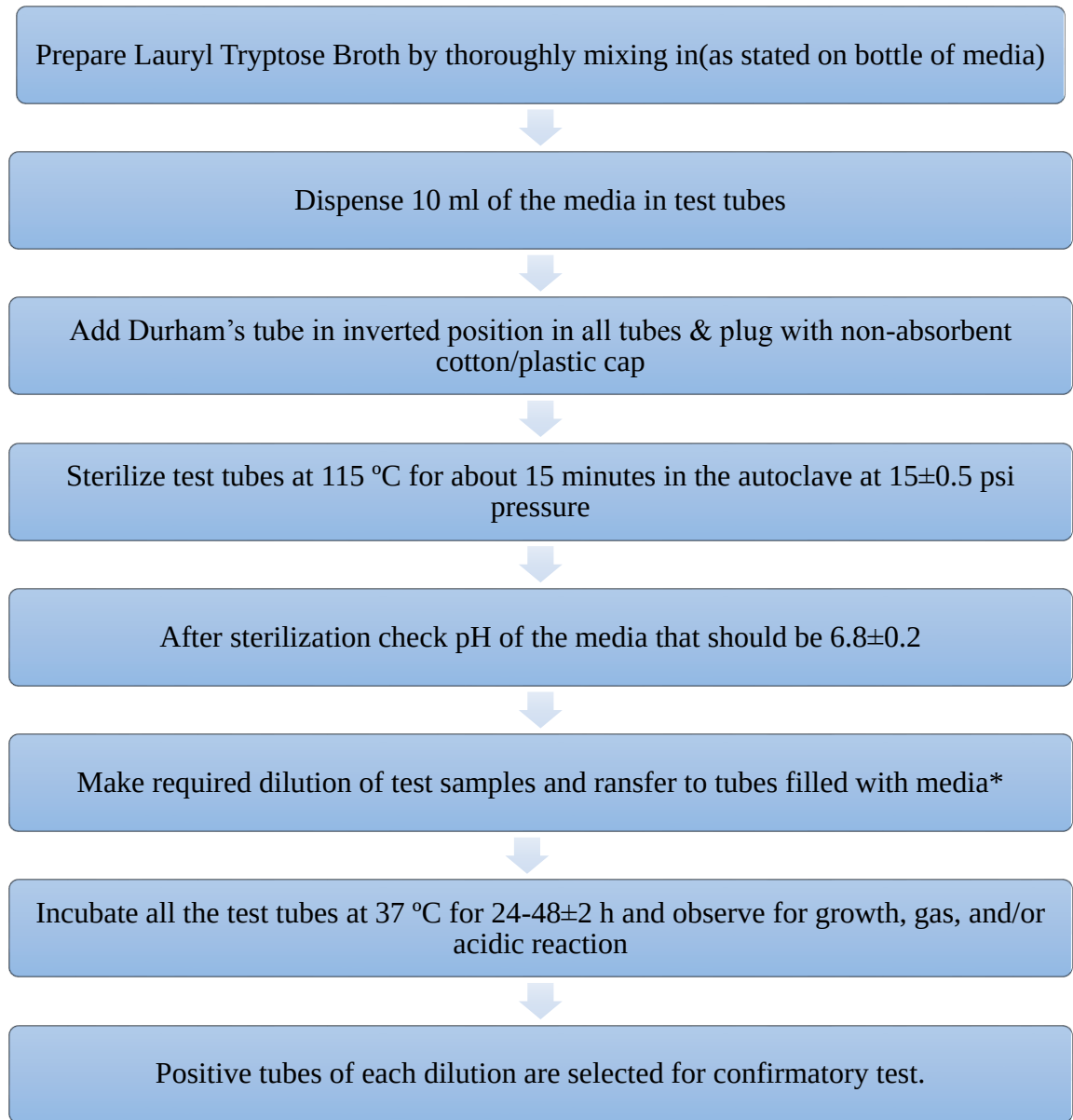


Fig: MPN test at different concentrations

PRESUMPTIVE TEST

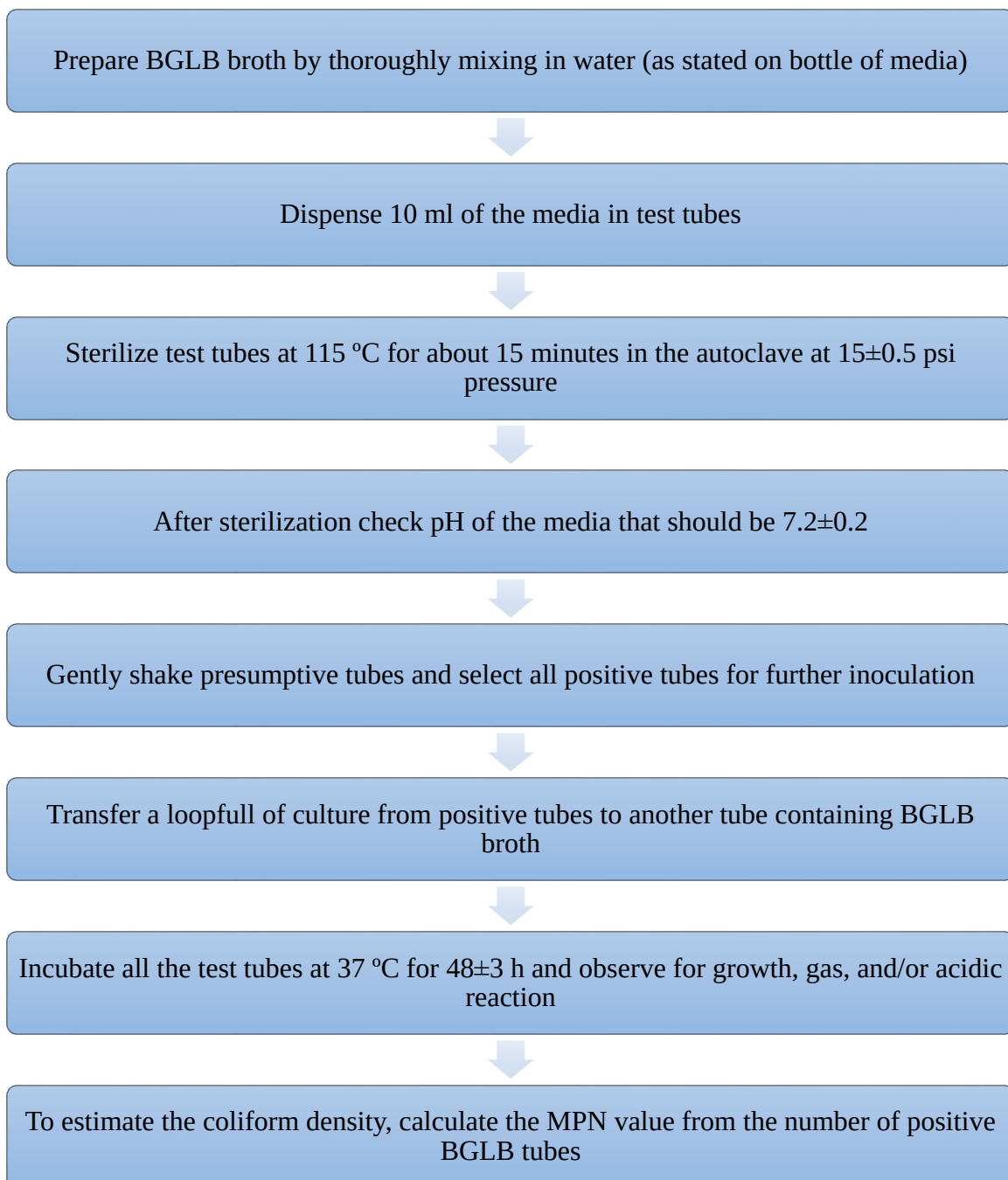


2. Confirmatory test

1. From each of the fermentation tubes with positive results transfer one loopful of medium to:
2. 3 ml lactose-broth or brilliant green lactose fermentation tube, to an agar slant and 3 ml tryptone water.
3. Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen, further incubate up to maximum of 48 ±3 hours to check gas production.
4. The agar slants should be incubated at 37°C for 24± 2 hours and Gram-stained preparations made from the slants should be examined microscopically.

5. The formation of gas in lactose broth and the demonstration of Gram negative, non-spore-forming bacilli in the corresponding agar indicates the presence of a member of the coliform group in the sample examined.

CONFIRMATORY TEST



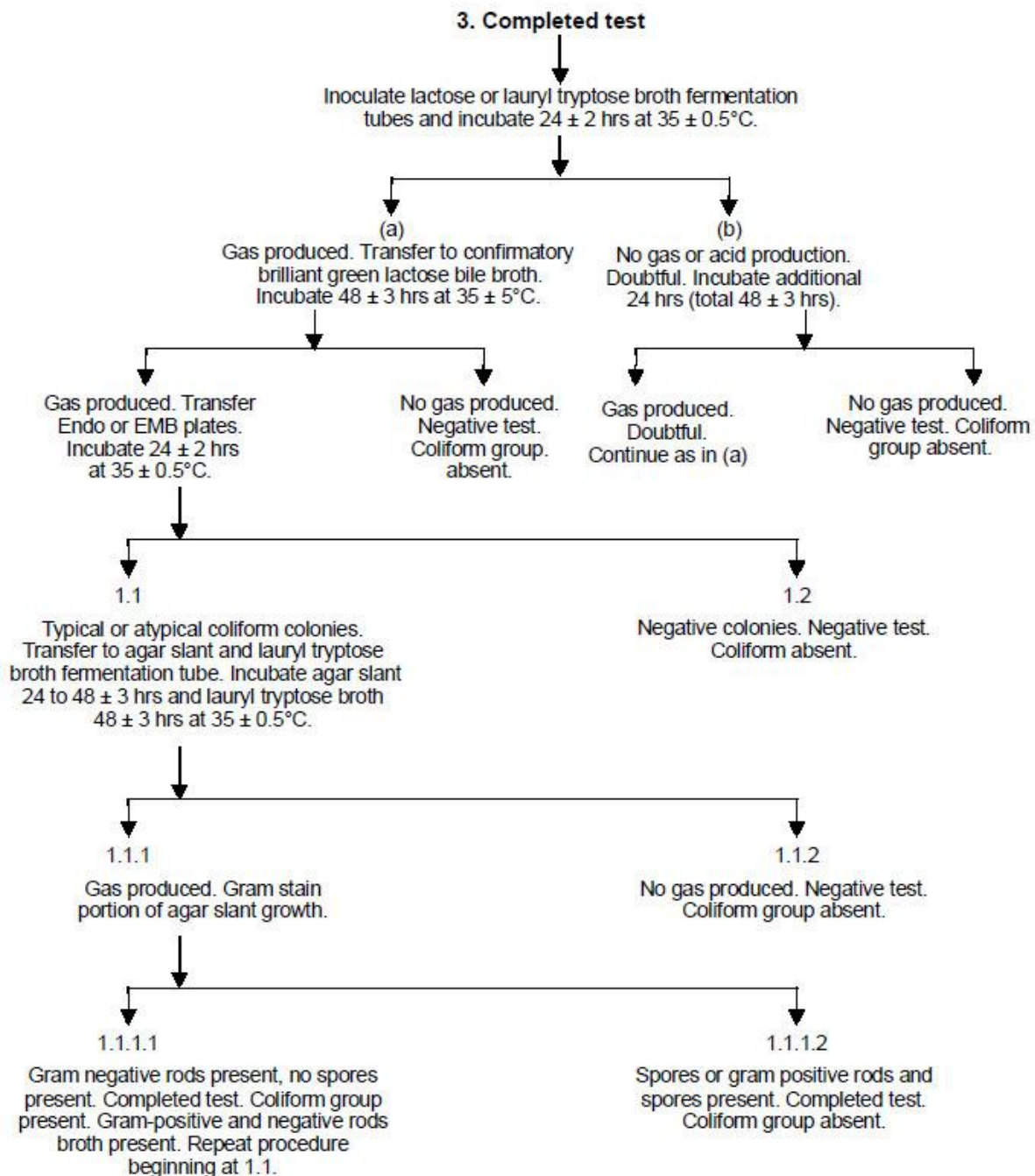


Fig: Summary of the full MPN test of completed test.

➤ **Advantages of MPN:**

- Ease of interpretation, either by observation or gas emission
- Sample toxins are diluted
- Effective method of analysing highly turbid samples such as sediments, sludge, mud, etc.
- That cannot be analysed by membrane filtration.

➤ **Disadvantages of MPN:**

- It takes a long time to get the results
- Results are not very accurate
- Requires more hardware (glassware) and media
- Probability of false positives

The number of positive findings of coliform group organisms resulting from the multiple portion decimal dilution planting should be computed as the combination of positives and recorded in terms of the Most Probable Number (MPN).

VI. QUESTIONS:

- What is MPN?
- Give the names of microorganisms present in water.
- Give the name of few waterborne diseases.
- What is presumptive test?
- What is potable water?

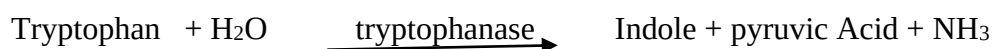
EXPERIMENT NO. 10(a)

I. AIM: IMViC test (Biochemical Identification)

II. PRINCIPLE: IMViC tests are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae. A series of tests used to study the physiological characteristics of bacteria from the family Enterobacteriaceae, especially *Escherichia* and *Enterobacter*. They are designed to differentiate Gram – negative intestinal bacilli of family *Enterobacteriaceae* which contains a large number of genera that are biochemically and genetically related to one another. IMViC tests consist of four different tests each of the letters in “IMViC” stands for one of these tests. I- Indole M – methyl red V- Voges- proskauer C- citrate

INDOLE TEST

• **Principle:** Tryptophan is an amino acid that can undergo deamination and hydrolysis by bacteria that express tryptophanase enzyme. Indole is generated by reductive deamination from tryptophan via the intermediate molecule indole pyruvic acid. Tryptophanase catalyzes the deamination reaction, during which the amine (-NH₂) group of the tryptophan molecule is removed. Final products of the reaction are indole, pyruvic acid, ammonium (NH₄⁺) and energy. Some bacteria can produce indole from amino acid tryptophan using the enzyme typtophanase.



Production of indole is detected using Ehrlich’s reagent or Kovac’s reagent. Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top.

III. REQUIREMENTS:

- Fresh growth of gram-negative rods (test organism)
- Sulphide indole motility (SIM) medium/ Motility indole urea (MIU) medium/ peptone water
- Kovac's reagent
- Incubator
- Inoculating wire and Bunsen burner

IV. PROCEDURE:

- Inoculate liquid tube medium or stab agar medium with the bacterial colony.
- Incubate for 18 to 24 hours in a BOD incubator at 37°C.
- Incubate one set of test tube with test organism and maintain one set as negative control without inoculation. Inoculate one set of test tube with *E.coli* as positive control.
- Add 3 drops of Kovac's reagent down the side of the tube and observe color change at the meniscus. Presence of red color indicates the positive results.
- If the test is negative, repeat the test after additional 24 hours of incubation if desired.

V. OBSERVATION:

- Positive: Appearance of a red or pink coloured ring at the top.
- Negative: Colorless or slightly yellow.

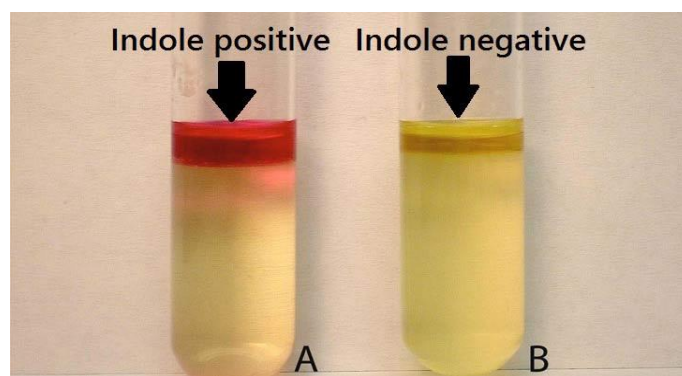


Fig: Positive and Negative result of Indole test (Source: Microbiologyinfo.com)

➤ Examples:

- **Indole positive organisms:**
 - *Escherichia coli*
 - *Klebsiella oxytoca*
 - *Proteus vulgaris*
 - *Citrobacter koseri*
 - *Morgenella morganii*
 - *Vibrio cholerae*
 - *Providencia species*
 - *Aeromonas species*

- *Plesiomonas species*
- *Pasteurella species*
- *Cardiobacterium hominis*
- *Propionibacterium acenes*

- **Indole negative organisms:**

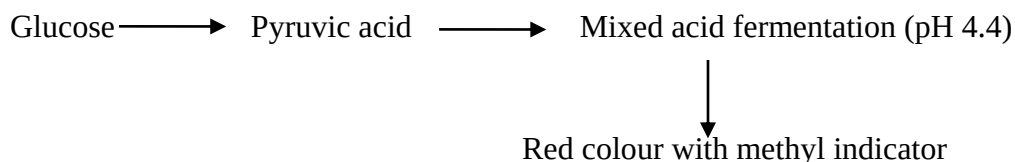
- *Klebsiella pneumoniae*
- *Enterobacter species*
- *Proteus mirabilis*
- *Citrobacter freundii*

➤ **Limitation of indole test:**

- Do not use media that contain dyes (e.g. Eosin methylene blue, MacConkey agar).

METHYL RED (MR) TEST

- **PRINCIPLE:** This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less.



- **REQUIRMENTS:**
 - MR-VP broth
 - Test organism
 - Inoculating wire
 - Bunsen burner
 - Incubator
 - Methyl red indicator
 - Control strains
- **PROCEDURE:**
 - Inoculate test organism and incubate at 35°C for at least 48 hours.
[Note: If the center of one colony is inoculated to a 0.5-ml volume of MR-VP broth, the test can be read at 18 to 24 hours.]
 - Remove approximately 1 ml of the 48-hours broth to a 13-by 100-mm tube. (The remainder should be reserved for testing at 3 to 5 days if necessary).
 - Add 3 to 6 drops (or 1 drop to 0.5 ml) of methyl red indicator to the aliquot.
 - Observe for red color immediately.
- **OBSERVATION:**
 - **Positive:** Appearance of a red colour is taken as positive result.
 - **Negative:** yellow colour is taken as negative result.

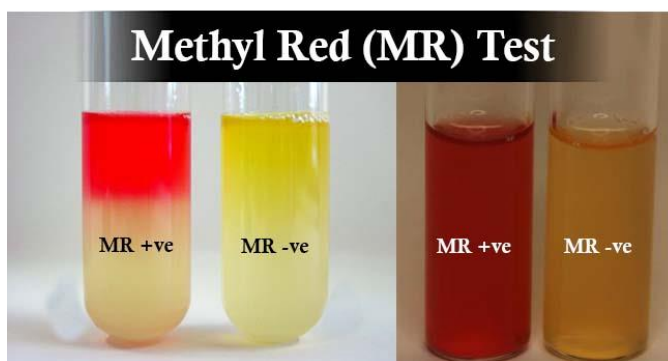


Fig: Negative and Positive result of MR test (Source: Microbiologyinfo.com)

- **Examples:**

- **MR +ve strains**

- *Escherichia coli*
- *Klebsiella ozanae*
- *Klebsiella rhinoscleromatis*
- *Klebsiella ornithiolytica*
- *Citrobacter*
- *Proteus*
- *Yersinia*
- *Edwardsiella*

- **MR-ve organisms**

- *Klebsiella pneumoniae*
- *Enterobacter* species

VOGES PROSKAUER (VP) TEST

- **PRINCIPLE:** While MR test is useful in detecting mixed acid producers, VP test detects butylene glycol producers. Acetyl-methyl carbinol (acetoin) is an intermediate in the production of butylene glycol. In this test two reagents, 40% KOH and alpha-naphthol are added to test broth after incubation and exposed to atmospheric oxygen. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alpha-naphthol to produce red colour. Role of alpha-naphthol is that of a catalyst and a colour intensifier.
- **REQUIREMENTS:**
 - MR-VP broth
 - Test organism
 - Inoculating wire
 - Bunsen burner
 - Incubator
 - 5%α- naphthol
 - 40% KOH
- **PROCEDURE:**
 - Inoculate test organism and incubate for at least 48 hours.
 - Add 0.6 ml of alpha-naphthol to the test broth and shake well.
 - Add 0.2 ml of 40% KOH to the broth and shake. Allow the tubes to stand for 15 minutes. Appearance of pink-red color is taken as a positive test.

[**Note:** The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents.]
- **OBSERVATION:**
 - **Positive:** A pink-red color at the surface is a positive reaction
 - **Negative:** A lack of a pink-red color is a negative reaction.

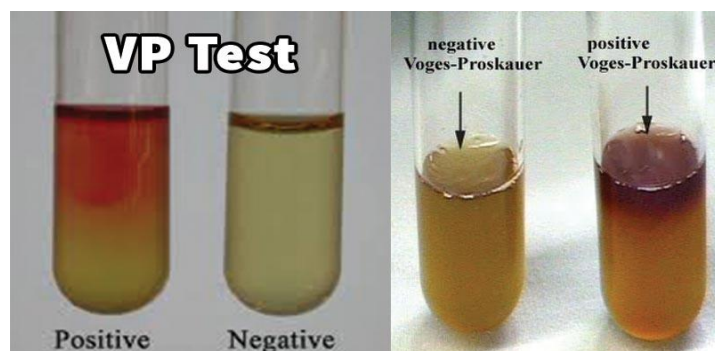
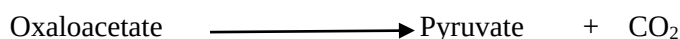
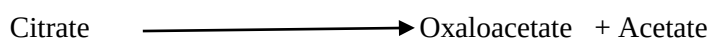


Fig: Positive and Negative result of VP test (Source: microbenote.com)

➤ **Examples:** *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive

CITRATE UTILIZATION TEST

- **PRINCIPLE:** This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen. Utilization of citrate involves the enzyme citrase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO_2 . Production of Na_2CO_3 as well as NH_3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium's color from green to blue.



- **REQUIREMENTS:**
 - Simmons citrate agar slant
 - Cotton plug
 - Sterile Inoculating wire/ sticks
 - Test organism
 - Bunsen burner
 - Incubator
 - Test tube rack
- **PROCEDURE:**
 - Streak the slant back and forth with a light inoculum picked from the center of a well-isolated colony.
 - Place cap loosely on the tube.
 - Incubate aerobically at 35°C to 37°C for 18 to 24 hours.
 - Observe a color change from green to blue along the slant.
- **OBSERVATION**
 - **Positive:** Growth on the medium with color change from green to intense blue.
 - **Negative:** Absence of growth and color change

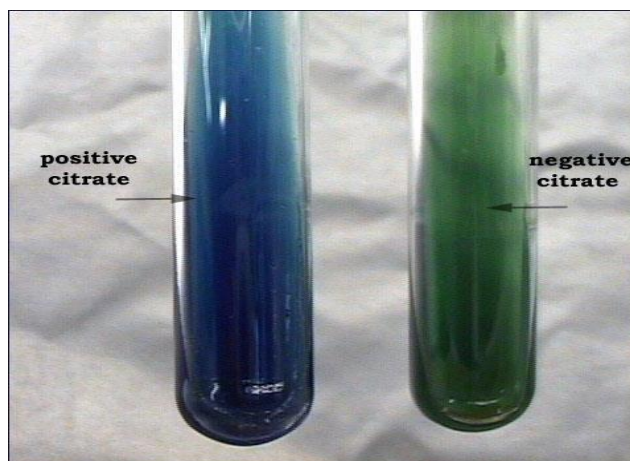


Fig: Citrate positive and negative result (Source: researchgate)

- **Examples:**

- **Positive citrate utilization test**

- *Klebsiella pneumoniae*
- *Citrobacter freundii*
- *Enterobacter cloacae* (a minority of strains gives negative results)
- *Salmonella* other than Typhi and Paratyphi A
- *Serratia marcescens*
- *Proteus mirabilis* (a minority of strains gives negative results)
- *Providencia alcalifaciens*
- *Vibrio vulnificus*
- *Euringella Americana*
- *Achromobacter oxylooxidans*

- **Citrate Test: variable (different strains give different results)**

- *Proteus vulgaris*
- *Vibrio cholerae*
- *Vibrio parahaemolyticus*

- **Negative citrate strains:**

- *Escherichia coli*
- *Shigella* species
- *Salmonella* Typhi
- *Salmonella* Paratyphi A
- *Morganella morganii*

- ❖ **Keynotes on Citrate utilization Test**

- Generally, incubate aerobically at 35°C to 37°C for 18 to 24 hours but if negative some organisms may require up to 4 days of incubation due to their limited rate of growth on citrate medium.
- Other methods of citrate utilization test available are-IMViC the test kit method and API test kit method
- As you know, *Escherichia coli* is citrate utilization test negative; although uncommon, natural *E. coli* variants that are citrate positive have been isolated. Citrate-negative strains of *E. aerogenes* have also been found.

- **QUESTIONS:**

- How's Kovac's Reagent prepared?
- What is the nature of methyl red?
- Name the MR positive and VP negative bacteria.
- Name the MR negative and VP positive bacteria.
- What is the role of citric acid?

EXPERIMENT NO. 11

I. **AIM:** Isolation and enumeration of microorganisms from Air: by exposure plate method

II. **PRINCIPLE:** 'AIR' is considered to be one of the major microbial reservoirs. In air microbes are suspended alone or attached to the surfaces of fine particles. They include intact cells/colonies, spore bodies and/or mycelium or filament fragments. These components can be isolated by the air samples or by the exposure plate technique. Air current is also important in the dispersal of microorganisms as it carries them over a long distance. In still air the particles with microorganisms tend to settle down. But a gentle air can keep them in suspension for long periods.

Air is not a medium in which organism grow but is a carrier of particulate matter, dust particles, spores etc. Air is mainly a transport medium for microorganisms. They occur in small numbers in the air, when compared with soil or water. The microflora of air can be studied under two headings outdoor and indoor microflora. Air is not a natural environment for microorganisms as it doesn't contain enough moisture and nutrients to support their growth and reproduction. One of the most common sources of air microflora is the soil. Microorganisms found in water may also be released into the air in the form of water droplets from plant or animal surfaces. The main sources of airborne microorganism is activities by human beings like coughing, sneezing, talking and laughing.

III. **REQUIREMENTS:** Culture media, Petri dishes, Stop watch, Glass marker, Water-bath, incubator, Colony counters.

IV. PROCEDURE:

- About 15-20 ml. of suitable melted culture medium (Potato-dextrose-agar medium for isolation of fungi, Nutrient agar medium or isolation of bacteria, Thornton medium for isolation of actinomycetes) is plated on a sterilized petri dish
- Petri plates are then allowed to settle for 30 min. on the laboratory desk or in the laminar chamber.
- Each petri dish is marked for the specific kind of isolation and period of aerial exposure.
- The petri dishes are exposed in the air separately for a definite period of 1, 2, 3, 4 and 5 mins, respectively (as per their marking).
- During exposure of each plate the upper cover is fully removed for the desired period.
- Each petri dish is incubated for 2 – 5 days or more for the appearance of colonies.
- Colonies are observed and counted by the colony counter. For each re-enactment, there should be at least five replicas.

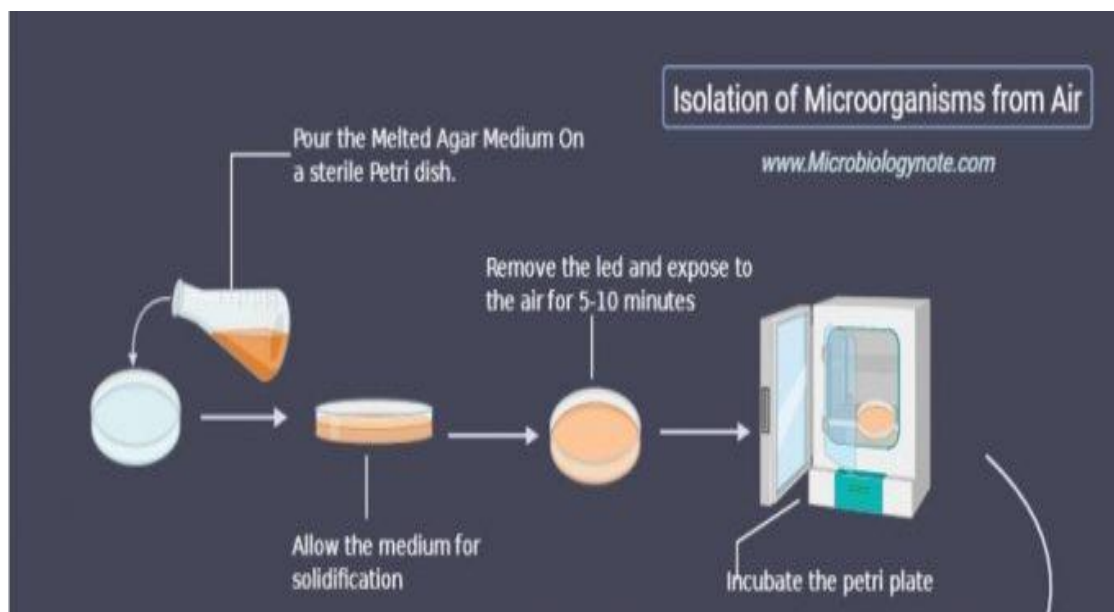


Fig : Isolation of microorganisms from air (Source: www.Microbiologynote.com)

V. OBSERVATION TABLE:

Exposure Time (Min)	Number of colonies					Average
	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	
1						
2						
3						
4						
5						
6						
7						

VI. RESULTS AND DISCUSSION: Observe the growth of bacterial and fungal colonies. Count the colony forming unit (CFUs) of bacteria and fungi which indicate the viable number of bacteria and fungi present in air.

I. QUESTIONS:

- Why do we study Aeromicrobiology?
- How is air sampled for microbiological analysis?
- Name other methods for examining air.

EXPERIMENT NO. 12

- I. AIM:** Isolation and enumeration of microorganisms from soil by serial dilution pour plating methods and Heterotrophic bacterial counts by colony counter
- II. PRINCIPLE:** Pour plate method is usually the method of choice for counting the number of colony-forming bacteria present in a liquid specimen. In this method, fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the centre of sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours. Microorganisms will grow both on the surface and within the medium. Colonies that grow within the medium generally are small in size and may be confluent; the few that grow on the agar surface are of the same size and appearance as those on a streak plate. Each (both large and small) colony is carefully counted (using magnifying colony counter if needed). Each colony represents a “colony forming unit” (CFU).

The number of microorganisms present in the particular test sample is determined using the formula: **CFU/mL = CFU * dilution factor * 1/aliquot**

For accurate counts, the optimum count should be within the range of 30-300 colonies/plate. To insure a countable plate a series of dilutions should be plated.

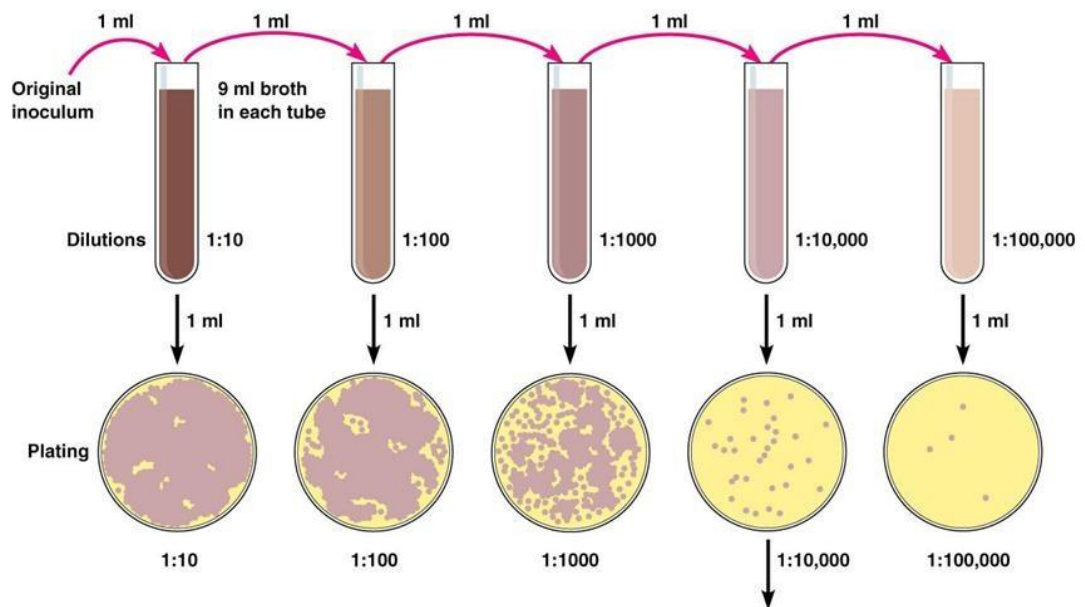
The HPC (heterotrophic plate count) method is used to make an estimate of the number of aerobic and facultative anaerobic heterotrophic bacteria in water. The concentration of heterotrophic bacteria in water gives information about the quality of the water and how much bacteria is removed during treatment. Different nutritional broths and agars are available to supply the necessary nutrients to bacteria in different types of water. The pour plate procedure, also known as the standard plate count, is an easy procedure and is commonly used to determine heterotrophic bacteria density. The sample mixes with a nutritional agar at a high temperature. The agar cools and becomes solid. During incubation, the bacteria in the sample grow and form colonies in the agar. After incubation, the agar is examined with a colony counter for bacteria colonies. The heat of the melted agar can cause heat shock to stressed bacteria, and the nutritionally rich agar can decrease the recovery of starved bacteria. The heterotrophic plate count is a good way to measure the efficiency of water treatment plants, growth in distribution lines and the general bacterial composition of source water. No single method, growth medium or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. The colonies that grow in the agar are smaller and less likely to grow into each other than colonies that are grown on membrane filters.

- II. MATERIALS REQUIRED:** Beaker, glass test tube, Colony counter, Petri dish, test sample, Plate Count Agar (PCA) or Nutrient Agar, Hot water bath, Flame.

IV. METHODOLOGY:

➤ **Procedure of Pour plate technique:**

1. Prepare the dilution of the test sample expected to contain between 30-300 CFU/mL. (*Follow serial dilution technique*)
2. Inoculate labeled empty petri dish with specified mL (0.1 or 1.0 mL) of diluted specimen
3. Collect one bottle of sterile molten agar (*containing 15 mL of melted Plate Count Agar or any other standard culture media*) from the water bath (45°C).
4. Hold the bottle in the right hand; remove the cap with the little finger of the left hand.
5. Flame the neck of the bottle.
6. Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish and replace the lid.
7. Flame the neck of the bottle and replace the cap.
8. Gently rotate the dish to mix the culture and the medium thoroughly and to ensure that the medium covers the plate evenly. Do not slip the agar over the edge of the petri dish.
9. Allow the agar to completely gel without disturbing it, it will take approximately 10 minutes.
10. Seal and incubate the plate in an inverted position at 37°C for 24-48 hours.

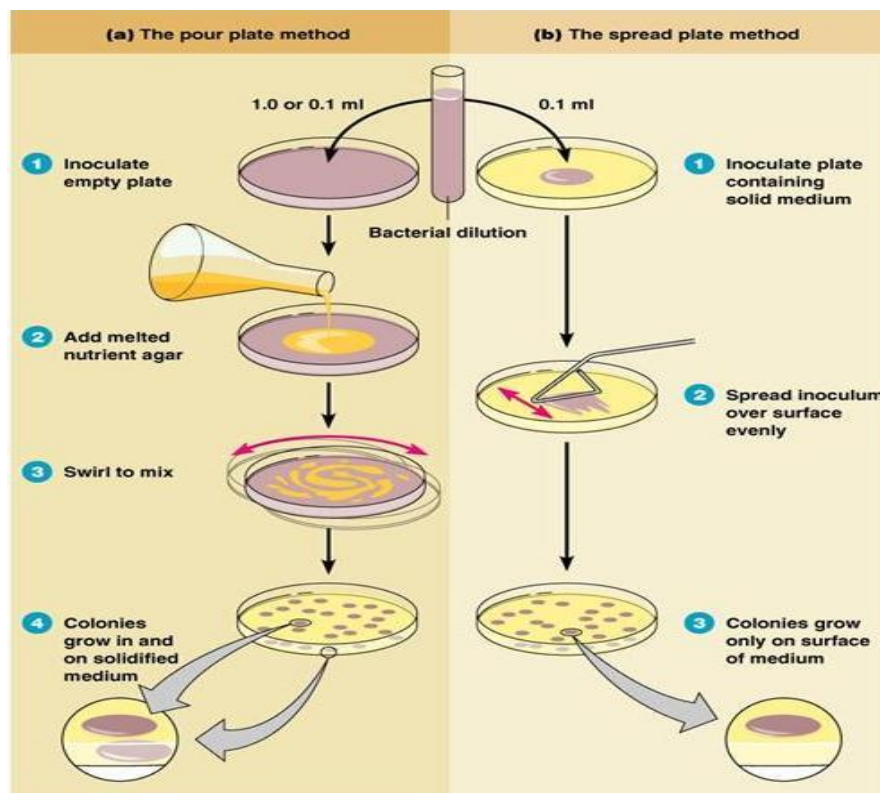


Calculation: Number of colonies on plate $\times$ reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $1/10,000$ dilution, then the count is $32 \times 10,000 = 320,000$ bacteria/ml in sample.)

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Colony count guidelines Count the colonies on the agar plate soon after the specified incubation time. If immediate analysis is not possible, keep the plates at 5–10 °C (41–50 °F) for a maximum of 24 hours.

- To determine the average colony count, add the count from each plate of the same dilution, then divide by the number of plates.
- Use two significant digits to report the CFU/mL.
- The colony counter has a grid to help count the colonies.
- The easiest way to count the colonies is to use a left-to-right pattern when it is necessary to count the colonies in a specified number of squares, use the squares that are representative of the colony distribution on the plate.
- Colonies can grow together to form large irregular shapes known as spreaders. If spreaders occur, count the colonies in spreader-free areas when less than one-half of the plate area has spreaders.
- When it is necessary to count the spreaders, count the spreader types that follow as one colony.
- A chain of colonies that can occur from a clump of bacteria that breaks apart when the agar and sample are mixed.
- A spreader that shows as a film of growth between the agar and the bottom of the petri dish.
- A colony that forms in a film of water at the edge or over the agar surface.



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- Count colonies that touch each other as individual colonies when they are different in shape or color.

- Count colonies that are near to each other and look similar as individual colonies when the distance between them is at minimum diameter of the smallest colony.

VI. **RESULTS:** The number of colonies can be calculated by counting in the terms of CFU per ml of samples.



Fig. Bacterial isolates on different dilution

QUESTIONS:

- Which method is used for sampling of soil microorganisms?
- Does microscopy helps in the study of soil microorganism?

Department of Environmental Science & Engineering

B. Tech. Environmental Engineering

Environmental Geotechnology Practical (ESC 355)

Environmental Geotechnology Laboratory. (Room No: 025)

INDEX OF EXPERIMENTS

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1.	Identification of soils; IS 1498-1970
2.	Sampling and Grain Size Distribution : IS 2720: Part IV : 1985
3.	Determination of Specific Gravity of Soil : IS 2720: Part III : Sec-1 & 2: 1980
4.	Determination of Plastic Limit of Soil : IS 2720: Part V : 1985
5.	Determination of Plastic Limit of Soil : IS 2720: Part V : 1985
6.	Determination of Shrinkage Limit of Soil : IS 2720: Part VI : 1972
7.	Determination of Density of Soil by Core Cutter Method : IS 2720: Part IX : 1992
8.	Sedimentation analysis of soil using pipette method: IS 2720: Part IV : 1985
9.	Determination of Permeability of soil : IS 2720: Part XVII : 1986 (Falling Head); Part XXXVI:1987 (Constant Head)
10.	Compaction Test-(Light Weight/Standard Proctor Compaction) (IS 2720-PART VII-1980) Reaffirmed-2011.
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12.	Triaxial Test of Soil : IS 2720 : Part 11 : 1993
13	Direct Shear Test of Soil : IS 2720 : Part 13 : 1986
14	Some Examples of Practical Examination Questions

Experiment No -1 : Identification of soils; IS 1498-1970

THEORY: Soils are broadly classified into two divisions :-

Coarse grained soils: In these soils, 50% or more of the total material by weight is larger than 75 micron IS sieve size.

Fine grained soils: In these soils, 50% or more of the total material by weight is smaller than 75 micron IS sieve size.

1. Coarse grained soils are further divided into two sub-divisions:

a) **Gravels (G):** In these soils more than 50% of the coarse fraction (+75 micron) is larger than 4.75 mm sieve size.

b) **Sands (S):** In these soils, more than 50% of the coarse fraction is smaller than 4.75mm IS sieve size.

Each of the above sub-divisions are further divided into four groups depending upon grading and inclusion of other materials.

1) W : Well Graded

2) C : Clay binder

3) P: Poorly graded

4) M : Containing fine materials not covered in other groups.

2. Fine grained soils

Fine grained soils are further divided into three sub-divisions:

a) Inorganic **silts** and very fine sands: M

b) Inorganic **clays**: C

c) Organic silts and clays and organic matter: O.

The fine grained soils are further divided into the following groups on the basis of the following arbitrarily selected values of liquid limit which is a good index of compressibility:

i) Silts and clays of low compressibility:

Having a liquid limit less than 35 and represented by symbol L.

ii) Silts and clays of medium compressibility:

Having a liquid limit greater than 35 and less than 50 and represented by symbol I.

iii) Silts and clays of high compressibility:

Having a liquid limit greater than 50 and represented by a symbol H.

COARSE-GRAINED SOILS

(more than 50% of material is larger than No. 200 sieve size.)

Clean Gravels (Less than 5% fines)			
GRAVELS More than 50% of coarse fraction larger than No. 4 sieve size		GW	Well-graded gravels, gravel-sand mixtures, little or no fines
		GP	Poorly-graded gravels, gravel-sand mixtures, little or no fines
	Gravels with fines (More than 12% fines)		
		GM	Silty gravels, gravel-sand-silt mixtures
		GC	Clayey gravels, gravel-sand-clay mixtures
Clean Sands (Less than 5% fines)			
SANDS 50% or more of coarse fraction smaller than No. 4 sieve size		SW	Well-graded sands, gravelly sands, little or no fines
		SP	Poorly graded sands, gravelly sands, little or no fines
	Sands with fines (More than 12% fines)		
		SM	Silty sands, sand-silt mixtures
		SC	Clayey sands, sand-clay mixtures

FINE-GRAINED SOILS

(50% or more of material is smaller than No. 200 sieve size.)

SILTS AND CLAYS Liquid limit less than 50%		ML	Inorganic silts and very fine sands, rock flour, silty or clayey fine sands or clayey silts with slight plasticity
		CL	Inorganic clays of low to medium plasticity, gravelly clays, sandy clays, silty clays, lean clays
		OL	Organic silts and organic silty clays of low plasticity
SILTS AND CLAYS Liquid limit 50% or greater		MH	Inorganic silts, micaceous or diatomaceous fine sandy or silty soils, elastic silts
		CH	Inorganic clays of high plasticity, fat clays
		OH	Organic clays of medium to high plasticity, organic silts
HIGHLY ORGANIC SOILS		PT	Peat and other highly organic soils

SIGNIFICANCE

The farmer's soil is arguable their most important asset, because it will be easier to grow higher yielding crops or pastures on soils that have good structure and chemistry, high levels of organic

matter and the ability to hold moisture. On larger farms, soil types can vary considerably from one part of the property to the other. It is possible to farm on poorer soils, but input costs will always be higher and yields lower, which is why the soil asset is so valuable and the ongoing management of that asset so important.

Experiment No -2 : Sampling and Grain Size Distribution : IS 2720: Part IV : 1985

APPARATUS REQUIRED:

Thermometer, Water Bath., Stirring Apparatus, I.S Sieves Apparatus, Balance-accurate to 0.01 g, Stop Watch, Desiccators, Centimeter Scale, Porcelain Evaporating Dish. Wide Mouth Conical Flask or Conical Beaker of 1000 ml capacity, Thick Funnel-about 10 cm in diameter, Filter Flask-to take the funnel, Measuring Cylinder-100 ml capacity, Wash Bottle-containing distilled water, Filter Papers, Glass Rod-about 15 to 20 cm long and 4 to 5 mm in diameter. Hydrogen Peroxide-20 volume solution, Hydrochloric acid N solution-89 ml of concentrated hydrochloric acid and Sodium Hexametaphosphate solution-dissolve 33g of Sodium Hexametaphosphate.

Procedure :

- I.S sieves are selected and arranged in the order as shown in the table
- The soil sample is separated into various fractions by sieving through various sieves placed in the descending size.
- The weight of soil retained on each sieve is recorded.
- The moisture content of soil if above 5% it is to be measured and recorded.

Table

IS Sieve Designation	Mass of Soil Retained + Mass Container	Mass of Container	Mass of Soil Retained	Cumulative mass Retained	Soil retained As percentage of soil taken	Soil passing as Percentage of soil taken	Combined percentage passing as percentage of total soil sample

Graph:

Gradation curve is obtained by plotting percentage passing on y-axis and log of sieve size on x-axis using a semi-log paper. Gradation curves are the best representation of soil nature i.e. it is well graded uniformly graded or poorly graded soil. Uniformity coefficient (C_u) and Coefficient of gradation (C_g or C_c Coeff. of Curvature) can also give us an idea of soil nature.

$$C_u = D_{60}/D_{10}$$

$$C_g = (D_{30})^2 / D_{60} * D_{10}$$

Where, D_{10} , D_{30} , and D_{60} are diameters for 10%, 30% and 60% passing respectively.

Experiment No -3 : Determination of Specific Gravity of Soil : IS 2720: Part III : Sec-1 & 2: 1980

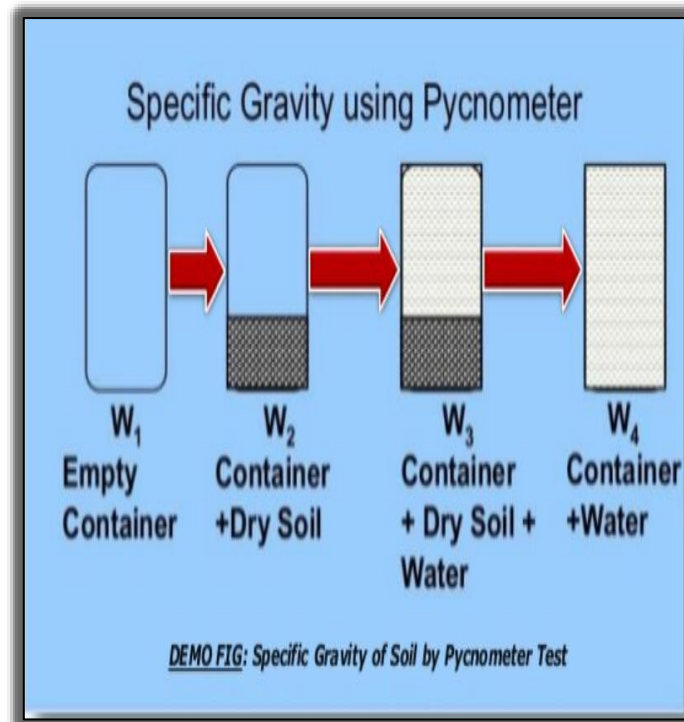
APPARATUS REQUIRED:

1. Sieve
2. Balance
3. Electric Oven
4. Pycnometer
5. Desiccator
6. Spatula
7. Wash Bottle

Procedure :

- Dry the density bottle (pycnometer) with stopper at 105° to 110°C, cool in the desiccator and weigh it to the nearest 0.001 g (W_1)
- Take 5 to 10 g sub-sample after passing through 2 mm sieve and oven dry at 105° to 110°C. Transfer this sample to the specific gravity bottle after cooling in the desiccator. Weigh the bottle and contents together with the stopper to the nearest 0.001 g (W_2).
- Add adequate water and immerse the stoppered bottle in the constant-temperature bath for approximately 1 h or until it has attained the constant temperature of the bath. Take the stoppered bottle out of the bath, wipe dry and weigh it to nearest 0.001 g (W_3)
- Clean the bottle, fill with water and insert the stopper. Allow it to attain constant temperature by immersing in the constant temperature bath for 1 hour and Take the bottle out of bath, wipe dry and weigh to the nearest 0.001g (W_4)
- Calculate the specific gravity by the following formula

$$G = \frac{(W_2 - W_1)}{(W_4 - W_1) - (W_3 - W_2)}$$



Table

Sl No	Particulars	Test No 1 (G1)	Test No 2 (G2)	Test No 3 (G3)
1	Weight of Pycnometer bottle (W1), g			
2	Weight of Pycnometer + dry soil (W2), g			
3	Weight of Pycnometer + soil + water, (W3), g			
4	Weight of Pycnometer + water (W4), g			
5	Calculation of specific gravity, G			
6	Average $G = (G1+G2+G3)/3$			

Significance:

The specific gravity of a soil is used in the phase relationship of air, water and solids in a given volume of the soil.

General Remarks:

The specific gravity of the soil particles lie with in the range of 2.65 to 2.85. Soils containing organic matter and porous particles may have specific gravity values below 2.0. Soils having heavy substances may have values above 3.0.

Precautions:

- The graduated cylinder used should be cleaned.
 - Dry the coarse aggregate so that it does not absorb moisture otherwise it will not give the desired results.
 - All the readings of mass should be noted carefully.
-

Experiment No -4 : Determination of Plastic Limit of Soil : IS 2720: Part V : 1985

APPARATUS REQUIRED:

1. Porcelain Evaporating Dish
2. Spatula
3. Surface for Rolling
4. Containers
5. Balance
6. Oven
7. Rod

Procedure :-

- Take a sample weighing about 20 g from thoroughly mixed portion of the material passing 425 micron IS Sieve.
- The soil sample shall be mixed thoroughly with distilled water in an evaporating dish or on the flat glass till the soil mass becomes plastic enough to be easily moulded with fingers.
- A ball shall be formed with about 8 g of this plastic soil mass and rolled between the fingers and the glass plate with just sufficient pressure to roll the mass into a thread of uniform diameter throughout its length.
- The rate of rolling shall be between 80 and 90 strokes/min counting a stroke as one complete motion of hand forward and back to the starting position again.
- The rolling shall be done till the threads are of 3 mm diameter. The soil shall then kneaded together to a uniform mass and rolled again.
- This process shall be continued until the thread crumbles under the pressure required for rolling and the soil can no longer be rolled into a thread.
- The pieces of crumbled soil thread shall be collected and moisture content is determined.

Table

SR NO	PARTICULARS	SAMPLE 1.	SAMPLE 2	SAMPLE 3
1.	Weight of the container in g			
2.	Weight of the container + wet soil in g			
3.	Weight of the container + dry soil in g			
4.	Weight of dry soil (3-1)			
5.	Weight of water (2-3)			
6.	Water Content % (5/4)			

Plastic limit of given soil sample =..... %

1. Plasticity index

The plasticity index is calculated as the difference between its liquid limit and plastic limit:

Plasticity index (I_p) = liquid limit (w_L) - plastic limit (w_p).

2. Toughness index

The toughness index shall be calculated as follows:

$$\text{Toughness index (} I_T \text{)} = \frac{I_p}{I_f}$$

where, I_p = plasticity index and I_f = flow index.

3. Liquidity index

The liquidity index is calculated as follows:

$$\text{Liquidity index (} I_L \text{)} = \frac{w_o - w_p}{I_p}$$

where, w_o = natural moisture content of soil , w_p = plastic limit of soil.

4. Consistency index

The consistency index shall be calculated as follows:

$$\text{Consistency index (} I_c \text{)} = \frac{w_o - w_p}{I_p}$$

General Remarks :-

SOIL TYPE	PLASTIC LIMIT (approx.)
SANDS	0
SILTS	20
CLAYS	45
COLLOIDAL CLAYS	46

SIGNIFICANCE:

The liquid and plastic limits of soils are both dependent on the amount and type of clay in a soil and form the basis for the soil classification system for cohesive soils based on the plasticity tests. Besides their use for identification, the plasticity tests give information concerning the cohesion properties of soil and the amount of capillary water which it can hold. They are also used directly in specifications for controlling soil for use in fill. These index properties of soil have also been related to various other properties of the soil.

PRECAUTIONS:

- (i) Rolling should be stopped when the thread is just starts crumbling.
 - (ii) Diameter of rolled thread should be of 3 mm diameter.
 - (iii) Weights of container, wet soil and dry soil should be taken accurately
-

Experiment No -5 : Determination of Plastic Limit of Soil : IS 2720: Part V : 1985

APPARATUS USED:

1. Mechanical Liquid Device
2. Grooving Tool
3. Porcelain Evaporating Dish
4. Flat Glass Plate
5. Spatula
6. Palette Knives
7. Balance
8. Oven
9. Wash Bottle
10. Containers

Procedure :-

- About 120 g of soil sample passing 425-micron IS Sieve shall be mixed thoroughly with distilled water in the evaporating dish
- A portion of soil paste shall be placed on the cup above the spot where the cup rest on the base. The paste shall have a consistency that would require 30 to 35 drops of cup to cause closer of standard groove for sufficient length.)
- Place a portion of the paste in the cup of Liquid Limit device and spread it with a few strokes of spatula.
- Trim it to a depth of 1 cm at the point of maximum thickness and return excess of soil to the dish.
- Using the grooving tool, cut a groove along a distance about 12 mm the center line of soil pat in the cup, so that clean sharp groove of proper dimension is formed.
- Lift and drop the cup by turning crank at the rate of two revolutions per second until the two halves of soil cake come in contact with each other for a length of about 12 mm by flow only, and record the number of blows, N.
- Take a representative portion of soil from the cup for moisture content determination and repeat the process thrice

CALCULATION

Determination number	1	2	3
Number of drops			
Container number			
Weight of container + wet soil, g			
Weight container + oven dry soil, g			
Weight of water, g			
Weight of empty container, g			
Weight of oven dry soil, g			
Moisture present			

Plot the relationship between water content (on y-axis) and number of blows (on x-axis) on semi-log graph. The curve obtained is called flow curve. The moisture content corresponding to 25 drops (blows) as read from the represents liquid limit. It is usually expressed to the nearest whole number.

GENERAL REMARKS:

SOIL TYPE	LIQUID LIMIT (approx.)
SANDS	20
SILTS	27
CLAYS	100
COLLOIDAL CLAYS	399

SIGNIFICANCE:

The liquid limits of soils is dependent on the amount and type of clay in a soil and form the basis for the soil classification system for cohesive soils based on the plasticity tests. Besides it use for identification, the plasticity tests give information concerning the cohesion properties of soil and the amount of capillary water which it can hold. It is also used directly in specifications for controlling soil for use in fill. These index properties of soil have also been related to various other properties of the soil.

PRECAUTIONS:

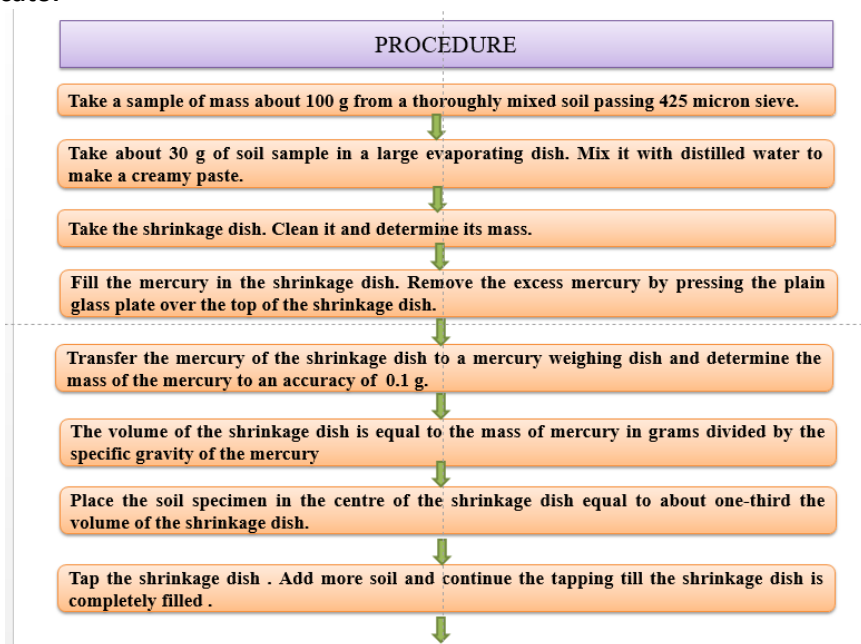
This test should proceed from the drier (more drops) to the wetter (less drops) condition of the soil.

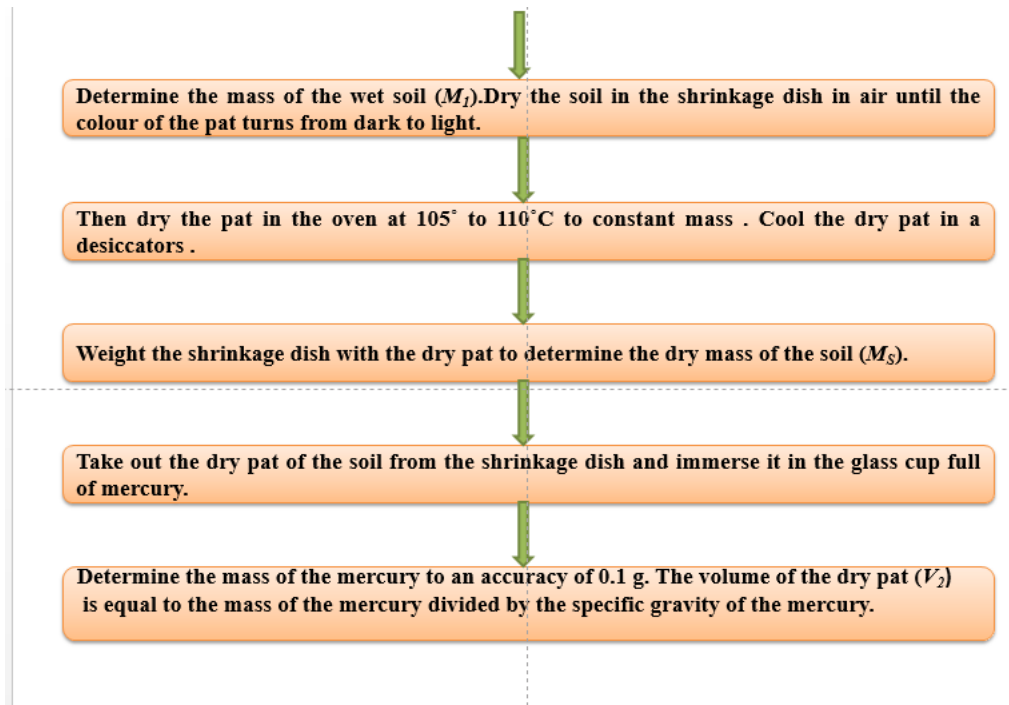
This test may also be conducted from wetter to drier condition provided drying is achieved by kneading the wet soil and not by adding dry soil.

Experiment No -6 : Determination of Shrinkage Limit of Soil : IS 2720: Part VI : 1972

APPARATUS USED:

1. Evaporating Dish
2. Spatula
3. Shrinkage Dish
4. Straight Edge
5. Glass Cup
6. Glass Plate
7. Oven
8. Sieve
9. Balances
10. Mercury
11. Desiccator





Calculations

1. Moisture Content (w): Calculate the moisture content of wet soil pat as a percentage of the dry weight of soil as follows:

$$w = \frac{W - W_0}{W_0} \times 100$$

Where

w = moisture content of the pat

W = weight of wet soil pat obtained by subtracting the weight of shrinkage dish from the weight of the dish and wet pat.

W_0 = weight of dry soil pat obtained by subtracting the weight of the shrinkage dish from the weight of the dish and dry pat.

2. Shrinkage Limit (Remolded Soil): Calculate the shrinkage limit using the following formula:

$$w_s = w - \left(\frac{V - V_0}{W_0} \right) \times 100$$

w_s = shrinkage limit in percent

V = volume of wet soil pat in ml,

V_0 = volume of dry soil pat in ml

3. Shrinkage index (I_s): Calculate the shrinkage using the following formula

$$I_s = I_p - w_s$$

I_p = plasticity index

Observation Table

1. Determination no. 2. Shrinkage Dish no. 3. Weight of shrinkage dish in g. 4. Weight of shrinkage dish in + wet soil pat in g. 5. Weight of shrinkage dish in + dry soil pat in g. 6. Weight of oven dry oven dry soil pat (W_o) in g. 7. Moisture content (w) of soil pat, percentage. 8. Weight of water in g. 9. Evaporating dish no. 10. Weight of mercury filling shrinkage disk + weight of evaporating disk. 11. Weight of evaporating dish 12. Weight of mercury filling shrinkage dish in g. 13. Volume of the soil pat (V) in ml 14. Weight of mercury displaced by the dry soil pat + weight of evaporating dish in g. 15. Weight of mercury displaced by dry soil in g 16. Volume of soil pat (V_o)	1	2	3
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General Remarks:-

SOIL	SHRINKAGE LIMIT FROM MERCURY METHOD(%) [approx.]
Red soil	21.5
Black soil	22.7
Bentonite	13.2

SIGNIFICANCE:

Shrinkage limit is needed for studying the swelling and shrinkage properties of cohesive soil. The shrinkage factor helps in the design problems of structure made up of this soil or resting on such soil. It helps in assessing the suitability of soil as a construction material in foundations, roads, embankments, and dams. The state of the given soil can also be found from the shrinkage limit.

PRECAUTIONS:

- Clean the sieves with the help of a brush, after sieving.
- While weighing put the sieve with soil sample on the balance in a concentric position.
- Check the electric connection of the sieve shaker before conducting the test.

Experiment No -7 : Determination of Density of Soil by Core Cutter Method : IS 2720: Part IX : 1992

APPARATUS REQUIRED:

1. Cylindrical Core Cutter. 100 mm internal diameter.
2. Steel Dolly of 130 mm long, 25 mm high and 100 mm internal diameter.
3. Steel Rammer mass 9 kg, overall length with the foot and staff about 900 mm.
4. Balance, with an accuracy of 1 g,
5. Palette Knife.
6. Straight Edge.
7. Steel Rule etc.
8. Square Metal Tray – 300mm x 300mm x 40mm.
9. Towel.

Procedure :-

- First we measure the internal diameter (D_i) and height (H) of the core so that the volume (V) of the steel tube can be determined in the laboratory.
- We also take note of the weight (W_c) of the core by using physical balance.
- We then take the apparatus to the site and the soil, to be tested, was leveled and cleaned.
- After that we remove the soil surrounding the core so that it can be pulled off easily.
- After that we remove the soil surrounding the core so that it can be pulled off easily.
- Then we fix the dolly Then we fix the dolly on the core and start pressing the core in the soil. The ramming was done till only 15 mm of the dolly remains above the soil.
- We take note of the weight of the soil including the core W_s .
- After that we take representative sample for moisture determination of soil.

Calculations :-

Table

1.Determination No.	Sample 1	Sample 2	Sample 3
2.Weight of core-cutter + wet soil(W_s) in g			
3. Weight of core-cutter (W_c) in g.			
4.Weight of wet soil (W_s-W_c), in g.			
5.Volume of core cutter (V_c) in cm^3			
6.Water container no.			
7.Weight of container with lid (W_1), in g			
8. Weight of container with lid and wet soil (W_2), in g			
9.Weight of container with lid and dry soil (W_3), in g			
10. Water content (w) in percentage $w = \frac{W_2 - W_3}{W_3 - W_1} \times 100$			

The bulk density γ_b , that is, the weight of the wet soil per cubic centimeter shall be calculated in g/cm^3 from the following formula:

$$\gamma_b = \frac{W_s - W_c}{V_c}$$

The dry density γ_d , that is, the weight of the dry soil per cubic centimeter shall be calculated in g/cm^3 from the following formula

$$\gamma_d = \frac{100 \gamma_b}{100 + w}$$

SIGNIFICANCE:

- We can be obtained dry density and bulk density of soil.
- Various properties of soil like void ratio, water content, degree of Saturation can be obtained from this method.

PRECAUTIONS:

- Steel dolly should be placed on the top of the cutter before ramming it down into the ground.
 - Core cutter should not be used for gravel, boulder or any hard ground.
 - Before removing cutter, soil should be removed around cutter to minimize disturbances.
 - While lifting cutter no soil should drop down.
-

Experiment No -8 : Sedimentation analysis of soil using pipette method: IS 2720: Part IV : 1985

APPARATUS REQUIRED:-

Sampling Pipette, Glass Sedimentation Tubes, Weighing Bottle, Constant Temperature Bath, Stirring Apparatus, Sieves, Balance, Oven, Stop Watch, Desiccator, Evaporating Dish, Conical Beaker, Funnel, Filter Flask, Pipette, Glass Filter Funnel, Measuring Cylinder, Wash Bottle, Glass Rod, Thermometer

REAGENTS REQUIRED:

1. Hydrogen Peroxide
2. Hydrochloric Acid
3. Sodium Hexametaphosphate Solution.

Procedure :

- If the soil fraction passing 75-micron IS Sieve during wet sieving has been collected, this can be oven-dried and used for pipette analysis, provided the soil has been pre-treated. 25 to 50 g (depending on the soil type) of this soil shall be used for the analysis.
- Pre-treatment of soil -The percentage of soluble salts in the soil shall be determined as specified in IS : 2720 (Part 21) - 1977. In case it is more than one percent, the soil shall be washed with water before further treatment taking care to see that soil particles not lost.
- Dispersion of soil- 25 ml sodium hexametaphosphate solution shall be added to the soil in the evaporating dish together with about 25 ml of distilled water and the soil brought into suspension by stirring with a glass rod. The mixture shall be warmed gently for 10 minutes and then transferred to the cup of the mechanical mixer using a jet of distilled water to wash all traces of the soil out of the evaporating dish. Any soil adhering to the dish shall be rubbed off with the rubber policeman. The amount of water used shall not exceed 150 ml. The soil suspension shall then be stirred well for 15 minutes or longer in the case of highly clayey soils. The suspension shall then be transferred to 75-micron IS Sieve placed on a receiver and the soil shall be washed on this sieve using a jet of distilled water from a wash-bottle. Particular care shall be taken to wash off all traces of suspension. adhering to the dispersion cup.
- The amount of distilled water used during the operation shall not exceed 150 ml. The suspension, that has passed through this sieve shall then be transferred to the sedimentation tube using the glass funnel and the volume of liquid is made up to 500 ml with distilled water. The treated soil fraction material retained on the 75-micron IS Sieve may be oven-dried and treated and the cumulative percentages of the soil retained on each sieve shall be calculated.
- Sedimentation -25 ml of sodium hexametaphosphate solution shall be added from a pipette to a graduated 500-ml sedimentation tube (comparison tube) and diluted with distilled water to exactly 500 ml. This sedimentation tube together with the tube containing the soil suspension shall be immersed in the constant temperature bath (where used), the temperature of the bath noted, the rubber bungs inserted and the tubes allowed to stand until they have reached the temperature of the bath. The tubes with their contents shall then be thoroughly shaken by inverting the tubes several times. They shall then be replaced in the apparatus, simultaneously starting the stop watch. The rubber bungs shall then be carefully removed without agitating the tubes.
- The pipette with the tap E closed shall be lowered vertically into the soil suspension until the end is 100 ± 1 mm below the surface of the suspension. It shall be lowered with great care some 15 seconds before the sample is due to be taken. Approximately 10 seconds shall be taken to complete this operation. The tap E shall be opened and a sample (V_p) drawn up into the pipette. The pipette and the bore in the tap E shall be filled with solution and tap E then closed. This operation shall take 10 seconds to complete. After each sampling operation the pipette shall be withdrawn from the suspension, taking approximately 10 seconds to complete the operation.

- During the sampling a small amount of the suspension may have been drawn up into the bulb D above the bore of the tap E. This surplus shall be washed away into the beaker down the outlet tube F by opening the tap E in such a way as to connect D and F. Distilled water shall then be allowed to run from the bulb funnel A into D and out through F until no solution remains in the system. A tared weighing bottle shall be placed under the end of the pipette and the tap E opened so that the contents of the pipette are delivered into the bottle.
- Any suspension left on the inner walls of the pipette shall be washed into the weighing bottle by allowing distilled water from the bulb A to run through B, D and E into the pipette. This procedure shall be repeated at the end of each time corresponding to particle diameters 0.02 mm, 0.006 mm, 0.002 mm, 0.001 mm. The weighing bottles and contents shall be placed in the oven maintained at 105° to 110°C and the sample evaporated to dryness
- After cooling in a desiccator the weighing bottle and contents shall be weighed to the nearest 0.001 g and the mass of the solid material in the sample determined (W_1, W_2, W_3 and W_4 for each respective sampling time). Between any of the times in which the above sampling is taking place, a sample (V_p ml) shall be taken from the tube containing the sodium hexametaphosphate solution. The mass of solid material in the sample shall be determined (W_s).

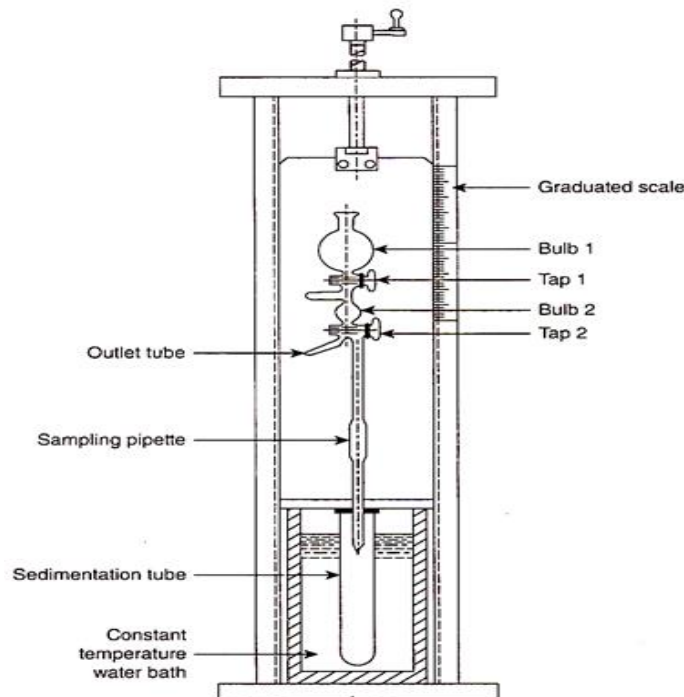


Figure 6.8 Pipette method apparatus.

Calculation:-

1-Loss of weight in pre-treatment- The loss of mass in pre-treatment of the soil in percentage shall be calculated as follows:

$$P = 100 - \frac{W_b (100 + w)}{W_a}$$

where, P = loss in mass in percentage, g

W_b = mass of soil after pre-treatment, g

w = air dry moisture content of the soil taken for analysis,

W_a = mass of air dry soil used, g

2- Diameter of particle - The diameter of the any sampling time t shall be calculated particle in suspension from the formula:

$$D = \sqrt{\frac{30 \mu}{980 (G - G_l)}} \sqrt{\frac{H}{t}}$$

where, D = diameter of particle in suspension, in mm;

μ = coefficient of viscosity of water in poises temperature of suspension at the time of sampling

G = specific gravity of the soil fraction used in the sedimentation analysis, in g/cm³;

G_1 = specific gravity of water, in g/cm³;

H = height of fall of the particles or sampling depth, in cm;

t = time elapsed before sampling, in minutes.

3-Percentage finer than D -The mass of solid material in 500 ml of suspension for each respective sampling time shall be calculated from the formula:

M_1 or M_2 or M_3 or M_4 , etc.,

$$\text{or } M_s \text{ in g} = \frac{W_1 \text{ or } W_2 \text{ or } W_3 \text{ or } W_4, \text{ etc., or } W_s}{V_p} \times 500$$

where, M_1, M_2, M_3, M_4 , etc. = mass of material in 500 ml from respective samplings,

M_s = mass of sodium hexametaphosphate in 500 ml of solution,

W_1, W_2, W_3, W_4 , etc. = mass of material in V_p ml from respective samplings,

W_s = mass of sodium hexametaphosphate in V_p ml of solution,

V_p = volume in ml of sample pipetted for respective sampling.

Mass of air dry soils (W_a)=.....g

Water content of air dry soil (w)=.....%

Mass of oven dry soil after pretreatment (W_b)=...g

Loss in mass in pre-treatment in percent=.....%

Specific gravity of soil=

Volume of suspension taken in pipette (V_a)=....ml

Depth at which sample was taken=cm

Mass of dispersing agent in the volume of -

suspension sampled=g

OBSERVATION TABLE:

Time Elapsed Before sampling	Temperature of Suspension	Mass Of Container + Oven Dry Soil fraction	Mass Of bottle	Mass Of Solid Material In Bottle In Volume V_p	Mass Of Solid Material In 500ml	Correct Mass Of Soil Material in 500ml	Diameter of Soil Grain in mm	% Finer Than D as % of W_b	Combined % Finer Than D As % of Total Sample

SIGNIFICANCE:

The pipette method is a modified sedimentation method for classifying finely divided material according to particle size. It is readily adaptable to the determination of the fineness of molding sand and has been satisfactorily used for this purpose at the National Bureau of Standards. The equipment required is easily assembled, the operation is simple, the time required for a determination is short, the information obtained is wider in scope, and the reproducibility of results is greater than is obtained by other methods now in use.

PRECAUTIONS:

- There should not no air bubble present in the pipette.
- During experiment the tap of should be air tight.
- The amount of distilled water used during the operation shall not exceed 150 ml
- The pipette with the tap E closed shall be lowered vertically into the soil suspension until the end is 100 ± 1 mm below the surface of the suspension

Experiment No -9 Determination of Permeability of soil : IS 2720: Part XVII : 1986 (Falling Head); Part XXXVI:1987 (Constant Head)

APPARATUS REQUIRED:

1. Set of Stand Pipes- Glass stand pipes for falling head test arrangement, varying in diameter from 5 to 20mm
2. Vacuum Pump
3. Compaction Rammer
4. Permeameter Mould
5. Mixing Pan
6. Graduated Cylinder
7. Meter Scale
8. Stop Watch
9. Thermometer
10. IS Sieve
11. Detachable Collar

Procedure:-

A 2.5kg sample shall be taken from a thoroughly mixed air-dried or oven-dried material. The moisture content of the 2.5 kg sample shall be determined as described in IS:2720(Part 1)- 1983. The permeameter mould shall be weighed empty to the nearest gram. After greasing lightly the inside of the mould it shall be clamped between the compaction base plate and the extension collar. The dry density for remoulding of soil sample shall be either the field density or the value of maximum dry density estimated by the compaction test. Connect the stand pipe of suitable diameter to the inlet at the top. Fill the stand pipe with water. Open the stop cock at the top and allow the water to flow out till all the air in the mould is removed. Close the stop cock and allow the water from the stand pipe to flow through the soil specimen. Open the valve and start the stop watch. Record the time interval for the head to fall from h_1 to $\sqrt{h_1 h_2}$ and also from $\sqrt{h_1 h_2}$ to h_2 . These two time intervals will be equal if the steady conditions have established. Repeat the previous step at least twice after changing the heights h_1 and h_2 .

Calculation:-

At a temperature T of water, the permeability k_T is calculated as

$$k_T = 2.303 \frac{aL}{A(t_f - t_i)} \log_{10} \frac{h_1}{h_2}$$

and the permeability at 27°C is given by]

$$k_{27} = k_T \frac{\gamma_T}{\gamma_{27}}$$

Other parameters to be calculated are the water content, void ratio and degree of saturation shall be determined according to IS:2720 (Part 2) -1973.

Diameter of specimen (D)=cm Length of specimen(L)=.....cm
 Area of specimen (A) = cm² Volume of specimen (V)=.....cm³
 Area of stand -pipe (a)= cm²

OBSERVATION TABLE:

SR No.	Initial time t_i (seconds)	Final time t_f (seconds)	Initial Head h_1 (cm)	Final Head h_2 (cm)

SIGNIFICANCE

The variable head permeameter is used to measure the permeability of relatively less pervious soils. The falling head permeability test has a high dispersion and is not sensitive to changes in porosity. The falling head permeability test is a common laboratory testing method used to determine the permeability of fine grained soils with intermediate and low permeability such as silts and clays.

PRECAUTIONS

- Soil sample should be oven dried properly there should no water present in the soil sample.
 - Take the reading of initial and final head with respect to initial and final time accurately.
-

Experiment No -10 Compaction Test-(Light Weight/Standard Proctor Compaction) (IS 2720-PART VII-1980) Reaffirmed-2011.

APPARATUS REQUIRED:

1. Proctor mould having a capacity of 944 cc with an internal diameter of 10.2 cm and a height of 11.6 cm. The mould shall have a detachable collar assembly and a detachable base plate.
2. Rammer: A mechanical operated metal rammer having a 5.08 cm diameter face and a weight of 2.5 kg. The rammer shall be equipped with a suitable arrangement to control the height of drop to a free fall of 30 cm.
3. Sample extruder, mixing tools such as mixing pan, spoon, towel, and spatula.
4. A balance of 15 kg capacity, Sensitive balance, Straight edge, Graduated cylinder, Moisture tins.

PROCEDURE:

1. Take a representative oven-dried sample, approximately 5 kg in the given pan. Thoroughly mix the sample with sufficient water to dampen it with approximate water content of 4-6 %.
2. Weigh the proctor mould without base plate and collar. Fix the collar and base plate. Place the soil in the Proctor mould and compact it in 3 layers giving 25 blows per layer with the 2.5 kg rammer falling through. The blows shall be distributed uniformly over the surface of each layer.
3. Remove the collar; trim the compacted soil even with the top of mould using a straight edge and weigh.
4. Divide the weight of the compacted specimen by 944 cc and record the result as the bulk density ρ_{bulk} .
5. Remove the sample from mould and slice vertically through and obtain a small sample for water content.
6. Thoroughly break up the remainder of the material until it will pass a no.4 sieve as judged by the eye. Add water in sufficient amounts to increase the moisture content of the soil sample by one or two percentage points and repeat the above procedure for each increment of water added. Continue this series of determination until there is either a decrease or no change in the wet unit weight of the compacted soil.

OBSERVATIONS:

Mould Diameter cm, Height cm, Volume cc, Weight gm

Density						
Determination No.	1	2	3	4	5	6
Weight of water added, W_w (gm)						
Weight of mould + compacted soil (gm)						
Weight of compacted soil, W (gm)						
Average moisture content, w %						
Bulk density (gm /cc) = $W / (\text{Mould volume})$						
Dry density (gm/cc) = Bulk density/(1+w)						
Water content						
Container No.						
Wt. of container (gm) = W_c						
Wt. Of container + wet soil (gm) = W_1						
Wt. Of container + dry soil (gm) = W_2						
Watercontent, $w = (W_2 - W_1) / (W_1 - W_c) \times 100\%$						

Note: Plot dry density vs. moisture content and find out the max dry density and optimum moisture for the soil.

GENERAL REMARKS:

- The peak point of the compaction curve: The peak point of the compaction curve is the point with the maximum dry density ρ_d max. Corresponding to the maximum dry density ρ_d max is a water content known as the optimum water content (also known as the optimum moisture content, OMC). Note that the maximum dry density is only a maximum for a specific compactive effort and method of compaction. This does not necessarily reflect the maximum dry density that can be obtained in the field.
- Zero air voids curve: The curve represents the fully saturated condition ($S = 100\%$). (It cannot be reached by compaction).

Experiment No -11 Consolidation Test of Soil-(IS 2720-PART-15-1986) Reaffirmed-2002

APPARATUS REQUIRED:

1. Consolidometer consisting essentially; a) A ring of diameter = 60mm and height = 20mm, b) Two porous stones c) Guide ring. d) Outer ring. e) Water jacket with base. f) Pressure pad.
2. Loading device consisting of frame, lever system, loading yoke dial gauge fixing device and weights.
3. Dial gauge (accuracy of 0.01 mm), Thermostatically controlled oven, Stopwatch, sample extractor, balance, soil trimming tools, spatula, filter papers, sample containers.

1. **Undisturbed Sample:** From the sample tube, eject the sample into the consolidation ring. The sample should project about one cm from outer ring. Trim the sample smooth and flush with top and bottom of the ring by using wire saw. Clean the ring from outside and keep it ready for weighing.

2. **Remoulded sample:** a. Choose the density and water content at which sample has to be compacted from the moisture-density curve, and calculate the quantity of soil and water required to mix and compact. b. Compact the specimen in compaction mould in three layers using the standard rammers. c. Eject the specimen from the mould using the sample extractor.

PROCEDURE:

1. Saturate two porous stones either by boiling in distilled water about 15 minute or by keeping them submerged in the distilled water for 4 to 8 hrs. Fittings of the Consolidometer which is to be enclosed shall be moistened.
2. Assemble the Consolidometer, with the soil specimen and porous stones at top and bottom of specimen, and providing a filter paper between the soil specimen and porous stone.
3. Position the pressure pad centrally on the top porous stone. Mount the mould assembly on the loading frame, and center it such that the load applied is axial. Make sure that the porous stone and pressure pad are not touching the walls of mould on their sides.
4. Position the dial gauge to measure the vertical compression of the specimen. The dial gauge holder should be set so that the dial gauge is in the beginning of its releases run, and also allowing sufficient margin for the swelling of the soil, if any.
5. Fill the mould with water and apply an initial load to the assembly. The magnitude of this load should be chosen by trial, such that there is no swelling. It should be not less than 50 g/cm² for ordinary soils & 25 g/cm² for very soft soils. The load should be allowed to stand until there is no change in dial gauge readings for two consecutive hours or for a maximum of 24 hours.
6. Note the final dial reading under the initial load. Apply first load of intensity 0.1 kg/cm² (Approx.) and start the stop watch simultaneously. Record the dial gauge readings at various time intervals. The dial gauge readings are taken until 90% consolidation is reached. Primary consolidation is gradually reached within 24 hrs.
7. At the end of the period, specified above take the dial reading and time reading. Double the load intensity and take the dial readings at various time intervals. Repeat this procedure for successive load increments. The usual loading intensity is as follows (Approx.): 0.1, 0.2, 0.5, 1, 2, 4 and 8 kg/cm² .
8. After the last loading is completed, reduce the load to ¼ of the value of the last load and allow it to stand for 24 hrs. Reduce the load further in steps of ¼ the previous intensity till an intensity of 0.1 kg/cm² is reached. Take the final reading of the dial gauge.
9. Reduce the load to the initial load, keep it for 24 hrs and note the final readings of the dial gauge.
10. Quickly dismantle the specimen assembly and remove the excess water on the soil specimen in oven, note its dry weight.

CALCULATIONS:

1. Height of solids (HS) is calculated from the equation $HS = W_s / (G_s \cdot \gamma_w A)$

2. Void ratio. Voids ratio at the end of various pressures are calculated from equation $e = (H - H_s)/H_s$
3. Coefficient of consolidation.

The Coefficient of consolidation at each pressure increment is calculated by using the following equations:

- i. $C_v = 0.197 d^2 / t_{50}$ (Log fitting method)
- ii. $C_v = 0.848 d^2 / t_{90}$ (Square fitting method)

In the log fitting method, a plot is made between dial readings and logarithmic of time, and the time corresponding to 50% consolidation is determined. In the square root fitting method, a plot is made between dial readings and square root of time, and the time corresponding to 90% consolidation is determined. The values of C_v are recorded in Table II.

4. Compression Index. To determine the compression index, a plot of voids ratio (e) Vs log (t) is made. The virgin compression curve would be a straight line and the slope of this line would give the compression index C_c .

5. Coefficient of compressibility. It is calculated as follows $a_v = \Delta e / \Delta \sigma'$ Δe – Change in void ratio $\Delta \sigma'$ - Change in vertical stress

6. Coefficient of permeability. It is calculated as follows $k = C_v \cdot a_v \cdot \gamma_w / (1 + e_0)$.

GRAPHS:

1. Dial reading VS log of time or
2. Dial reading VS square root of time.
3. Voids ratio VS log σ' (average pressure for the increment).

General Remarks:

1. While preparing the specimen, attempts has to be made to have the soil strata orientated in the same direction in the consolidation apparatus.
2. During trimming care should be taken in handling the soil specimen with least pressure.
3. Smaller increments of sequential loading have to be adopted for soft soils.

OBSERVATION AND READING (LOADING):

Table I: Data Sheet for Consolidation Test: Time-Displacement Relationship

Ring Dimensions: Diameter (cm): _____ Area (cm²): _____ Height (cm): _____
 Initial Data: Specimen Ht (cm): _____ Specific Gravity of Soil: _____
 Weight of wet soil + Ring (g): _____ Weight of Ring (g): _____ Bulk Density (g/cc): _____

Table _ 1

Pressure Intensity (Kg/cm ²)	0.1	0.2	0.5	1	2	4	8
Time (min)							
0							
0.25							
1							
2							
4							
8							
15							
30							
1 hr							
2 hrs							
4 hrs							
8 hrs							
24 hrs							

OBSERVATION AND READING (UNLOADING):

Removed Pressure (kg/cm ²)	Retained Pressure (kg/cm ²)	Dial Gauge reading
0	8	
4	4	
2	2	
1	1	
0.5	0.5	
0.3	0.2	
0.1	0.1	
0.1	0.05 (Seating pressure)	

Water Content determination:

Weight of Saturated Sample + Ring (g): _____

Weight of oven dried soil +Ring (g): _____

Water Content (%): _____

Table II: Data Sheet for Consolidation Test: Pressure-Voids Ratio

Applied Pressure	Final dial reading	Change in Specimen Height	Final Specimen Height	Height of solids	Height of voids	Void ratio	Average Height during Consolidation	Fitting Time, t_{90}	Coefficient of Consolidation, c_v
0									
0.1									
0.2									
0.5									
1.0									
2.0									
4.0									
8.0									
2.0									
0.5									
0.1									

Experiment No -12 Triaxial Test of Soil: IS 2720 : Part 11 : 1993

Apparatus Required

- 1: Triaxial Shear Test Apparatus
- 2: Triaxial Shear Test Setup
- 3: sample tubes 3.8 cm (1.5 inch) internal diameter 12.5 cm (5 inches) long.
- 4: Rubber Ring
- 5: Open ended cylindrical section
- 6: Weighing balance

Procedure

Triaxial Test on Cohesive Soil:

Consolidated Undrained test:

- A de-aired, coarse porous disc or stone is placed on the top of the pedestal in the triaxial test apparatus. A filter paper disc is kept over the porous stone. The specimen of the cohesive soil is then placed over the filter paper disc. The usual size of the specimen is about 37.5mm diameter and 75.0mm height. A porous stone is also placed on the top of the specimen. De-aired vertical filter strips are placed at regular spacing around the entire periphery such that these touch both the porous stones. The sample is then enclosed in a rubber membrane, which is slid over the specimen with the help of a membrane stretcher. The membrane is sealed to the specimen with O-rings.
- The triaxial cell is placed over the base and fixed to it by tightening the nuts. The cell is then filled with water by connecting it to the pressure supply. Some space in the top portion of the cell is filled by injecting oil through the oil valve. When excess oil begins to spill out through the air-vent valve, both the valves (oil valve and air-vent valve) are closed. Pressure is applied to the water filled in the cell by connecting it to the mercury-pot system. As soon as the pressure acts on the specimen, it starts consolidating. The specimen is connected to the burette through pressure connections for measurement of volume changes. The consolidation is complete when there is no more volume change.
- When the consolidation is complete, the specimen is ready for being sheared. The drainage valve is closed. The pore water pressure measurement device is attached to the specimen through the pressure connections. The proving ring dial gauge is set to zero. Using the manual control provided in the loading frame, the ram is pushed into the cell but not allowed to touch the loading cap. The loading machine is then run at the selected speed. The proving ring records the force due to friction and the upward thrust acting on the ram. The machine is stopped, and with the manual control, the ram is pushed further into the cell bringing it in contact with the loading cap. The dial gauge for the measuring axial deformation of the specimen is set to zero.
- The sample is sheared by applying the deviator stress by the loading machine. The proving ring readings are generally taken corresponding to axial strains of 1/3%, 2/3%, 1%, 2%, 3%, 4%, 5%, until failure or 20% axial strain.
- Upon completion of the test, the loading is shut off. Using the manual control, all additional axial stress is removed. The cell pressure is then reduced to zero and the cell is emptied. The triaxial cell is unscrewed and removed from the base. O-rings are taken out, and the membrane is removed. The specimen is then recovered after removing the loading cap and the top porous stone. The filter paper strips are peeled off. The post-shear mass and length are determined. The water content of the specimen is also found.

Unconsolidated Undrained test:

- The procedure is similar to that for a consolidated-undrained test, with one basic difference that the specimen is not allowed to consolidate in the first stage. The drainage valve during the test is kept closed. However, the specimen can be connected to the pore-water pressure measurement device if required.
- Shearing of the specimen is started just after the application of the cell pressure. The second stage is exactly the same as in the consolidated-undrained test described above.

4.1.3

Consolidated Drained test:

The procedure is similar to that for a consolidated-undrained test, with one basic difference that the specimen is sheared slowly in the second stage. After the consolidation of the specimen in the first stage, the drainage valve is not closed. It remains connected to the burette throughout the test. The volume changes during the shearing stage are measured with the help of the burette. As the permeability of cohesive soils is very low, it takes 4-5 days for the consolidated drained test.

Triaxial tests on Cohesionless Soils:

- Triaxial tests on specimens of cohesionless soils can be conducted using the procedure as described for cohesive soils. As the samples of cohesionless soils cannot stand of their own, a special procedure is used for preparation of the sample as described below.
- A metal former, which is a split mould of about 38.5mm internal diameter, is used for the preparation of the sample. A coarse porous stone is placed on the top of the pedestal of the triaxial base and the pressure connection is attached to a burette (not shown). One end of a membrane is sealed to the pedestal by O-rings. The metal former is clamped to the base. The upper metal ring of the former is kept inside the top end of the rubber membrane and is held with the help of clamp before placing the funnel and the rubber bung in position.
- The membrane and the funnel are filled with de-aired water. The cohesionless soil which is to be tested is saturated by mixing it with enough water in a beaker. The mixture is boiled to remove the entrapped air. The saturated soil is deposited in the funnel, with a stopper in position, in the required quantity. The glass rod is then removed and the sample builds up by a continuous rapid flow of saturated soil in the former. The funnel is then removed. The sample may be compacted if required. The surface of the sample is leveled and a porous stone is placed on its top. The loading cap is placed gently on the top porous stone. O-rings are fixed over the top of the rubber membrane.
- A small negative pressure is applied to the sample by lowering the burette. The negative pressure gives rigidity to the sample and it can stand without any lateral support. For sample of 37.5mm diameter, a negative pressure of 20cm of water (or 2 kN/m²) is sufficient. As soon as the negative pressure is applied, the consolidation of the sample occurs and it slightly shortens. The diameter of the upper porous stone should be slightly smaller than that of the specimen so that it can go inside when the sample shortens; otherwise, a neck is formed.
- The split mould is then removed and the diameter and the height of the sample are measured. The thickness of the membrane is deducted from the total diameter to get the net diameter of the sample. The cell is then placed over the base and clamped to the base. It is then filled with water.
- The rest of the procedure is the same as for cohesive soils.

Computation of Various Parameters:

Post-Consolidation Dimensions: In consolidated-drained and consolidated-undrained tests, the consolidation of the specimen takes place during the first stage. As the volume of the specimen decreases, its post-consolidation dimensions are different from the initial dimensions. The post consolidation dimensions can be determined approximately assuming that the sample remains cylindrical and it behaves isotropically.

Let L_i , D_i and V_i be the length, diameter and the volume of the specimen before consolidation. Let L_0 , D_0 and V_0 be the corresponding quantities after consolidation. Volumetric change, $\Delta V_i = V_i - V_0$ Volumetric change is measured with the help of burette.

Volumetric strain, $\epsilon_v = \Delta V_i / V_i$

For isotropic consolidation, the volumetric strain is three times the linear strain (ϵ_l), thus: $\epsilon_l = \epsilon_v / 3$ $L_0 = L_i - \Delta L_i = L_i - L_i \times \epsilon_l$ $L_0 = L_i (1 - \epsilon_l) = L_i (1 - \epsilon_v/3)$ Similarly, $D_0 = D_i (1 - \epsilon_v/3)$

The post consolidation diameter D_0 can also be computed after L_0 has been determined from the relation: $(\pi/4 \cdot D_0^2) \times L_0 = V_0$ Or $D_0 = \sqrt{V_0 / [(\pi/4) \times L_0]}$

5.2 Cross-sectional area during Shear Stage: As the sample is sheared, its length decreases and the diameter increases. The cross-sectional area A at any stage during shear can be determined assuming that the sample remains cylindrical in shape. Let ΔL_0 be the change in length and ΔV_0 be the change in volume. The volume of the specimen at any stage is given by $V_0 \pm \Delta V_0$.

Therefore, $A (L_0 - \Delta L_0) = V_0 \pm \Delta V_0$ Or, $A = V_0 \pm \Delta V_0 / L_0 - \Delta L_0 = V_0 (1 \pm \Delta V_0 / V_0) / L_0 (1 - \Delta L_0 / L_0)$

This is the general equation which gives the cross-sectional area of the specimen and it can be written as: $A = A_0 L_0 (1 \pm \Delta V_0 / V_0) / L_0 (1 - \epsilon_l)$

$A = A_0 (1 \pm \Delta V_0 / V_0) / (1 - \epsilon_l)$

Where, ϵ_l is the axial strain in the sample.

For an undrained test, the volumetric change (ΔV_0) is zero and then the equation above becomes: $A = A_0 / (1 - \epsilon_l)$ The stresses in the specimen at various stages of shear should be calculated using the cross-sectional area A as found above.

Stresses: Deviator Stress:

The deviator stress (σ_d) acting on the specimen when the axial load applied by the loading machine is P can be obtained as: $\sigma_d = P/A$ The deviator stress (σ_d) is equal to $(\sigma_1 - \sigma_3)$. It may be noted that the load indicated by the proving ring is slightly more than P because of friction on the ram and the upward thrust on the ram due to pressure of the water in the cell. The correction can be determined separately. A more convenient procedure is to lift the ram above the specimen when the cell pressure has been applied. The machine is started keeping the strain rate the same as to be used in the actual test. The proving ring records the load. To account for correction, the dial gauge on the proving ring is set to zero to indicator zero load. This automatically compensates the ram friction and the upward thrust on the ram due to cell pressure. Thus the load indicated by the proving ring during shear would be equal to the load P applied to the specimen. The minor principal stress (σ_3) is equal to the cell pressure (σ_c). The major principal stress (σ_1) is equal to the sum of the cell pressure and the deviator stress. Thus, $\sigma_1 = \sigma_3 + (\sigma_1 - \sigma_3) = \sigma_3 + \sigma_d$ The deviator stress at failure ($\sigma_1 - \sigma_3$) is known as the compressive strength of the soil.

Experiment No -13 Direct Shear Test of Soil : IS 2720 : Part 13 : 1986

APPARATUS

Direct shear device, Load and deformation dial gauges, Balance.

PROCEDURE

Test Procedure:

- (1) Weigh the initial mass of soil in the pan.
- (2) Measure the diameter and height of the shear box. Compute 15% of the diameter in millimeters.
- (3) Carefully assemble the shear box and place it in the direct shear device. Then place a porous stone and a filter paper in the shear box.
- (4) Place the sand into the shear box and level off the top. Place a filter paper, a porous stone, and a top plate (with ball) on top of the sand
- (5) Remove the large alignment screws from the shear box! Open the gap between the shear box halves to approximately 0.025 in. using the gap screws, and then back out the gap screws.
- (6) Weigh the pan of soil again and compute the mass of soil used.
- (7) Complete the assembly of the direct shear device and initialize the three gauges (Horizontal displacement gage, vertical displacement gage and shear load gage) to zero.
- (8) Set the vertical load (or pressure) to a predetermined value, and then close bleeder valve and apply the load to the soil specimen by raising the toggle switch.
- (9) Start the motor with selected speed so that the rate of shearing is at a selected constant rate, and take the horizontal displacement gauge, vertical displacement gage and shear load gage readings. Record the readings on the data sheet. (Note: Record the vertical displacement gage readings, if needed).
- (10) Continue taking readings until the horizontal shear load peaks and then falls, or the horizontal displacement reaches 15% of the diameter.

Analysis:

- (1) Calculate the density of the soil sample from the mass of soil and volume of the shear box.
- (2) Convert the dial readings to the appropriate length and load units and enter the values on the data sheet in the correct locations. Compute the sample area A , and the vertical (Normal) stress s_v . $A N s_v =$ Where: N_v = normal vertical force, and s_v = normal vertical stress
- (3) Calculate shear stress (t) using $A h F t =$ Where F_h = shear stress (measured with shear load gage)
- (4) Plot the horizontal shear stress (t) versus horizontal (lateral) displacement H .
- (5) Calculate the maximum shear stress for each test.
- (6) Plot the value of the maximum shear stress versus the corresponding vertical stress for each test, and determine the angle of internal friction (ϕ) from the slope of the approximated Mohr-Coulomb failure envelope.

SOME EXAMPLES OF PRACTICAL EXAMINATION QUESTIONS
B. Tech (Environmental Engineering)
Subject: Environmental Geotechnology(ESC355) - Practical Examination

Q-1. Determine void ratio and porosity of the supplied soil. Write details of lab experiment performed by you. Derive the relation between void ratio and porosity?

Q-2 A sampler with a volume of 45 cm³ is filled with soil sample. When the soil sample is poured into a measuring cylinder, it displaced 25 cm³ of water. What is the porosity and the void ratio of the soil sample?

Q.3. Determine the Specific Gravity of supplied soil sample, by Specific Gravity bottle method. Write details of lab experiment performed by you.

Q-4 Write down the significance of Specific Gravity of soil in Geotechnical Engineering.

Q.5. Determine the shrinkage limit of supplied soil sample. Write details of lab experiment performed by you.

Q-6 A soil sample has void ratio of 0.50 in the dry condition. The grain specific gravity has been determined as 2.72. What will be the Shrinkage limit of this soil?

Q.7. Determine liquid limit / plastic limit of the given soil samples. Write details of lab experiment performed by you.

Q-8. Calculate the plasticity index and what do you infer from the results ?

Q.9. Determine the coefficient of permeability of supplied soil by falling head method. Write details of lab experiment performed by you.

Q-10. Determine the coefficient of permeability from the following data :
Length of sand sample – 25 cm, Area of cross section of the sample- 30cm². Head of water = 40cm, Discharge = 200 in 110s.

Q.11. Determine the shear strength of soil by direct shear test method in laboratory. What do you understand by shear stress and strain of soil.

Q.12. Determine the particle size distribution of soil sample by Sieve method. Draw the particle size distribution curve from your data sheet.

Q-13. What do you understand by D₆₀, D₃₀ and D₁₀.

Q14. Determine the settlements due to primary consolidation of soil by conducting one dimensional test. In consolidation test the void ration of a soil sample decrease from 1.20 to 1.10 when the pressure is increased 160 to 320 kN/ m². Calculate the coefficient of consolidation of the sample if the coefficient of permeability (k) is 8.0×10^{-7} .

Department of Environmental Science and Engineering

B. Tech. Environmental Engineering

Wastewater Engineering Practical (ESC 366)

Wastewater Engineering Laboratory (Room No: 211-A)

Wastewater Engg. (Practical ESC 366)

INDEX OF EXPERIMENTS

Sl. No.	Name of Practical
1.	Determination of pH, acidity, alkalinity of wastewater sample
2.	Determine TSS, TDS, TVS & TS of given wastewater sample. Also determine the settleable solids & non-settleable SS in given wastewater sample.
3.	Demonstration of Nitrate-nitrogen ($\text{NO}_3\text{-N}$) Ammonical Nitrogen & TKN of given wastewater sample
4.	Determination of BOD & COD in municipal wastewater and confer on the biodegradability of wastewater.
5.	Determination of Phenol & SO_4 in municipal wastewater
6.	Determine MLSS, MLVSS concentration, sludge volume index (SVI) of sludge sample
7.	Determination of TOC, DOC, UV_{254} & SUVA of given wastewater sample
8.	Determination of organic pollutant by High Performance Liquid Chromatography
9.	Determination of VFA using of given wastewater sample by Gas Chromatography
10.	Determination of dye concentration in given wastewater sample
11.	Exam

Experiment No- 1

Aim: Determination of pH, Acidity and Alkalinity of given water sample.

A. pH (Electrometric method)

1.1 Principle

The pH is determined by measurement of the electromotive force (emf) of a cell comprising of an indicator electrode (an electrode responsive to hydrogen ions such as glass electrode) immersed in the test solution and a reference electrode (usually a calomel electrode). Contact is achieved by means of a liquid junction, which forms a part of the reference electrode. The emf of this cell is measured with pH meter.

Since the pH is defined operationally on a potentiometric scale, the measuring instrument is also calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using standard buffers having assigned pH value so that

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

where pH_B = assigned pH of standard buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$\text{pH}_s = \text{pH}_B + F (E_s - E_B) / 2.303 RT$$

where, pH_s = potentiometrically measured sample Ph

F = Faraday 9.649×10^4 coulomb/mole

E_s = Sample emf V

E_B = Buffer emf V

R = Gas constant 1.987 cal deg⁻¹ mole⁻¹

T = absolute temperature, °K

1.2 Apparatus and equipment

- pH meter: Consisting of potentiometer, a glass electrode, a reference electrode and a temperature compensating device. A balanced circuit is completed through potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolt.
- Reference electrode: Consisting of a half cell that provides a standard electrode potential. Generally calomel, silver-silver chloride electrodes are used as reference electrode.
- Sensor (glass) electrode: Several types of glass electrodes are available. The glass electrode consists essentially of a very thick walled glass bulb, made of low melting point glass of high electrical conductivity, blown at the end of a glass tube. This bulb contains an electrode, which has a constant potential, e.g. a platinum wire inserted in a solution of H^+ hydrochloric acid saturated with quinhydrone. The bulb is placed in the liquid where pH is to be determined.

- d. Beakers: Preferably use polyethylene or TFE beakers.
- e. Stirrer: Use a magnetic TFE coated stirring bar.

1.3 Reagents and standards

- a. pH 4 buffer solution: Dissolve 10.12g potassium hydrogen phthalate, $\text{KHC}_8\text{H}_4\text{O}_9$ in distilled water. Dilute to 1L.
- b. pH 7 buffer solution: Dissolve 1.361g anhydrous potassium dihydrogen phosphate, KH_2PO_4 , and 1.42g anhydrous disodium hydrogen phosphate, Na_2HPO_4 , which have been dried at 110°C . Use distilled water which has been boiled and cooled. Dilute to 1L.
- c. pH 9.2 buffer solution: Dissolve 3.81gm borax, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in distilled water, which has been previously boiled and cooled. Dilute to 1L.

1.4 Calibration

Before use, remove the electrodes from the water and rinse with distilled or demineralised water. Dry the electrodes by gentle wiping with a soft tissue. Calibrate the electrode system against standard buffer solution of known pH. Because buffer solution may deteriorate as a result of mould growth or contamination, prepare fresh as needed for work or use readily available pH buffers. Use distilled water a conductivity of less than 2μ siemens at 25°C and distilled and pH 5.6 to 6.0 for the preparation of all standard solutions. For routine analysis, commercially available buffer tablets, powders or solutions of tested quality also are permissible. Buffer having pH 4.0, 7.0 and 9.2 are available. In preparing buffer solutions from solid salts, dissolve all the material; otherwise, the pH calibration will be incorrect. Prepare and calibrate the electrode system with buffer solutions with pH approximating that of the sample, to minimise error resulting from nonlinear response of the electrode.

1.5 Procedure

- a. Before use, remove electrodes from storage solutions (recommended by manufacturer) and rinse with distilled water.
- b. Dry electrodes by gently blotting with a soft tissue paper, standardise instrument with electrodes immersed in a buffer solution within 2 pH units of sample pH.
- c. Remove electrodes from buffer, rinse thoroughly with distilled water and blot dry.
- d. Immerse in a second buffer below pH 10, approximately 3 pH units different from the first, the reading should be within 0.1 unit for the pH of second buffer. (If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or pH meter)
- e. For samples analysis, establish equilibrium between electrodes and sample by stirring sample to ensure homogeneity and measure pH.
- f. For buffered samples (or those with high ionic strength), condition the electrodes after cleaning by dipping them into the same sample, and read pH.
- g. With poorly buffered solutions (dilute), equilibrate electrodes by immersing in three or four successive portions of samples. Take a fresh sample and record the pH.

1.6 Calculation

The pH value is obtained directly from the instrument.

1.7 Environmental Significance

The pH of a solution is measured as negative logarithm of hydrogen ion concentration. At a given temperature, the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion concentration. pH values from 0 to 7 are diminishing acidic, 7 to 14 increasingly alkaline and 7 is neutral.

Measurement of pH in one of the most important and frequently used tests, as every phase of water and wastewater treatment and waste quality management is pH dependent.

The pH of natural water usually lies in the range of 4 to 9 and mostly it is slightly basic because of the presence of bicarbonates and carbonates of alkali and alkaline earth metals. pH value is governed largely by the carbon dioxide/ bicarbonate/ carbonate equilibrium. It may be affected by humic substances, by changes in the carbonate equilibria due to the bioactivity of plants and in some cases by hydrolysable salts.

(B) Measure mineral and phenolphthalein acidity

1.8 Principle

Acids contribute to corrosiveness and influence chemical reaction rates, chemical speciation and biological processes. Acidity of water is its quantitative capacity to react with a strong base to a designated pH. The measured value may vary significantly with the end point pH used in the determination. When the chemical composition of the sample is known study mineral acids, weak acids such as carbonic and acetic and hydrolyzing salts such as iron or aluminum sulfate may contribute to the measured acidity according to the method of determination.

Mineral acidity: It is measured by titration to a pH of about 3.5, the methyl orange end point (also known as methyl orange acidity). **Total acidity:** Titration of a sample to the phenolphthalein end point of pH 8.3 measures mineral acidity plus acidity due to weak acids, thus this is called as total acidity (or phenolphthalein acidity). In water analysis, this test does not bear significant importance because methyl orange acidity invariably remains absent in the raw water and even phenolphthalein acidity (that too principally due to the excessive-prevalence of dissolved carbon dioxide and carbonic acids) normally does not exist to a significant extent in the raw water.

Importance: As far as water analysis is concerned, acidity test does not bear significant importance because methyl orange acidity invariably remains absent in the raw water and even phenolphthalein acidity (that too principally due to the excessive-prevalence of dissolved carbon dioxide and carbonic acids) normally does not exist to a significant extent in the raw water.

1.9 Reagents

pH meter; Reagents: Sodium hydroxide titrant (0.02 N); Phenolphthalein Indicator; Methyl Orange Indicator

1.10 Procedure

1. Take 50 ml sample in a conical flask and add 2-3 drops of methyl orange indicator solution.
2. Fill the burette with 0.02 N NaOH solution and titrate till the colour of solution just

changes to faint **orange** colour, indicating the end point. Record the volume of titrant consumed as V_1 in ml. Calculate the methyl orange acidity using Eq (1a):

When the 0.02 N NaOH solution, used in titration is not standardized, mineral acidity is calculated using following Eq (1b):

3. For phenolphthalein acidity test, add 2-3 drops of phenolphthalein indicator solution to water sample from step 2 and continue the titration till the faint **pink** colour develops in the solution (i.e., the end point of titration). Record the volume of titration consumed as V_2 (mL) and calculate total acidity or phenolphthalein acidity using Eq.(2):

1.11 Calculation

Methyl orange acidity (or Mineral Acidity) = $(V_1 \times 1000) / (\text{Sample volume})$ (1a)

Methyl orange acidity = $(V_1 \times N \times 50 \times 1000) / (\text{Sample vol.})$ (1b)

Total acidity (or Phenolphthalein Acidity) = $(V_2 \times N \times 50 \times 1000) / (\text{Sample vol.})$ (2)

(C) Alkalinity

1.12 Principle

Alkalinity of sample can be estimated by titrating with standard sulphuric acid (0.02N) at room temperature using phenolphthalein and methyl orange indicator. Titration to decolourisation of phenolphthalein indicator will indicate complete neutralization of OH^- and $\frac{1}{2}$ of CO_3^{--} , while sharp change from yellow to orange of methyl orange indicator will indicate total alkalinity (complete neutralisation of OH^- , CO_3^{--} , HCO_3^-)

1.13 Apparatus

- a. Beakers: The size and form will depend upon the electrode and the size of the sample to be used for determination of alkalinity.
- b. Pipettes (volumetric)
- c. Flasks (volumetric): 1000mL, 200mL, 100mL

1.14 Reagents

- a. Standard H_2SO_4 , 0.02 N: Prepare 0.1N H_2SO_4 by diluting 3mL conc. H_2SO_4 to 1000mL. Standardise it against standard 0.1N Na_2CO_3 solution. Dilute appropriate volume of H_2SO_4 to 1000mL to obtain standard 0.02 H_2SO_4 .
- b. Phenolphthalein indicator: Dissolved 0.5g in 500mL 95% ethyl alcohol. Add 500mL distilled water. Add dropwise 0.02N NaOH till faint pink colour appears (pH 8.3).
- c. Methyl orange indicator: Dissolve 0.5g and dilute to 1000mL with CO_2 free distilled water (pH 4.3-4.5).

1.15 Procedure

- a. Take 25 or 50mL sample in a conical flask and add 2-3 drops of phenolphthalein indicator.
- b. If pink colour develops titrate with 0.02N H_2SO_4 till disappears or pH is 8.3. Note the volume of H_2SO_4 required.
- c. Add 2-3 drops of methyl orange to the same flask, and continue titration till yellow colour changes to orange. Note the volumes of H_2SO_4 required.
- d. In case pink colour does not appear after addition of phenolphthalein continue as above.

- e. Alternatively, perform potentiometric titration to preselected pH using appropriate volume of sample and titration assembly. Titrate to the end point pH without recording intermediate pH.

1.16 Calculation

Calculate total (T), phenolphthalein (P) alkalinity as follows:

P-alkalinity, as mg CaCO₃/L = A x 1000/mL sample

T-alkalinity, as mg CaCO₃/L = B x 1000/mL sample

In case H₂SO₄ is not 0.02 N apply the following formula:

Alkalinity, as mg CaCO₃/L = A/B x N x 50000 / mL of sample

Where,

A = mL of H₂SO₄ required to bring the pH to 8.3

B = mL of H₂SO₄ required to bring the pH to 4.5

N = normality of H₂SO₄

Once carbonate and bicarbonate alkalinities are known, then their conversions to milligrams CO₃ -- or HCO₃ - /L are possible.

mg CO₃ --/L = Carbonate alkalinity mg CaCO₃/L x 0.6

mg HCO₃ = Bicarbonate alkalinity mg CaCO₃/L x 1.22

from above, molar concentration may be obtained as follows:

[CO₃ --] = mg/L CO₃ / 60000

[HCO₃ -] = mg/L HCO₃ - / 61000

1.17 Environmental Significance

Alkalinity is a chemical measurement of a water's ability to neutralize acids. Alkalinity is also a measure of water buffering capacity or its ability to resist changes in pH upon the addition of acids or bases. Alkalinity of natural waters is due primarily to the presence of weak acid salts although strong bases may also contribute (i.e. OH⁻) in extreme environments. Bicarbonates represent the major form of alkalinity in natural waters; its source being the partitioning of CO₂ from the atmosphere and the weathering of carbonate minerals in rocks and soil. Other salts of weak acids, such as borate, silicates, ammonia, phosphates, and organic bases from natural organic matter, may be present in small amounts. Alkalinity, by convention, is reported as mg/L CaCO₃ since most alkalinity is derived from the weathering of carbonate minerals.

1.18 References

Standard Methods for the Examination of Water and Wastewater, APHA, AWWA and WEF, 21st Edition, 2005.

Please see the practical through

<https://youtu.be/vwY-xWMam7o>

<https://youtu.be/vwY-xWMam7o?t=254>

<https://www.youtube.com/watch?v=LzE-cwRFuoA>

(B) Measure mineral and phenolphthalein acidity

1.8 Principle

Acids contribute to corrosiveness and influence chemical reaction rates, chemical speciation and biological processes. Acidity of water is its quantitative capacity to react with a strong base to a designated pH. The measured value may vary significantly with the end point pH used in the determination. When the chemical composition of the sample is known study mineral acids, weak acids such as carbonic and acetic and hydrolyzing salts such as iron or aluminum sulfate may contribute to the measured acidity according to the method of determination.

Mineral acidity: It is measured by titration to a pH of about 3.5, the methyl orange end point (also known as methyl orange acidity). **Total acidity:** Titration of a sample to the phenolphthalein end point of pH 8.3 measures mineral acidity plus acidity due to weak acids, thus this is called as total acidity (or phenolphthalein acidity). In water analysis, this test does not bear significant importance because methyl orange acidity invariably remains absent in the raw water and even phenolphthalein acidity (that too principally due to the excessive-prevalence of dissolved carbon dioxide and carbonic acids) normally does not exist to a significant extent in the raw water.

Importance: As for as water analysis is concerned, acidity test does not bear significant importance because methyl orange acidity invariably remains absent in the raw water and even phenolphthalein acidity (that too principally due to the excessive-prevalence of dissolved carbon dioxide and carbonic acids) normally does not exist to a significant extent in the raw water.

1.9 Reagents

pH meter; Reagents: Sodium hydroxide titrant (0.02 N); Phenolphthalein Indicator; Methyl Orange Indicator

1.10 Procedure

4. Take 50 ml sample in a conical flask and add 2-3 drops of methyl orange indicator solution.
5. Fill the burette with 0.02 N NaOH solution and titrate till the colour of solution just changes to faint **orange** colour, indicating the end point. Record the volume of titrant consumed as V_1 in ml. Calculate the methyl orange acidity using Eq (1a):
When the 0.02 N NaOH solution, used in titration is not standardized, mineral acidity is calculated using following Eq (1b):
6. For phenolphthalein acidity test, add 2-3 drops of phenolphthalein indicator solution to water sample from step 2 and continue the titration till the faint **pink** colour develops in the solution (i.e., the end point of titration). Record the volume of titration consumed as V_2 (mL) and calculate total acidity or phenolphthalein acidity using Eq.(2):

Please see the practical through: <https://www.youtube.com/watch?v=Yiq3FoykArQ> and https://www.youtube.com/watch?v=UL56Lr_TKTI

1.11 Calculation

Methyl orange acidity (or Mineral Acidity) = $(V_1 \times 1000) / (\text{Sample volume})$ (1a)

Methyl orange acidity = $(V_1 \times N \times 50 \times 1000) / (\text{Sample vol.})$ (1b)

Total acidity (or Phenolphthalein Acidity) = $(V_2 \times N \times 50 \times 1000) / (\text{Sample vol.})$ (2)

(C) Alkalinity

1.12 Principle

Alkalinity of sample can be estimated by titrating with standard sulphuric acid (0.02N) at room temperature using phenolphthalein and methyl orange indicator. Titration to decolourisation of phenolphthalein indicator will indicate complete neutralization of OH^- and $\frac{1}{2}$ of CO_3^{--} , while sharp change from yellow to orange of methyl orange indicator will indicate total alkalinity (complete neutralisation of OH^- , CO_3^{--} , HCO_3^-)

1.13 Apparatus

- d. Beakers: The size and form will depend upon the electrode and the size of the sample to be used for determination of alkalinity.
- e. Pipettes (volumetric)
- f. Flasks (volumetric): 1000mL, 200mL, 100mL

1.14 Reagents

- d. Standard H_2SO_4 , 0.02 N: Prepare 0.1N H_2SO_4 by diluting 3mL conc. H_2SO_4 to 1000mL. Standardise it against standard 0.1N Na_2CO_3 solution. Dilute appropriate volume of H_2SO_4 to 1000mL to obtain standard 0.02 H_2SO_4 .
- e. Phenolphthalein indicator: Dissolved 0.5g in 500mL 95% ethyl alcohol. Add 500mL distilled water. Add dropwise 0.02N NaOH till faint pink colour appears (pH 8.3).
- f. Methyl orange indicator: Dissolve 0.5g and dilute to 1000mL with CO_2 free distilled water (pH 4.3-4.5).

1.15 Procedure

- f. Take 25 or 50mL sample in a conical flask and add 2-3 drops of phenolphthalein indicator.
- g. If pink colour develops titrate with 0.02N H_2SO_4 till disappears or pH is 8.3. Note the volume of H_2SO_4 required.
- h. Add 2-3 drops of methyl orange to the same flask, and continue titration till yellow colour changes to orange. Note the volumes of H_2SO_4 required.
- i. In case pink colour does not appear after addition of phenolphthalein continue as above.
- j. Alternatively, perform potentiometric titration to preselected pH using appropriate volume of sample and titration assembly. Titrate to the end point pH without recording intermediate pH.

Please see the practical through <https://www.youtube.com/watch?v=zXvEmlFqicw>,

<https://www.youtube.com/watch?v=vFzP-iqEpxY>
https://www.youtube.com/watch?v=7ldFmvix_C8

and

1.16 Calculation

Calculate total (T), phenolphthalein (P) alkalinity as follows:

P-alkalinity, as mg CaCO₃/L = A x 1000/mL sample

T-alkalinity, as mg CaCO₃/L = B x 1000/mL sample

In case H₂SO₄ is not 0.02 N apply the following formula:

Alkalinity, as mg CaCO₃/L = A/B x N x 50000 / mL of sample

Where,

A = mL of H₂SO₄ required to bring the pH to 8.3

B = mL of H₂SO₄ required to bring the pH to 4.5

N = normality of H₂SO₄

Once carbonate and bicarbonate alkalinities are known, then their conversions to milligrams CO₃ -- or HCO₃ - /L are possible.

mg CO₃ --/L = Carbonate alkalinity mg CaCO₃/L x 0.6

mg HCO₃ = Bicarbonate alkalinity mg CaCO₃/L x 1.22

from above, molar concentration may be obtained as follows:

[CO₃ --] = mg/L CO₃ / 60000

[HCO₃ -] = mg/L HCO₃ - / 61000

1.17 Environmental Significance

Alkalinity is a chemical measurement of a water's ability to neutralize acids. Alkalinity is also a measure of water buffering capacity or its ability to resist changes in pH upon the addition of acids or bases. Alkalinity of natural waters is due primarily to the presence of weak acid salts although strong bases may also contribute (i.e. OH⁻) in extreme environments. Bicarbonates represent the major form of alkalinity in natural waters; its source being the partitioning of CO₂ from the atmosphere and the weathering of carbonate minerals in rocks and soil. Other salts of weak acids, such as borate, silicates, ammonia, phosphates, and organic bases from natural organic matter, may be present in small amounts. Alkalinity, by convention, is reported as mg/L CaCO₃ since most alkalinity is derived from the weathering of carbonate minerals.

1.18 References

Standard Methods for the Examination of Water and Wastewater, APHA, AWWA and WEF, 21st Edition, 2005.

Experiment No.: 2

Name of the Practical Determination of TSS, TDS, TVS, TS, settleable solids & non-settleable SS of wastewater sample.

Objectives Estimation of total, dissolved, suspended and volatile solids along with settleable & non-settleable biomass in given wastewater sample.

Theoretical Concepts: Solids analysis provides one of the fundamental measurements used for control of the activated sludge process and for the regulation of wastewater discharges. Gravimetric analysis is based on the determination of constituents or categories of materials by measurement of their weight. The experiment illustrates the principles of weighing and demonstrates separation and categorization techniques used to define the various types of solids in wastewaters. These techniques involve three analytic operations in addition to weighing. These are: **filtration, evaporation, and combustion**. Filtration is used to separate suspended or particulate (non filterable) fraction from dissolved or soluble (filterable) fractions. Evaporation separates water from material dissolved or suspended in it. Combustion differentiates between organic and inorganic matter. Organic matter will be destroyed completely by burning at 550°C for 30 min.

Total solids are the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids (TS) includes total suspended solids (TSS), the portion of total solids retained by a filter, and total dissolved solids (TDS), the portion that passes through the filter. Residues dried at $180 \pm 2^{\circ}\text{C}$ will lose almost all mechanically occluded water. Some water of crystallization may remain, especially if sulfates are present. Organic matter may be lost by volatilization, but not completely destroyed. Loss of CO_2 results from conversion of bicarbonates to carbonates and carbonates may be decomposed partially to oxides or basic salts. Some chloride and nitrate salts may be lost. In general, evaporating and drying water samples at 180°C yields values for dissolved solids.

Dissolved solids - Solids which are dissolved in the water and would pass through a filter of $2.0\ \mu\text{m}$ (or smaller) nominal pore size under specified conditions. Dissolved solids are measured by passing the sample water through a filter, then drying the water which passes through. The solids remaining after the filtered water is dried are the dissolved solids.

Suspended solids - Solids which are suspended in the water and would be caught by a filter. Suspended solids are measured by passing sample water through a filter. The solids caught by the filter, once dried, are the suspended solids. Suspended solids can include but are not limited to silt and clay particles, decaying plant and animal matter, industrial waste and sewage.

- **Settleable solids** - Suspended solids which would settle out of the water if given enough time. Settleable solids are measured by allowing the sample water to settle for fifteen minutes, then by recording the volume of solids which have settled to the bottom of the sample.
- **No settleable solids** - Suspended solids which are too small and light to settle out of the water, also known as colloidal solids. No settleable solids are measured by subtracting the amount of settleable solids from the amount of suspended solids.

Procedure:

1. TS: A well-mixed sample (25ml) is evaporated in a pre-weighed empty dish and dried to constant weight in an oven at 103 to 105°C. The increase in weight over that of the empty dish represents the TS.
2. TDS: A well-mixed sample (25ml) 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°C.
3. TSS: A well-mixed sample (25ml) is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids.
4. Total volatile solids (TVS): The residue from TS is ignited to constant weight at 550°C, the weight lost on ignition is the TVS.
5. Settable solids & non-settleable SS:

Gravimetric method:

- Determine TSS as described above.
- Pour a well-mixed sample into a glass vessel of not less than 9 cm dia. using not less than 1 L and sufficient sample to give a depth of 20 cm. Alternatively use a glass vessel of greater diameter and a larger volume of sample. Let stand quiescent for 1 h and, without disturbing the settled or floating material, siphon 250 mL from centre of container at a

point halfway between the surface of the settled material and the liquid surface. Determine TSS (mg/l) of this supernatant liquor. These are the nonsettleable solids.

Observations

Record the following observations in the table:

1. Weight of empty dish =
2. Weight of residue (dried at 105°C) + dish =
3. Weight of residue (dried at 180°C) + dish =
4. Weight of filter paper =
5. Weight of filter + dried residue (at 105°C) =
6. Weight of residue + dish before ignition =
7. Weight of residue + dish or filter after ignition =

Observation Table:

Sample code	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7
1.							
2.							
3.							

Calculations:

a) For **TS**:

$$mg\ TS / L = \frac{(A - B) * 1000}{sample\ volume,\ ml} \quad (1)$$

Where: A = weight of dried residue + dish, mg, and

B = weight of empty dish, mg

b) For **TDS**:

$$mg\ TDS / L = \frac{(A - B) * 1000}{sample\ volume,\ ml} \quad (2)$$

Where:

A = weight of dried residue + dish, mg, and

B = weight of dish, mg

c) For **TSS**:

$$mg\ TSS / L = \frac{(A - B) * 1000}{sample\ volume,\ ml} \quad (3)$$

Where:

A = weight of filter + dried residue, mg, and

B = weight of filter, mg.

d) For **TVS**:

$$mg\ TVS / L = \frac{(A - B) * 1000}{sample\ volume,\ ml} \quad (4)$$

Where:

A = weight of residue + dish before ignition, mg,

B = weight of residue + dish or filter after ignition, mg, and

e) For **Settable solids & non-settleable SS**

$$mg\ settleable\ solids/L = mg\ TSS/L - mg\ no\ settleable\ solids\ (TSS\ of\ supernatant\ liquor)/L$$

Results & Discussion:

1. The TS of the given wastewater sample is
2. The TDS of the given wastewater sample is
3. The TSS of the given wastewater sample is
4. The TVS of the given wastewater sample is
5. The settleable solids in the given wastewater sample is

6. The non-settleable solids of the given wastewater sample is

Environmental Significance:

- Total suspended solids (TSS) is a major parameter used to evaluate the quality & strength of domestic wastewater and to determine the efficiency of treatment unit. It cuts down the light transmission through the water and lowers the rate of photosynthesis in aquatic flora.
- A high content of total dissolved solids (TDS) elevates the density of water, influences osmo-regulation of freshwater organisms, reduces solubility of gases (like oxygen) and also reduces utility of water for drinking, irrigation and industrial purposes.
- The term settleable solids are applied to distinguish solids in suspension state which can be separated by means of gravity settling. It helps in designing the primary settling tank, and also depicts the sediment load of waste streams entering the natural water bodies.

Sample Questions to Answer:

- 1) What are the permissible limits of TSS & TDS in wastewater?
- 2) Discuss the sources and impacts of suspended solids?
- 3) Why solid determination is important?

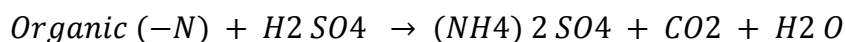
Experiment No. 3

Name of the Practical Determination of Nitrate-nitrogen (NO₃-N) Ammonical Nitrogen (NH₄-N) & TKN of given wastewater sample.

Objectives To determine TKN, Ammonia Nitrogen & Nitrate concentration in given wastewater sample.

Theory: -The Kjeldahl method is used to determine the nitrogen content in organic and inorganic samples. For longer than 100 years the Kjeldahl method has been used for the determination of nitrogen in a wide range of samples. The determination of Kjeldahl nitrogen is made in foods and drinks, meat, feeds, cereals and forages for the calculation of the protein content. Also, the Kjeldahl method is used for the nitrogen determination in wastewaters, soils and other samples. The Kjeldahl procedure involves three major steps:

- 1) Digestion -Organic nitrogen is converted into NH₄⁺
 - 2) Distillation -NH₃ is distilled and retained in a receiver vessel
 - 3) Titration - Nitrogen is determined
- 1) **Digestion:** -The aim of the digestion procedure is to break all nitrogen bonds in the sample and convert all of the organically bonded nitrogen into ammonium ions (NH₄⁺). Organic carbon and hydrogen form carbon dioxide and water. In this process the organic material carbonizes which can be visualized by the transformation of the sample into black foam. During the digestion the foam decomposes and finally a clear liquid indicates the completion of the chemical reaction. For this purpose, the sample is mixed with sulfuric acid at temperatures between 350 and 380 °C. The higher the temperature used; the faster digestion can be obtained. The speed of the digestion can be greatly improved by the addition of salt and catalysts. Potassium sulphate is added in order to increase the boiling point of sulfuric acid and catalysts are added in order to increase the speed and efficiency of the digestion procedure. Oxidizing agents can also be added to improve the speed even further.



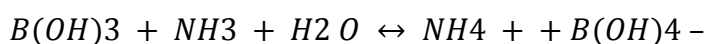
After digestion is completed the sample is allowed to cool to room temperature, then diluted with water and transferred to the distillation unit.

- 2) Distillation:** - During the distillation step the ammonium ions (NH_4^+) are converted into ammonia (NH_3) by adding alkali (NaOH). The ammonia (NH_3) is transferred into the receiver vessel by means of steam distillation.

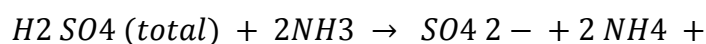


The receiving vessel for the distillate is filled with an absorbing solution in order to capture the dissolved ammonia gas.

- Common absorbing solutions involve aqueous boric acid [$\text{B}(\text{OH})_3$] of 2-4% concentration. The ammonia is quantitatively captured by the boric acid solution forming solvated ammonium ions.

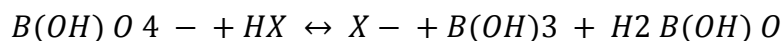


Also, other acids can be used as precisely dosed volume of sulfuric acid or hydrochloric acid that captures the ammonia forming solvated ammonium ions.



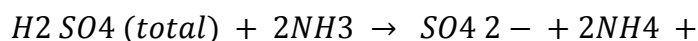
- 3) Titration:** - The concentration of the captured ammonium ions can be determined using two types of titrations:

- When using the boric acid solution as absorbing solution, an acid-base titration is performed using standard solutions of sulfuric acid or hydrochloric acid and a mixture of indicators. Depending on the amount of ammonium ions present, concentrations in the range of 0.01N to 0.5N are used. Alternatively, the end point can be determined potentiometrically with a pH-electrode. This titration is called direct titration.



HX = strong acid ($\text{X} = \text{Cl}^-$, etc.)

- When using sulfuric acid standard solution as absorbing solution, the residual sulfuric acid (the excess not reacted with NH_3) is titrated with sodium hydroxide standard solution and by difference the amount of ammonia is calculated. This titration is called back titration.

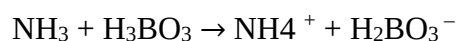


Water which has been polluted by organic wastes contains ammonia nitrogen (free gas NH_3 or ionic specie NH_4^+), organic nitrogen (proteins, urea) & Both forms influence water quality. Organic nitrogen compounds excreted by animals or released when animals and plants die - Present in sewage effluents & Bacterial decomposition of organic wastes produces ammonia.

- Ammonia - conversion to gaseous form



distillation and absorption



titration $\text{H}_2\text{BO}_3^- + \text{H}^+ \rightarrow \text{H}_3\text{BO}_3$

Nitrate is the most abundant form of inorganic nitrogen. It is formed during the nitrification step when municipal waste water is aerated. Continuous monitoring of nitrate is desired during both the nitrification and the denitrification steps.

The nitrate ion absorbs strongly in the UV range with a maximum absorbance at 205 nm. When such a sensor is used in municipal waste water samples, a filtering step has to be included before the UV measurement can be performed to account for turbidity. Furthermore, if the method is employed for continuous monitoring, the optical parts immersed in this type of sample must be regularly cleaned due to biofilm formation. To compensate for variations in sample turbidity, measurements are usually performed at two wavelengths predominantly at 220 and 275 nm. The absorbance registered at the latter wavelength is subtracted from the absorbance value observed at 220 nm.

Apparatus required: kjeldahl apparatus, conical flask, pipette, glass beads, measuring cylinder spectrophotometer, beaker, pipette, sample tubes

Reagents required

A) Total Kjeldahl Nitrogen

- **Digestion reagent:** - mix 13.4g K_2SO_4 & 7.3g $CuSO_4$ in DW (800 ml) and add 134 ml conc. H_2SO_4 . Makeup final volume with DW up to 1000ml.
- **Sodium thiosulphate solution:** - mix 500g NaOH & 25g $Na_2S_2O_3 \cdot 5H_2O$ in DW and makeup final volume 1000ml.
- **Standard Boric Acid Solution:** - 20g H_3BO_3 in DW, add 10ml mix indicator & Dilute to 1L.
- **Mix indicator:** - add 200mg methyl red indicator and 100 ml 95% ethyl alcohol in a flask and 100mg methylene blue and 50ml 95% ethyl alcohol in another flask and finally mix both solution in another volumetric flask.
- **0.02N H_2SO_4 solution:** - add 0.54ml 98% concentrated H_2SO_4 in 1L DW.

B) Ammonical Nitrogen

- **Reagent water:** - Ammonia free: Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions. Note: All solutions must be made with ammonia-free water.
- **Standard Boric Acid Solution:** - 20g H_3BO_3 in DW, add 10ml mix indicator & Dilute to 1L.
- **Mix indicator:** - add 200mg methyl red indicator and 100 ml 95% ethyl alcohol in a flask and 100mg methylene blue and 50ml 95% ethyl alcohol in another flask and finally mix both solution in another volumetric flask.
- **0.02N H_2SO_4 solution:** - add 0.54ml 98% concentrated H_2SO_4 in 1L DW.
- **Borate buffer solution:** - add 88ml 0.1N NaOH (4g NaOH in 1L DW) & 500ml 0.025M $Na_2B_4O_7$ (9.5g $Na_2B_4O_7$ in 1L DW). Makeup final volume with DW up to 1L.

C) Nitrate Nitrogen-

- 1) **Stock nitrate solution:** - First dry potassium nitrate (KNO_3) in an oven at 105°C for 24 hr & mix 0.7218gm in DW (1L). Preserve with 2ml CHCl_3/L .

Procedure for TKN determination

- Take 50 ml of sample in 800 ml Kjeldahl flask. Add a few glass beads.
- Add carefully 50 ml of digestion reagent and heat.
- As digestion continues colored and turbid samples will become transparent and pale green. After digestion, cool, dilute sample to 300 ml with water and mix.
- Tilt the flask away and carefully add 50 ml of sodium hydroxide thiosulphate reagent to form an alkaline layer at flask bottom. Swirl the Kjeldahl flask to ensure complete mixing. The pH of the solution should exceed 11.
- Take 50 ml of boric acid cum indicator solution in 500 ml of flask and place it below the condenser of the distillation assembly so that the lowered open end of the condenser is dipped in solution.
- Collect at least 200 ml of distillate in flask. Remove the flask with the distillate.
- Titrate the distillate with 0.02N H_2SO_4 to its original color, i.e. green to pink color indicates the end point of filtration.
- Run a distilled water blank in the same manner.

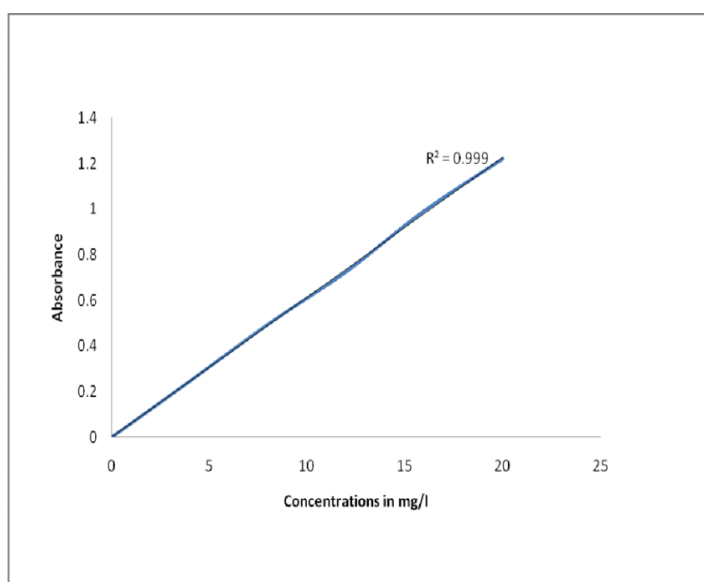
Procedure for Ammonical Nitrogen determination

- Take 100 ml of sample in a 800 ml Kjeldahl flask. Add a few glass beads.
- Add carefully 400ml Distill water & 25ml borate buffer and adjust pH to 9.5 with 6N NaOH.
- Arrange the Kjeldahl assembly & take 50 ml of boric acid cum indicator solution in 500 ml of flask and place it below the condenser of the distillation assembly so that the lowered open end of the condenser is dipped in solution.
- Collect at least 200 ml of distillate in flask (Total volume 250ml). Remove the flask with the distillate.
- Titrate the distillate with 0.02N H_2SO_4 to its original color, i.e. pale lavender.
- Run a distilled water blank in the same manner.

Procedure for Nitrate Nitrogen determination

- Filter the sample if it is turbid then measure 50 ml sample into a volumetric flask.
- Add carefully 1ml concentrated HCl and mix thoroughly.
- Clean sample tube properly with distil water & then rinse with sample also.
- First set blank in UV spectroscopy and set the absorbance range 220 & 225nm.
- Fill sample tube with prepared sample, dry outside wall of sample tube with tissue paper properly and read absorbance.

Calibration curve: - Plot calibration curve by taking absorbance at different known concentration (prepared by stock solution).



Observation

A - Volume of Standard 0.02N H₂SO₄ solution used for sample titration.

B - Volume of Standard 0.02N H₂SO₄ solution used for blank.

	Reading at 220nm	Reading at 275nm
Blank sample		
Sample1		

Calculations

$$\text{TKN /L} = [(A-B) * 280] / \text{ml of sample}$$

$$\text{NH}_3\text{-N /L} = [(A-B) * 280] / \text{ml of sample}$$

Calculation of $\text{NO}_3^- \text{N}$: - for sample and standards, subtract two times the absorbance reading at 275nm reading from the reading at 220 nm to obtain absorbance due to $\text{NO}_3^- \text{N}$. Construct a standard curve by plotting absorbance due to $\text{NO}_3^- \text{N}$ against $\text{NO}_3^- \text{N}$ concentration of standard. Using corrected sample absorbance obtained sample concentration directly from standard curve

Result & discussion:

1. The concentration of Nitrate-nitrogen ($\text{NO}_3\text{-N}$) in the given sample ismg/l.
2. The concentration of Ammonical Nitrogen ($\text{NH}_4\text{-N}$) in the given sample ismg/l.
3. The concentration of TKN in the given sample ismg/l.

Environmental Significance:

Nitrogen is an essential nutrient for the growth of photosynthetic organisms, thus it is important to monitor and control the discharge into the environment. Four forms of Nitrogen namely; Nitrate Nitrogen ($\text{NO}_3\text{-N}$), Nitrite Nitrogen ($\text{NO}_2\text{-N}$), Ammonical Nitrogen ($\text{NH}_3\text{-N}$) and organic nitrogen may be present in the water and wastewater. All these form of nitrogen as well as nitrogen gas (N_2), are biochemically interconvertible and are components of nitrogen cycle. Nitrate nitrogen is the highest oxidisable form of nitrogen and occur in the trace quantities in the surface water but may attain some high level in groundwater. The high concentration of nitrate nitrogen in the drinking water may cause blue baby disease. In addition, excessive nitrogen may lead to depletion of dissolve oxygen and results eutrophication in the receiving water body.

Sample Questions to Answer:

- i. How the TKN analysis and Ammonical Nitrogen analysis give different results for the same waste?
- ii. Explain the difference between Organic nitrogen and Ammonical nitrogen?
- iii. what is environmental significance of Nitrate nitrogen?
- iv. What kind of precision should be taken during spectroscopically analysis & why, briefly explain?

Experiment No.: 4

Name of the Practical Determination of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in municipal wastewater and confer on the biodegradability of wastewater.

Objectives To understand the biodegradability and organic strength of the wastewater.

Theoretical Concept: The most widely used test indicating organic pollution of both wastewater and surface water is the 5-day BOD (BOD₅). This determination involves the measurement of the dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter. Thus, BOD₅ is the total amount of oxygen consumed by microorganisms during the first five days of biodegradation. Oxygen demand is associated with the biodegradation of the carbonaceous portion of wastes and oxidation of nitrogen compounds such as ammonia.

The following equations simplify the process of biodegradation:



Chemical oxygen demand (COD) is termed as the amount of a specific oxidizing agent that reacts with sample under controlled conditions and it is expressed as oxygen equivalence. This parameter indicates the extent of organic matter contamination of water and is always higher than the biochemical oxygen demand (BOD). It is used to indicate organic matter contamination and it helps in knowing overall organic load to the receiving body. This test is widely used to determine:

- a) Degree of pollution in water bodies and their self-purification capacity,
- b) Efficiency of treatment plants,
- c) Pollution loads, and
- d) Provides rough idea of Biochemical oxygen demand (BOD) which can be used to determine sample volume for BOD estimation.

The limitation of the test lies in its inability to differentiate between the biologically oxidizable and biologically inert material and to find out the system rate constant of aerobic biological stabilization.

Most of the organic matters are destroyed when boiled with a mixture of potassium dichromate and sulphuric acid producing carbon dioxide and water. A sample is refluxed with a known amount of potassium dichromate in sulphuric acid medium and the excess of dichromate is titrated against ferrous ammonium sulphate. The amount of dichromate consumed is proportional to the oxygen required to oxidize the oxidizable organic matter.

Selection of Method

There are two methods available for COD determination namely open reflux and closed reflux.

Open Reflux Principle

- Suitable for a wide range of wastes with a large sample size.
- Due to its higher oxidizing ability dichromate reflux method is preferred over other procedures using other oxidants (e.g. potassium permanganate).
- Oxidation of most organic compounds is up to 95-100% of the theoretical value.

Closed Reflux Principle

- This method is conducted with ampoules and culture tubes with pre-measured reagents.
- Measurement of sample volume and reagent volume are critical
- This method is economical in the use of metallic salt reagents and generate smaller quantity of hazardous wastes.
- Volatile organic compounds (VOC) gets completely oxidized in a closed system than the open because of longer contact time with oxidants.

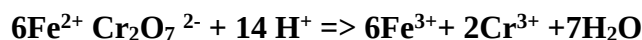
Reaction with dichromate solution of sample

Potassium dichromate is a strong oxidizing agent and it can be used to prepare solution of exact normality.



Here $d = (2n/3) + (a/6) - (b/3) - (c/2)$

During experiment, excess dichromate concentration is determined by titrating it with ferrous ammonium sulphate (FAS). The reaction is given by:



Here $d = (2n/3) + (a/6) - (b/3) - (c/2)$

Ferriin Indicator (ferrous 1, 10-phenanthroline sulphate) It is used to indicate change in oxidation-reduction potential of the solution and it indicates the condition when all dichromate has been reduced by ferrous ion. It gives a very sharp brown color change which can be seen in spite of blue color generated by the Cr^{3+} ions formed on reduction of the dichromate.

Procedure:

Apparatus required for BOD:

- Incubation bottle 300mL volume; Air compressor, 20°C incubator.
- Digestion vessels; block heater; micro burette; ampule sealer. Borosilicate culture tubes (16mm*100 mm or 20 mm*150mm) with TFE lined-screw caps are used. The block heater is required to operate at $150 \pm 2^\circ\text{C}$ with holes to accommodate digestion vessels. **Do not use an oven because of the possibility of leaking samples generating corrosive and explosive atmosphere.**

Reagents for DO measurement:

- **Manganese sulphate solution** Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 400 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ or 364g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water, filter, and dilute to 1L. The MnSO_4 solution should not give a colour with starch when added to an acidified potassium iodide (KI) solution.
- **Alkali-iodide-azide reagent:**
- For saturated or less than saturated samples: Dissolve 500g NaOH (or 700g KOH) and 150g KI (or 135g NaI) in distilled water and dilute to 1000mL. Add 10 g sodium azide, NaN_3 dissolved in 40 mL distilled water. This solution should not give colour with starch solution when diluted and acidified.
- For supersaturated samples: Dissolve 10g NaN_3 in 500mL distilled water. Add 480g NaOH and 750g NaI and stir to dissolve the contents.

- **Sulfuric acid** One mL is equivalent to ~ 3 ml alkali-iodide-azide reagent.
- **Starch solution** Dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid as preservative in 100 ml hot distilled water.
- **Standard sodium thiosulfate titrant** Dissolve 6.205 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in distiller water and add 1.5 ml 6N NaOH or 0.4 g solid NaOH and dilute to 1000 ml. Standardize with bi-iodate solution.
- **Standard potassium bi-iodate solution (0.0021M)** Dissolve 812.4 mg $\text{KH}(\text{IO}_3)$ in distilled water and dilute to 1000 mL.
- **Standardization** Dissolve ~ 2 g KI, free from iodate in an Erlenmeyer flask with 100 to 150 mL distilled water; add 1 mL 6N H_2SO_4 or a few drops of conc. H_2SO_4 and 20.00 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw colour is reached. When the solution is of equal, 20.00 mL 0.025M $\text{Na}_2\text{S}_2\text{O}_3$ should be required. If not, adjust the $\text{Na}_2\text{S}_2\text{O}_3$ solution to 0.025M.

Reagents for COD:

- **Standard potassium dichromate digestion solution, 0.01667M** Add to about 500 mL distilled water 49.03 g $\text{K}_2\text{Cr}_2\text{O}_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc. H_2SO_4 , and 33.3 g HgSO_4 . Dissolve, cool to room temperature, and dilute to 1000 ml.
- **Sulfuric acid reagent** Add H_2SO_4 at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4 or 10.12 g silver sulphate/L H_2SO_4 . Let stand 1 to 2 d to dissolve and mix. This accelerates the oxidation of straight chain aliphatic and aromatic compounds. (1 Kg = 543.47826 mL of H_2SO_4 and take 20.24 g of Ag_2SO_4 to 2 L of H_2SO_4 or 22.264 g of Ag_2SO_4 to 2.2 L of H_2SO_4)
- **Standard ferrous ammonium sulphate titrant (FAS), approximately 0.10M** Dissolve 39.2 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in distilled water. Add 20 mL Conc. H_2SO_4 , cool, and dilute to 1000 ml. Standardize solution daily against standard $\text{K}_2\text{Cr}_2\text{O}_7$ digestion solution as follows: Pipet 5.00 mL digestion solution into a small beaker. Add 10 mL reagent water to substitute for sample. Cool to room temperature. Add 1 to 2 drops diluted Ferroin indicator and titrate with FAS titrant.

$$\text{Molarity of FAS solution} = [\text{V}_{\text{K}_2\text{Cr}_2\text{O}_7} \times 0.1] / (\text{V}_{\text{FAS}})$$

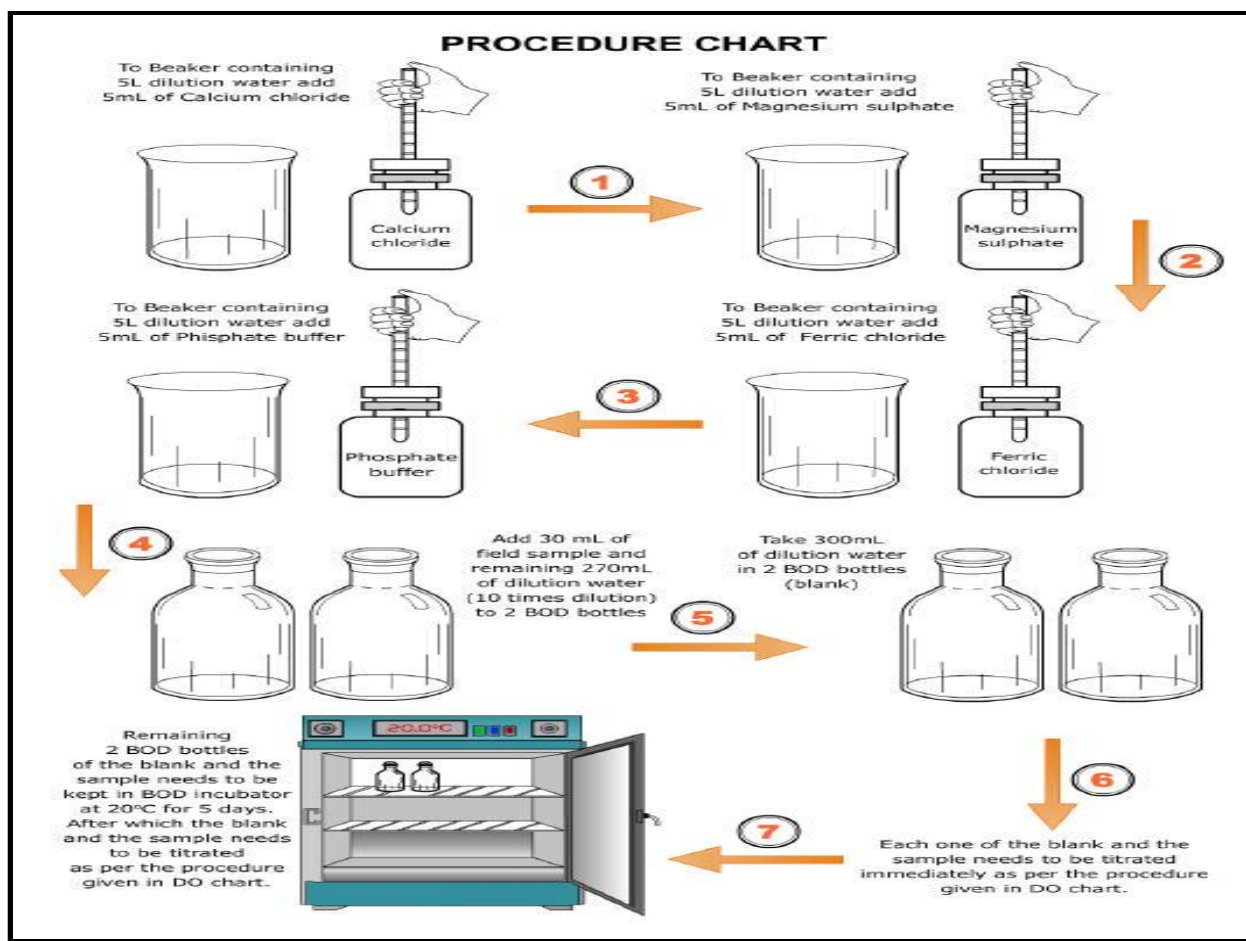
Where: $\text{V}_{\text{K}_2\text{Cr}_2\text{O}_7}$ = volume of $\text{K}_2\text{Cr}_2\text{O}_7$ (mL); V_{FAS} = volume of FAS (mL)

- **Ferroin indicator** This indicator is used to indicate change in oxidation-reduction potential of the solution.

Steps for DO calculation

- Make dilution water by adding 2mL/L of following reagents in distilled water:
 - Phosphate buffer solution
 - Magnesium sulphate solution
 - Calcium chloride solution
 - Ferric chloride solution
 - Sodium Sulphite solution
- For a given sample bottle, add 1 mL of alkali azide and then 1 mL manganous sulphate solution. Shake well the bottle and keep it open for 5 minutes to settle the precipitate. Add 2 mL concentrated H_2SO_4 and place the cap on the bottle. Shake well the bottle till all the precipitate is dissolved.
- Take 203 mL of sample in conical flask and titrate with standard sodium thiosulfate solution (0.025N) till the colour changes from dark yellow to light yellow. Then add few drops of starch indicator and continue to titrate till the colour of the solution becomes either colourless or changes to its original sample colour. Note down volume of 0.025N sodium thiosulfate consumed.
- Calculate DO value of the sample. Remember that in 200 mL sample, 1 mL of sodium thiosulfate of 0.025N equals to 1 mg/L dissolved oxygen:

➡ Dissolved oxygen (DO) (in mg/L) = mL of sodium thiosulfate (0.025N) consumed

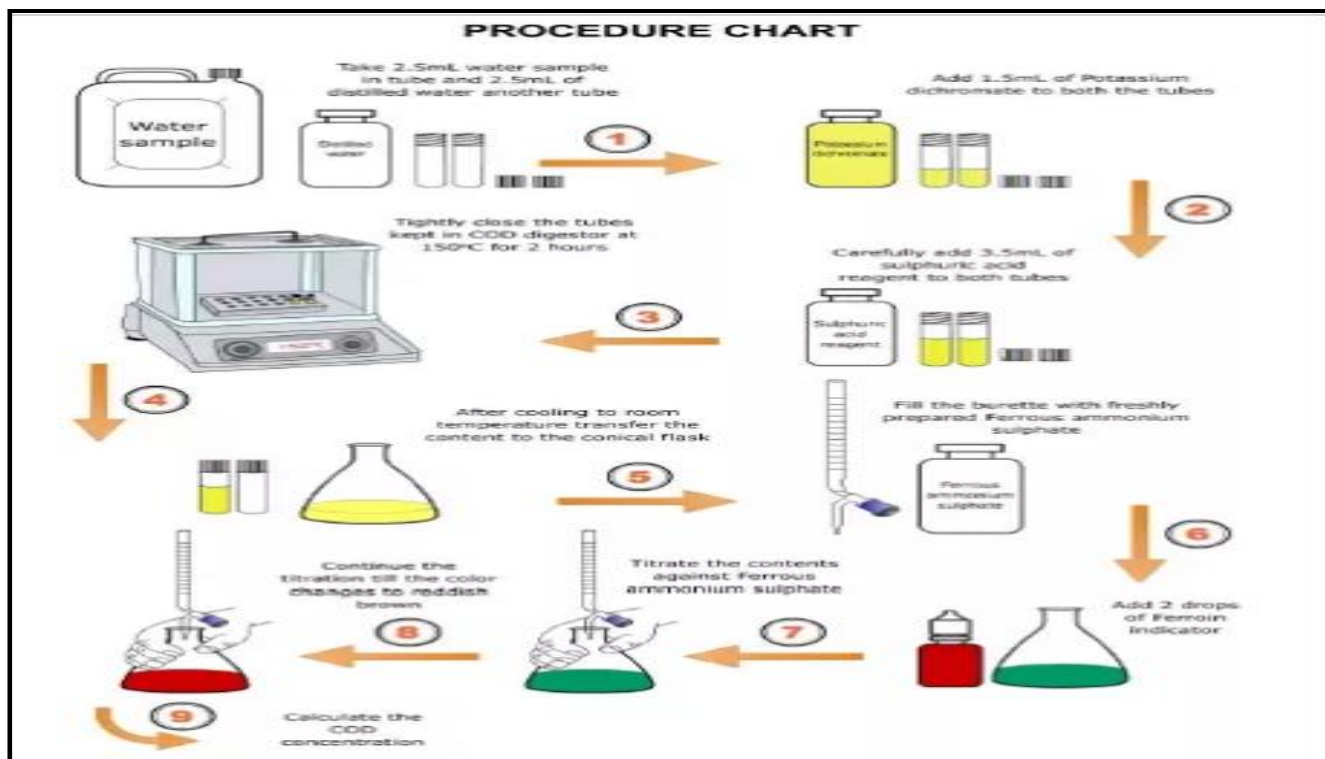


Procedure for BOD determination

- i. Prepare BOD dilutions. Use dilution water (it contains nutrients, the exact contents are described in Standard Methods): Blank (only dilution water); 5 mL sample in 300 mL BOD bottle, fill up with dilution water; 15 mL sample in 300 mL BOD bottle, fill up with dilution water; 20 mL sample in 300 mL BOD bottle, fill up with dilution water.
- ii. Take 300 mL sample in BOD bottle. Prepare two sets of this sample. Keep one set for DO analysis for day 0 (i.e. Sample0Day) and another sample in BOD incubator for 5 days at 20°C (Sample5Day).
- iii. Measure DO in different samples at $t=0$.
- iv. Incubate samples in 20°C for 5 days.
- v. Come back in the lab after 5 days and record dissolved oxygen.
- vi. Record data in following manner:

Procedure for COD determination

- i. Wash culture tubes and caps with 20% H_2SO_4 before using to prevent contamination.
- ii. Place sample in culture tube or ampule and add digestion solution. Carefully run sulfuric acid reagent down inside of vessel so an acid layer is formed under the sample-digestion solution layer and tightly cap tubes or seal ampules, and invert each several times to mix completely.
- iii. Place tubes or ampules in block digester preheated to 150°C and reflux for 2 hr. behind a protective shield. **CAUTION: These sealed vessels may be under pressure from gases generated during digestion. Wear face and hand protection when handling and dangerous pressures will be generated at 150°C .**
- iv. Cool to room temperature and place vessels in test tube rack. Some mercuric sulphate may precipitate out but this will not affect the analysis.
- v. Add 0.05 to 0.10 mL (1 to 2 drops) Ferroin indicator and stir rapidly on magnetic stirrer while titrating with standardized 0.10M FAS. The end point is a sharp color change from blue-green to reddish brown, although the blue green may reappear within minutes. In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.



Observation table for DO

Bottle no.	Wastewater sample (mL)	Initial DO (mg/L) (DO ₀)	DO at 5-day (mL) (DO ₅)

Observations table for COD

Sample	Burette Reading	COD value (mg/l)
1.		
2.		
3.		

Calculations

Calculate 5-day BOD value of the sample at 20°C

$$\text{t-day BOD} = [\text{DO}_t - \text{DO}_0] / (P)$$

Where P = Dilution factor = 300mL / (sample volume in mL)

COD is given by

$$\text{COD as mg/L} = (A - B) \times M \times 8000 / (V_s)$$

Where: A = volume of FAS used for blank (mL);

B = volume of FAS used for sample (mL);

M = molarity of FAS; 8000 = milliequivalent weight of oxygen $\times 1000$ mL/L

V_s = Volume of sample

Note: Analyze samples in duplicates because of small sample size.

Results and discussion:

- i. The calculated BOD value the given sample = mg/l
- ii. The calculated value of COD of the given sample = mg/l

Environmental Significance:

- Biochemical Oxygen Demand (BOD), is a measurement of the amount of dissolved oxygen (DO) that is used by aerobic microorganism when decomposing organic matter in water. This test has its widest application in measuring waste loadings to wastewater treatment plants and evaluating the BOD removal efficiency. The higher the BOD value, greater the amount of organic matter or “food” available for the oxygen consuming bacteria. If the rate of DO consumption by the bacteria exceeds the supply of DO from aquatic plants, algae photosynthesis for diffusion from air, unfavorable condition occur. Depletion of DO causes stress on aquatic organism, making the environment unsuitable for life.

- Chemical Oxygen Demand (COD) test determines the oxygen required for chemical oxidation of most organic matter and oxidizable inorganic substances with the help of strong chemical oxidant. COD determination has an advantage over BOD determination as the result of COD test can be obtained in about 3 hours, as compared to 5 days required for BOD test.

Sample Questions to Answer:

- If 5-day BOD at 20°C=150mg/L ($k=0.23/\text{day}$), calculate 5-day BOD at 15°C and 22°C? Use following dependence of (k) with temperature: $k_T = k_{20} \{\theta\}^{(T-20)}$
- Why do the COD analysis and BOD analysis give different results for the same wastewater?

Experiment No.: 5

Name of the Practical Determination of phenol and sulphate in municipal wastewater.

Objectives To determine Phenol & Sulphate concentration in wastewater sample.

Theoretical concept: Phenols are defined as hydroxyl derivatives of benzene, and its condensed nuclei occur in domestic and industrial wastewaters, natural wastes and potable water supplies. Odoriferous and objectionable tasting chlorophenols are formed as a result of chlorination of water containing phenol. Phenols may be present in raw water owing to the discharge of wastewaters from coke distillation plants, the petrochemical industry and numerous other industries where phenols serve as intermediates. They are also present in municipal wastewaters. Presence of phenols may lead to objectionable taste in chlorinated drinking water and hence its monitoring is essential. Phenols can be removed from drinking water by super-chlorination (chlorine-dioxide or chloramines treatment) ozonation and activated carbon adsorption.

Sulphate is widely distributed in nature and may be present in natural water in concentrations ranging from a few to several thousand milligrams/L. Sulphates are of considerable concern because they are indirectly responsible for two serious problems often associated with the handling and treatment of wastewater. Odour and sewer corrosion problem result from the reduction of sulphates to hydrogen sulphide under anaerobic conditions. Sulphate ion is precipitated in an acetic acid medium with barium chloride so as to form barium sulphate crystals of uniform size. The crystal formation is enhanced in the presence of an acetic acid buffer solution containing magnesium chloride, potassium nitrate, sodium acetate and acetic acid. Light absorbance of the BaSO₄ suspension is measured by photometer and SO₄⁻ concentration is determined by comparison of the reading with a standard curve.

Apparatus required: Magnetic stirrer, Nephelometer or Spectrophotometer, Distillation apparatus, pH meter, Funnels, Nessler tubes, short or long form

Reagents required:

A) Sulphate

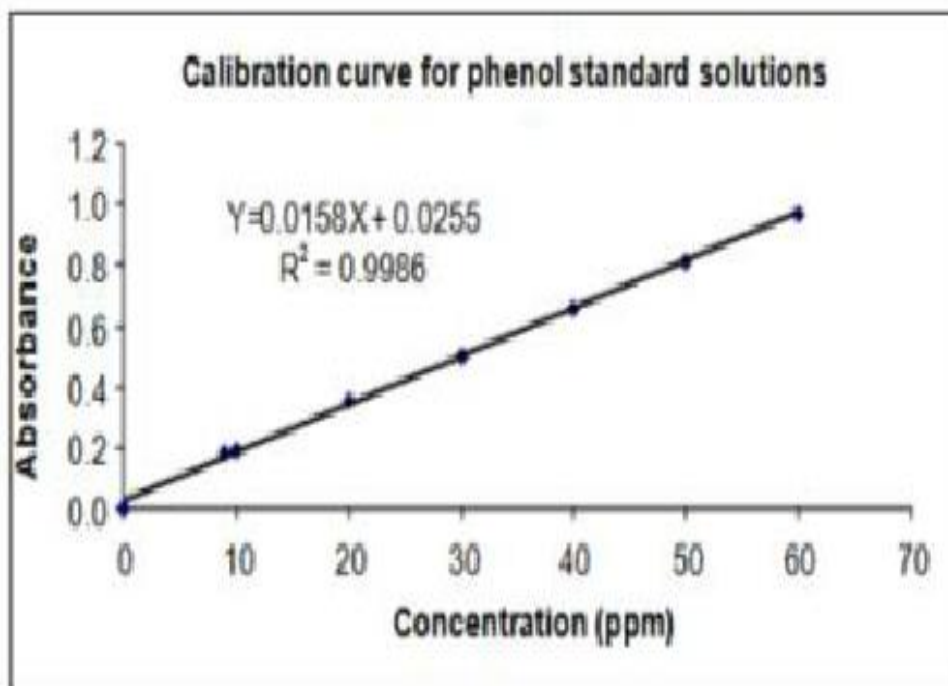
- **Buffer solution A:** Dissolve 30 g magnesium chloride, 5 g sodium acetate, 1 g potassium nitrate and 20 ml acetic acid in 500 ml distilled water and make up to 1000 ml.
- **Buffer solution B** (required when the sulphate concentration of sample is less than 10 mg/l): Dissolve 30 g magnesium chloride, 5 g sodium acetate, 0.111 g sodium sulphate and 20 ml acetic acid in 500 ml distilled water and make up to 1000 ml.
- **Barium chloride:** crystals of 20 to 30 mesh size. In standardization uniform turbidity is produced with this mesh range and the appropriate buffer.
- **Standard sulphate solution:** Dissolve 0.1479 g anhydrous Na_2SO_4 in distilled water and dilute to 1000 ml.

B) Phenol

- **Phosphoric acid solution:** Dilute 10 mL of 85% H_3PO_4 to 100 mL with distilled water.
- **Copper sulfate solution:** Dissolve 100 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in distilled water and dilute to 1L.
- **Buffer solution:** Dissolve 16.9 g NH_4Cl in 143 mL conc. NH_4OH and dilute to 250 mL with distilled water. Two ml should adjust 100 ml of distillate to pH.
- **Amino antipyrine solution:** Dissolve 2 g of 4AAP in distilled water and dilute to 100 ml.
- **Potassium ferricyanide solution:** Dissolve 8 g of $\text{K}_3\text{Fe}(\text{CN})_6$ in distilled water and dilute to 100 ml.
- **Stock phenol solution:** Dissolve 1.0 g phenol in freshly boiled and cooled distilled water and dilute to 1 liter. 1 ml = 1 mg phenol.
- **Working solution, A:** Dilute 10 ml stock phenol solution to 1 liter with distilled water. mL = 10 μg phenol.
- **Working solution B:** Dilute 100 ml of working solution A to 1000 mL with distilled water. 1 mL = 1 μg phenol.
- **Ferrous ammonium sulfate:** Dissolve 1.1 g ferrous ammonium sulfate in 500mL distilled water containing 1 mL conc. H_2SO_4 and dilute to 1 liter with freshly boiled and cooled distilled water.

C) Preparation of calibration curve

- Take 100 mL distilled water in a 500 mL glass stoppered conical flask or iodine flask. Add 50 mL stock phenol solution and 10 mL 0.1N bromate-bromide solution followed by 1 mL concentrated HCl and swirl gently.
- If brown colour of free bromine does not persist, add 10.0 mL portions bromate-bromide solution until it does. Put the stopper tightly and keep it for 10 minutes.
- Add 1 g KI, keep flask stoppered, swirl and again keep it for some time.
- Prepare a blank in the same manner using distilled water and 10 mL 0.1N bromate-bromide solutions.
- Titrate blank and sample with 0.025 N sodium thiosulphate using starch as an indicator.



Procedure for sulphate determination

- Filter the sample if it is turbid and adjust the temperature between 15 to 30°C.
- Measure 100 ml sample into a 250 ml Erlenmeyer flask.
- Add 20 ml buffer solution and mix in a stirring apparatus.
- While stirring add a spoonful of BaCl₂ crystal and the timing immediately.
- Stir for 60 ± 2 sec at constant speed.
- After stirring period is over, pour solution into a absorption cell of photometer and measure turbidity at 5 ± 0.5 min.
- Estimate SO₄ concentration in sample by comparing turbidity reading with a calibration curve prepared by carrying SO₄²⁻ standards through the entire procedure.
- Space standards at 5 mg/L increments in the 0 to 40 mg/L SO₄²⁻ range.
- Correct for sample color and turbidity by running blanks to which BaCl₂ is not added.

Procedure for phenol determination

- Measure 500 mL sample into a beaker, add 50 mL phenol-free distilled water, lower the pH to 4.0 with H₃PO₄ solution using methyl orange as an indicator.
- Add 5 mL CuSO₄ solution & Transfer to distillation flask and collect 500 mL distillate using measuring cylinder as receiver.
- If the distillate is turbid repeat the same procedure as above. Omit addition of H₃PO₄ and CuSO₄ if the preserved sample is used.
- Take 100 mL distillate or an aliquot of the distillate containing not more than 0.5 mg phenol and dilute to 100 mL in a 250 mL beaker.
- Prepare a 100 mL distilled water blank and a series of 100 mL phenol standards containing 0.1, 0.2, 0.3, 0.4 and 0.5 mg phenol.
- Add 2.5 mL 0.5N NH₄OH solution in each and adjust the pH to 7.9 ± 0.1 with phosphate buffer and dilute to 100 mL in a 250 mL beaker.
- Add 1 mL 4-aminoantipyrine solution and 1 mL K₃Fe (CN)₆ solution in each flask and mix well.
- Measure the absorbance of each after 10 minutes against blank at 500 nm.

Calculations:

$$\text{SO}_4 \text{ in mg/L} = \text{mg SO}_4^{2-} \times 1000 / \text{ml of sample}$$

Calculation of the concentration of phenol solution as follows:

$$\text{mg/L phenol} = 7.842 [(A \times B) - C]$$

A = mL thiosulphate for blank

B = mL bromate-bromide solution used for sample divided by 10

C = mL thiosulphate used for sample

Use of calibration curve,

$$\mu\text{g/L, phenol} = [(A / B) \times 1000]$$

Where:

A = mg phenol in sample (estimated from calibration curve)

B = mL original sample

Results and discussion:

Concentration of SO_4 in mg/L =

Concentration of phenol in $\mu\text{g/L}$ =

Environmental Significance:

- Discharge of phenolic compounds without treatment may lead to serious health risks to humans, animals, and aquatic systems. Phenolic compounds are highly toxic even at low concentrations. Their presence in streams and other waterways will cause foul smell and will adversely affects the fish tissues and other aquatic lifes.
- Water containing appreciable amount of sulphates forms hard scale in boilers and heat exchangers. Sulphates also causes odour and corrosion of sewer in anaerobic conditions because it gets converted to hydrogen sulphide.

Sample Questions to Answer:

- 1) Why the presence of even trace phenolic compounds in drinking water is totally undesirable?
- 2) List the major sources of phenolic compounds that pollute the aquatic environment?
- 3) What are the permissible limits of phenol and SO_4 concentration in wastewater?

Experiment No.: 6

Name of the Practical Determination of MLSS, MLVSS & SVI of sludge sample.

Objectives To characterize the biological sludge produced from biological treatment process & viable and nonviable biomass and sludge volume index.

Theoretical concept:

Mixed Liquor Suspended Solids (MLSS) represents microbes mixed with inorganic substances like, clay, silt, fine sands etc. in a sample of mixed liquor. This test is essentially the same as the test for TSS, except for the use of mixed liquor as the water sample. In addition, the concentration of suspended solids found in the mixed liquor is typically much greater than that found in the raw or treated water.

Mixed Liquor Volatile Suspended Solids (MLVSS) gives a rough approximation of the amount of biomass present in activated sludge. Volatile solids are those solids which are burnt up when a sample is heated to 550°C. Most of the volatile solids in a sample of mixed liquor will consist of microorganisms and organic matter.

Procedure:

- Collect a grab sample of sludge.
- Filter a well-mixed sample (25ml) through a weighed standard glass-fibre filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the MLSS. Calculate MLSS using equation 1.
- Ignite the filter and the MLSS residue from step 2 in a muffle furnace at 550°C. An ignition time of 15 to 20 minutes is usually sufficient for 200 mg residue. However, when igniting more than one sample or when igniting heavier samples, the ignition time may need to be increased.
- Let the filter cool partially in the air until most of the heat has dissipated. Then transfer the filter to a desiccator to cool the rest of the way to air temperature.
- Weigh the filter and record the weight.

- Repeat the cycle of igniting, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% or 0.5 mg, whichever is less.
- Calculate the MLVSS in the sample using equation 2.
- SVI is determined by settleability test in which 1 liter of the mixed liquor sample is poured into measuring cylinder. The sludge is allowed to settle for 30 minutes, and the volume of the settled sludge is measured in mL/L. Calculate the SVI using equation 3.

Observations:

Record the following observations in the table:

1. Weight of empty filter paper =
2. Weight of dried filter paper with residue =
3. Weight of residue + filter before ignition =
4. Weight of residue + filter after ignition =
5. Vol. of settled sludge after 30mins =

Observation Table:

Sample. Code	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5
1.					
2.					
3.					

Calculations:

a) For MLSS:

$$mg \text{ MLSS} / L = \frac{(A - B) * 1000}{\text{sample volume, ml}} \quad (1)$$

A = weight of filter + dried residue, mg, and

B = weight of filter, mg.

b) For MLVSS:

$$mg \text{ MLVSS} / L = \frac{(C - D) * 1000}{\text{sample volume, ml}} \quad (2)$$

C = weight of residue + dish before ignition, mg, and

D = weight of residue + dish or filter after ignition, mg,

c) For SVI:

$$SVI \left(\frac{mL}{gm} \right) = \frac{\text{Settled sludge volume, } \frac{mL}{L} * 1,000 \text{ mg}}{\text{MLSS concentration, } \frac{mg}{L}} \quad (3)$$

Results & Discussion:

1. The MLSS of the given sludge sample ismg/l
2. The MLVSS of the given sludge sample ismg/l
3. The SVI of the given sludge sample isml/gm

Environmental Significance:

MLSS and MLVSS terms are used to express the microbial concentration in biological wastewater treatment process. MLSS contains microbes mixed with inorganic substances like, clay, silt, fine sands etc., whereas MLVSS measure true microbial concentration. MLVSS gives a rough approximation of the amount of biomass present in activated sludge and is used for calculation of design parameters of biological treatment systems, like substrate utilisation rate, F/M ratio and recirculation ratio etc. The ratio of MLVSS: MLSS should be around 0.80 for satisfactory performance of wastewater treatment plant.

Sample Questions to Answer:

- 1) Why MLVSS is always less than MLSS?
- 2) What does SVI signify?
- 3) What do you expect about volatile and fixed suspended solids in raw wastewater? Discuss using their solid characteristics.

Experiment No.: 7

Name of the Practical Determination of Total Organic Carbon (TOC), Dissolve Organic Carbon (DOC), UV₂₅₄, and SUVA.

Objectives Quantitative assessment of Natural organic Matter (NOM) in terms of TOC and DOC & Hydrophobic (high molecular weight) and Hydrophilic (low molecular weight) characterization of NOM presence in aqueous solution.

Theoretical concept: The yellow or brown color commonly associated with natural water is often the result of the decomposition of naturally occurring organic matter (NOM). The most abundant sources of this material include decaying vegetation, algae, and microscopic organisms. These NOM cause problem in the production of drinking water. It has adverse effects on the aesthetic water quality and may result in the befouling of the pipeline with negative hygienic consequences. It may also react with residual disinfectants (chlorine) to produce potentially toxic and carcinogenic disinfection by-products (DPBs) especially Trihalomethane (THMs). NOM in water usually expressed in terms of total organic carbon (TOC), dissolved organic carbon (DOC) (sample filtered through a 0.45 µm filter), ultra-violet absorbance (UV₂₅₄), and Specific ultraviolet absorption (SUVA). These four parameters are the direct and convenient measurement of the total organic content present in the water sample. It is also capable of providing information regarding the organic character of water becomes an important element in water monitoring programs. Measurement of NOM is of vital importance to the operation of drinking water treatment plant to improve water quality and to minimize the production of DPBs.

Principle of TOC and DOC analysis: The measurements of TOC and DOC is performed using Total Organic Carbon Analyzer - model no TOC-L CSH, made by Shimadzu, Japan. Two types of carbonaceous are present in water: total organic carbon (TOC) and inorganic carbon (IC). Organic carbon binds with hydrogen or oxygen to form organic compounds. Collectively, the two forms of carbon are referred to as total carbon (TC) and the relationship between them is expressed as:

$$\text{TOC} = \text{TC} - \text{IC}$$

Prior to the TOC determination, it is essential to remove IC by acidifying the sample. The remaining TC is then measured to determine TOC, and the result is generally referred to as TOC. This value is referred to as a non-purgeable organic carbon that is present in a sample in a non-volatile form (NPOC), thus,

$$\text{TOC} = \text{NPOC}$$

Principle of UV₂₅₄ and SUVA analysis: UV₂₅₄ and SUVA are another important key surrogate of NOM provide an insight into its nature. As the organic content (humic acids) of water are known to absorb ultraviolet light at specific wavelengths of 254, which reveal the humic or non-humic character of NOM. Whereas aromaticity and hydrophobicity of the organic carbon are determined in terms of SUVA. The value of SUVA > 4 Lmg⁻¹ m⁻¹ indicate the humic nature (hydrophobicity) with high molecular weight, and value < 2 Lmg⁻¹ m⁻¹ indicate nonhuman nature (hydrophilic) with low molecular weight whereas range value between 2 to 4 Lmg⁻¹ m⁻¹ indicate a mixture of hydrophobic and hydrophilic of NOM fraction.

The determination of UV₂₅₄ is performed by using UV visible spectrophotometer at a wavelength of 254 and can express in cm⁻¹. Where the SUVA is the ratio of UV₂₅₄ and DOC concentration of the sample expressed in the unit of L.mg⁻¹.m⁻¹.

Apparatus required: TOC analyzer, Samples vials, Filter assembly, Spectrophotometer, Glassware's Filter assembly

Reagents required:

Standard Potassium hydrogen phthalate (KHP) (C₈H₅KO₄): Weight accurately 0.2125 gm of C₈H₅KO₄ and dissolve in 100 ml of distill water to get 100 ppm carbon concentration. Use this solution as the mother solution.

Now, A series of VFA standards for the calibration curves were prepared (as required) for the calibrating the instrument.

Procedure for TOC and DOC:

- Switch on the TOC analyser and calibrate it by using standard Potassium hydrogen phthalate (KHP) prepared.
- Take 50 ml of the filtered water sample, it must be filtered through the 0.45 μm filter paper if DOC is to analyse.
- Then the sample is inserted to TOC analyser where it acidified to pH 2 to 3.
- Now the acidified sample is introduced to the combustion tube, which is filled with an oxidation catalyst and heated to 680°C where it converted to CO_2 by the combustion furnace.
- The carrier gas (Zero air) flows to the combustion tube and carries the sample combustion products from the combustion tube to an electronic dehumidifier, where the gas is cooled and dehydrated.
- The gas then carries the sample combustion products through a halogen scrubber to remove chlorine and other halogens.
- Finally, the carrier gas takes the sample to a non-dispersive infrared (NDIR) detector, which is sensitive to the absorption frequency of CO_2 . the NDIR generates a non-linear signal that is proportional to the instantaneous concentration of CO_2 in the carrier gas. That signal is then plotted versus the sample's analysis time. The peak area is proportional to the TOC concentration of the sample.

A new calibration curve was established whenever a new range was used, prior to the measurement of the TOC in the sample.

Procedure for SUVA:

- Sample volume: For most applications a the 50-mL sample is adequate. Use 100 mL sample if a 10-cm cell path length is required.
- Sample preparation: Wash filter and filter assembly bypassing at least 50 mL organic-free water through the filter. For specific applications and correlations, sample pH may be adjusted with HCl or NaOH.
- Spectrophotometric measurement: Let spectrophotometer equilibrate according to manufacturer's instructions. Set wavelength to 254 nm and adjust spectrophotometer to

read zero absorbance with the organic-free water blank. Measure UV absorbance at 254 nm of at least two filtered portions of the sample at room temperature.

- Report mean UV absorption in units of cm^{-1} .

Observations

Record the observations in the table

Sample. Code	Obs. 1 TOC/DOC (ppm)	Obs. 2 TOC/DOC (ppm)	Obs. 3 TOC/DOC (ppm)	Average Concentration
1.				
2.				

Record the observations in the table:

Sample. Code	Obs. 1 SUVA ($\text{L.mg}^{-1}.\text{m}^{-1}$)	Obs. 2 SUVA ($\text{L.mg}^{-1}.\text{m}^{-1}$)	Obs. 3 SUVA ($\text{L.mg}^{-1}.\text{m}^{-1}$)	Average Concentration ($\text{L.mg}^{-1}.\text{m}^{-1}$)
1.				
2.				

Calculation:

- The value of SUVA can be calculated with the following formula

$$SUVA = \frac{UV_{254}}{DOC} * 100$$

Results & Discussion:

- The TOC of the given sludge sample isppm
- The DOC of the given sludge sample isppm
- Take the average of all the three observation as a final result ($\text{L.mg}^{-1}.\text{m}^{-1}$)

Environmental Significance:

The amount of natural organic matter (NOM) in water is determined usually by Total organic carbon (TOC), Dissolved organic carbon (DOC), and UV-absorbance. The presence of NOM, significantly affect the drinking water treatment and give rise to formation of several disinfection by-products which are carcinogens and need to be regulated.

Sample Questions to Answer:

- 1) What do you understand by the term “TOC” in wastewater?
- 2) What is the significance of SUVA?

Experiment No. 8

Name of the Practical Determination of organic pollutant by High Performance liquid chromatography (HPLC).

Objectives To determine the concentration of phenol in municipal wastewater through HPLC.

Theoretical Concept: HPLC is an important analytical method commonly used to separate and quantify components of liquid samples. In this technique, a solution (first phase) is pumped through a column that contains a packing of small porous particles with a second phase bound to the surface. The different solubilities of the sample components in the two phases cause the components to move through the column with different average velocities, thus creating a separation of these components. The pumped solution is called the mobile phase, while the phase in the column is called the stationary phase. The main components of HPLC is shown in figure 1.

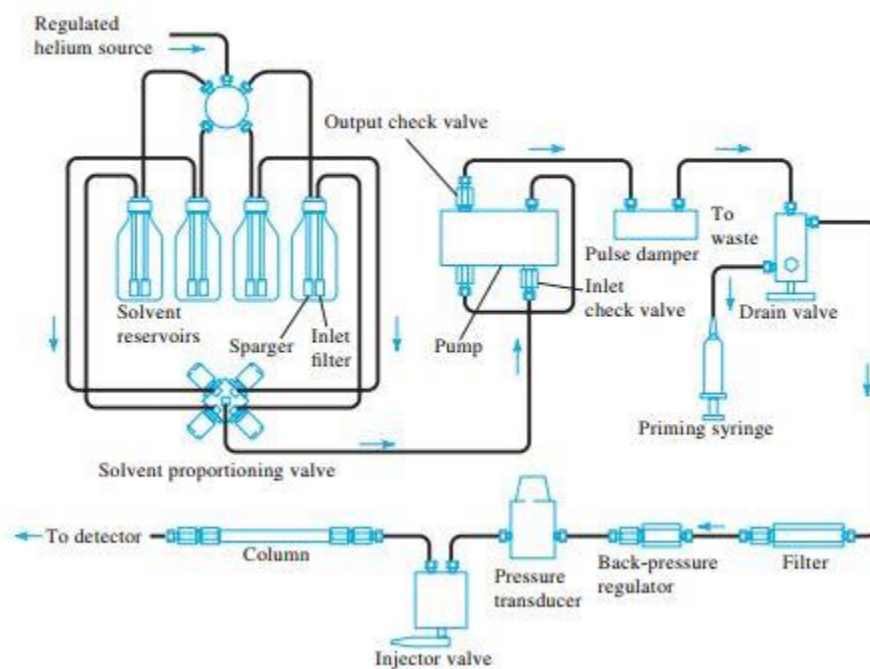


Figure 1. Block diagram showing components of typical HPLC

Procedure

Preparation of the Mobile Phase: Prepare the mobile phase by mixing methanol and Milli-Q water in the ratio of 70:30 v/v. For this add 120 ml of Milli-Q water in 280 mL of methanol solution.

Start the instrument and set the column conditions.

- Column conditions for phenol analysis: run standard in an isocratic mode by fixing mobile phase ratio at 95% methanol and 5% acetonitrile.
- Maintain the flow rate at 1 ml/min and injection volume of 20 μ L.
- Set the detection wavelength of UV at 280 nm and set column temperature at 25°C.
- Autozero the baseline and start the run the standards for 10 minutes.

Preparation of standard solutions of phenol for calibration:

- Prepare 1000 ppm stock solution of phenol by adding 1g of crystalline phenol powder into 1000 ml of volumetric flask and makeup the volume by pouring Milli-Q water.
- Prepare standards for calibration from 100, 200, 300, 400 and 500 ppm through serial dilution method in a 100ml volumetric flasks.
- Filter each standard solution through 45 μ m syringe filter into HPLC vials.
- Place the sample vials in the HPLC tray and initiate the analysis.
- Lastly run the filtered wastewater sample to determine the unknown concentration of phenol.

Calculations

- The peak for phenol will generate at retention time of 3 minute.
- Determine peak areas on the chromatograms for each standard and the unknown samples by the triangular method, which equals peak height times the width at $\frac{1}{2}$ height (Figure 2). After determining which peak corresponds to each component based upon the time it takes for each component to show their respective peak, enter these peak areas into a computer spreadsheet.

- Create calibration curves of peak area vs. concentration (mg/L) in the standards for all three components.
- Determine the least-squares fit for each calibration curve.
- Calculate the concentration of phenol in wastewater sample from the peak areas shown from the HPLC trials for the samples. Calculate the amount, in mg/L.

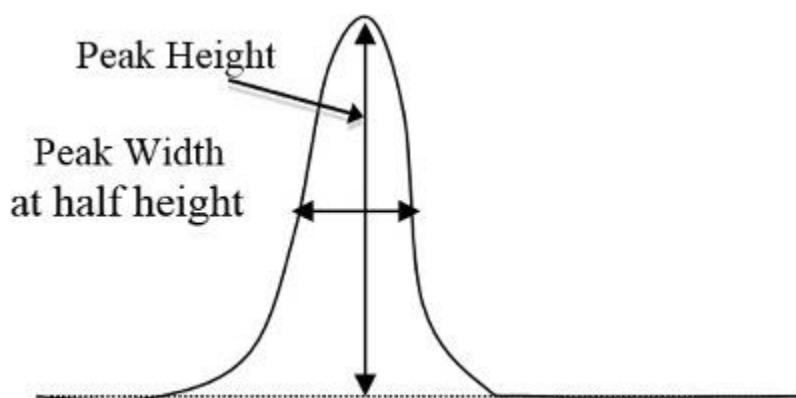


Figure 2. A basic example of a curve's peak height and width, which are to be multiplied (peak height times width at $\frac{1}{2}$ height).

Result & Discussion

The concentration of phenol in the given sample is.....mg/l.

Environmental Significance:

Organic pollutants enter the surface water from various sources such as municipal, industrial effluents and agricultural activities etc. Wastewater with organic pollutants like pesticides, organic dyes, priority pollutants impair the water use and give rise to generation of intermediates and by-products during treatment and hence need to remove from the water.

Sample Questions to Answer:

1. What is the working principle of HPLC?
2. What do you understand by mobile phase in HPLC?

Experiment No.: 9

Name of the Practical Determination of VFA using of given wastewater sample by Gas Chromatography (GC).

Objectives Chromatographic separation and isolation of individual VFAs from an original aqueous sample.

Theoretical concept: The measurement of organic acids, either by adsorption and elution from a chromatographic column or by distillation, can be used as a control test for anaerobic digestion. Volatile fatty acids are classified as water-soluble fatty acids that can be distilled at atmospheric pressure. These volatile acids can be removed from aqueous solution by distillation, despite their high boiling points, because of co-distillation with water. This group includes water-soluble fatty acids with up to six carbon atoms i.e., acetic acid, propionic acid, valeric acid, butyric, and iso-valeric.

The distillation method is empirical and gives incomplete and somewhat variable recovery. Factors such as heating rate and proportion of sample recovered as distillate affect the result, requiring the determination of a recovery factor for each apparatus and set of operating conditions. However, it is suitable only for routine control purposes.

Principle of VFA analysis by the gas chromatographic method: Chromatography is a physical method for separating components in a mixture. The basis of separation lies within the separating column, which is small-diameter tubing packed with a medium of large surface area. A mobile phase percolates through the stationary medium. The name gas chromatography denotes that the mobile phase is a gas.

The gas-liquid chromatography (GLC) has a stationary phase, a liquid spread over the surface of solid support. In GLC, a stream of carrier gas flows through the column. A sample is injected into the carrier gas near the head of a packed chromatographic column. Separation of various components of the sample occurs as a result of multiple forces by which the column material tends to retain these components. In the gas phase, the components move towards the outlet, but they are selectively retarded by the stationary phase. Consequently, all components pass through the column at varying speeds and emerge in the inverse order of their retention by the column material. Upon emerging from the column, the gaseous phase immediately enters a

detector attached to the column. Here the individual components register a series of signals which appear as peaks on the chromatogram.

The chromatographic peaks are used for qualitative and quantitative detection of the components. A typical chromatogram consists of a baseline and the peaks. The distance between peaks is influenced by the rate of flow of gas, the column conditions and column temperature. However, the peak area is related to the concentration of component. Therefore, under constant conditions, a comparison between a standard reference with that of the sample will tell about the concentration of a component.

Apparatus: The gas chromatography

Reagents:

- **VFA (C₂ –C₅ standard solution (25 mg/ml)** – Transfer 2.5 g of individual volatile fatty acid (acetic, propionic, butyric, valeric and iso-valeric), in separate 100 ml volumetric flask and make up the volume with distilled water.
- **Lactic acid standard solution (100 mg/ml)** – Transfer 2.5 g lactic acid in a 25 ml volumetric flask and make up the volume.
- **Pivalic acid internal standard (2.5 mg/ml)** – Dissolve 0.5 g pivalic acid in distilled water and dilute to 200 ml.
- **Oxalic acid (0.12 M)** – Dissolve 3.782 g oxalic acid dehydrate in water and dilute to 250 ml.
- **Composite working standard** – Pipette 1 ml of C₂C₅ standard solution (reagent 1), 2.5 ml lactic acid standard solution (reagent 2), 10 ml pivalic acid (reagent 3) and 25 ml oxalic acid solution (reagent 4) into a 100 ml volumetric flask and dilute to volume.

Procedure:

- A series of VFA standards for the calibration curves were prepared in the same manner as described above. Calibration curves were obtained using five aqueous solutions of acids: acetic, propionic, butyric, valeric, and caproic, in the different concentration range.
- Now, the GC analyses were performed on a gas chromatograph equipped with a flame ionization detector (FID) and a DB-25 capillary column (30 m, 0.25 mm I.D., 0.25 µm film thickness)
- Set the GC as following operating conditions:

Gas flows

Nitrogen 20 ml/min
Hydrogen 50 ml/min
Zero Air 500 ml/min

Temperature

Injection 180°C
Detector 240°C
Oven 150°C

- In each case, 2 µL of the sample (acidified to pH 2 using 65% of nitric acid) was injected.

Calculation:

Calculate mg/ml of each acid in the sample from the expression:

Conc. of unknown = $(RF_1 / RF_2 \times \text{standard conc.} \times 2)$

Where,

$$RF_1 = \frac{\text{Area of sample component peak}}{\text{Area of internal standard}}$$
$$RF_2 = \frac{\text{Area of known peak}}{\text{Area of internal standard}}$$

Observations:

Record the following observations in the table:

Observation Table:

Sample. Code	Obs. 1 (mg/ml)	Obs. 2 (mg/ml)	Obs. 3 (mg/ml)	Average Concentration
1.				
2.				

Results & Discussion:

The average concentration of VFA is.....

Environmental significance:

Volatile fatty acids (VFAs) originate from anaerobic biodegradation of organic matters and is responsible for unpleasant odour. This lowers down the pH and reactors gets failure if pH is not neutralised by increasing alkalinity. VFAs are widely used parameters to assess the establishment of acidogenic/methanogenic phase in an anaerobic digester and helps in improving the process stability.

Sample Questions to Answer:

- 1) How would you categorise VFA?
- 2) How VFAs are formed?
- 3) Why the level of VFA must be controlled?

Experiment no. 10

Name of the Practical Determination of dye concentration in given wastewater.

Objective Characterization of dye concentration in a given wastewater sample.

Theoretical Concept: Dyes like direct dyes, reactive dyes, azo dyes, acidic dyes, inorganic dyes, organic dyes are used during the dyeing process. These dyes have a conglomerate molecular structure which is resistant to breaking their chemical bond by simple mechanisms like coagulation, adsorption, oxidation etc.

In this experiment, we will use spectrophotometry to determine the concentration of an unknown solution. In order to accomplish this goal, we must first determine what wavelength of light the solution best absorbs (the wavelength of maximum absorbance). Then we will prepare solutions of known concentration, measure their absorbances, and create a calibration plot. We can then use information from that plot to determine the concentration of our unknown.

Spectrophotometry is the study of light as a function of its wavelength. Light is energy, in the form of electromagnetic radiation. That light has the capability to do work. Plastic left out in the sun degrades and becomes brittle. If you put a bleaching agent in your hair and go sit in the sun, sunlight causes your hair to bleach. Solar cells change light energy into electricity. We can understand the energy contained in light by understanding the light wave. If we look at the wave, we can define the length of the repeat units (wavelength), how many waves repeat per second (frequency) and the height of the wave (amplitude). We can relate frequency and wavelength for an electromagnetic wave by: $c = \lambda \cdot \nu$, or the speed of light is equal to the frequency of the wave times the wavelength. This equation works for the whole electromagnetic wave series. Refer to your textbook for a more complete description. We are looking at a small portion of this spectrum, between the ultra violet and infrared sections, which we call visible light. We see visible light. We can feel infrared radiation as heat. We cannot visually see ultraviolet light but we feel the sunburn caused by overexposure to UV light. Violet light, at a wavelength of about 400 nm has higher energy than red light, at about 700 nm.

This visible light can cause chemical changes or reactions in compounds. The compounds absorb specific wavelengths of light. These reactions can be permanent or reversible. Different molecules absorb different wavelengths of light. The color of an object is due to the combination of those wavelengths that an object did not absorb. Plants appear green because they absorb the

wavelengths in the blue and red regions, leaving the greens, to be seen. The absorbed light energy is used in photosynthesis, the conversion of CO₂ and water into sugars and carbohydrates.

We can differentiate compounds based on their light absorption. We can look at blue or green mineral samples and predict that there are copper compounds present. Dark red/orange rocks often have iron compounds. A red ruby can be identified as chromium doped alumina. We can more precisely understand these compounds by burning a small amount and examining the light spectra given off by the flame. This is called flame emission spectroscopy. This information is tabulated into tables.

You can estimate the wavelength of the color absorbed visually by samples by use of the following table:

Wavelength absorbed (nm)	Color absorbed	Approximate color observed
410	violet	green-yellow
430	violet-blue	yellow
480	blue	orange
500	blue-green	Red
530	green	purple
560	green-yellow	violet
580	yellow	violet-blue
610	orange	blue
680	red	blue-green
720	purple-red	green

Procedure:

a) Preparation for the experiment

Plug in and turn on a spectrophotometer. Allow it to warm up and go through its automated calibration.

b) Determine the wavelength of maximum absorbance

- i. Fill one cuvette approximately half-full with deionized water (blank). Fill a second cuvette approximately half-full with the stock solution (sample). Record the concentration of this stock solution (from the label) in your lab notebook. Wipe both cuvettes clean with a Kim wipe tissue.

ii. **Load the samples:**

- Be sure the smooth (clear) sides of the cuvettes are on the same sides as the holes in the slots. This is where the light passes through! If you are not sure, ask your instructor.
- Load the BLANK into slot.
- Load STOCK solution sample into other slot.
- Close the cover.

iii. **Set the wavelength:**

- Press the “set nm” button.
- Enter the desired wavelength (first wavelength should be 460 nm).
- Press “enter”.

iv. **Zero the Spectrophotometer, Measure the absorbance of the sample**

- Put the sample into the slot in spectrophotometer for the measurement of reading.
- Press “measure blank”. This will set the absorbance reading to zero, and then measure the absorbance of the sample.
- Read and record the absorbance (once the noise stops).

v. Repeat steps 4-6 for each wavelength to be measured – every 20 nm wavelength increment up to 680 nm (e.g. 480, 500, 520...). Be sure to zero for each wavelength!

vi. Once you find your wavelength of maximum absorbance, record the absorbance at 10 nm above and below this wavelength to better determine the maximum.

vii. Remove your cuvettes, dump your sample, and clean the cuvettes.

Data Analysis

- Plot the wavelength (x-axis) versus absorbance in your notebook.
- From your graph, determine and clearly label the wavelength of maximum absorbance. Show the graph to your instructor for approval before proceeding.
- Obtain a labelled unknown and record its number in your lab notebook.

c) Standards and Unknown Solution

Prepare Standard Solutions

- i. The stock solution will be one of your 5 solutions of known concentration. Label it known #5. Deliver about 5 mL solution to test tube #5
- ii. To prepare known #1, pipette or deliver (by volumetric pump) 5.00 mL of the stock solution into a 25.0 mL volumetric flask, and add deionized water to the mark on the neck of the flask. Mix well. Save approximately 10 mL in a test tube.
- iii. To prepare knowns #2-4, repeat the above step, increasing the volume of stock solution by 5.00 mL each time. (e.g. #2 will use 10.0 mL stock solution as calculated in the prelab)
- iv. Once you have prepared the 4 solutions, fill the numbered test tubes approximately three quarters full with their corresponding solutions. Dump the remaining solutions from the volumetric flasks into the waste, rinse them thoroughly, and return them immediately for others to use.
- v. In the prelab, you calculated the concentration of each standard solution (knowns #1-5). Copy the solution numbers and concentrations into your data section, with an additional column for absorbances.

Data Analysis:

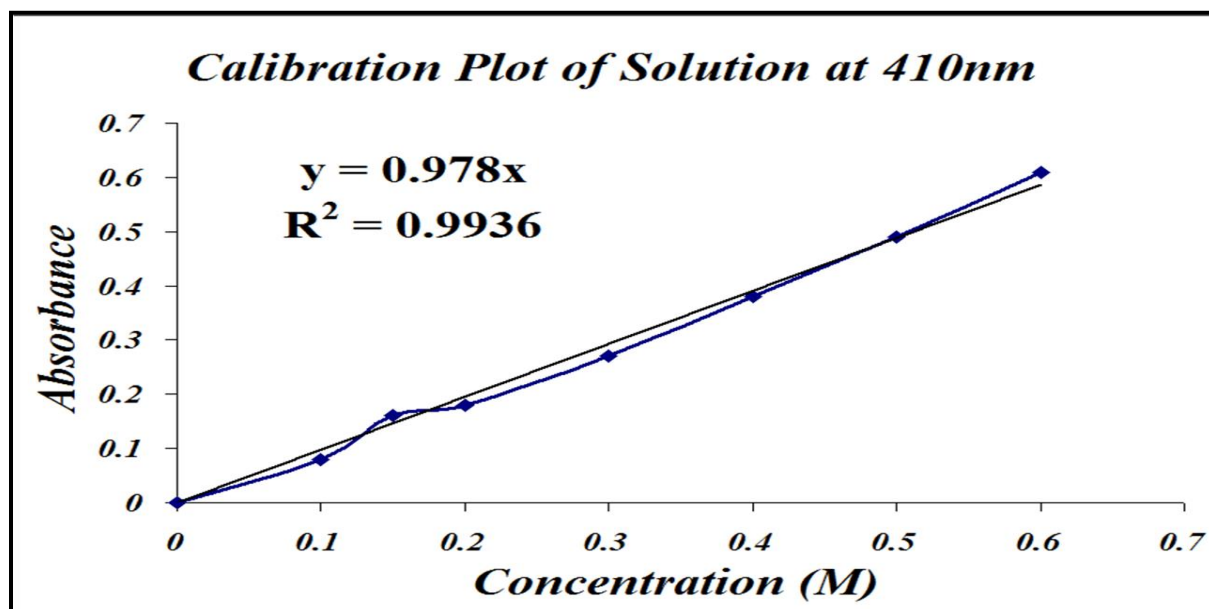
- i. Plot your calibration data, [conc] (on the x-axis) vs. Absorbance in your lab notebook. Use nearly a full page to do this. Follow the guidelines for good graphing, including drawing a best fit line.
- ii. Calculate the slope (k) by choosing the two points from your plot that would best estimate the slope of your best fit line and plug them into the slope equation.
- iii. Express the relationship in Beer's law form, substituting your slope for k.
- iv. Add your unknown data point to the best fit line based on its absorbance and clearly label it. Drop a dotted line to the concentration axis to estimate the concentration of your unknown.
- v. Use the Beer's law equation to calculate the concentration of your unknown. It should be close to the estimate in the step above.

Observations:

Solution	Volume of stock solution (ml)	Volume of diluted solution (ml)	Absorbance at maximum wavelength
Blank			
#1			
#2			

Graphs and calculations:

Draw the graphs and find the unknown concentration of the dye present in the wastewater.

Graph for calibration curve**Results:**

The dye concentration in the given sample: mg/l

Environmental Significance:

- Dyes exhibited a group of organic compounds that could have detrimental impacts on the environment, as well as, some of them can cause hazards to humans depending on their concentration and duration of exposure.

- Removal of dyes from wastewater is a major concern, due to its colour and associated toxicity to human health and life.

Sample Questions to answer:

- 1) Why do we close the cover of the spectrophotometer during absorbance measurements?
- 2) Briefly explain what information you obtained from the calibration plot and why we needed it. Be specific. How did you use it to find the concentration of the unknown?

Department of Environmental Science and Engineering

B. Tech. Environmental Engineering

Solid Waste Management Practical (ESC 357)

Solid Waste Management Laboratory (Under Construction – 1st Floor)

Solid Waste Management. (Practical ESC 357)

INDEX OF EXPERIMENTS

Sl. No.	Name of Practical
1.	Sample preparation, sampling techniques, coning and quartering method, overburden and other waste sampling
2.	Characterization of solid wastes and proximate analysis I.
2 A.	To determine the moisture percent of the waste sample.
2 B.	To determine the volatile matter percentage in the sample
3.	Practical use of Bomb Calorimeter
4.	To perform the ultimate analysis of solid waste
5.	Estimation of Kjeldahl Nitrogen in solid waste sample
6.	Estimates of total organic carbon (OC expressed as C) in the solid waste
7.	To determination of available phosphorus in solid waste samples
8.	To determine various components of Municipal Solid Waste in your location
9.	Determination of field capacity of soil sample

Experiment No.: 1

Aim: - Sample preparation, sampling techniques, coning and quartering method, overburden and other waste sampling.

Introduction

Sampling is the process of collecting a portion of an environmental medium as representative of the locally remaining medium. It is one of the crucial steps as the reliability and the applicability of subsequent chemical analysis and recommendations will depend on it. The characteristics measured in those samples and any consequent subsamples (a sample of a sample), including laboratory subsamples, are then considered as “representative” of the site from which they were collected.

Various sampling techniques that can be used for sampling solid wastes include coning and quartering, random sampling, stratified sampling and cluster sampling methods.

Coning and quartering method is one of the sampling techniques used in case of sampling of solid wastes. It is basically a systematic splitting process which can be performed on both dry and moist materials virtually of any size. The coning and quartering method is used to reduce the sample size without creating a systematic bias. This process also preserves the particle correlations within each quadrant.

Apparatus & Reagents

Soil corer, plastic sheets, shovel, cross cutter, mortar and pestle, sieves, shallow tray.

Procedure

- 1) **Selection of sampling location:** The sampling area can be subdivided into grids or sampling units if considerable difference in texture, elevation and color is observed. This is done to map parameters and to identify problematic areas in the study area. If no considerable difference in soil parameters is observed then the whole area is treated as one sampling unit. Composite sampling is performed on each of the subarea or grid.
- 2) **Coning and Quartering method**
 - a) The surface litter is scrapped and vegetation cover is removed from the sample location.
 - b) Soil sample is extracted from a depth of 15 cm from the top surface; in case of OB sampling depth of 50-100 cm is considered.
 - c) The soil is then mixed and shoveled into a uniform conical pile. The pile must be made so that natural segregation in the cone is radially symmetrical.
 - d) The cone is then spread from the center to form a flattened disc of material.
 - e) This disc is then divided into four sections (quarters) with a cross cutter having 90° angles.
 - f) One pair of opposite quarters is removed and the other pair is used for the sample.
 - g) If the sample is too large then it can be coned and quartered again until the desired sample size is obtained.

3) Preparation of Sample

- The sample is air dried by laying it in a shallow trays or plastic sheets for up to 7 days in case of room drying and 2-4 days in case of sun drying (maximum temperature 35 °C).
- The dried sample is then weighed and passed through 2 mm sieve. The sieved sample is then reweighed and the proportion of soil with size less than 2 mm size is recorded.
- In case, some clods are formed, the clods are crushed and slowly grinded with the help of mortar and pestle.
- A sample weighing 10 g is then oven dried at 105 °C for overnight (minimum 6 h) to determine the moisture content of the sample.
- The sample is then stored inside a bag with the details of location and depth of sampling.

Precautions

- A collected sample must be accurate and precise within the set of specifications, and at the same time, it should be representative of the lot.
- High temperature drying of sample should be avoided as many constituents of the sample are sensitive to temperature.

Experiment No.: 2

Aim: Characterization of solid wastes and proximate analysis I.

The Experiment No. 2 has been divided into two distinct sections based on their measurable parameters. The Sections are: Experiment No. 2A & 2B.

Theory: Characterization of waste is the process by which the composition of different waste streams is analysed. The waste stream may include wastes such as paper, glass, food waste, etc. The characterization of solid waste is generally done by evaluating the elemental composition of the solid waste, the proximate analysis, the leachate composition and the calorific value of the solid waste samples. This characterization of solid waste components can serve as a foundation, when decisions on waste management are made and should be included in a total life cycle evaluation of a product. Waste characterization information helps in planning how to reduce waste, set up recycling programs, and conserve money and resources. This also plays an important part in the treatment of waste.

Proximate analysis is important in evaluating the combustion properties of waste or waste derived fuel. This serves as a simple means for determining the behavior of a solid waste when it is heated. It determines the contents of moisture, volatile matter, ash and fixed carbon of the waste or waste derived fuel. Proximate analysis includes the following tests:

- Moisture content, determined by loss of moisture after heating at 105 °C for 1 h.
- Volatile combustible matter, the additional loss of weight after ignition at 950 °C for 7 min in a covered crucible (oxygen is excluded).
- Fixed carbon, the combustible residue left after volatile matter is removed; ignition at 600–900 °C.
- Ash, the weight of residue after combustion in an open crucible.

In proximate analysis I, the content of moisture and volatile matter of the waste will be determined; while in the proximate analysis II, the ash and fixed carbon of the waste will be determined.

Experiment No.: 2A

Aim: To determine the moisture percent of the waste sample.

Theory: Moisture content and availability in a solid waste landfill are among the most important environmental factors affecting waste degradation. The presence or lack of moisture in a landfill can ultimately change the time frame for waste degradation and landfill stabilization from a few years to several decades. To obtain the greatest benefit for waste degradation and gas generation, moisture conditions need to be near optimal, or increased to optimal through moisture infiltration, as early as possible in the degradation processes

Apparatus and reagents: Crucible, weighing machine, oven, desiccator

Procedure:

1. 10 g of the sample is weighed into a crucible.
2. The sample is then heated at 110°C for 1 hour in an oven and dried using a desiccator.
3. The percentage of moisture content (MC) is calculated as a fraction loss in weight of sample before and after drying.

Calculation:

M1 = mass of empty crucible (g)

M2 = mass of empty crucible + sample (g)

M3 = mass of empty crucible + sample after heating (g)

Moisture content = M2 – M3

$$\% \text{ moisture content} = \frac{M2 - M3}{M2 - M1} \times 100$$

Result:

Precautions:

1. Appropriate insulating gloves and tongs should be used to hold or remove the sample from the oven.
2. The hot sample should be carefully placed inside the desiccator after removal from the hot air oven.

Experiment No.: 2B

Aim: To determine the volatile matter percentage in the sample.

Theory: Volatile matter is that portion of the waste that is converted to gas before and during combustion. It is measured as the weight percent of gas (emissions) from a waste sample that is released during heating to 950° C in an oxygen-free environment, except for moisture (which will evaporate as water vapor), at a standardized temperature.

Apparatus and reagents: crucible, muffle furnace, dessicator, weighing machine

Procedure:

1. The sample is heated at a temperature of 950° C for 7 minutes using a muffle furnace.
2. The crucible is cooled in the desiccator immediately after removal from the furnace and is then weighed.
3. The percentage loss of mass of the sample excluding the percentage moisture is the percentage of volatile matter.

Calculation:

M1 =mass of empty crucible (g)

M2 =mass of empty crucible + sample before heating (g)

M3 =mass of empty crucible + sample after heating (g)

MC =percentage of moisture content

$$\% \text{ Volatile matter} = \frac{M2 - M3}{M2 - M1} \times 100 - MC$$

Result:

Precautions:

1. Appropriate insulating gloves and tongs should be used to hold or remove the sample from the furnace.
2. The furnace door should be closed and opened gently to prevent damage to the machine parts.
3. Flammable materials should be kept away from the furnace, including things like paper, flammable liquid, old rags, etc.
4. Furnace should be turned off and all the equipments should be put away after use.

Experiment No.: 3

Aim: Practical use of Bomb Calorimeter

What is a Bomb Calorimeter?

The calorimeter used to accurately determine the energy change during a reaction is known as a bomb calorimeter. The modern Bomb calorimeter is a development of the original calorimeter of Berthelot. The modern bomb calorimeter is made of corrosion-resisting steel like which the combination Bomb Calorimeter.

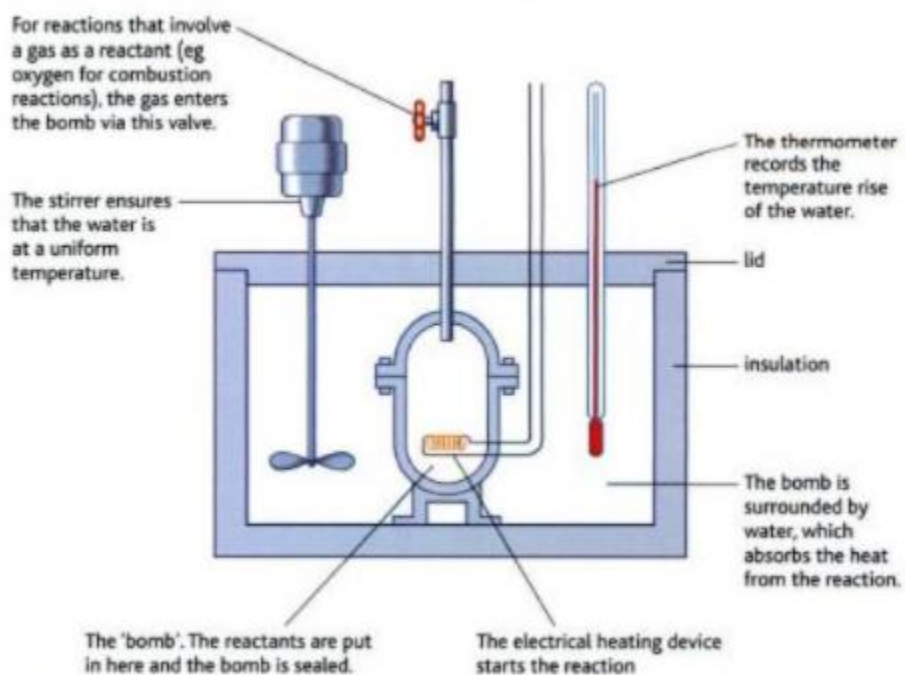
The bomb calorimeter is an instrument used to measure the heat of a reaction at a fixed volume and the measured heat is called the change of internal energy (ΔE). In chemistry, the changes in heat of a reaction can be measured at fixed pressure or volume.

Working on Bomb Calorimeter

The bomb calorimeter is a type of constant-volume calorimeter used to measure the combustion heat of oxygen-burnable samples. Four critical parts are needed in every bomb calorimeter.

The bomb calorimeter is a laboratory instrument used to measure the amount of a sample's combustion heat or heat power when excess oxygen combustion occurs. This research aims to determine the effect of using the bomb calorimeter on the ability of physics students to process science. Influences involve the efficacy of using the devices and learning how to develop the abilities of the scientific method of students before and after using materials.

If the heat of the capacity calorimeter C_{cal} of to the calorimeter is known, then one determines the heat generated by and only needs to note the change in the temperature process. Calorimetry is widely used in present-day laboratories.



Construction of Bomb Calorimeter

The bomb calorimeter is used to determine the calorific values of solid and liquid fuels. It consists of a strong steel shell known as a bomb.

It consists of a base that supports the platinum crucible and is screwed to the body of the bomb. The top of the bomb carries an oxygen supply connection and a valve to release the product. One gram of powdered sample coal is taken for the test and the calorimeter is filled with 2000 cm³ of water. The sample is placed in the platinum crucible. The iron fuse wire which surrounds the sample of coal is connected to the lower end of the two electrodes. The electrodes extend through the base of the bomb and connect the fuse wire to an electric circuit. The coal can be ignited by closing the electric circuit.

The bomb is placed inside a copper vessel that contains water. There is a stirring device for agitating the water within the calorimeter. The calorimeter containing the bomb is placed in another container which acts as a heat insulator. The temperature of water in the calorimeter is measured by a thermometer.

The oxygen cylinder is coupled to the bomb and oxygen is admitted to the bomb through the valve until the pressure gauge in the cylinder indicates a pressure of 25 atmospheres. The fuel is ignited by passing a current through the fuse wire. The temperature of both starts increasing and the readings on the thermometer are taken at one-minute intervals for 10 minutes, after the maximum intervals, after the maximum temperature is reached. Thereafter the temperature starts falling slowly. When the temperature fall shows a steady rate the readings are taken at regular intervals for an additional five minutes.

Calculation

Weight of the fuel = m g

Weight of water taken in the calorimeter = w_1 g

Water equivalent of calorimeter = w_2 g

Initial temperature of water = t_1 °C

Final temperature of water = t_2 °C

Specific heat of water = 4.187 kJ/kg

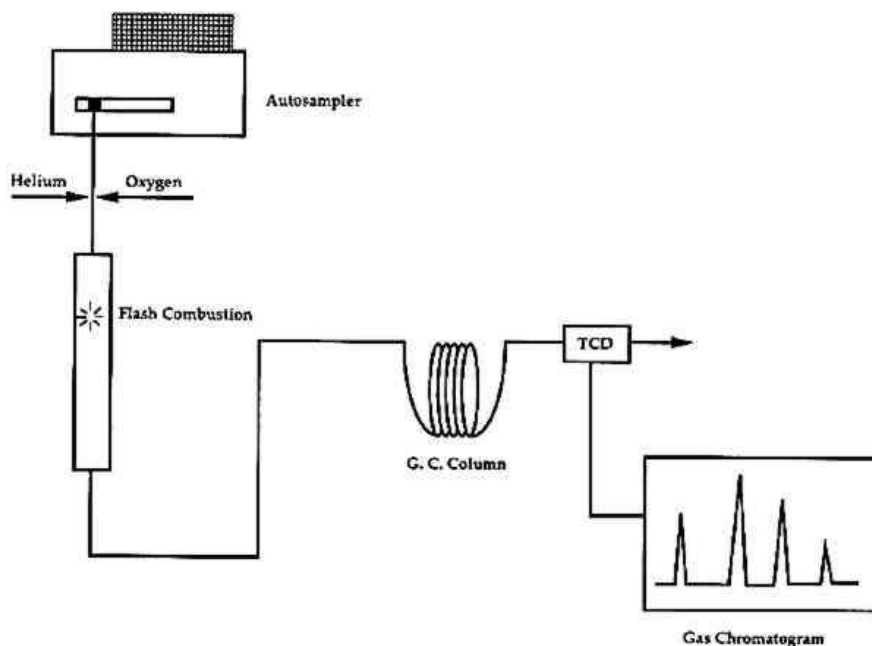
$$\text{GCV} = \frac{(W_1 + W_2)(t_2 - t_1) \times 4.184}{m} \quad \text{kJ/kg}$$

Experiment No.: 4

Aim: To perform the ultimate analysis of solid waste

Theory: CHNSO elemental analysis, also known as organic elemental analysis or elemental microanalysis, determines the amounts of carbon (C), hydrogen (H), nitrogen (N), sulfur (S) and oxygen (O) present in a sample. It is a reliable and cost-effective technique used to assess the purity and chemical composition of compounds and can be used on a wide range of different sample types including solid, liquid, volatile and viscous substances. Knowing the composition of the organic elements also helps analysts to determine the structure of the sample substance. The chemical characterization of organic compounds is used in research as well as for quality control (QC).

Elemental analysis is a highly reliable analytical technique that is used in a wide range of applications across many different industries (see below). The most common technique of CHNSO elemental analysis is based on the combustion of the sample. This can be performed in a dedicated instrument known as an Elemental Analyzer. Upon combustion, the sample generates uniform compound gases of the elements C, H, N and S. These combustion products (e.g. CO₂, H₂O, NO₂, etc.) are measured using gas chromatography and thus the ratio of the elements in the original sample is determined. C, H, N and S can all be determined simultaneously whereas O may be analyzed in a second step by pyrolysis.



The instrument is calibrated with the analysis of std compounds using the K-factors calculations. Thus the instrument ensures maximum reliability of the results because the combustion gases are not split or diluted but directly carried to build in GC system simultaneous determination of CHNS can be done in less than 10 mins. This method finds the greatest utility in finding out percentages of C, H, N, S, (O) in organic compounds which are generally combustible at 1800o C. Key components of CHNS are auto sampler, combustion reactors, chromatographic column, and T.C.D. detector.

Apparatuses: CHNS analyser, desiccator, sample

Procedure:

Waste is dried in a muffle furnace at 105 C and is ground into small fractions. Then the ground fraction is followed by the screening to get a size fraction between 1-2 mm. 5 mm sample is weighed in a very sensitive weighing machine of accuracy 3 decimal places and is placed in a pelletizer to make a pellet. The samples are placed in the autosampler of the chns analyzer and the result is noted down from the system.

Precaution:

Organic elemental analysis requires relatively small sample weights. The actual sample weights vary according to the nature of the substance, the detection limits, the level of accuracy required and the type of elemental analyzer used. As the final amounts of each element are typically given as a percentage of the original sample weight, it is critical that the sample is weighed accurately. Due to the very small sample size, a highly accurate microbalance is recommended.

Pellet should be made very cautiously.

Experiment No.: 5

Aim: Estimation of Kjeldahl Nitrogen in solid waste sample

What is the Kjeldahl method?

The Kjeldahl method is a means of determining the nitrogen content of organic and inorganic substances. Although the technique and apparatus have been altered considerably over the past 100 years, the basic principles introduced by Johan Kjeldahl endure today. The Kjeldahl method may be broken down into three main steps: Digestion - the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution. Distillation - adding excess base to the acid digestion mixture to convert NH_4^+ to NH_3 , followed by boiling and condensation of the NH_3 gas in a receiving solution. Titration - to quantify the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution.

Acid Considerations

Sulfuric acid has been used alone for the digestion of organic samples. (As a convenience to reduce bumping of the digestion mixture, Alundum boiling chips or pumice are often added.) The amount of acid required is influenced by sample size and relative amount of carbon and hydrogen in the sample, as well as amount of nitrogen. A very fatty sample consumes more acid. Also, heat input and digestion length influences the amount of acid loss due to vaporization during the digestion process. Remember that a Kjeldahl flask is essentially a bulb with a condenser neck off to the side for refluxing of the acid.

Salt Additions

The problem with using sulfuric acid alone for digestion is very long digestion times result with many samples due to the slow rate of organic decomposition. The addition of an inorganic salt to the digest elevates the boiling point of the H_2SO_4 . The solution temperature of concentrated sulfuric acid alone is about 330°C . Addition of a salt such as K_2SO_4 can elevate the solution temperature of the digestion mixture to 390°C or more, depending on the ratio of salt to acid. This

significantly increases the rate of organic decomposition in the digestion mixture, shortening the length of time required for digestion

Catalyst Additions

Several catalysts have been employed by Kjeldahl chemists over the years to increase the rate of organic breakdown during the acid digestion. Mercuric oxide has been the most effective and widely used. However, mercury forms a complex with ammonium ions during digestion. The addition of sodium thiosulfate or sodium sulfide after digestion and before distillation will break the complex and precipitate mercuric sulfide. This is also important from a safety point of view, as mercury vapor might escape to the atmosphere during the distillation process.

Heat Input and Digestion Length

Typically the heating elements used for Kjeldahl digestions have variable settings. Heat input is frequently specified as “that setting which brings 250 ml of water at 25° C to a rolling boil in 5 minutes.” Initially an organic sample usually chars and blackens. The reaction may at first be very vigorous depending on the matrix and the heat input. With organic decomposition the digestion mixture gradually clears as CO₂ evolves. Metallic ions might tint the clear digestion mixture. Note that solution clearing in itself is not an indication that all organic nitrogen has been broken down. Digestion length must be determined by recovery studies on known materials of similar matrix if a new method is being developed.

Rapid Steam Distillation Apparatus

The development of block digestors as an alternative to macro-Kjeldahl digestion has been paralleled by the development of benchtop steam distillation units. Basically, this equipment consists of a steam generator to inject steam into the alkaline digestion mixture and an evaporator-type condenser, where contact with water-cooled glass coils causes the vapors to condense and drip into the receiving solution via a delivery tube. Usually the unit is designed to accept straight digestion tubes from block digestors, though there is no reason in theory why any digestion techniques could not be combined with any distillation technique. Steam distillation is much more

rapid than classical macro-distillation, typically taking from 3 to 8 minutes. On the other hand, only one distillation at a time can be done. Rapid steam distillation units are available with different features, ranging from relatively manual models to highly automated models. Basic models dispense dilution water and base under control of a push button. Some models include a timer to control the length of distillation, shutting down automatically. Some units automate the entire distillation process once the digestion flask is in place, and others automatically titrate to endpoint after distillation and calculate and display the results in a printed report. Titrators Of course, receiving solutions may be individually hand titrated using an indicator solution and buret, but several models of benchtop automatic titrator instruments are also available. Some units will titrate one receiving solution at a time to a set endpoint. Others will automatically titrate a number of receiving flasks sequentially. Still, other models provide a printout of results along with a digital readout.

Chemicals required for digestion

Conc H₂SO₄: 10 ml

Catalyst mixture: CuSO₄ +K₂So₄ 3gm

Sample weight 0.1 to 1 gm

Digestion time: 45 min to 1 h

Chemical required for distillation

40%NaOH: 40 ml per sample

4% boric acid: 25 ml per sample

Mixed indicator: 4 drops methyl reds and bromocresol green

Chemicals required for titration

0.1N HCL or 0.02N H₂SO₄

Calculation

% of N = $14 \times \text{Normality of acid} \times \text{actual titrant value} \times 100 / \text{sample weight} / 1000$

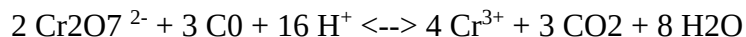
Experiment No.: 6

Aim: Estimates of total organic carbon (OC expressed as C) in the solid waste

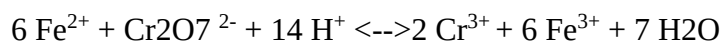
Principle

The determination of soil organic carbon is based on the Walkley & Black chromic acid wet oxidation method. Oxidizable organic carbon in the soil is oxidized by 0.167 M potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution in concentrated sulfuric acid. The heat of the reaction raises the temperature which is sufficient to induce substantial oxidation.

The chemical reaction is as follows:



The $\text{Cr}_2\text{O}_7^{2-}$ reduced during the reaction with soil is proportional to oxidizable organic C present in the sample. The organic carbon can then be estimated by measuring the remaining unreduced dichromate by back-titrating with ferrous sulfate or ammonium ferrous sulfate using diphenylamine or o-phenanthroline-ferrous complex as an indicator.



Alternately the organic carbon can be calculated from the amount of chromic ion (Cr^{3+}) formed, using a colorimetric procedure measuring absorbance at 588 nm. An advantage of this procedure over the titrimetric method is that accurate standardisation of the $\text{Cr}_2\text{O}_7^{2-}$ solution is not required

Reagents

1 N Potassium Dichromate: Dissolve 49.040 g $\text{K}_2\text{Cr}_2\text{O}_7$ AR (dried at 105 °C) in deionised water, transfer to a 1 L volumetric flask and make to volume with deionised water.

Sulphuric Acid

98% w/w This should be used fresh from the bottle and not left standing in a burette or beaker, as it rapidly picks up moisture from the air.

0.4N ferrous sulphate

Dissolve 112 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 800 mL deionised water containing 15 mL concentrated H_2SO_4 . Dilute to 1 L with deionised water and store in a dark bottle. Ferroin

Dissolve 1.485 g O-phenanthroline monohydrate and 0.695 g ferrous sulphate in approximately 80 mL deionised water, then dilute to 100 mL. Store in a dark bottle away from light.

Procedure

Determine the moisture content of the air-dry soil which has been ground to pass a 0.42 mm sieve. Weigh accurately enough soil to contain between 10 mg and 20 mg of carbon into a dry tared 250 mL conical flask (between 0.5 g and 1 g for topsoil and 2 g and 4 g for subsoil). (See Note 1.). Accurately add 10 mL 1 N $\text{K}_2\text{Cr}_2\text{O}_7$ and swirl the flask gently to disperse the soil in the solution. Add 20 mL concentrated H_2SO_4 , directing the stream into the suspension. Immediately swirl the flask until the soil and the reagent are mixed. Insert a 200 °C thermometer and heat while swirling the flask and the contents on a hot plate or over a gas burner and gauze until the temperature reaches 135 °C (approximately ½ minute). Set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without soil) must be run in the same way to standardize the FeSO_4 solution. When cool (20–30 minutes), dilute to 200 mL with deionised water and proceed with the FeSO_4 titration using either the "ferroin" indicator or potentiometrically with an expanding scale pH/mV meter or auto titrator.

Calculation

$$\text{OC}(\%) = 10(B-T) \times 0.003 \times 100 / B / \text{wt of sample}$$

Where B = volume of ferrous ammonium sulphate required for blank titration

T = volume of ferrous ammonium sulphate needed for the waste sample

Experiment No.: 7

Aim: To determination of available phosphorus in solid waste samples

Phosphorus is extracted from the soil using Bray No 1 solution as an extractant. The extracted phosphorus is measured colourimetrically based on the reaction with ammonium molybdate and the development of the 'Molybdenum Blue' colour. The absorbance of the compound is measured at 882 nm in a spectrophotometer and is directly proportional to the amount of phosphorus extracted from the soil.

Apparatus

- Centrifuge (6 000 rpm).
- Diluter/Dispenser
- High strength centrifuge tubes with caps (15 mL capacity).
- Spectronic 20 photometer with 10 mL tubes.

Reagents

Bray No 1 Extracting Solution

Dissolve 2.22 g Ammonium Fluoride A.R. (NH_4F) in deionised water and transfer to a 2 L volumetric flask. Add 5 mL concentrated hydrochloric acid and bulk to volume with deionised water.

Reagent A

Dissolve 17.14 g ammonium molybdate A.R. $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ in 200 mL of warm deionised water. Dissolve 0.392 g potassium antimonyl tartrate A.R. ($\text{KSbO} \cdot \text{C}_4\text{H}_4\text{O}_6$) separately in 150 mL deionised water. Place 500 mL deionised water in a 2 L volumetric flask and slowly add 200 mL concentrated sulphuric acid with mixing. When cooled, add the cooled molybdate and tartrate solutions, then mix and bulk to volume with deionised water.

Reagent C

Dissolve 0.53 g L-Ascorbic Acid A.R. (C₆H₈O₆) in deionised water and transfer to a 500 mL volumetric flask. Add 70 mL of Reagent A and bulk to volume with deionised water. Prepare fresh a volume of this solution sufficient for the day's work by proportioning the above quantities.

Standard Phosphorus Solution (P ≡ 50 mg/L)

Dissolve 0.2195 g potassium dihydrogen orthophosphate A.R. (KH₂PO₄) in 100 mL deionised water, transfer to a 1 L volumetric flask, add 5 mL concentrated sulphuric acid (A.R.) and bulk to volume with deionised water.

Phosphorus Working Standard (P ≡ 2.50 mg/L)

Pipette 5 mL standard phosphorus solution into a 100 mL volumetric flask and bulk to volume with deionised water.

Procedure

1. Dispense 7 mL Bray Extracting Solution into the oven-dry equivalent of 1 g of air-dry soil contained in a centrifuge tube. Include one tube containing the Bray Solution only for the blank.
2. Stopper the tube and shake vigorously for 1 minute.
3. Transfer the tubes to the centrifuge and spin at 6 000 rpm for 5 minutes.
4. Dispense 0.50 mL of the supernatant plus 2.0 mL Reagent C into a colourimeter tube. Mix and stand for 30 minutes.
5. Prepare a set of reference standards from the 2.50 mg/L phosphorus solution using the diluter/dispenser and the following table as a guide:

DILUTION TABLE FOR STANDARDS			
mls 2.5mg/L Ref.	mls Reagent C	Phos. Conc. (µg/2.5 mL)	Typical Absorbance Value

6. Set instrument zero (∞ Abs.) and then set full scale (zero Abs.) using the blank solution prepared above.
7. Measure and record the absorbance of the standards and samples at wavelength 882 nm.

8. Prepare a chart or graph from the standards data to plot phosphorus concentration against absorbance, or derive the equation of the line of best fit using linear regression. Use the chart or equation to determine the phosphorus concentration in the sample solutions.

Calculations

Calculate Available Phosphorus content.

$$\text{Available Phosphorus (mg/kg)} = C \times 14 / \text{ODW}$$

Where: C = Phosphorus concentration from chart/equation ($\mu\text{g}/2.5 \text{ mL}$) ODW = Oven-dry sample weight (g) 14 = Dilution factor

Experiment No.: 8

Aim: To determine various components of Municipal Solid Waste in your location.

The idea behind this experiment:

The information on the nature of wastes, their composition, physical and chemical characteristics – and the quantities generated are basic needs for the planning of a Solid Waste Management system. In order to plan, design, and operate a solid waste management system, a thorough knowledge of the quantities generated, the composition of wastes and their characteristics are essential. As a first step, a proper definition of the terms is necessary to avoid the general confusion that is common in the usage of these terms. Municipal waste includes wastes resulting from municipal activities and services such as street waste, dead animals, market waste and abandoned vehicles. However, the term is commonly applied in a wider sense to incorporate domestic wastes, institutional wastes and commercial wastes

Procedure:

Collect the waste from your location.

Weigh the total wastes and classify them according to the different components (as we discussed in the class)

Compare the data with other sources.

Understand the trends and how they could be reduced at the source or segregated properly for valuable products from the extracted wastes.

Discuss your observations.

Experiment No.: 9

Aim: Determination of field capacity of soil sample

Theory

Field capacity is the moisture content in percentage of soil on an oven-dry basis when it has been completely saturated and downward movement of the excess water has practically ceased. Such a stage is reached generally in 48 to 72 hours after saturation.

Materials/Equipment Required

A black polythene sheet,, water, physical balance, and drying oven.

Procedure

Select a representative spot in the field. Ensure that the water table is not within two meters from the layer of which field capacity is to be determined. Bund an area of about 2.5 sq. meters on all four sides and remove all weeds to avoid transpiration. Pour water till the desired layers get sufficiently wet. Spread a straw mulch of at least 40 cm thickness on the surface to prevent evaporation. A polyethylene sheet can be conveniently used instead of a mulch.

Observation

Weight the wet soil sample

Then weigh the dry soil sample after drying in the oven

The difference will give the field capacity of of the soil.

Department of Environmental Science and Engineering

B. Tech. Environmental Engineering

Geoinformatics Practical (ESC 358)

Remote Sensing & GIS Laboratory (Room No: 311)

INDEX OF EXPERIMENTS

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5	Practical 5: Vector Data Handling in QGIS
6	Practical 6: Image Mosaicking, PCA, Subset
7	Practical 7: Unsupervised Image Classification
8	Practical 8: Supervised Image Classification, Accuracy Assessment & Change Detection
9	Practical 9: Introduction to Digital Elevation Model using QGIS
10	Practical 10: Watershed Delineation

Practical 1: Introduction to QGIS

QGIS stands for Quantum Geographic Information System is a free and open-source GIS application. It was a result of Source Forge project, QGIS is developed using C++ and Qt toolkit. Initially QGIS developed for displaying the GIS data, now it evolved as full GIS software package. It is published under GNU Free Documentation License as an official project of Open-Source Geospatial Foundation (OSGeo). It is compatible with all the operating systems and can very well handle multiple raster, vector and databases functionalities. QGIS is also serves as a window for assessing numerous other Open-source GIS packages such as SAGA, GRASS, Post GIS, map server and also statistical package like 'R' etc. It also has very easy and convenient access to various tools and plugins. It is overall a very rich open-source Geospatial tool.

Link to download QGIS- <https://download.qgis.org/>

1. How to install QGIS

- Latest version of QGIS can be downloaded from the link given above.
- Now Navigate to downloaded setup file of QGIS and right click on it and then select "Run as Administrator".
- A pop-up window as shown below will appear. Click on "Next".
- Now accept the License Agreement and then click on "Next"
- After that installation process starts, it will take some time to complete.
- At last, click on the "Finish" and QGIS will be installed on your system.

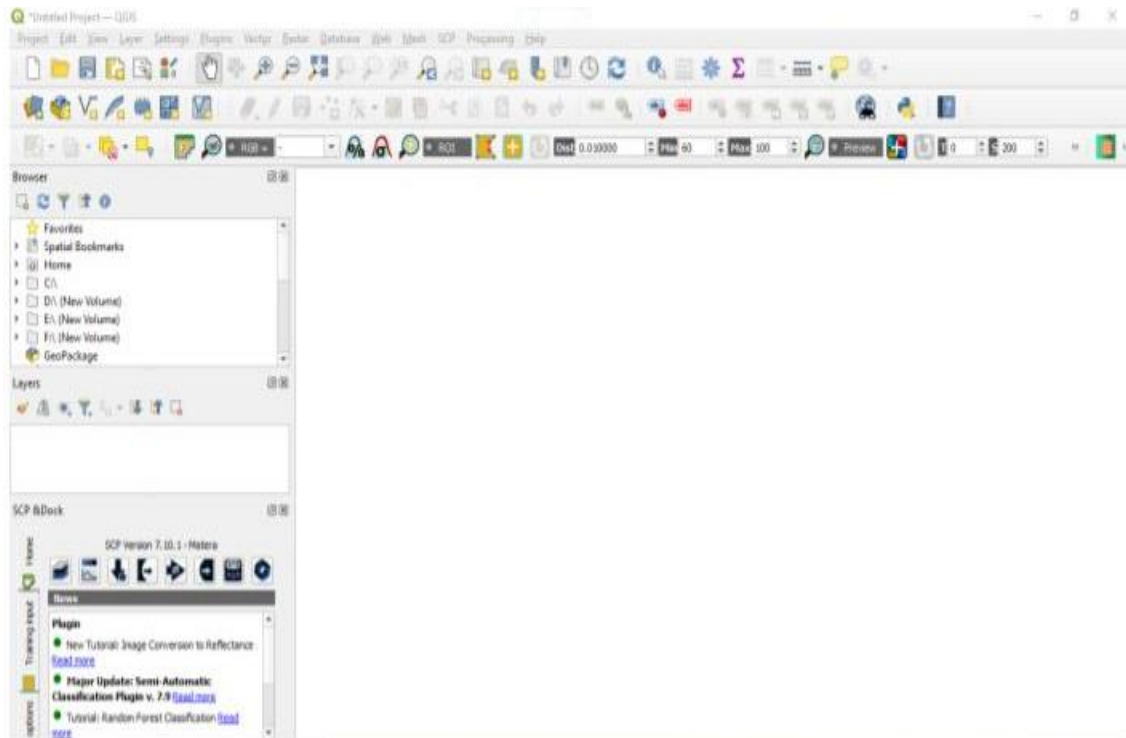


- Now QGIS is ready to use. Navigate to 'Start > All Programs > QGIS Desktop 3.22', to start QGIS Desktop.

2. Introduction to interface

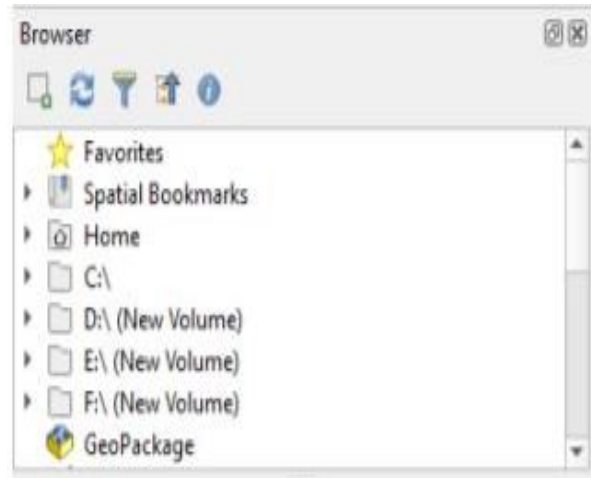
The elements identified in the figure below are:

- a) Browser Panel
- b) Layers List
- c) Toolbars
- d) Menu bar
- e) Map canvas
- f) Status Bar



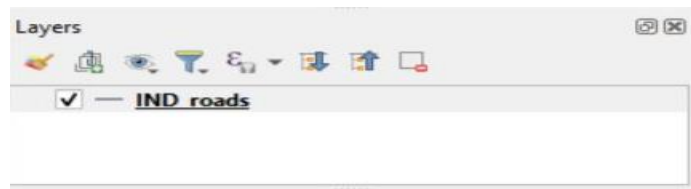
a) Browse Panel

Browser panel is used for browsing the data. Double click on the folder name where you have saved the data and you can navigate through the data. You can have access to common vector files (e.g., ESRI shapefile or MapInfo files), databases (e.g., PostGIS, Oracle, Spatialite or MSSQL Spatial) and WMS/WFS connections.

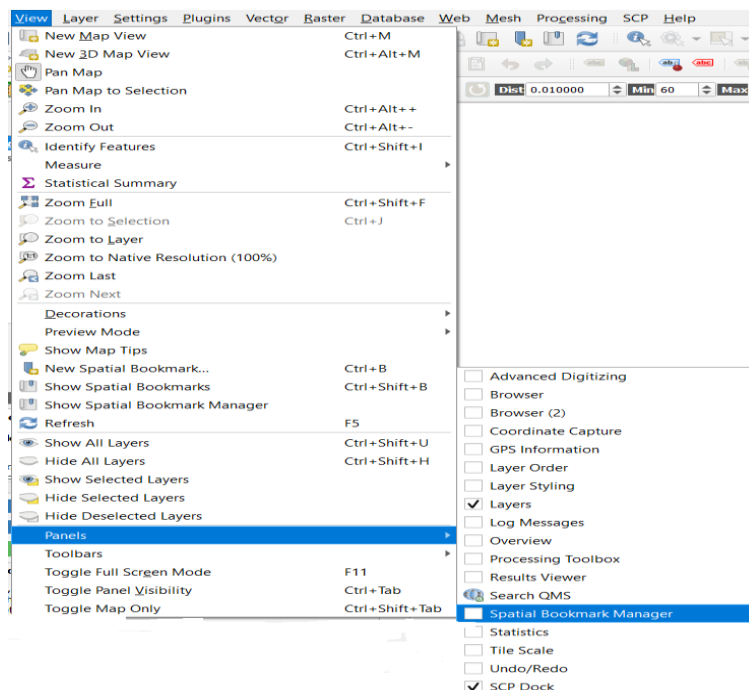


b) Layers Panel

In the Layers list, you can see a list, at any time, of all the layers available to you. Expanding collapsed items (by clicking the arrow or plus symbol beside them) will provide you with more information on the layer's current appearance. Right-clicking on a layer will give you a menu with lots of extra options.



One can activate/deactivate the panels from View>Panels

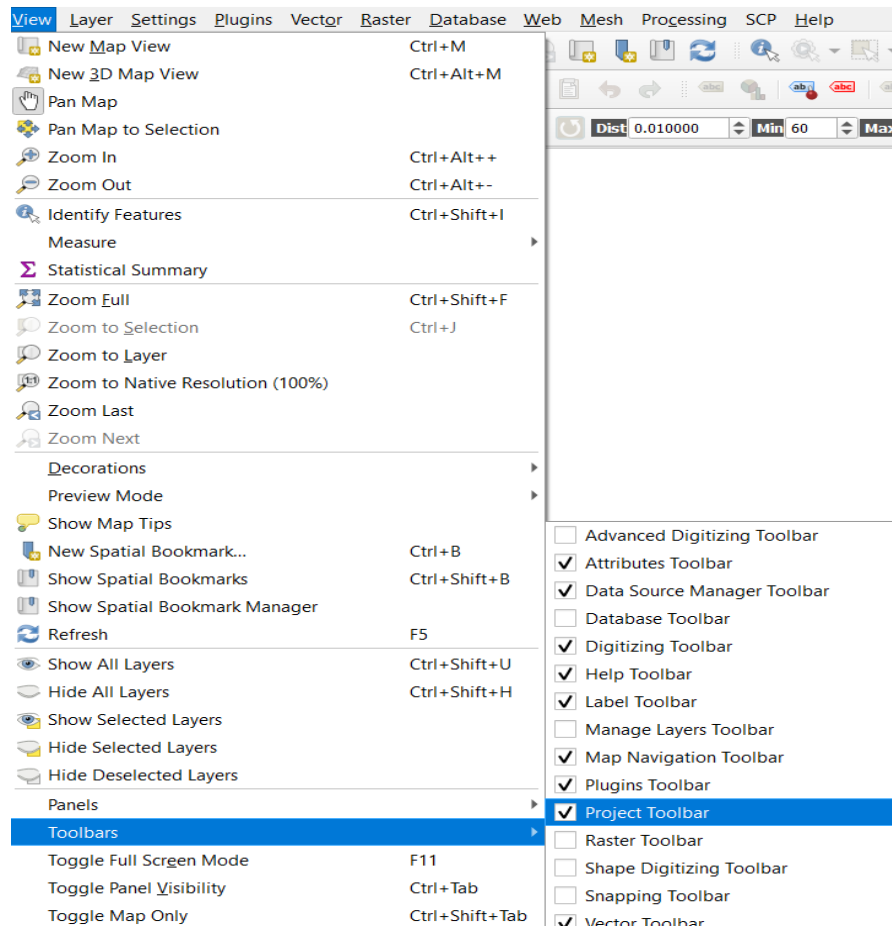


c) Toolbars

Most oft-used sets of tools can be turned into toolbars for basic access. For example, the File toolbar allows you to save, load, print, and start a new project.



You can easily customize the interface to see only the tools you use most often, adding or removing toolbars as necessary via View > Toolbars.



Even if they are not visible in a toolbar, all of your tools will remain accessible via the menus.

d) Menu Bar

It contains numerous pull-down menus to administrate the project files. It Provides access to various QGIS features.

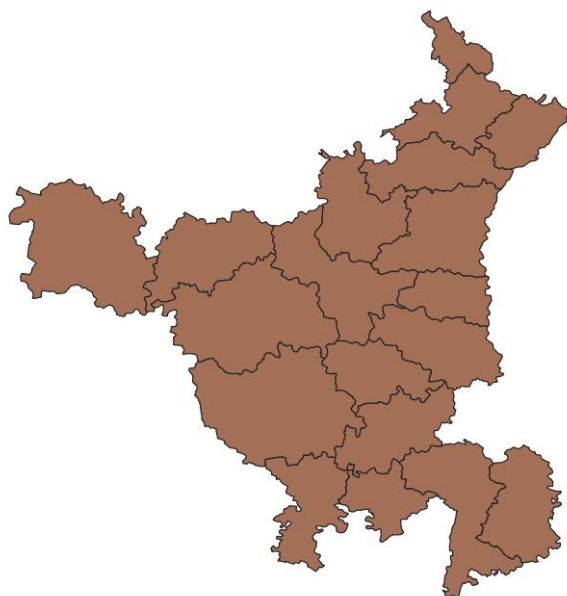


- Using **Project menu** we can open, save and create new quantum GIS projects. We can also make attractive map compositions using Composer Manager.
- **Edit menu** contains editing tools mostly corresponding to vector layers and these functions are enabled only when layers are toggled, in other words when we enabled the editing mode.
- **View menu** contains tools to map navigation, selecting features, identifying features, Measurement tools, contains tools to manage bookmarks, toolbars and panels.
- **Layer menu** contains various tools to load layers like Vector layer, Raster layer, PostGIS layer, spatialite layer etc. It also facilitates to create new shapefile layer. You can query the attribute data using Query feature. Convert layer from one vector layer format to another using save as feature. You can view layer properties and also possible to do labelling using Labeling feature and many other tools we can discuss about them later.
- **Settings menu** contains tools to set and manage custom CRS, keyboard shortcuts, style manager, customization and snapping options.
- **Plugins menu** actually makes QGIS more powerful. Plugins are small software components which add a specific ability to the QGIS application. Various core and third-party plug-ins can be accessed and managed by using this toolbar. We also have 'Python Console' from where we can execute python code.
- **Vector menu** contains various Research, Analysis, Geoprocessing, Geometry and Data management tools using which you can do extensive geospatial analyses on vector data sets. You can also download Open Street Map data [here](#).
- **Raster menu** contains various tools such as Georeferencer, Interpolation, tools for Terrain analysis, Zonal statistics, defining projection, Analysis tools and very important Raster calculator to perform raster operations.
- **Database menu** contains database manger which is a non-official part of QGIS core, you can drag and drop contents from QGIS browser into the DBmanager. It can execute SQL queries against spatial database. You can directly import shapefiles to PostgreSQL using spit.
- **Processing menu** is an added-up menu in this version of QGIS. It has a very useful Tool box (facilitates to use various Digital Image processing tools) and Graphical

builder. k. Help menu helps to access various help sections of all the functionalities available in the QGIS.

e) Map Canvas

This is where the map itself is displayed and where layers are loaded. In the map canvas you can interact with the visible layers: zoom in/out, move the map, select features and many other operations.



f) Status Bar

It displays the current coordinates, where the mouse pointer is pointed in map canvas. It also shows the scale of the map and map rendering option to enable or disable map rendering.

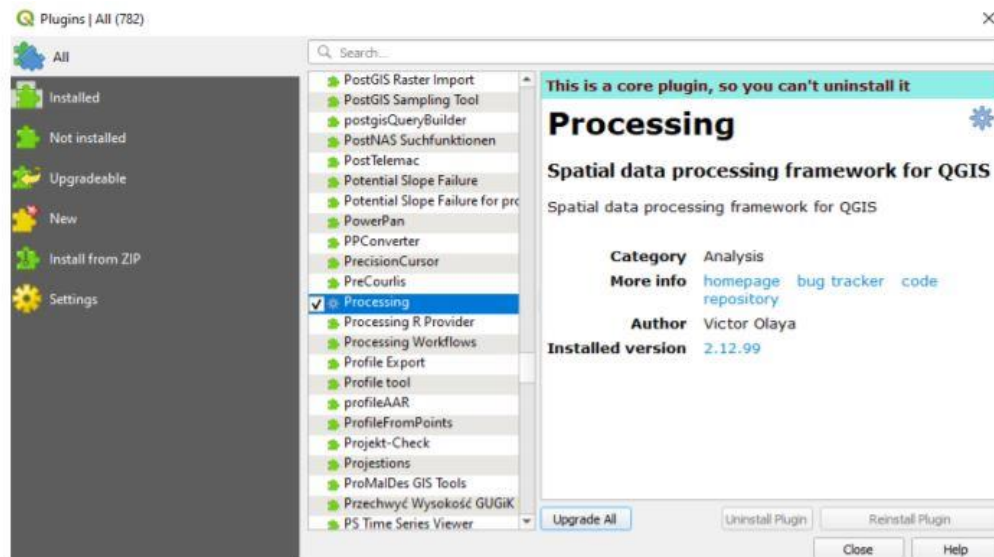


2.1 Plugins

To begin using plugins, you need to know how to download, install and activate them. To do this, you will learn how to use the “Plugin Installer” and “Plugin Manager”.

Managing Plugins - To open the Plugin Manager, click on the menu item Plugins > Manage and Install Plugins.

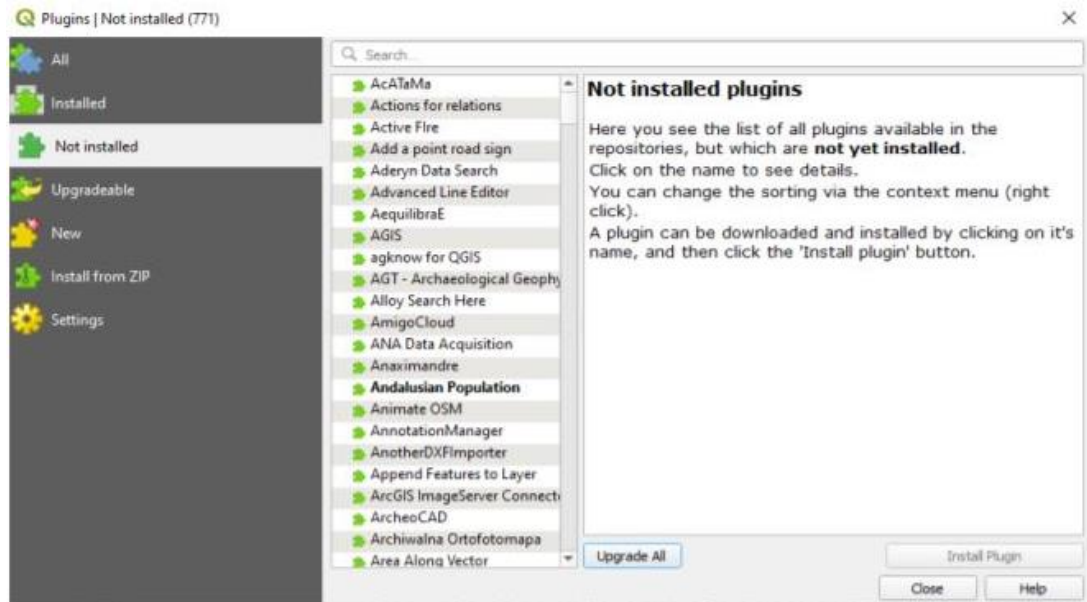
- In the dialog that opens, find the Processing plugin:



- Click in the box next to this plugin and uncheck it to uninstall it.
- Click Close.
- Looking at the menu, you will notice that the Processing menu is now gone. This means that many of the processing functions you have been using before have disappeared! This is because they are part of the Processing plugin, which needs to be activated for you to use them.
- Open the Plugin Manager again and reactivate the Processing plugin by clicking in the checkbox next to it and clicking Close.
- The Processing menu should be available again.

Installing New Plugins - The list of plugins that you can activate and deactivate draws from the plugins that you currently have installed.

- To install new plugins, select the Not Installed option in the Plugin Manager dialog. The plugins available for you to install will be listed here. This list will vary depending on your existing system setup.



- You can find information about each plugin by selecting it in the list of plugins displayed.



- A plugin can be installed by clicking the Install Plugin button below the plugin information panel.

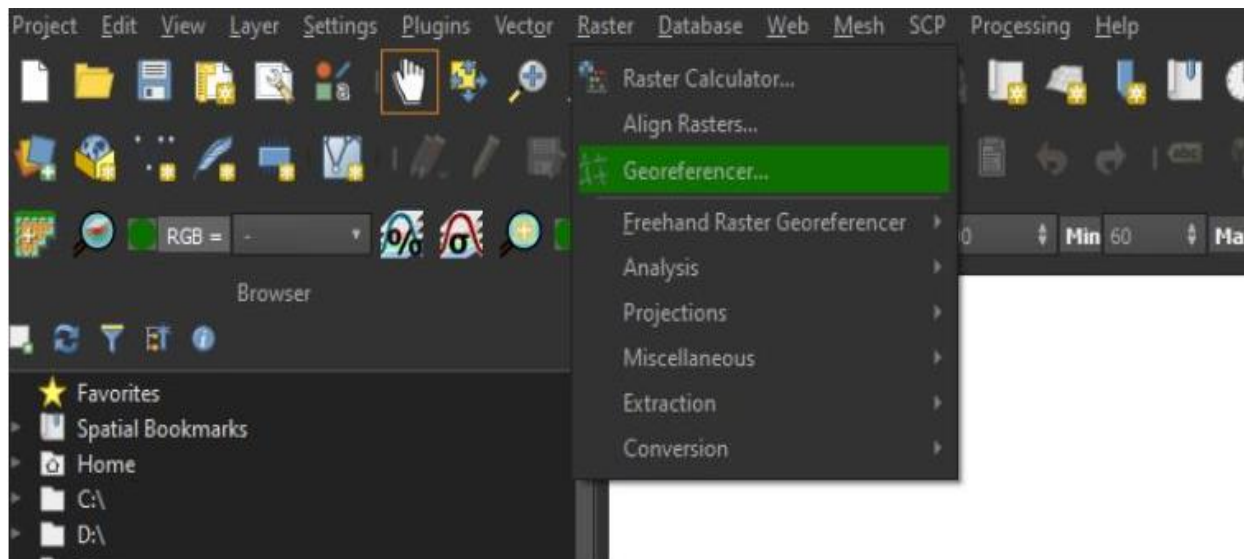
Practical 2: Georeferencing of Dataset

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 transformation to perform georeferencing/rectification of the Toposheet.

Dataset used: <https://dst-iget.in/index.php/tutorialdetails/1/24>

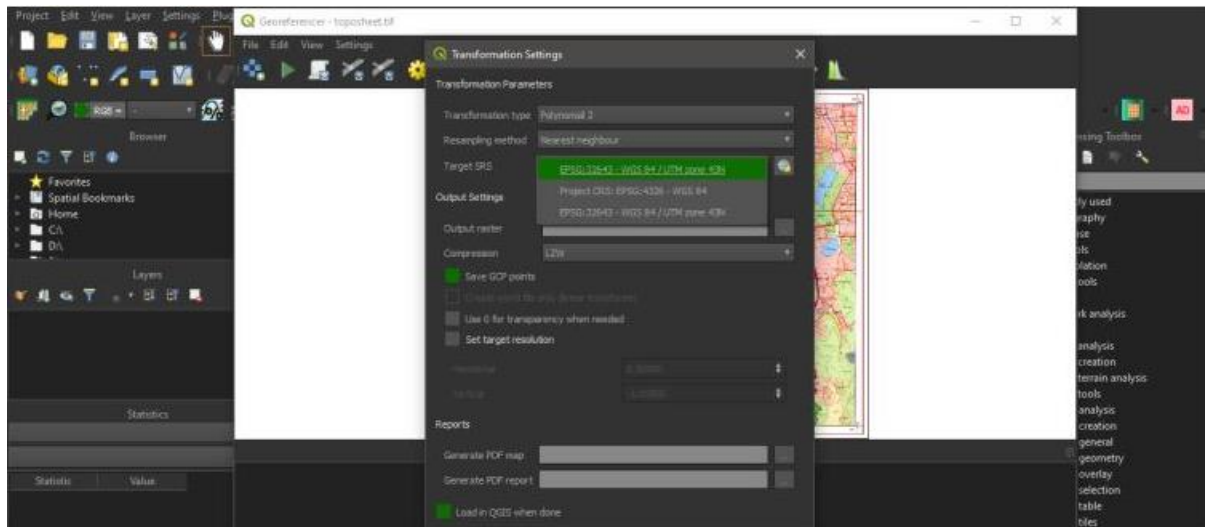
Step 1: First, we open Quantum GIS (QGIS) via the Start menu. (Start → All Programs → QGIS Dufour → QGIS Desktop 3.22)

Step 2: We then open QGIS Georeferencer via the menu bar. (Raster → Georeferencer → Georeferencer)

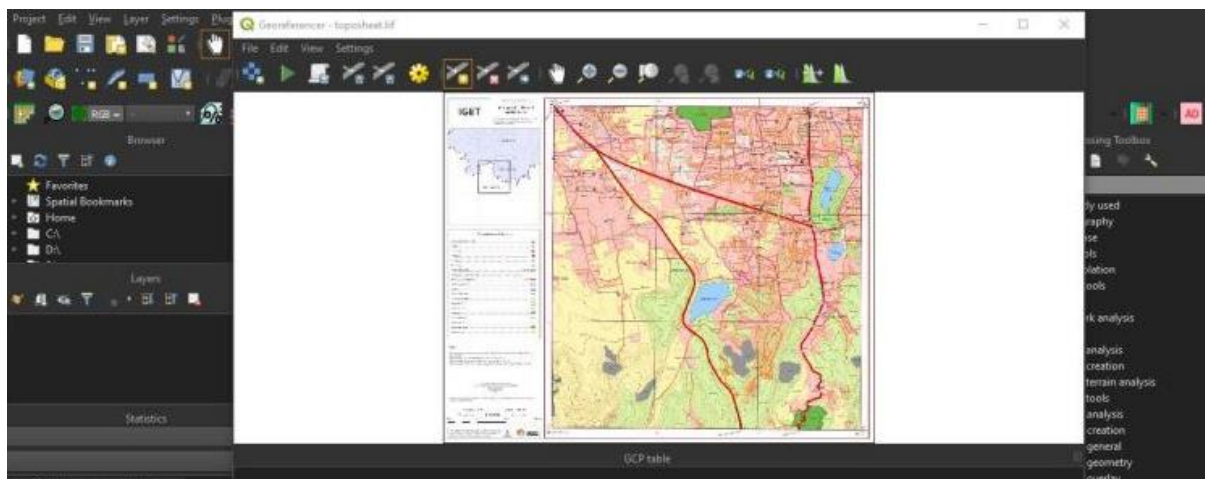


Step 3: The Georeferencer is in the form of a window with two parts to it. The upper part is called as 'Main Workspace' dedicated to display the Raster Map to be georeferenced and it allows the user to input the geographic or projected coordinates of control points. The lower part, titled 'GCP table', is where the Ground Control Point data and residuals will be displayed.

Step 6: In the Georeferencer window go to Settings → Transformation Settings it will then be presented with the Transformation Settings ' window, from which we will select the 'WGS 84/ UTM Zone 43N' under Target SRS option.

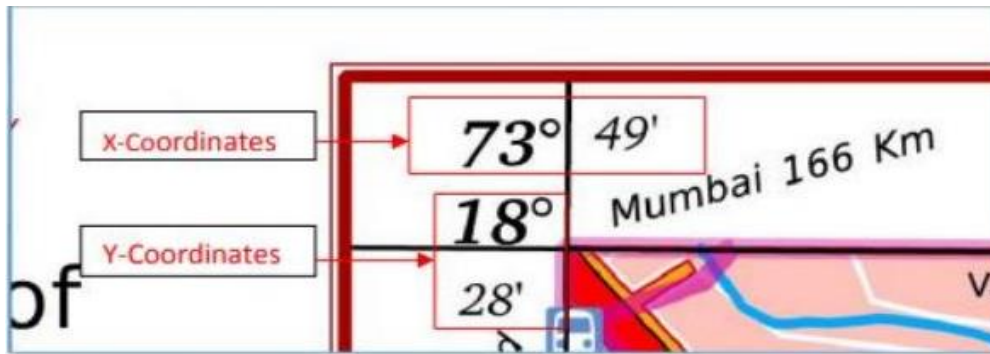


Step 7: The toposheet will be loaded in the Georeferencer window and should look like below

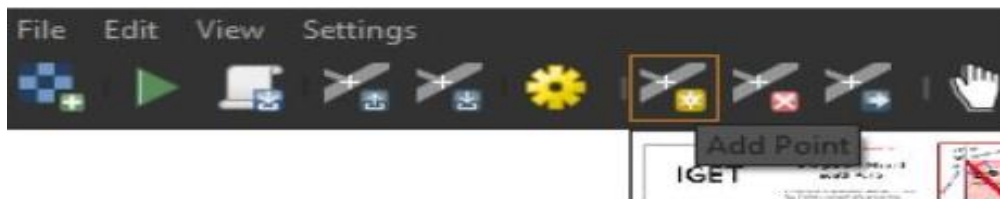


Step 8: To georeference an image we use Ground Control Points (GCPs). GCP is a location on the earth's surface with known coordinates on both earth and Toposheet/ imagery, i.e., geographic and pixel coordinates respectively. In this tutorial we use graticule intersections as GCPs.

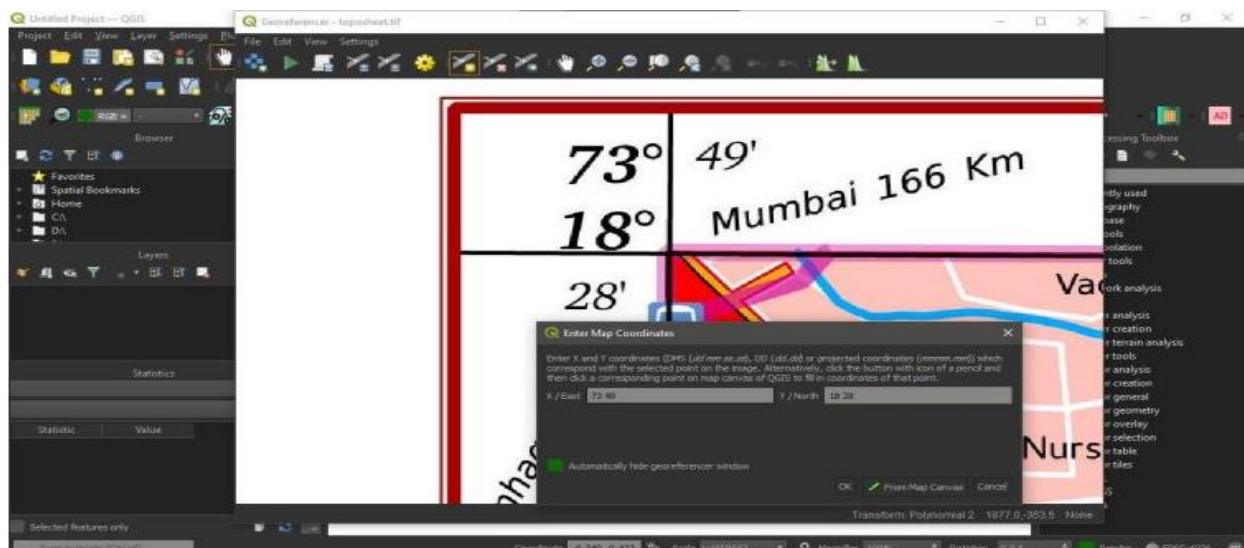
Step 9: To start adding GCPs to our map, we first zoom to a corner of the map where we can easily identify the intersection of the latitude and longitude. Use the scroll wheel of the mouse to zoom in and out of the map. Use the 'Pan' Button when needed

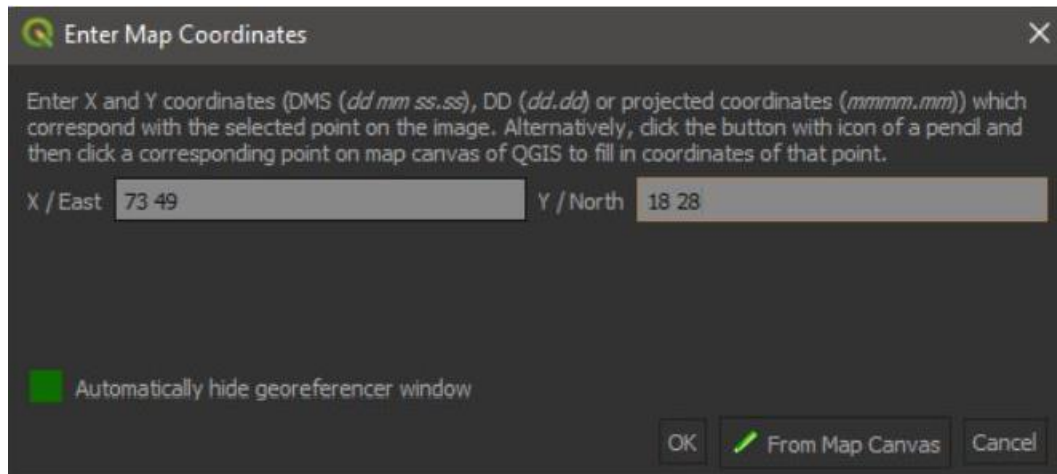


Step 10: To add a GCP click on the 'Add point' button, or go via the menu (Edit → Add Point). The mouse will transform into a '+' sign, which we use to click on the centre of the intersection. Use 'View tool' when needed.

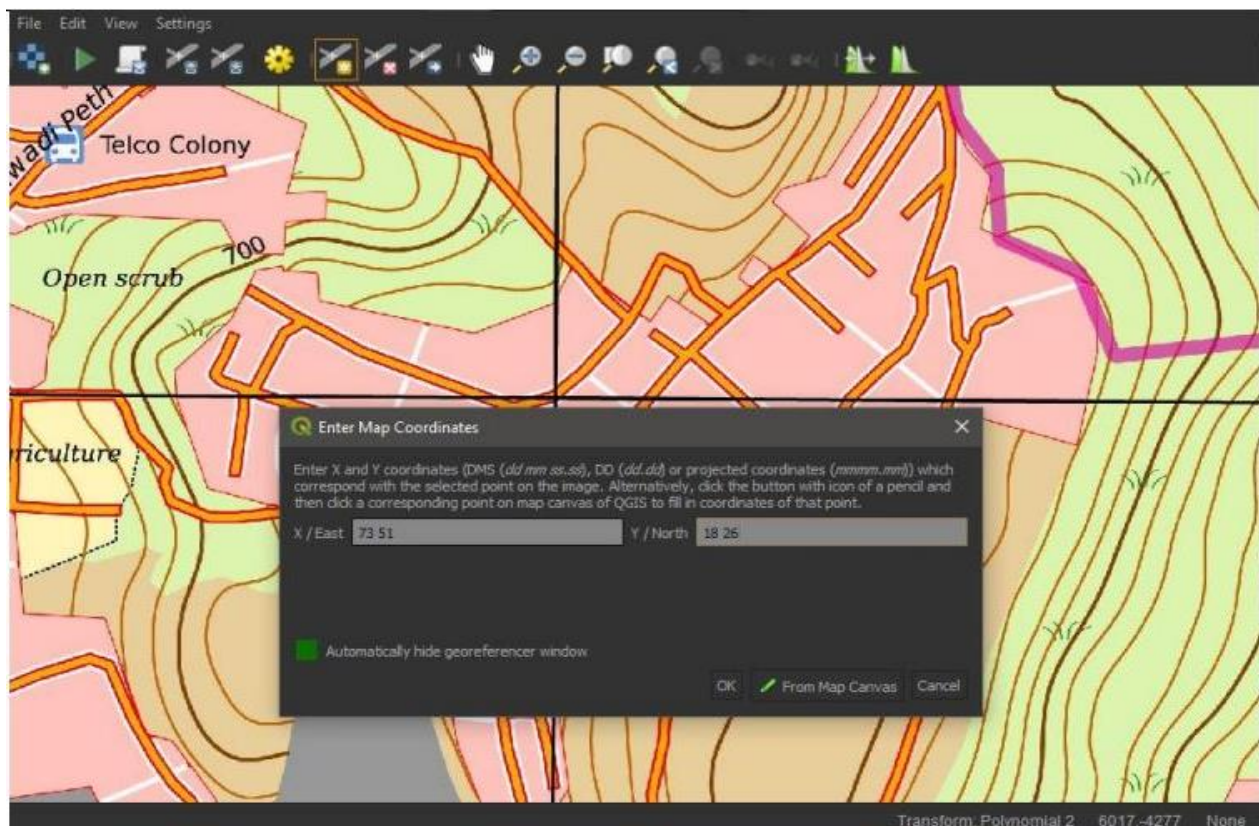


Step 11: A window 'Enter map coordinates' will pop up where we enter the coordinates of the point which we take from the map. Always enter 'Longitude or Easting' in X field and 'Latitude or Northing' in Y field. Use 'space bar' in the key board to separate the Degree value from Minutes value and then click 'OK'.

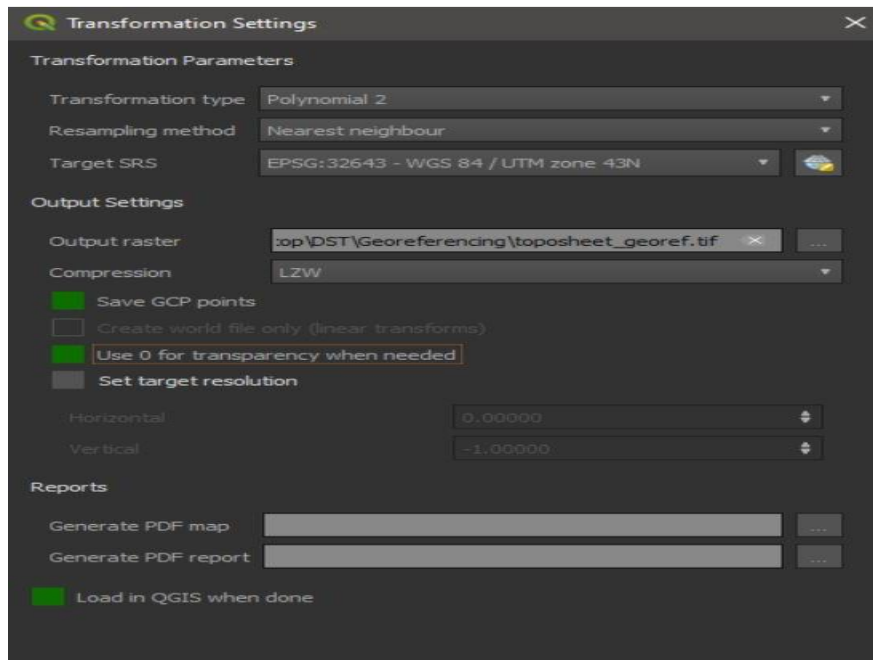




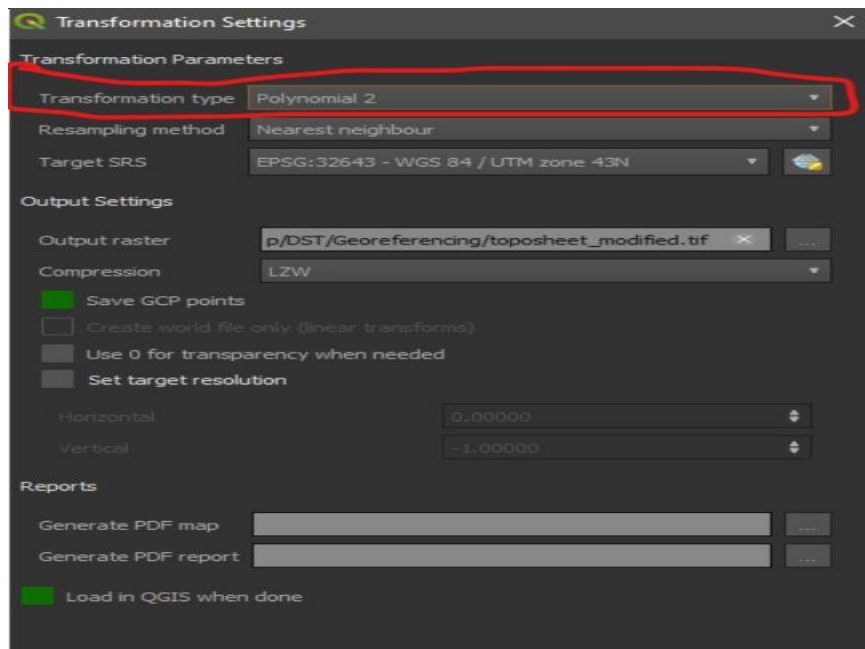
Step 12: In this exercise we are using the 'Polynomial 2' transformation to georeference the image. For the 'Polynomial 2' transformation we will require minimum 6 + 1(for check) i.e., 7 GCPs or more GCPs on the map. Therefore, we need to mark at least 7 GCPs. The GCPs locations should be spread out 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.



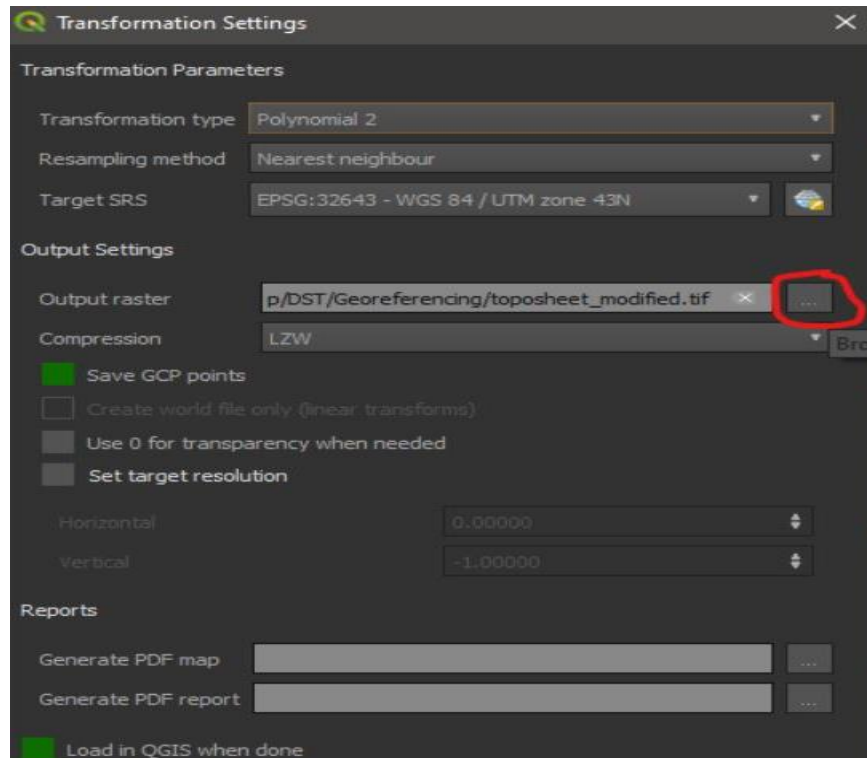
Step 13: We now set the spatial reference settings for the toposheet by clicking on the 'Transformation Settings' button. The 'Transformation Settings' window pops up in which we will enter the spatial information of our map.

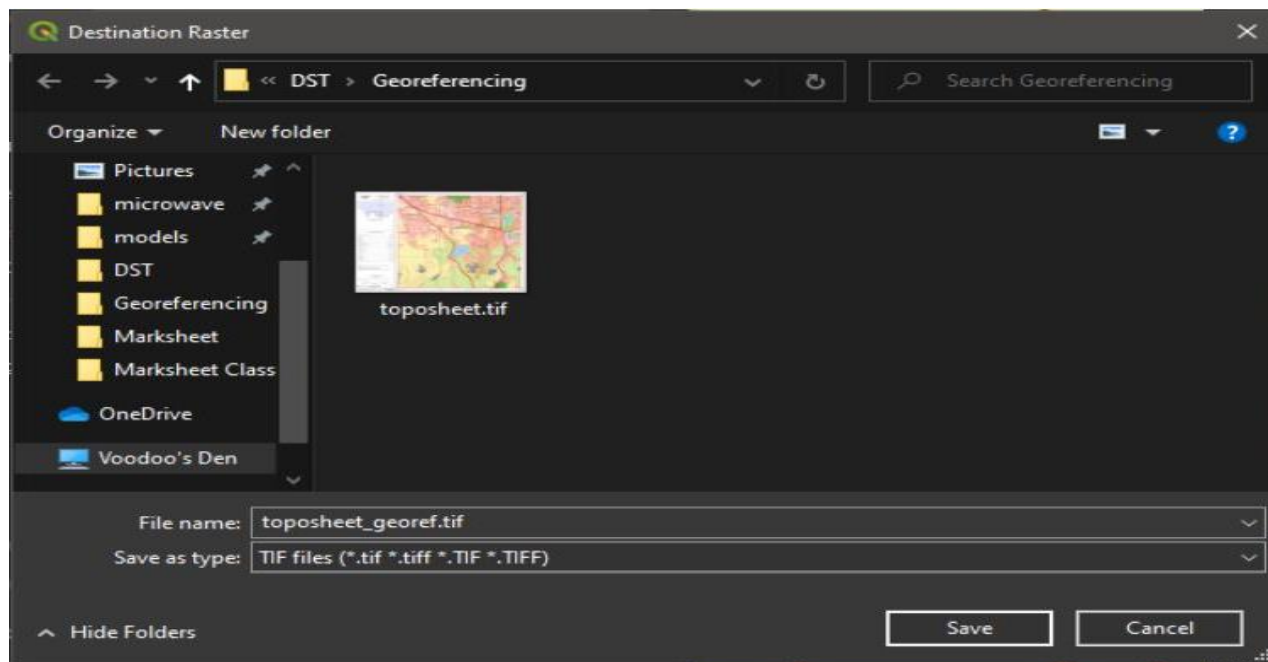


Step 14: Click on the 'Transformation type' drop-down menu and select 'Polynomial 2'. This means we will be using a second order polynomial transformation.



Step 15: Click on the button next to 'Output Raster'. A dialogue box will appear in which we enter the name of our output file. It is recommended to include the name of the original file in this file for example 'Toposheet_WGS84_georef.tif'.





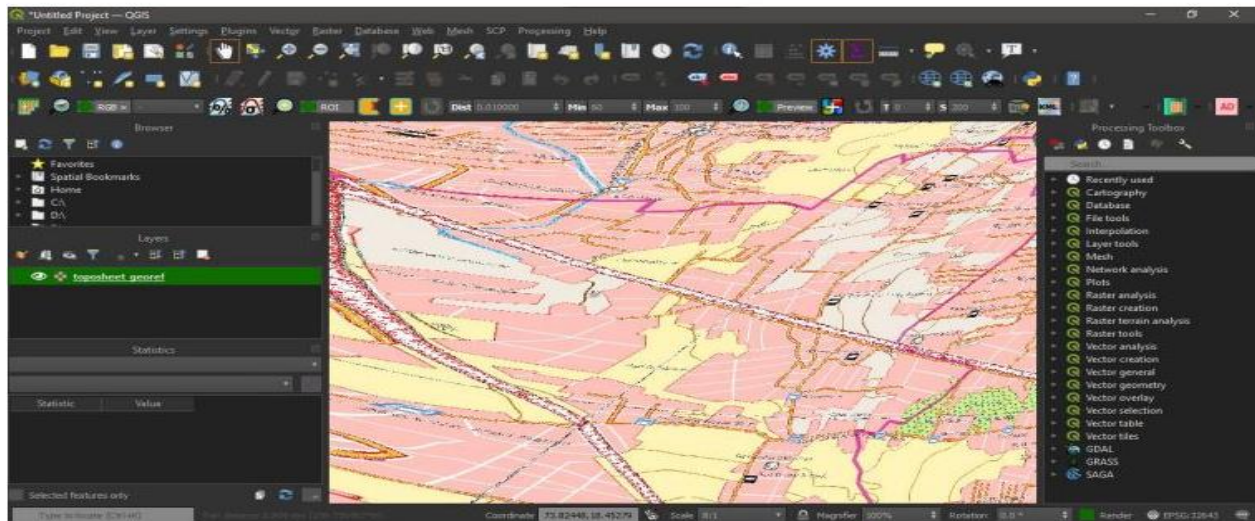
Step 16: 'Check' the check box 'Load in QGIS when done' and 'Use 0 for transparency when needed', leave the reset of the values as default and click 'OK'

Step 17: After Click 'OK', the last column 'residual [pixels]' displays some values. These are the error values associated with the GCPs. An error value of 1 or less would be satisfactory.

Visible	ID	Source X	Source Y	Dest. X	Dest. Y	dX (pixels)	dY (pixels)	Residual (pixels)
☒	0	2239.96	-273.61	73.8167	18.4667	-0.0417183	0.00933992	0.0427511
☒	1	4307.06	-2467.23	73.8333	18.45	-0.0990032	-0.0840593	0.129875
☒	2	8439.68	-6854.48	73.8667	18.4167	0.116438	0.00933992	0.116812
☒	3	6374.16	-4660.86	73.85	18.4333	-0.573471	-0.0840593	0.579599
☒	4	8480.8	-314.73	73.8667	18.4667	0.0747194	0.0186798	0.077019
☒	5	2196.47	-6815.73	73.8167	18.4167	-0.149439	-0.0373597	0.154038
☒	6	4292.04	-4647.41	73.8333	18.4333	0.672474	0.168119	0.693171
Transform: Polynomial 2 Mean error: 0.937241 -2388,-7101 None								

Step 18: Double check and adjust the GCP locations if the value is greater than 1. To adjust a point click on the 'Move GCP Point' button, and then click and drag the point to the desired location. Use 'Delete Point' button to delete an erroneous GCP. We can also enable and disable the GCPs by using check boxes under 'on/off' column in 'GCP Table'.

Step 19: Once the error is around or below 1, click the 'Start Georeferencing' Button. The processing will take at about 2 minutes. The georeferenced image will be found at the location specified for the 'Output File' in step 15. You can also notice, the output file loaded in QGIS canvas.



Step 20: It is useful to save the GCPs for later use if the Georeferencing needs to be done again, or if corrections are required. To save them, click on the 'Save GCPs as' button. In the pop up window enter an appropriate name for the GCP file, preferably the same as that of the image. This file can be loaded later on by clicking on the 'Load GCP points' button in 'Georeferencer' window after adding toposheet, and selecting it from the pop-up window.

Practical 3: Projection & Transformation

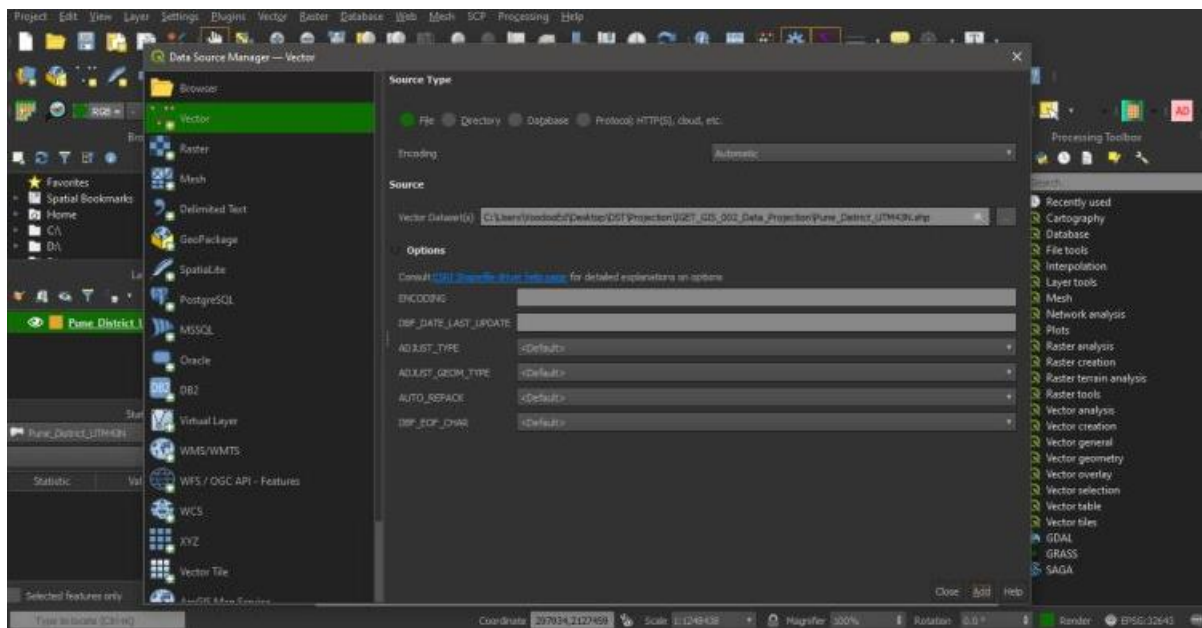
As we know the earth is spherical in shape but its surface is not regular at all places. It contains mountains, valleys and plains. Due to this, it is not suitable to perform direct mathematical calculations on it. Therefore, a mathematical surface of oblate spheroid best fitting the irregular surface of the earth is considered as a frame of reference for measuring locations on the surface of earth and is called as Datum. If the spheroid is best fitting a particular area, then it is called as Local Datum and if it is best fitting the entire globe then it is called as Global Datum. For example, Everest ellipsoid is the best fit to India and its adjacent countries but not to the entire world, similarly WGS 84 ellipsoid is the best fit to the entire world but not exactly to India. Generally Geographic Coordinate Systems (GCS) are associated with this datum, the measuring units are angular in nature. It is very hard to measure distance, area and other parameters by using spherical coordinates and also it is very difficult to represent this three-dimensional curved surface on a piece of paper. To overcome this difficulty, we use a method called as map projection. It aims to convert the earth's 3D curved surface to a map's two-dimensional flat surface i.e., on a piece of paper. For this purpose, we use projection surfaces like Plain, Cone and Cylinder (see the following figure). Since, it is impossible to convert a curved surface to a flat surface accurately, we have to compromise on some properties to preserve some other properties. For example, to preserve the Shape of a feature, we have to compromise on its area and distance. Therefore, a projection is never accurate. Choosing the right projection is very important for successful GIS projects.

Dataset used: <https://dst-iget.in/index.php/tutorialdetails/1/3>

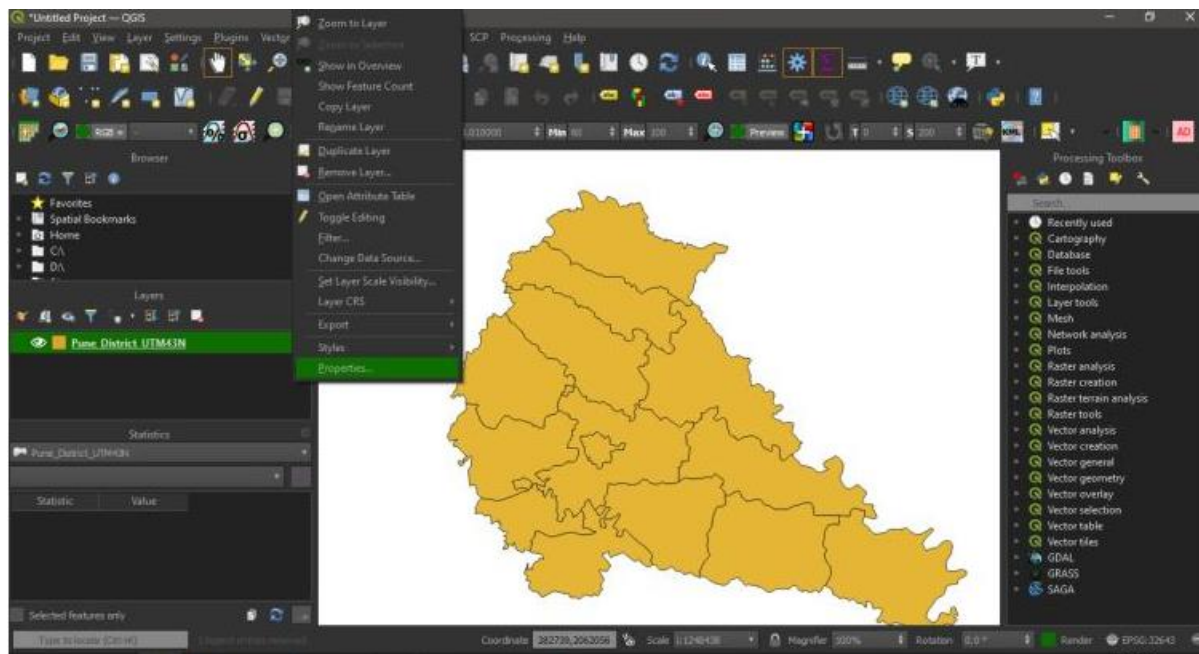
Step 1: Open the QGIS desktop via, 'Start All Programs QGIS Dufour QGIS Desktop 3.22'.

Step 2: Add the vector layer 'Pune_District_UTM43N.shp' via., 'Add Vector layer button' Click on 'Browse' in the popup window browse to the 'IGET_GIS_002_Data' folder. Make sure the file type should be 'ESRI Shapefiles' select the

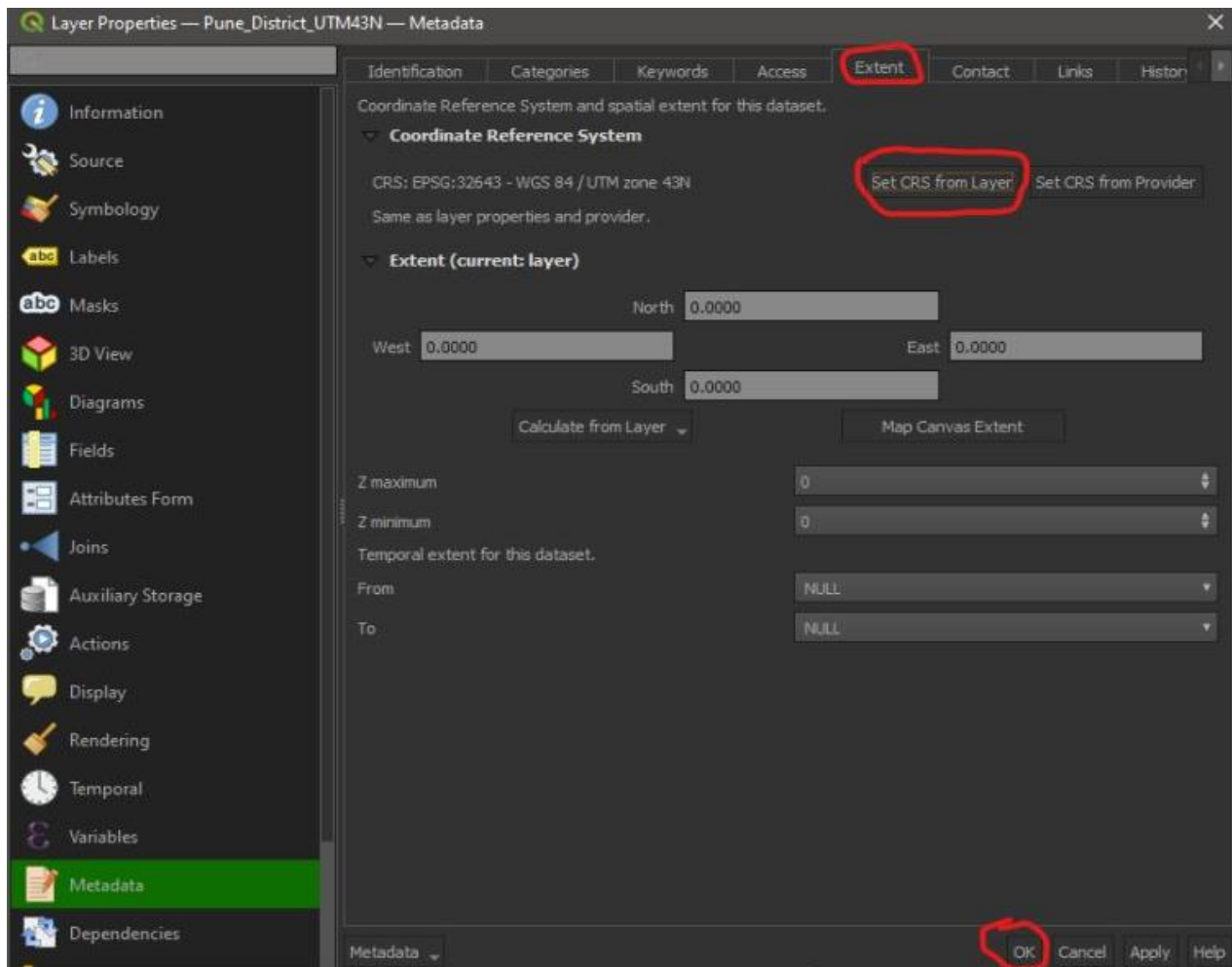
'Pune_District_UTM43N.shp' files Click on 'Open' and again on 'Open'



Step 3: Now Right-Click on the 'Pune_District_UTM43N.shp' layer under 'Layer' section Select 'Properties'



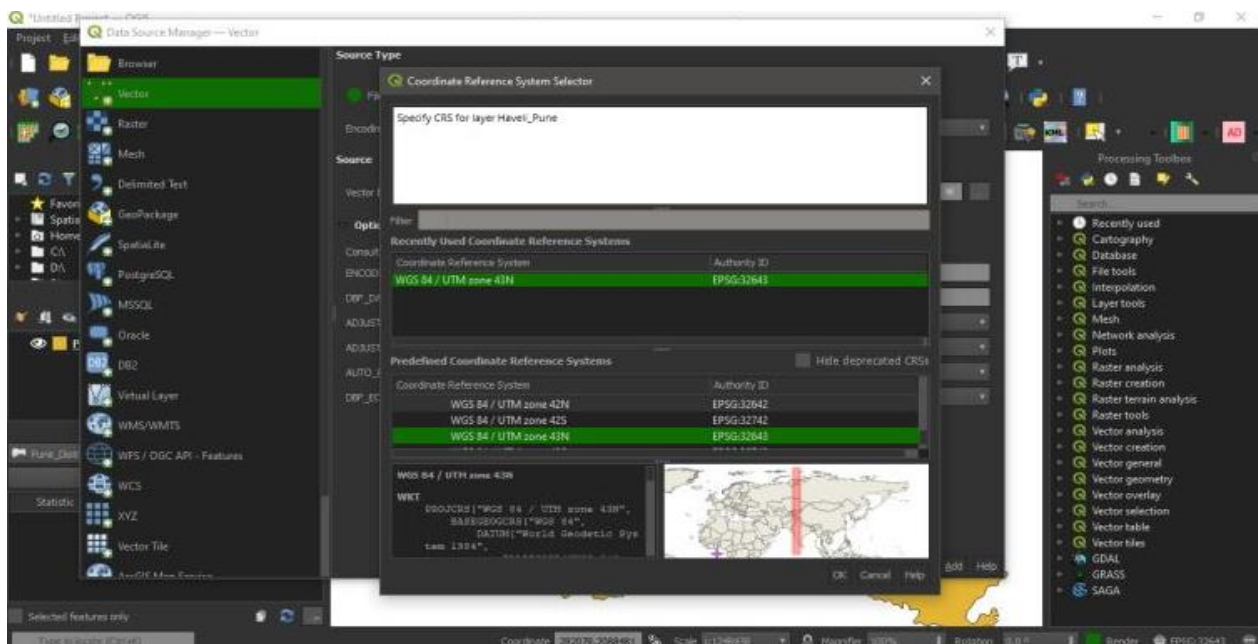
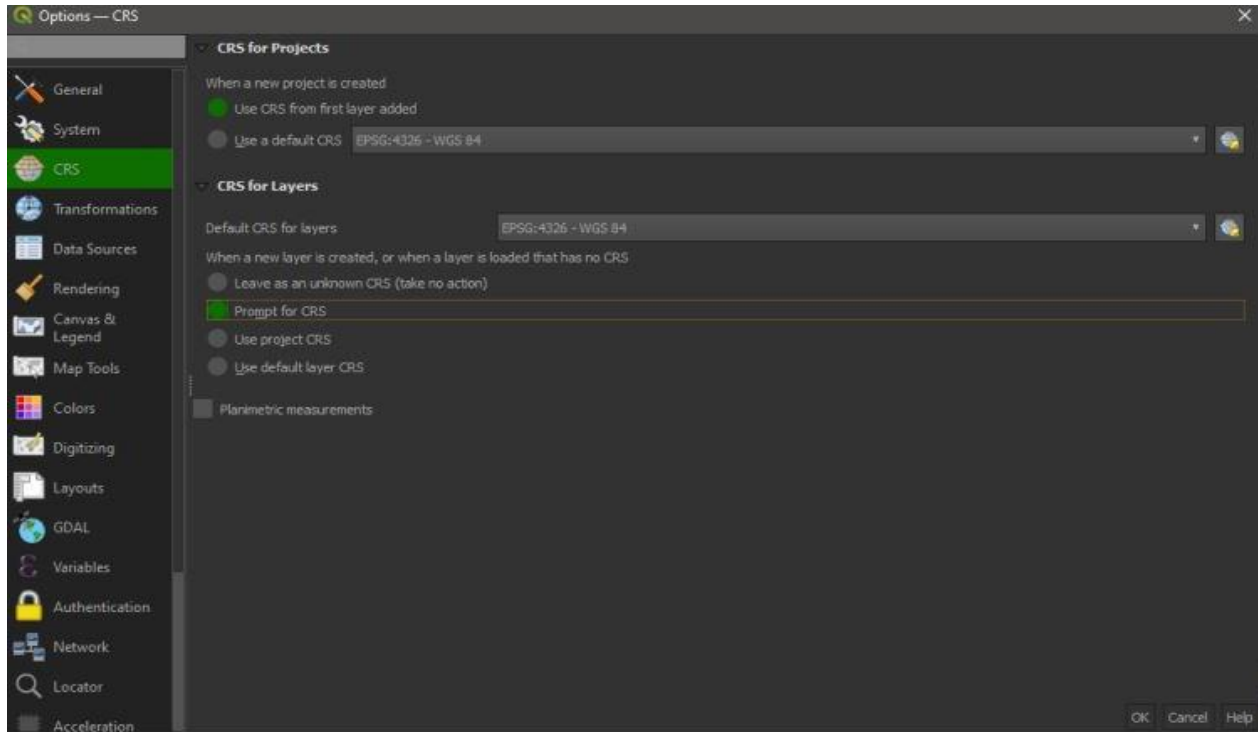
Step 4: In the 'Layer Properties' popup window Click on 'Metadata' tab Click on Extent Tab And select the Set CRS from Layer option which will show the Coordinate/Spatial Reference System (CRS/ SRS) of the layer. The layer we selected is in UTM projection's 43rd Zone based on WGS 84 datum in northern hemisphere. Click on 'OK'.



Step 5: If the map layer does not contain any projection (CRS) information, you will be prompted to select the CRS information via, 'Coordinate Reference System Selector' window. Now add the vector layer 'Haveli_Pune.shp' to the QGIS Map Canvas (refer Step 2)

Now you will be prompted to select the layer CRS select WGS84/ UTM zone 43N from 'coordinate reference systems of the world' section or you can use the Filter Options to find the projection easily click on 'OK'.

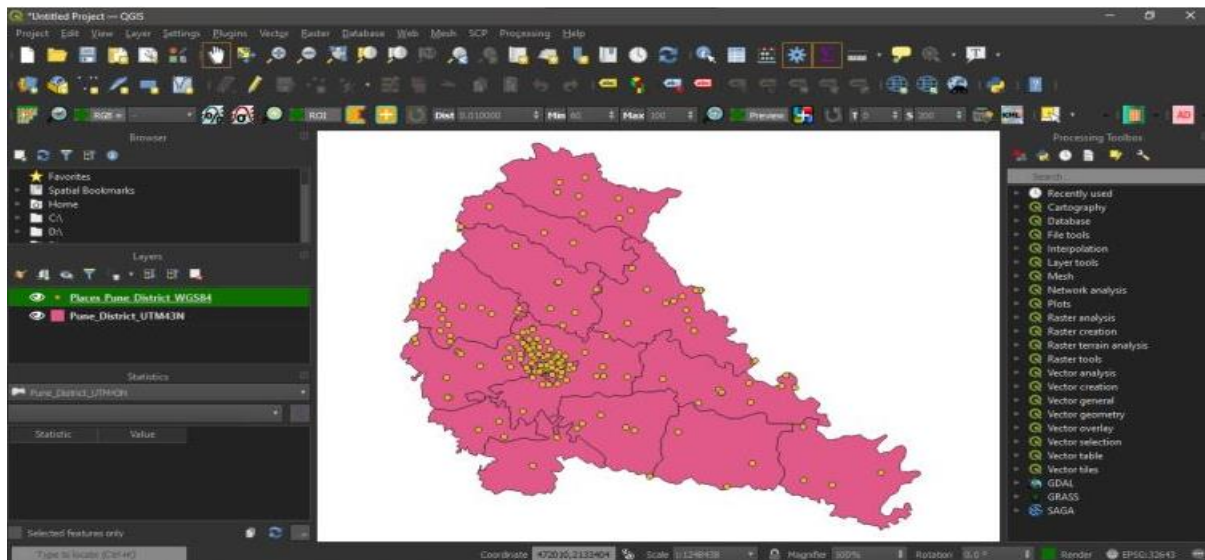
Now you can see 'Haveli' taluka added to the right place on Map. Note: This Prompt will work only if the radio button set to it under CRS tab. This can be accessed via, Main menu bar Settings Options CRS tab (see below figure). It is advised to set the radio button to 'Prompt for CRS' by default to avoid difficulties.



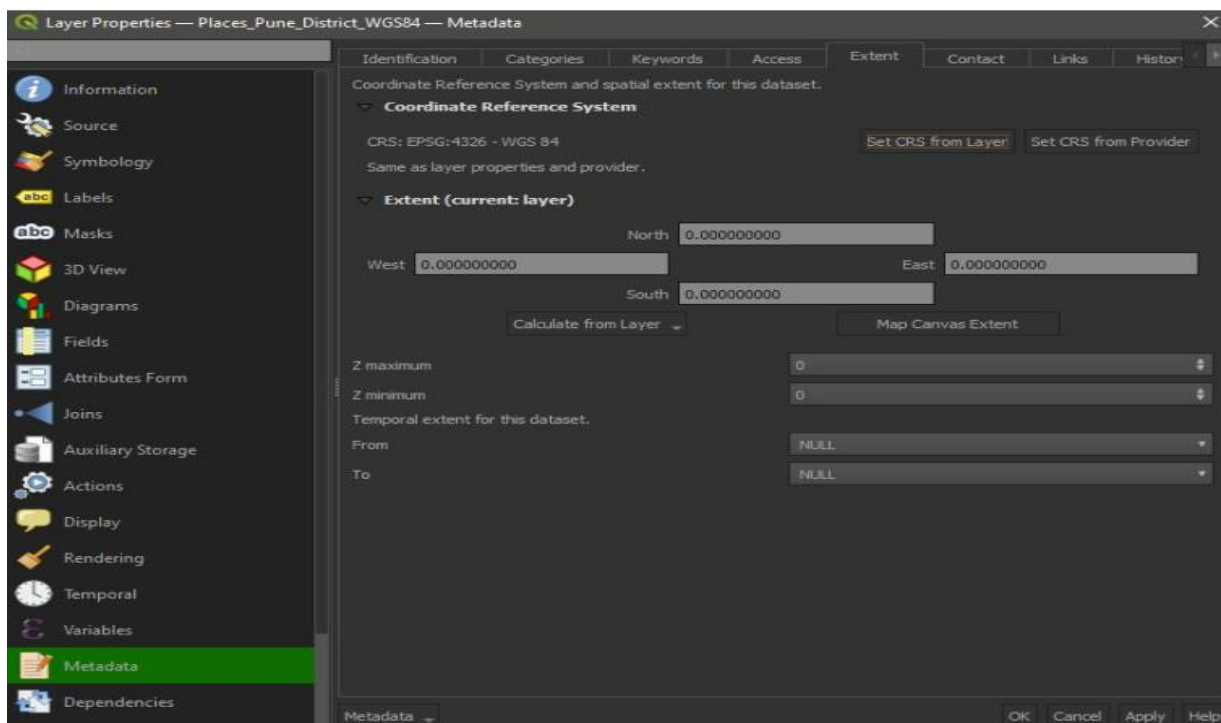
Reprojecting a Vector layer

Reprojection of vector layers from Geographic to the projected system is often required for various geoprocessing analyses. On the fly helps to visualize the layer on right location but doesn't alter

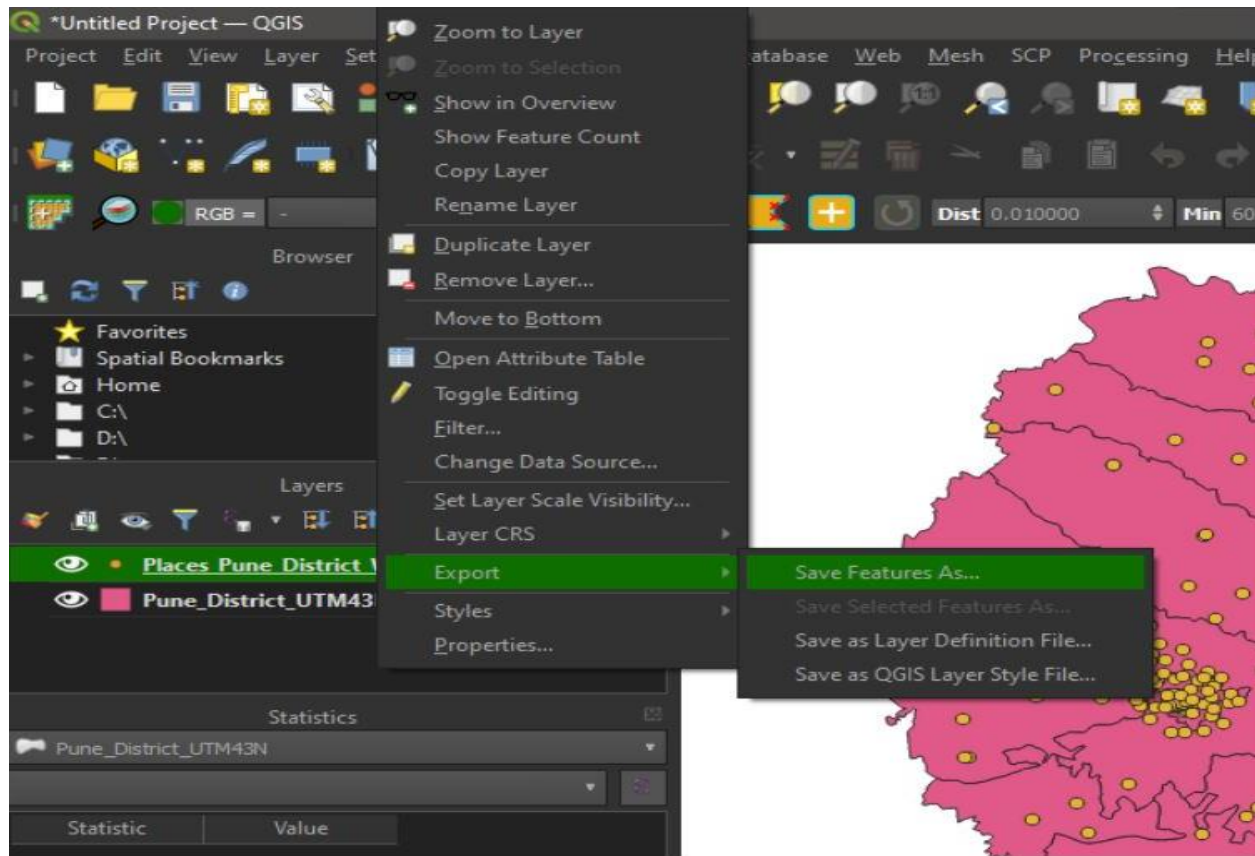
the projection information of the layers. In this section, we will learn how to reproject a shapefile in WGS84 (i.e., Geographic Coordinate System) containing place names of Pune district to the UTM projection. Step 6: Add 'Places_Pune_District_WGS84.shp' vector layer from tutorial data using (refer Step 2).



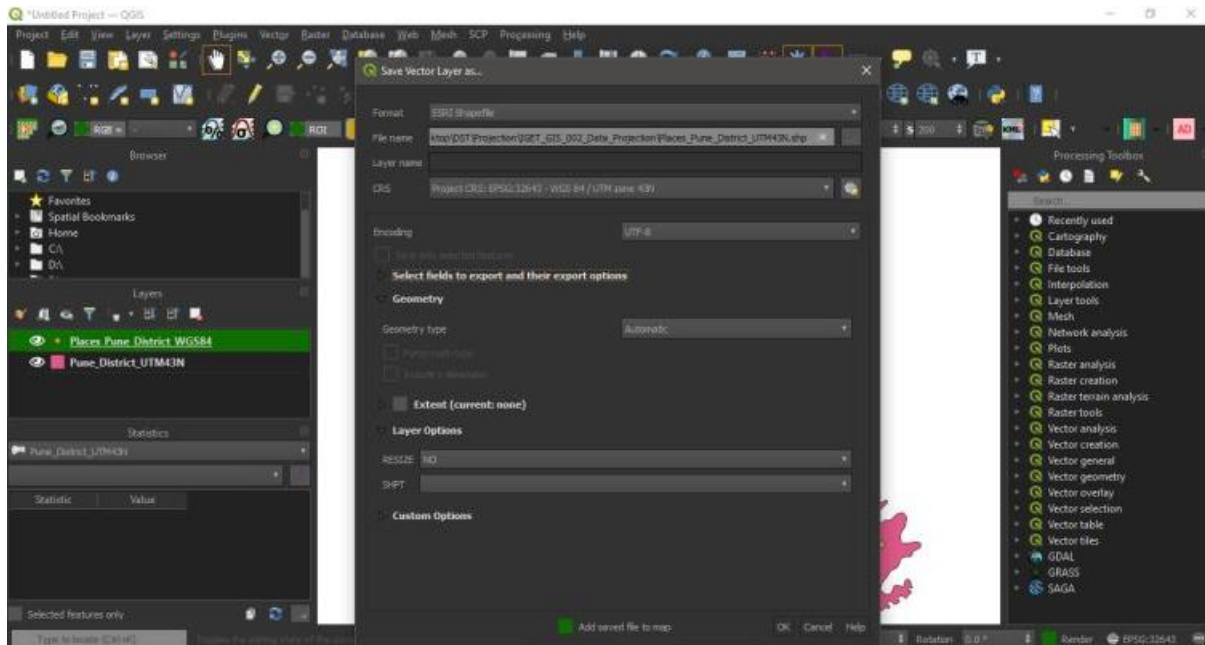
Step 7: Check the projection information refer Step 4, you can see the layer is in GCS: WGS84.



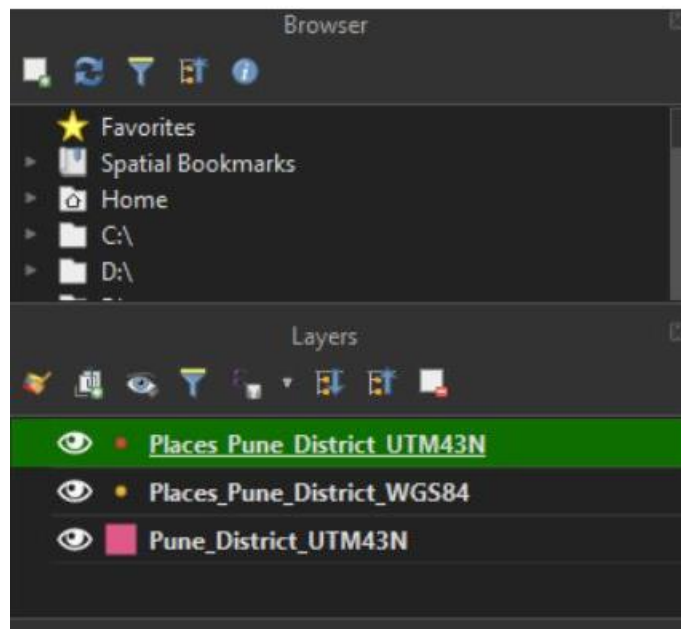
Step 8: Now Right-Click the 'Places_Pune_District_WGS84.shp' under 'Layers' selection. Click on 'Export' then select 'Save Feature As'.



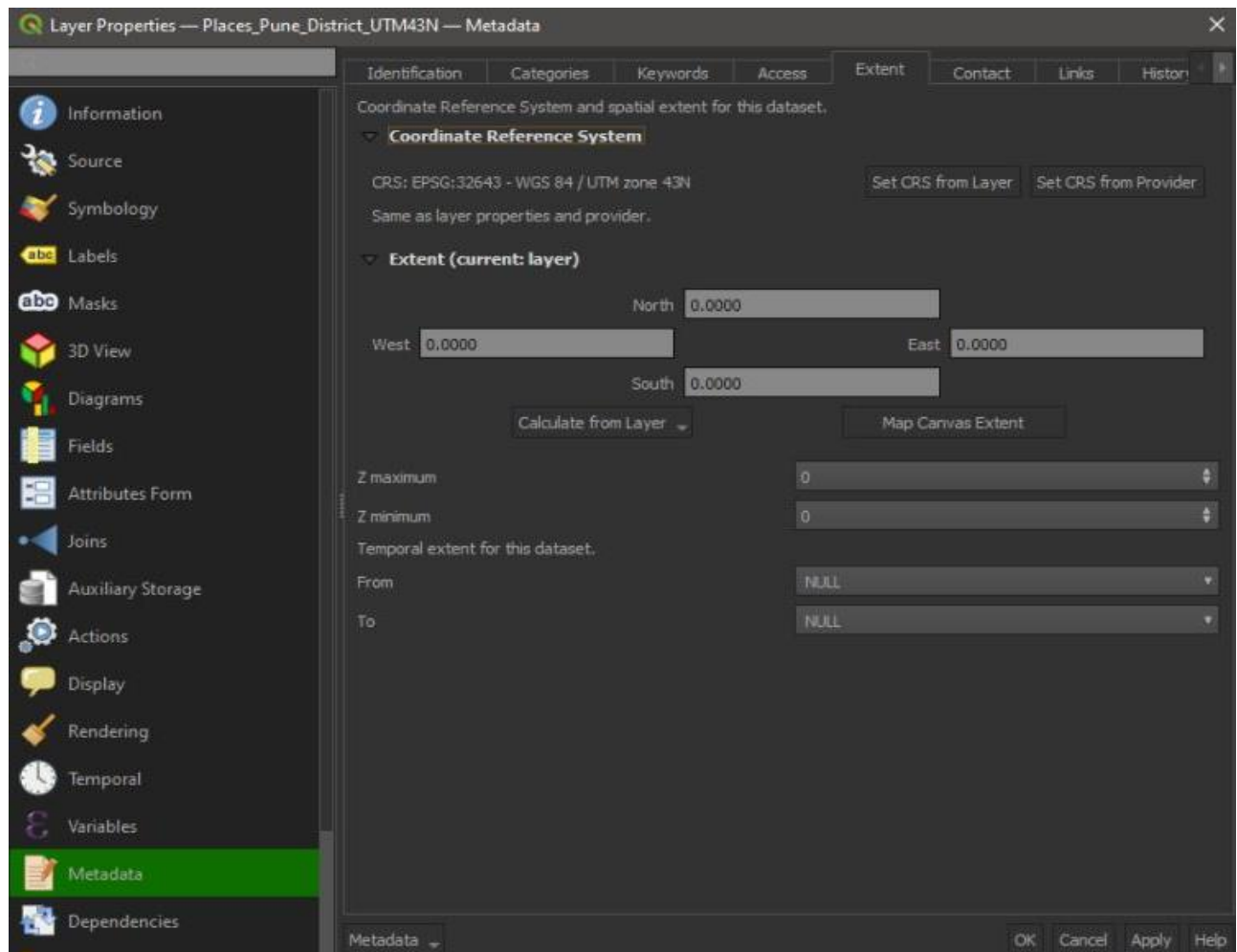
Step 9: In 'Save vector layer as' window select desired format you want, in this case 'ESRI Shapefile' click on 'Browse' right side to 'Save As' Navigate to the desired path that you want to save file. Name it as 'Places_Pune_District_UTM43N.shp' Click on 'Save' Under 'CRS' section select, 'Selected CRS' from the down drop list Now you can see CRS selection box will be enabled, click on 'Browse' Use filter to select the WGS84/ UTM zone 43N in the popup window (refer Step 5) Click 'OK'. Check' the check box of 'Add saved file to map' Click on 'OK'.



Step 10: You can notice the newly added layer under Map Layers section.



Step 11: Now check the 'Metadata' tab for information about CRS of 'Places_Pune_District_UTM43N.shp', it should look like below

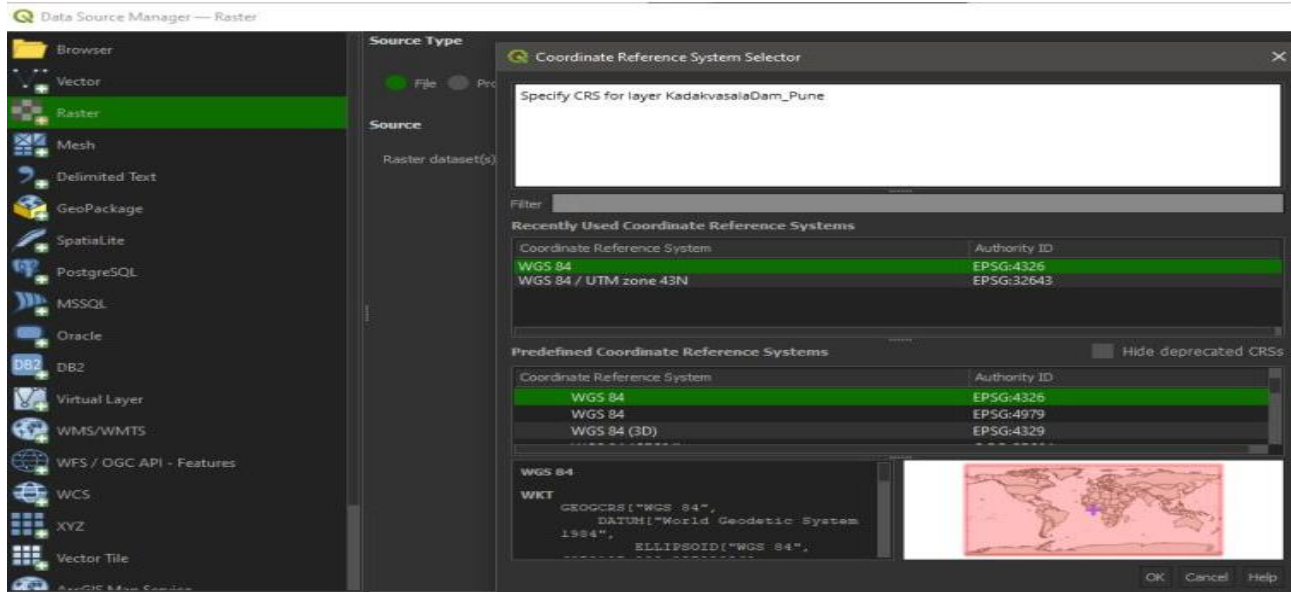


Projecting a raster dataset

Step 12: If the raster data set isn't associated with any CRS then you will be prompted to choose the 'CRS' like Step 3.

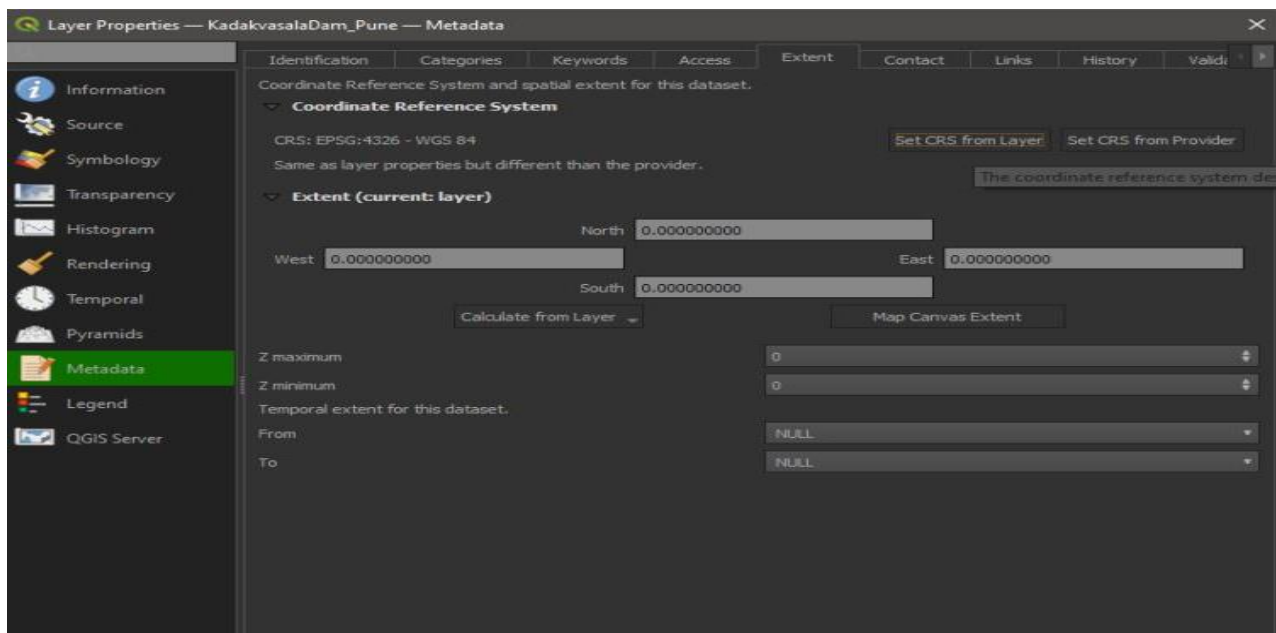
Step 13: Add the raster layer of Kadakvasala Dam via, 'Main menu bar Data Manager Add Raster Layer' or else directly click on icon from the toolbar browse and select the raster layer named 'KadakvasalaDam_Pune.tif' in tutorial data Click on 'Open' in the popup window.

Step 14: Since the 'KadakvasalaDam_Pune.tif' image doesn't contain any CRS information you will be prompt to select CRS Use filter to find 'WGS 84' datum, select it click 'OK'



Step 15: Now you can notice the ‘KadakvasalaDam_Pune.tif’ layer is added to the ‘Map canvas’ and listed under ‘Map Layers’ section.

Step 16: Check the Metadata of the raster layer via, ‘Right-click on the layer Properties’. Click over ‘Metadata’ tab in popup window Select Extent then ‘Set CRS from Layer’ to see the CRS details associated with it. You can see the layer was assigned to ‘WGS 84’ datum.

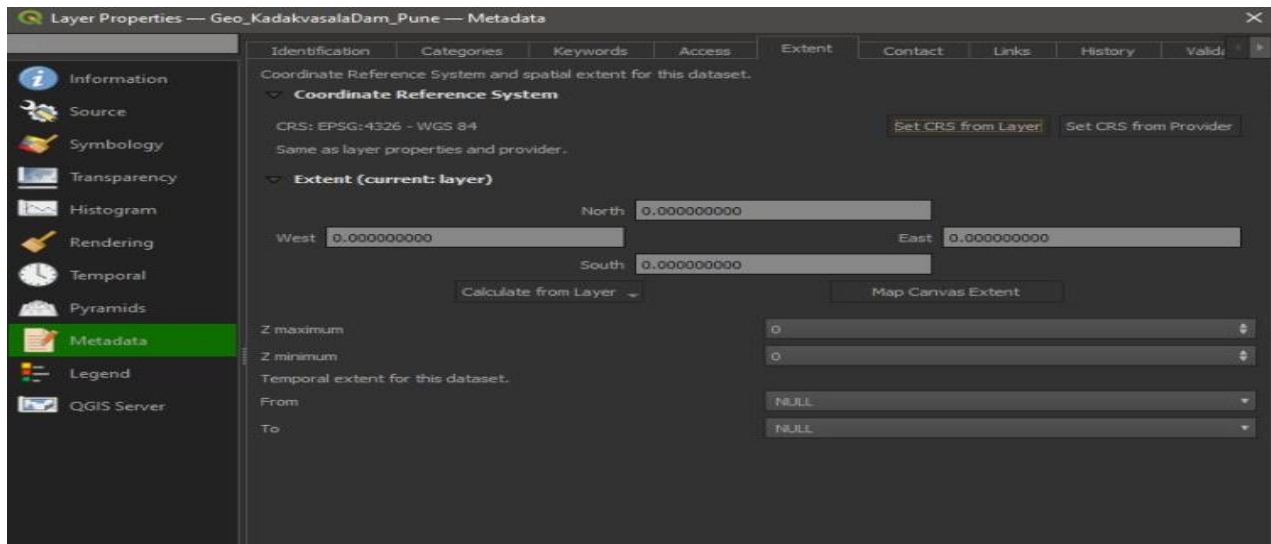


Reprojecting a raster dataset

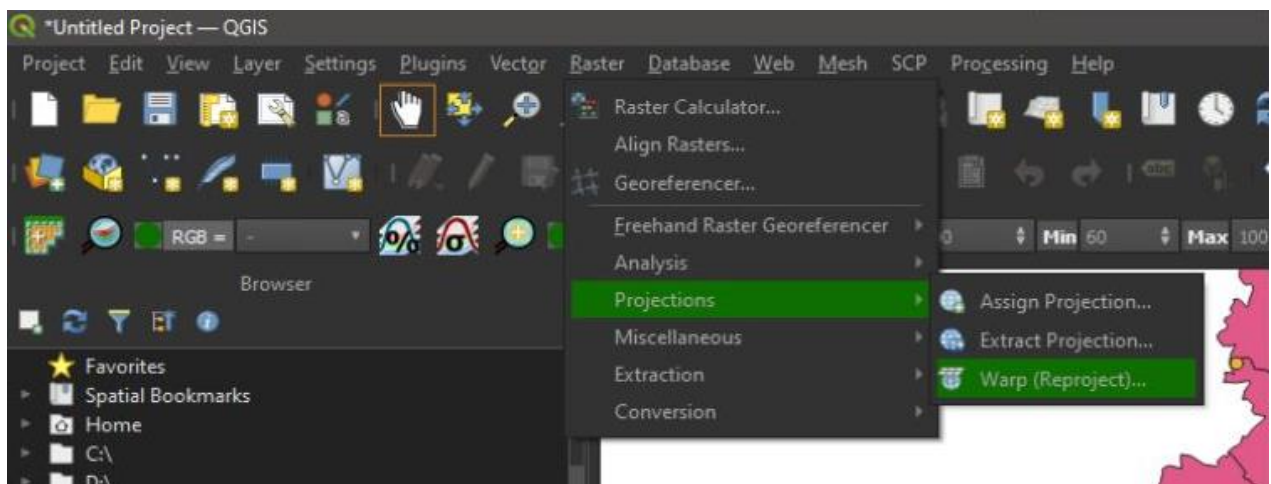
In this section we will reproject a raster layer from Geographic Coordinate System (GCS) to Projected Coordinate System (UTM).

Step 17: Open the georeference raster layer ‘Geo_KadakvasalaDam_Pune.tif’.

Step 18: Now check for Layer SRS. It is in WGS 84 GCS projection.



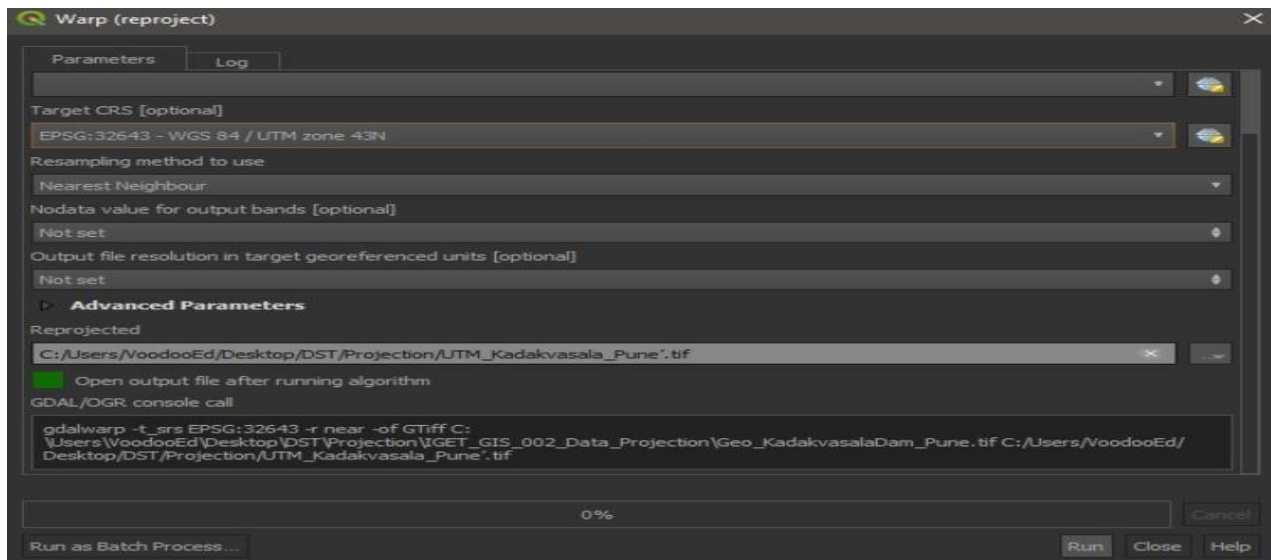
Step 19: Go to the ‘Main menu bar Raster Projections Warp (Reproject)’.



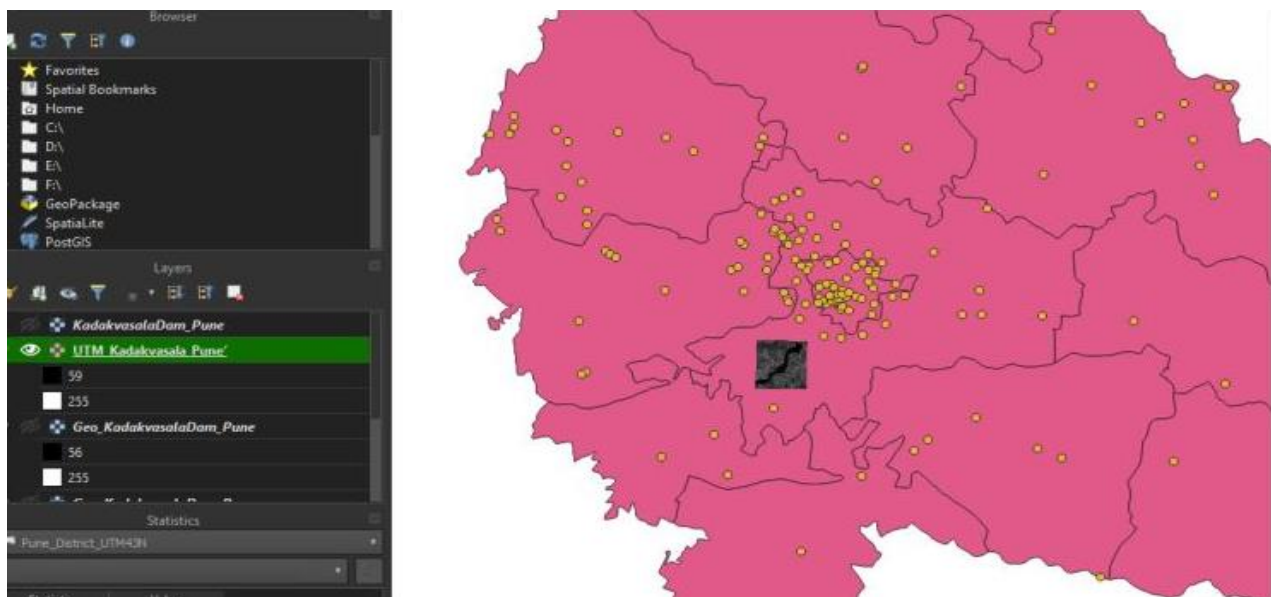
Step 20: In the popup window of ‘Warp (Reproject)’ Select Input file as ‘Geo_KadakvasalaDam_Pune.tif’ Go to Advance Parameters click on Reprojected then ‘Save to File’ ‘Select’ of Output file Browse to the desired path in popup window enter name as

‘UTM_Kadakvasala_Pune’ and select Files of Type as ‘ [GDAL] GeoTiff (*.tif, *.tiff, *.TIF, *.TIFF)’ click on ‘Save’.

Now ‘Check’ the check box of ‘Target CRS’ click on ‘Select’ located right side to Target CRS use Filter to find ‘WGS 84/ UTM zone 43N’ select it click on ‘OK’



Step 21: Click on ‘Run’ in Warp window you can see the ‘UTM_Kadakvasala_Pune.tif’ attached to the Map canvas.



Practical 4: Getting Started with Spatial Data

Spatial Data

Spatial, also known as geospatial data comprise the relative geographic information about the earth and its features. A pair of latitude and longitude coordinates defines a specific location on earth. It contains information about a physical object which is represented by numeric values of a geographic coordinate system. Spatial data consists of points, lines, polygons and other geographic and geometric data primitives, which can be mapped by location, stored with an object as metadata or used by a communication system to locate end-user devices.

Specialized software applications can be used to access, visualize, manipulate and analyse spatial data.

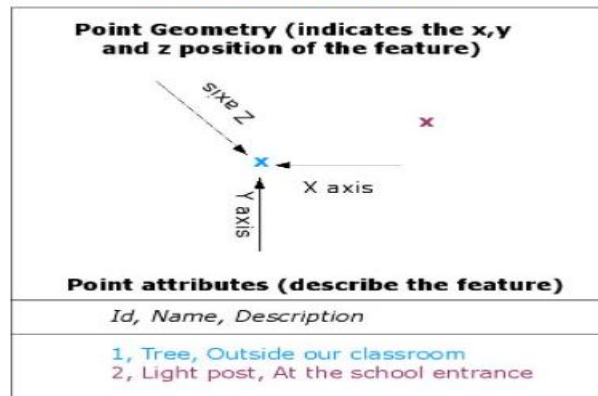
Spatial data are of two types according to the storing technique, namely, raster data and vector data. Raster data are composed of grid cells identified by row and column. The whole geographic area is divided into groups of individual cells, which represent an image. Satellite images, photographs, scanned images, etc., are examples of raster data.

Vector data are composed of points, polylines, and polygons. Wells, houses, etc., are represented by points. Roads, rivers, streams, etc., are represented by polylines. Villages and towns are represented by polygons.

Vector Data

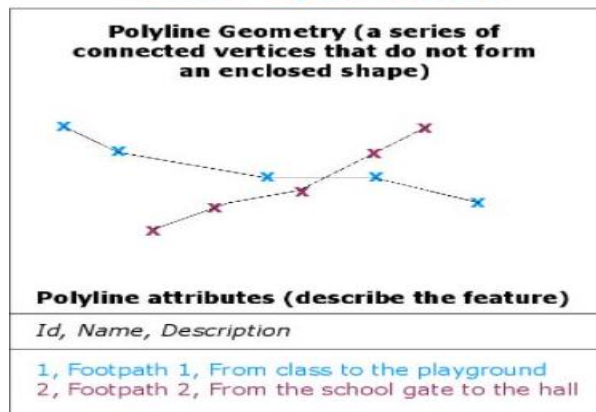
Vector data are the representation of the geographic features using points, lines, and polygons. Vector models are useful for storing data that has discrete boundaries, such as country borders, land parcels, and streets.

Vector Point Feature



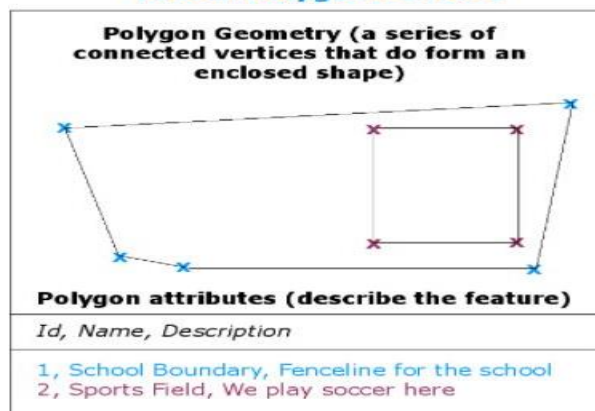
A point feature is described by its X, Y and optionally Z coordinate. The point attributes describe the point e.g. if it is a tree or a lamp post.

Vector Polyline Feature



A polyline is a sequence of joined vertices. Each vertex has an X, Y (and optionally Z) coordinate. Attributes describe the polyline.

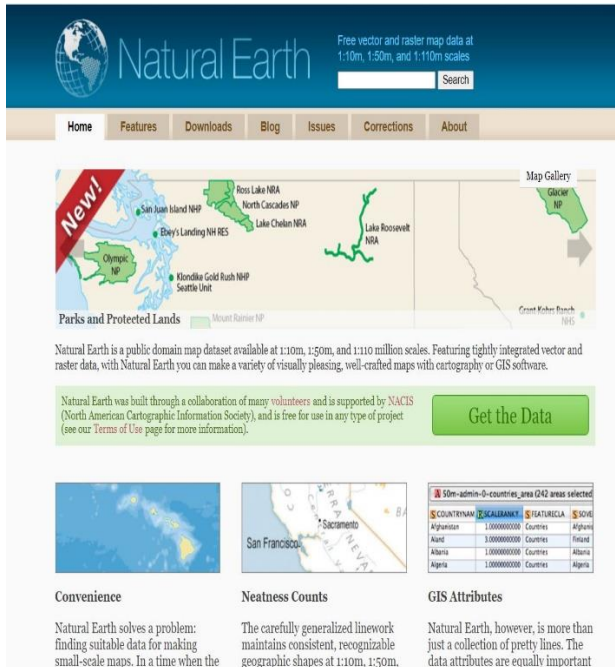
Vector Polygon Feature



A polygon, like a polyline, is a sequence of vertices. However, in a polygon, the first and last vertices are always at the same position.

Vector data for GIS usually comes with (.shp) file extension. For a shape file to function in ArcGIS some ancillary file types are required such as (.shx) and (.dbf), (.prj), (.xml), (.sbn) and (.sbx).

Some free platform for vector data are: Natural Earth (<http://www.naturalearthdata.com/>) , Planet OSM (<https://planet.openstreetmap.org/>) , Diva-GIS (<http://www.diva-gis.org/Data>).



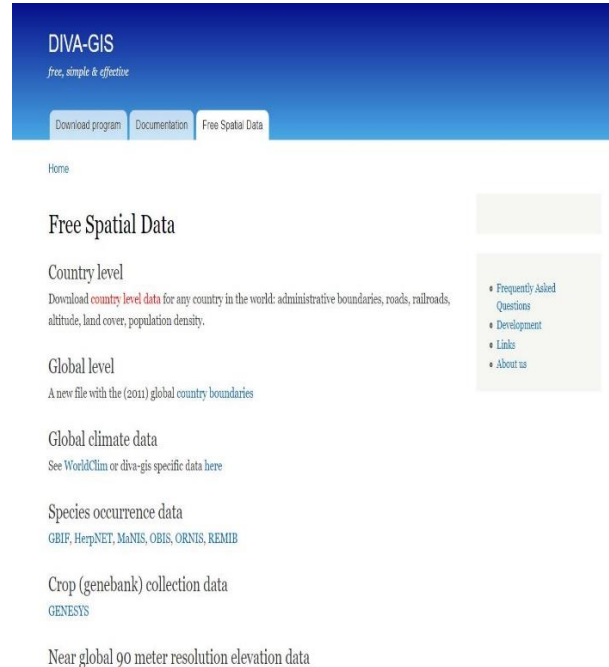
Natural Earth is a public domain map dataset available at 1:10m, 1:50m, and 1:110 million scales. Featuring tightly integrated vector and raster data, with Natural Earth you can make a variety of visually pleasing, well-crafted maps with cartography or GIS software.

Natural Earth was built through a collaboration of many volunteers and is supported by NACIS (North American Cartographic Information Society), and is free for use in any type of project (see our [Terms of Use](#) page for more information).

Convenience
Natural Earth solves a problem: finding suitable data for making small-scale maps. In a time when the

Neatness Counts
The carefully generalized linework maintains consistent, recognizable geographic shapes at 1:10m, 1:50m,

GIS Attributes
Natural Earth, however, is more than just a collection of pretty lines. The data attributes are equally important



Free Spatial Data

Country level
Download **country level** data for any country in the world: administrative boundaries, roads, railroads, altitude, land cover, population density.

Global level
A new file with the (2011) global country boundaries

Global climate data
See [WorldClim](#) or [diva-gis](#) specific data here

Species occurrence data
[GBIF](#), [HerpNET](#), [MaNIS](#), [OBIS](#), [ORNIS](#), [REMB](#)

Crop (genebank) collection data
[GENESYS](#)

Near global 90 meter resolution elevation data



Planet OSM

The files found here are regularly-updated, complete copies of the OpenStreetMap.org database, and those published before the 12 September 2012 are distributed under a Creative Commons Attribution-ShareAlike 2.0 license, those published after are Open Data Commons Open Database License 1.0 licensed. For more information,

Complete OSM Data

[Latest Weekly Planet XML File \(torrent\) \(RSS\)](#)

111 GB, created 2 days ago,
md5: ac82df5e9c6bc67db07510a9777d77d0.

[Latest Weekly Changesets \(torrent\) \(RSS\)](#)

4.7 GB, created 2 days ago,
md5: db37d6fd43bb54817d519ae5d8d135cc.

[Latest Weekly Planet PBF File \(torrent\) \(RSS\)](#)

61 GB, created 2 days ago,
md5: 48fa1106f81be7ee4ae91f2a302f3a60.

Each week, a new and complete copy of all data in OpenStreetMap is made available as both a compressed XML file and a custom PBF format file. Also available is the ["history"](#).

Using The Data

You are granted permission to use OpenStreetMap data by [the OpenStreetMap License](#), which also describes your obligations.

You can [process the file](#) or extracts with a variety of tools. [Osmosis](#) is a general-purpose command-line tool for converting the data among different formats and databases, and [Osm2pgsql](#) is a tool for importing the data into a Postgis database for rendering maps.

[Processed coastline data](#) derived from OSM data is also needed for rendering usable maps.

Extracts & Mirrors

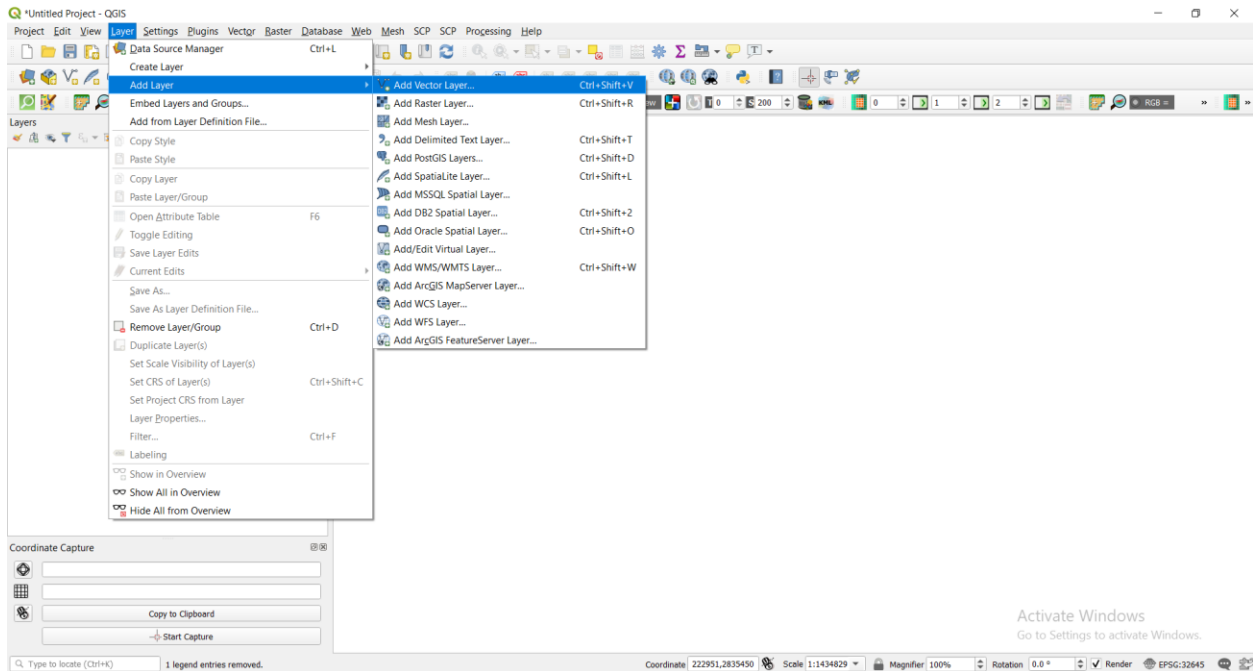
The complete planet is very large, so you may prefer to use one of [several periodic extracts](#) (individual countries or states) from third parties. [GeoFabrik.de](#) and [BBBike.org](#) are two providers of extracts with up-to-date worldwide coverage.

Reading a Vector File

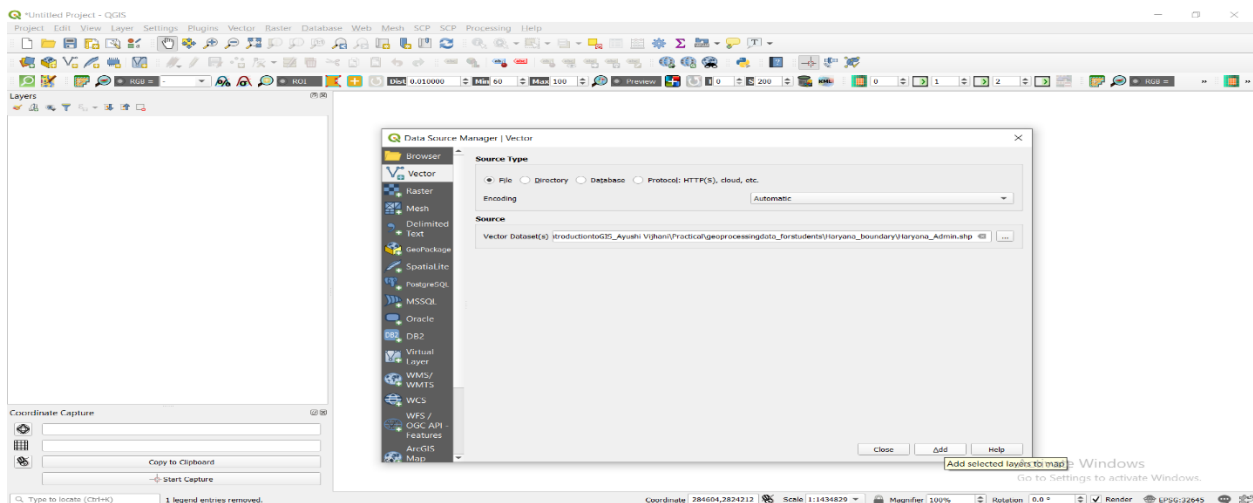
Dataset Used: Haryana_Boundary (<http://www.diva-gis.org/Data>)

Free Spatial Data > Country Level > Country (India), Subject (Choose Haryana).

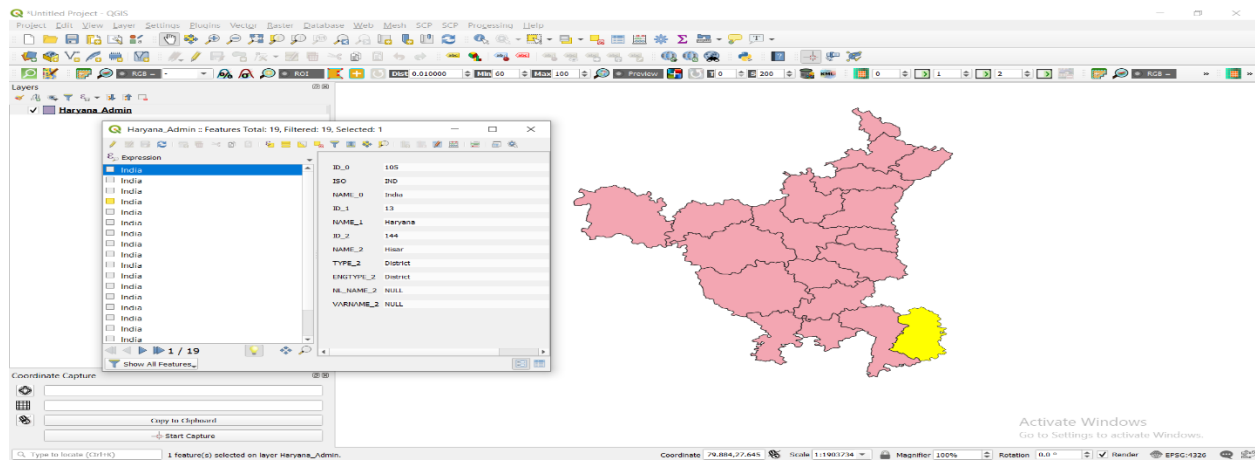
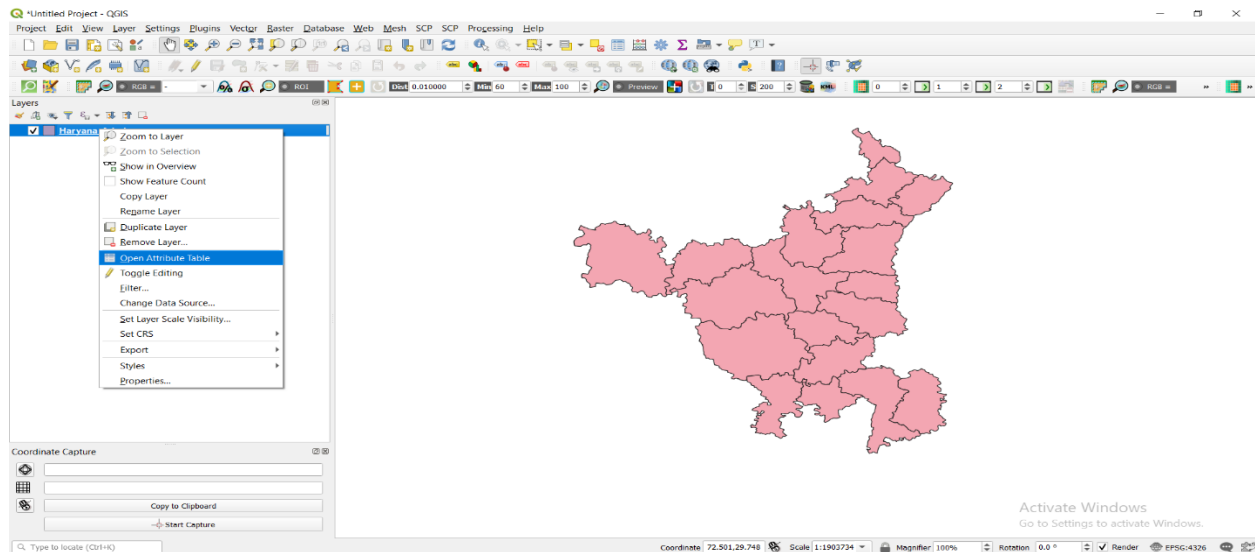
Step 1: To open the vector file in QGIS, click on Layer> Add Layer> Add Vector Layer



Step 2: Select the .shp file that you want to add and click on Add> Close.



Step 3: To check the attribute table, right-click on the .shp file that you have added in the Layer then choose the “Open Attribute Table”.

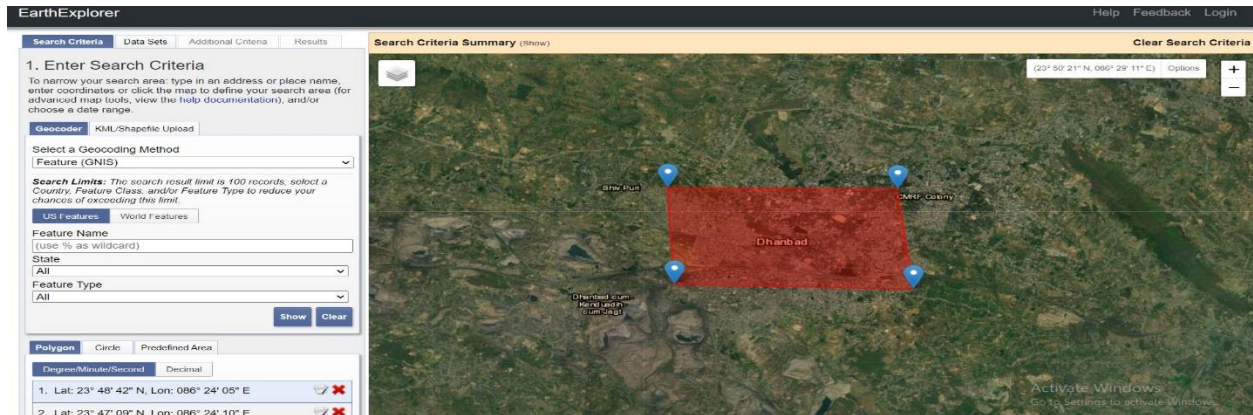


Raster Data

Scanned maps, images from satellites, digitalized aerial photographs, or digital pictures are classified as raster. Raster is a method for the storage, processing and display of spatial data. Each area is divided into rows and columns, which form a regular grid structure. Each cell must be rectangular, but not necessarily square. Each cell within this matrix contains location coordinates as well as an attribute value. The spatial location of each cell is implicitly contained within the ordering of the matrix, unlike a vector structure that stores topology explicitly. Areas containing the same attribute value are recognised as such, however, raster structures cannot identify the boundaries of such areas as polygons.

Raster data for GIS usually comes in TIFF(.tiff), ASCII (.asc) and Gridfile(.grd) format; Other than that, any digital image having pixel as its unit can be considered as raster.

Some of the sources for downloading raster data for GIS are USGS Earth Explorer (<https://earthexplorer.usgs.gov>), USGS GloVis (<https://glovis.usgs.gov/app?fullscreen=1>), UOPS (<https://uops.nrsc.gov.in/ImgeosUops/FinalImgeosUops/FinalImgeosUopsSSL.html>), NASA Earth Data (<https://earthdata.nasa.gov/>).



Downloading Raster File

The process for downloading free satellite imagery is following:

- Open Earth Explorer > Login with your personal Earth Data login > Navigate to your area of interest > Make a bounding box by double-clicking the mouse and adding four points surrounding the region > Set Date range for which satellite image to be searched > Click on Data sets
- Datasets > Select Landsat > Landsat Collection 1 Level 1 > Landsat 8 OLI/TIRS C1 Level 1 > Check the box > Go to Additional Criteria

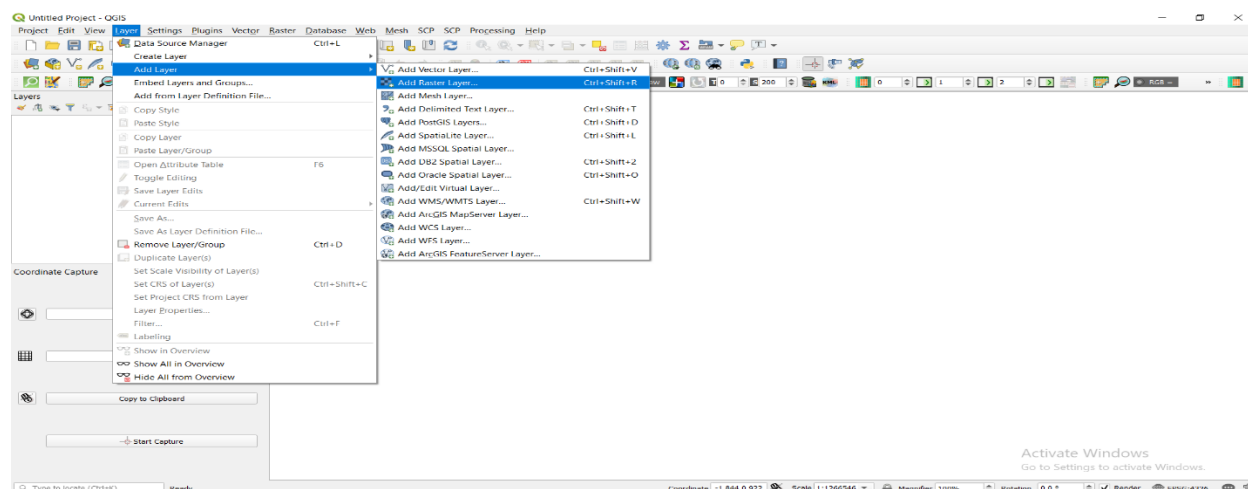
- Additional Criteria > Select Land Cloud Cover Less than 10% > Scene Cloud Cover Less than 10% > Go to Results
- Results > Show footprint > Check the scene covers the entire bounding box or not > Show Browse Overlay > Check the overview of the scene > Show Metadata (Check the metadata) > Download the imagery.

Reading a Raster File

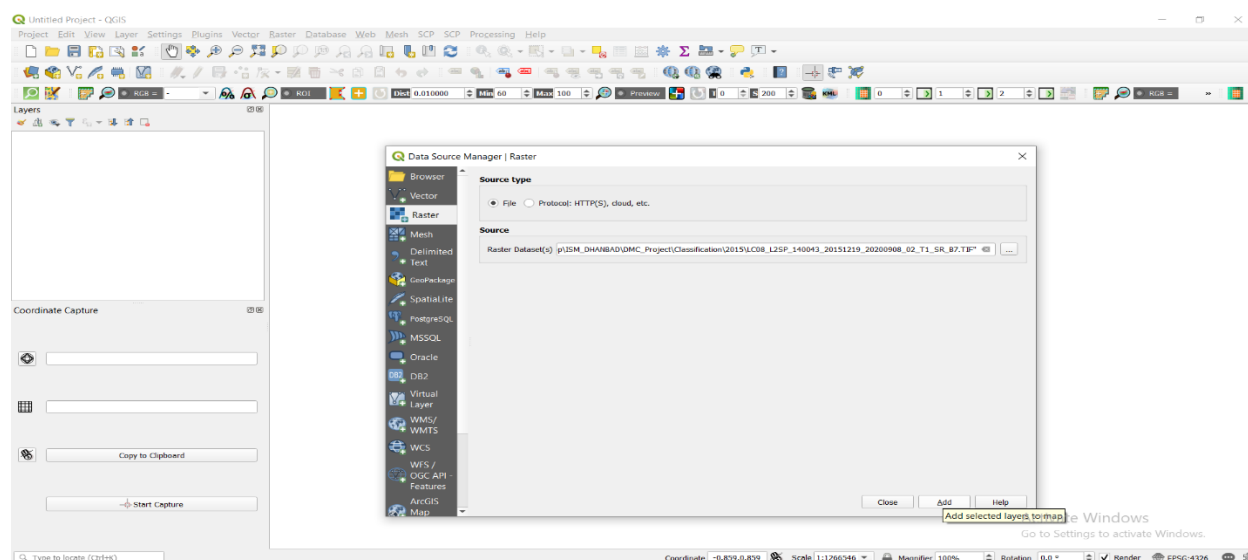
Downloaded raster file will be on zip format > Extract the files > All the bands will be unloaded.

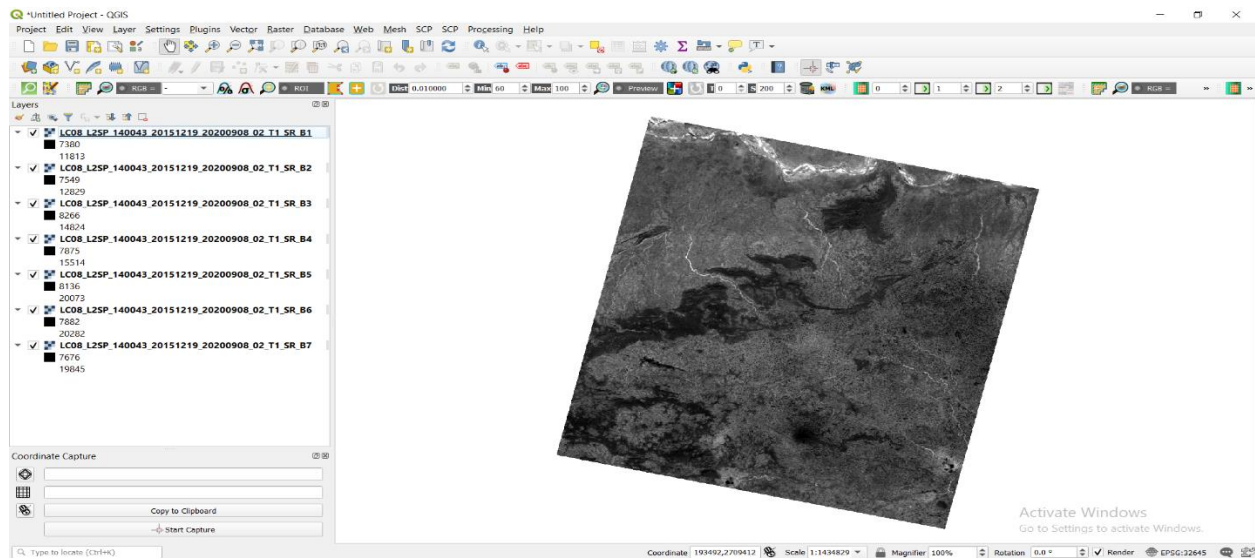
The number of bands in the imagery will depend upon the specific satellite and its sensor. The wavelength of the bands and their information will always be available with image metadata.

Step 1: Click on Layers> Add Layer> Add Raster Layer

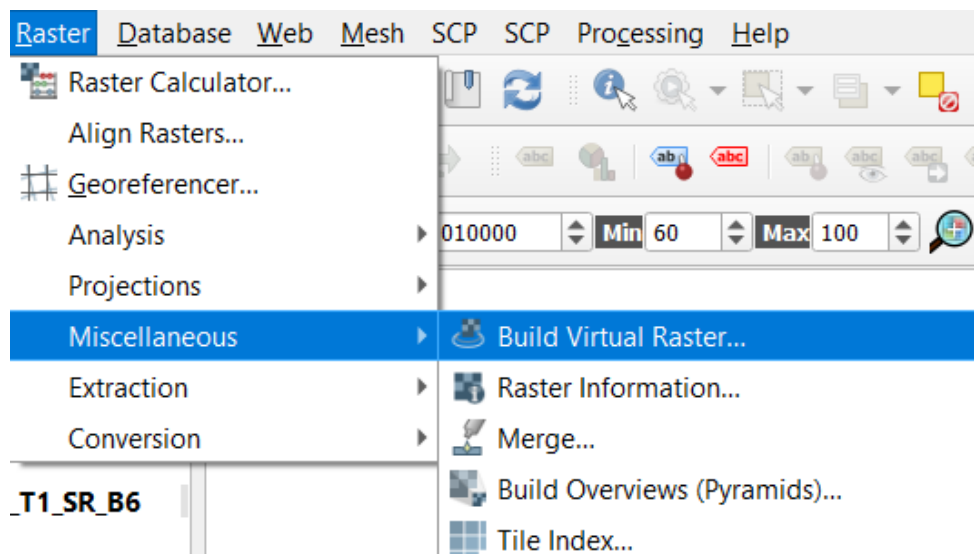


Step 2: Select all the bands, then click on Add > Close.

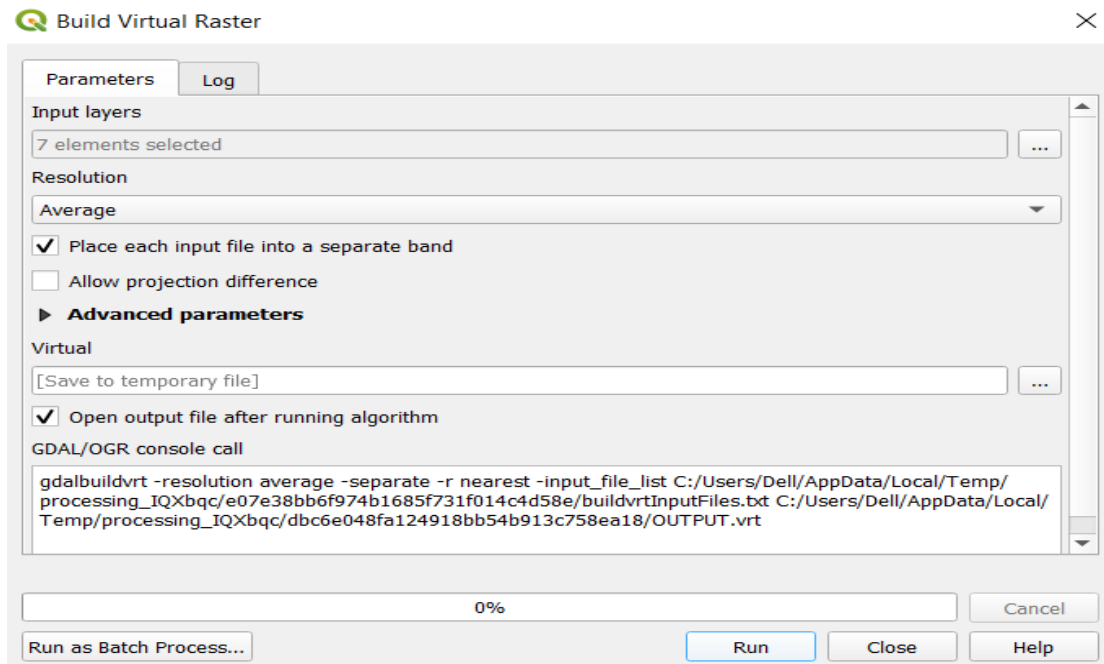




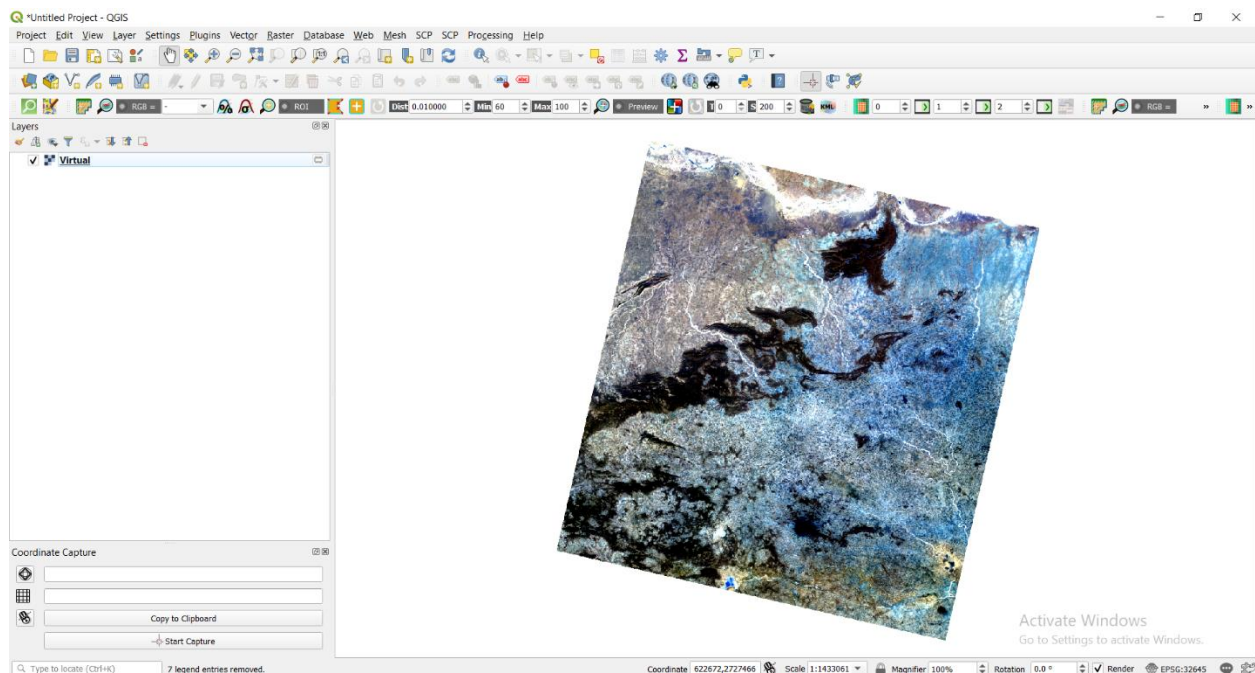
Step 3: To create a composite of all the bands click on Raster> Miscellaneous> Build Virtual Raster



In Input Layers choose the bands for which you have to make a composite. In the resolution, you choose “Average/ Highest/Lowest”. Then click on Run.

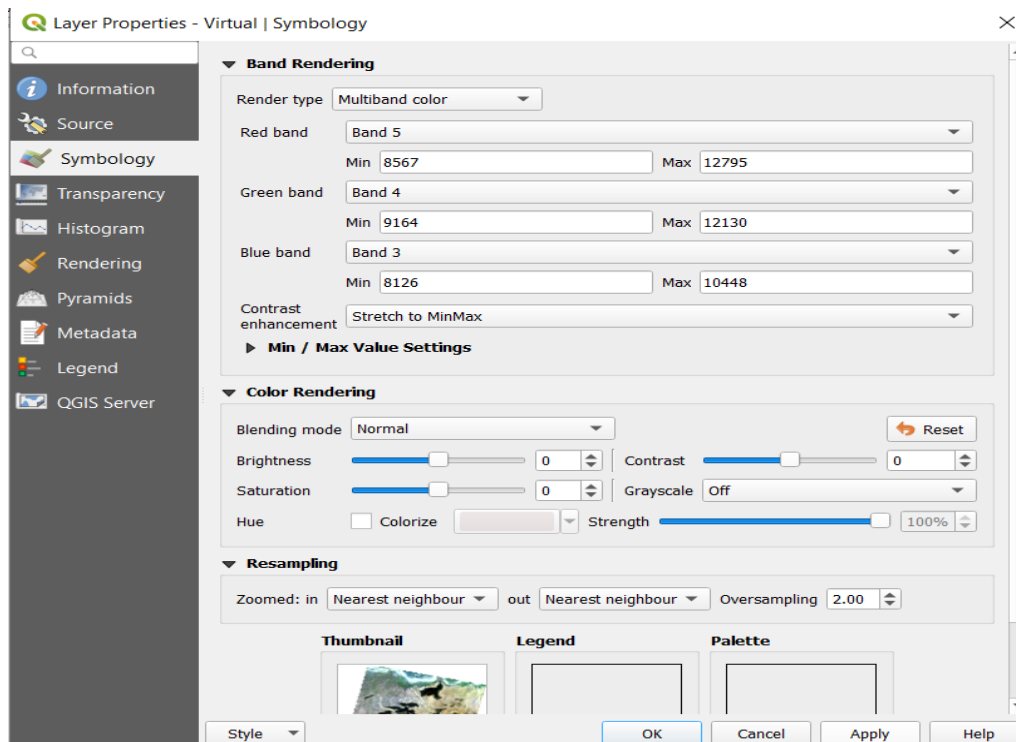


This is how the composite bands look like.

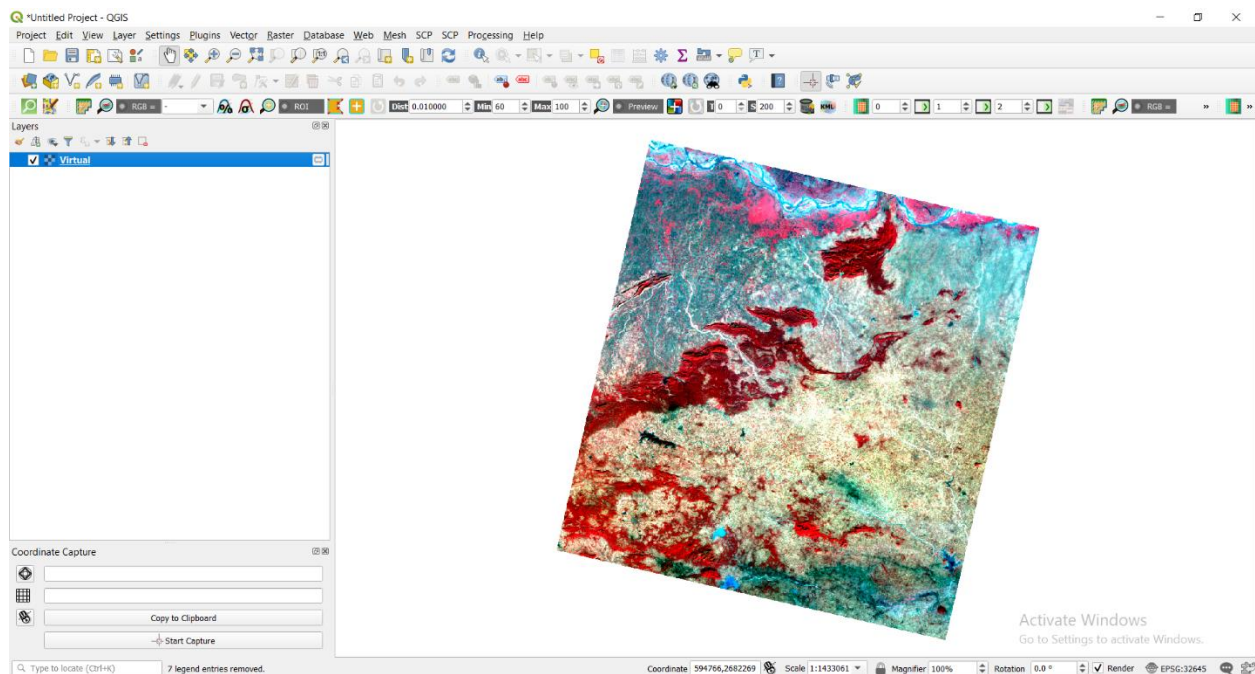


Step 4: To make a False Color Composite (FCC) of the image, right-click on the composite image file that has been added in the Layer then click on “Properties”.

Step 5: Click on “Symbology”. Add the bands and then click on “Apply”



The FCC would look like this as shown below.



Practical 5: Vector Data Handling in QGIS

Creating a shape file in QGIS

Step 1: Click on the “New Shapefile Layer”



Step 2: A window will pop-up where we can provide the location where ever we want to save the shapefile in the “File name”.

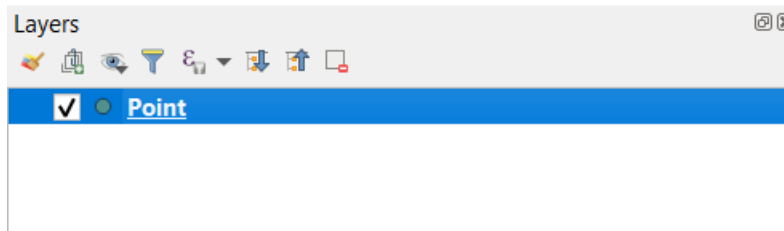
We can also choose the type of geometry i.e. point, line or polygon.

In the “New Field” we can provide the name of the geomerty we are choosing in the “Geometry type”.

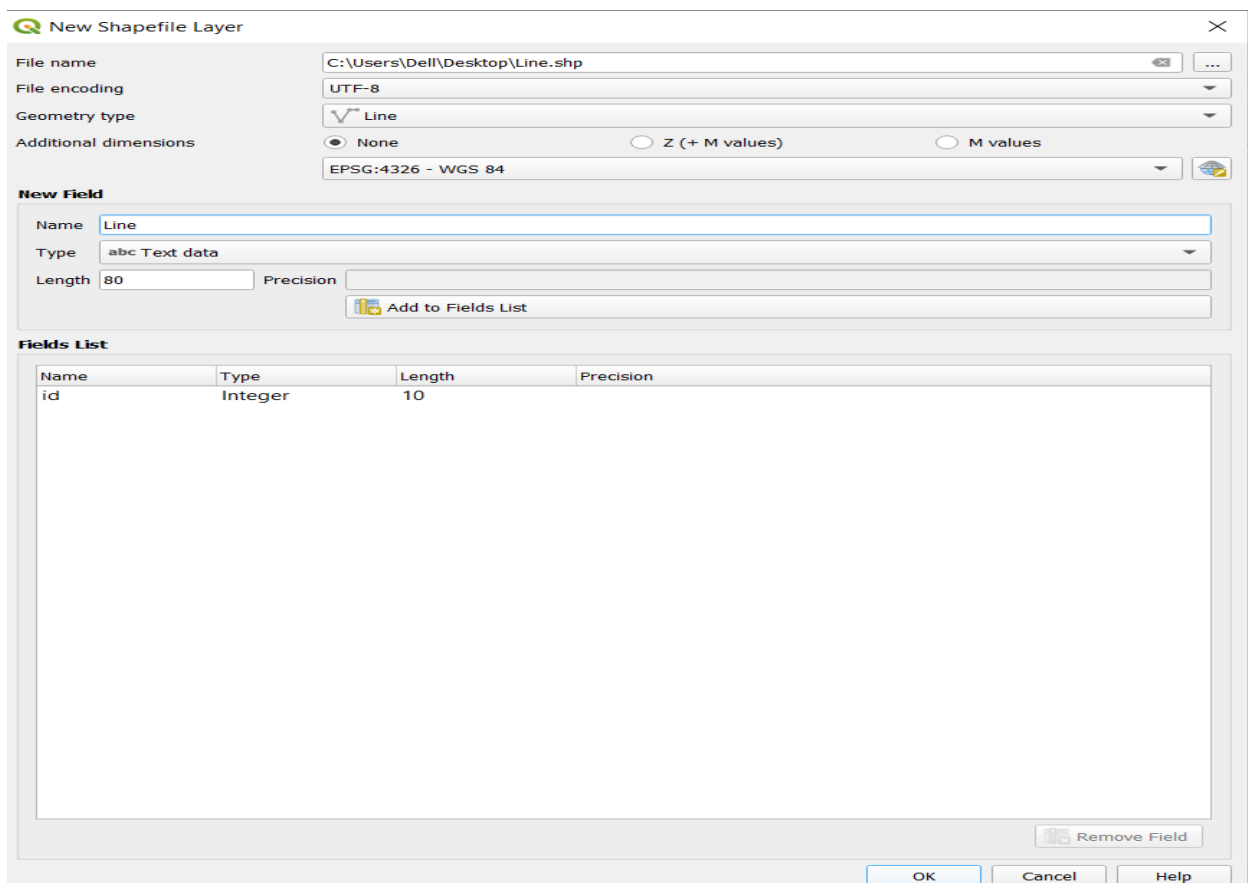
A screenshot of the 'New Shapefile Layer' dialog box. The 'File name' field is set to 'C:\Users\Dell\Desktop\Point.shp'. The 'File encoding' is set to 'UTF-8'. The 'Geometry type' is set to 'Point'. Under 'Additional dimensions', 'None' is selected. The 'New Field' section shows a field named 'Point' with type 'abc Text data' and length '80'. The 'Fields List' table contains one field: 'id' with type 'Integer' and length '10'. The dialog has 'OK', 'Cancel', and 'Help' buttons at the bottom right.

Name	Type	Length	Precision
id	Integer	10	

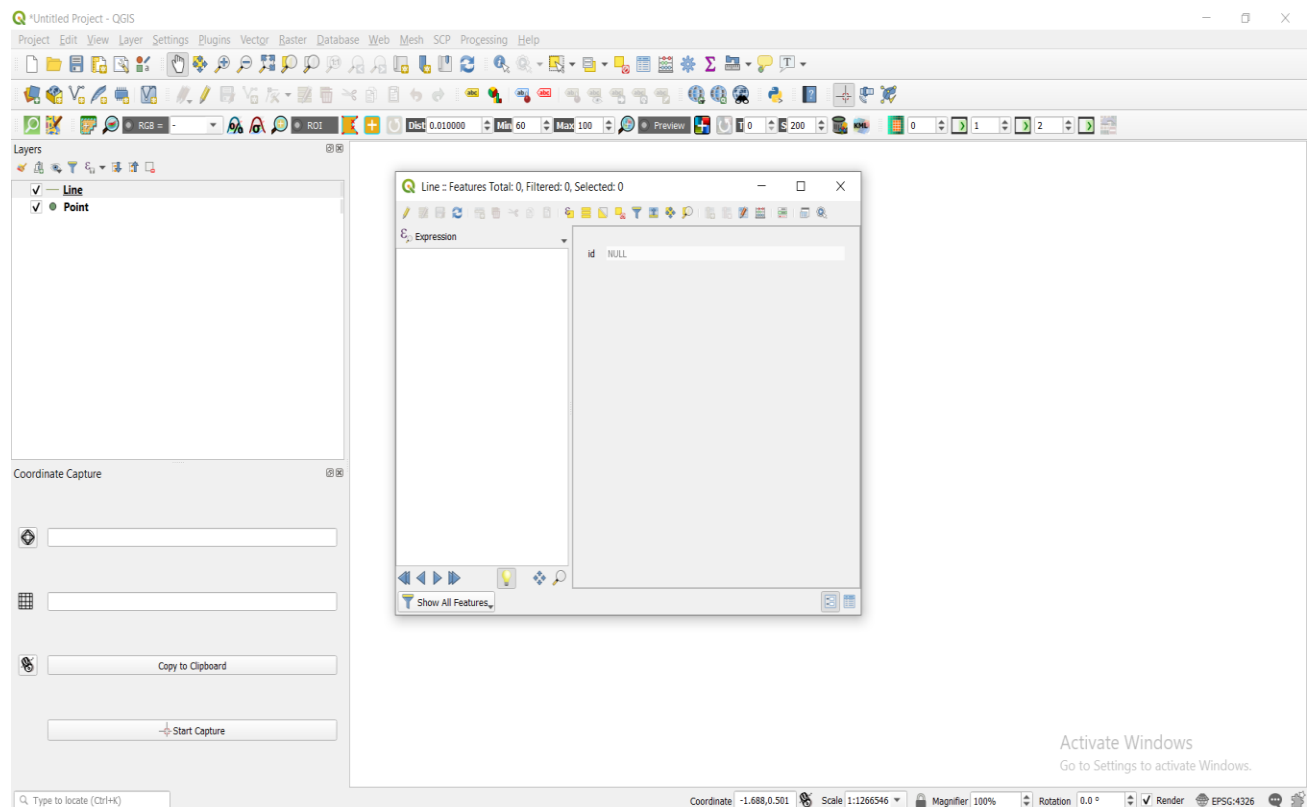
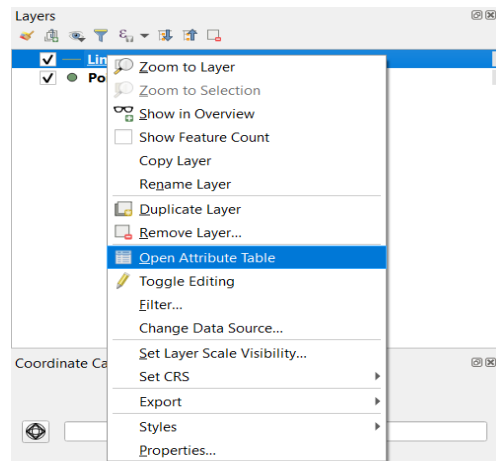
After giving all the necessary information click on “OK”. After clicking on “OK” the shapefile will get added to the Layer.



Similarly, we can create the shapefile of line and polygon.

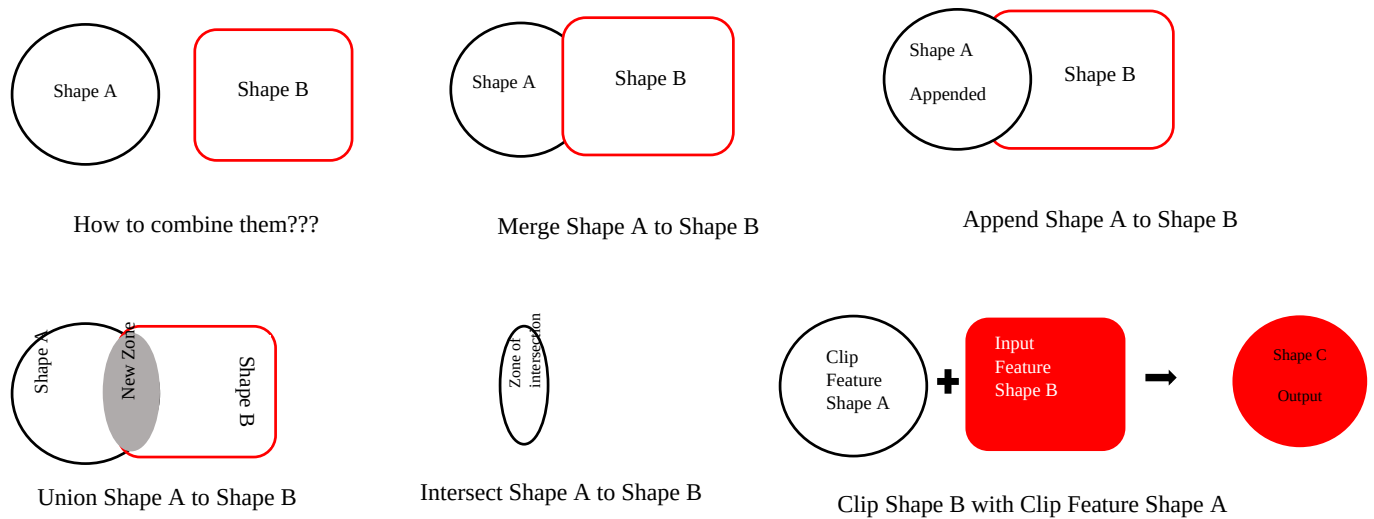


Step 3: To open the attribute table select the shapefile which you have created, then right-click on it and choose the “Open Attribute Table” option. Since, we have only created the shapefile so nothing will be there in the attribute table.



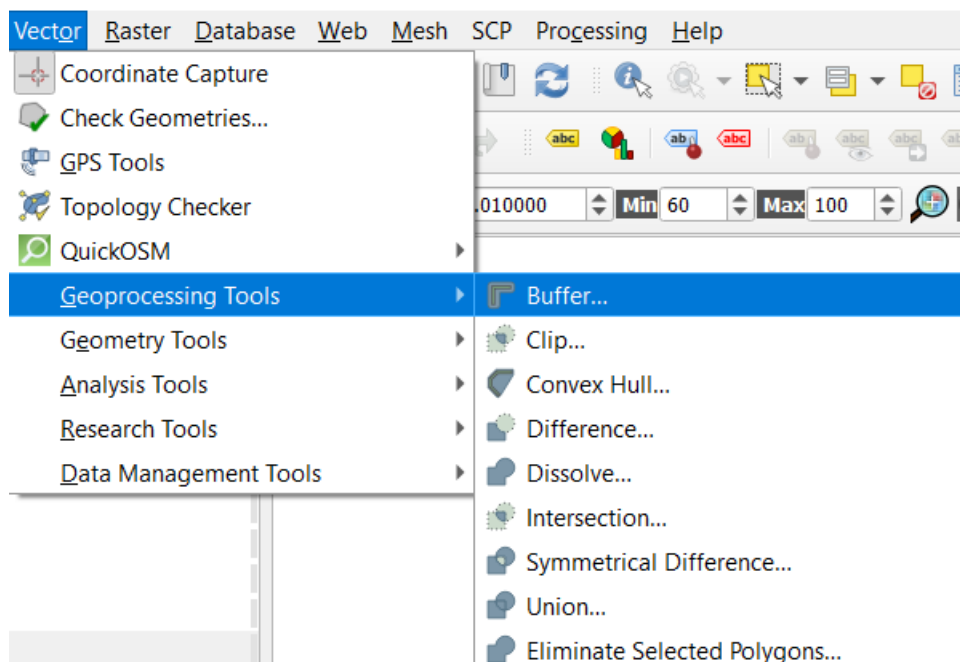
Combining Spatial Data in QGIS

Let us imagine that we have two shapefiles A and B. We will see different options for combining these two shape files.



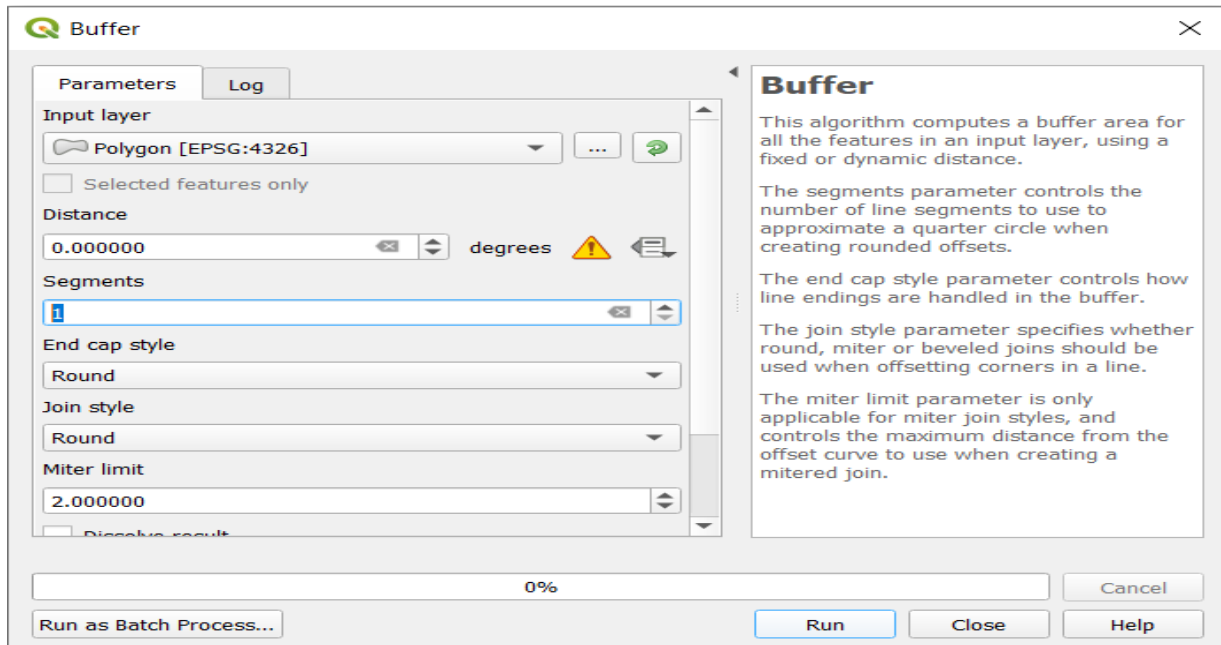
Buffer

Step 1: To create a buffer, we have to choose Vector> Geoprocessing Tools> Buffer

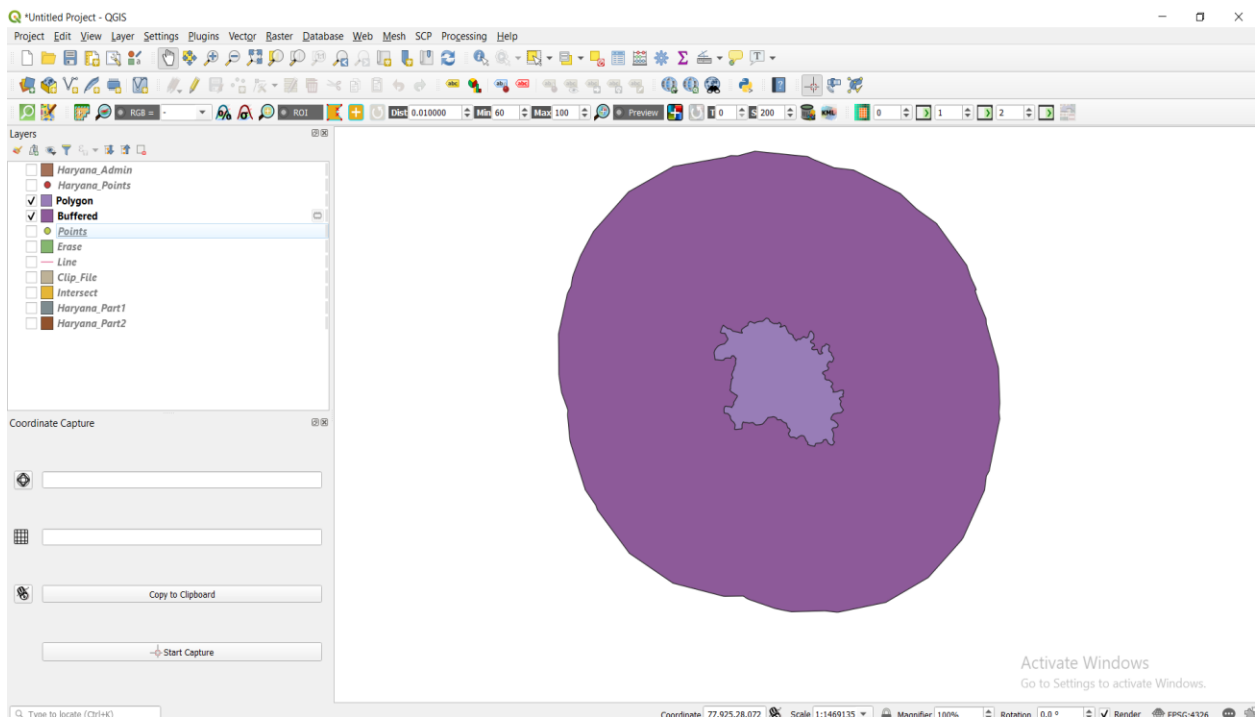


Step 2: A window will of Buffer will pop-up. In this choose the file of which you have to create a buffer. Distance can be provided in terms of degrees or meters or km.

Then click on “Run” option.



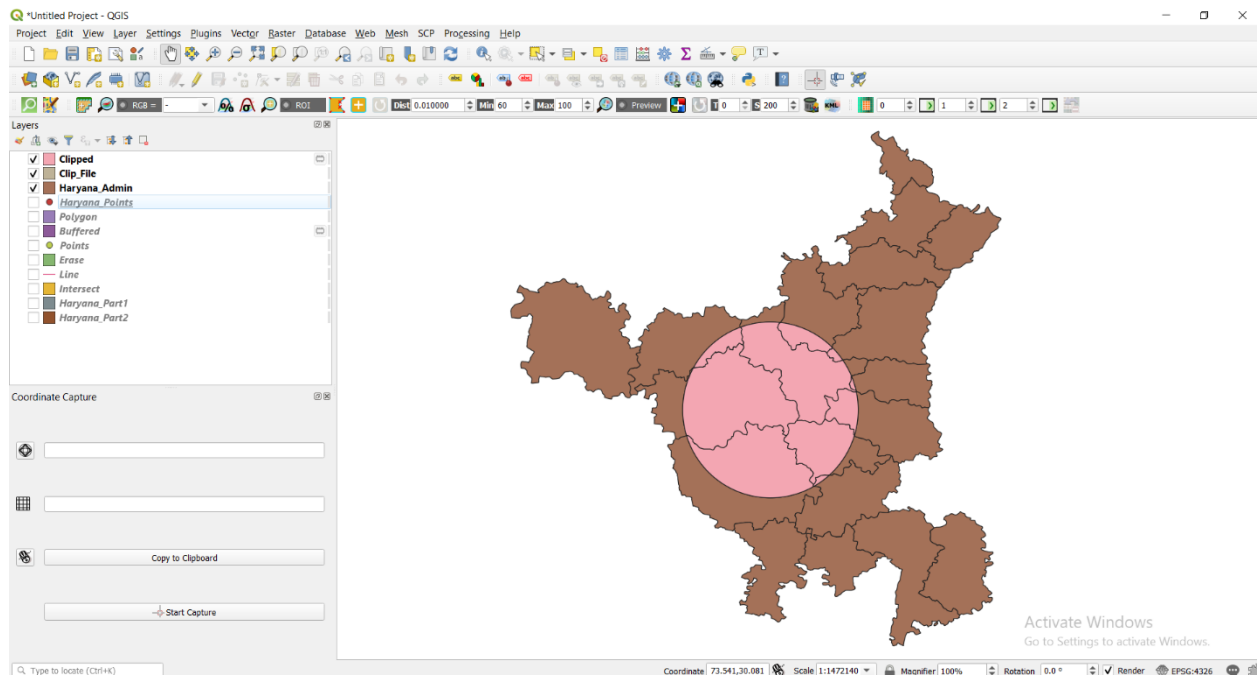
The buffer is created around the polygon.



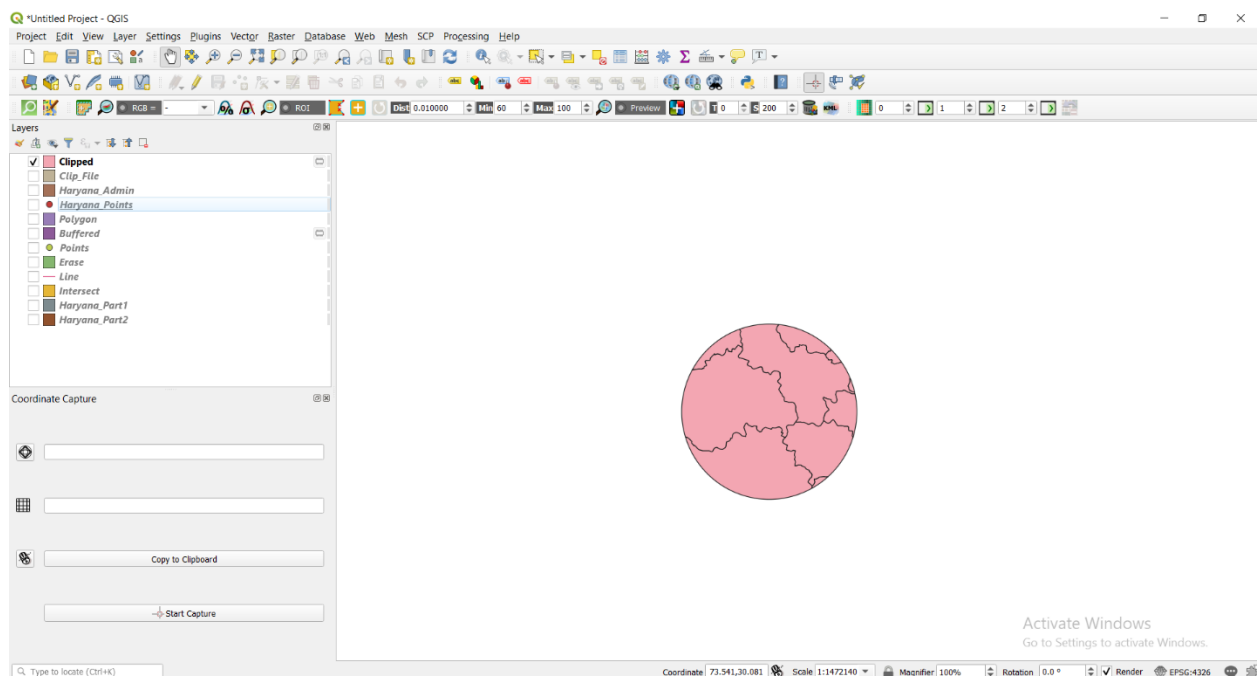
Clip

Step 1: To clip a shapefile, we need to have two shapefiles at the same time. Then choose Vector> Geoprocessing Tools> Clip

Like in this, we have one shapefile of “Haryana_Admin” from which we have to clip an area and another one is a “Clipped” shapefile.



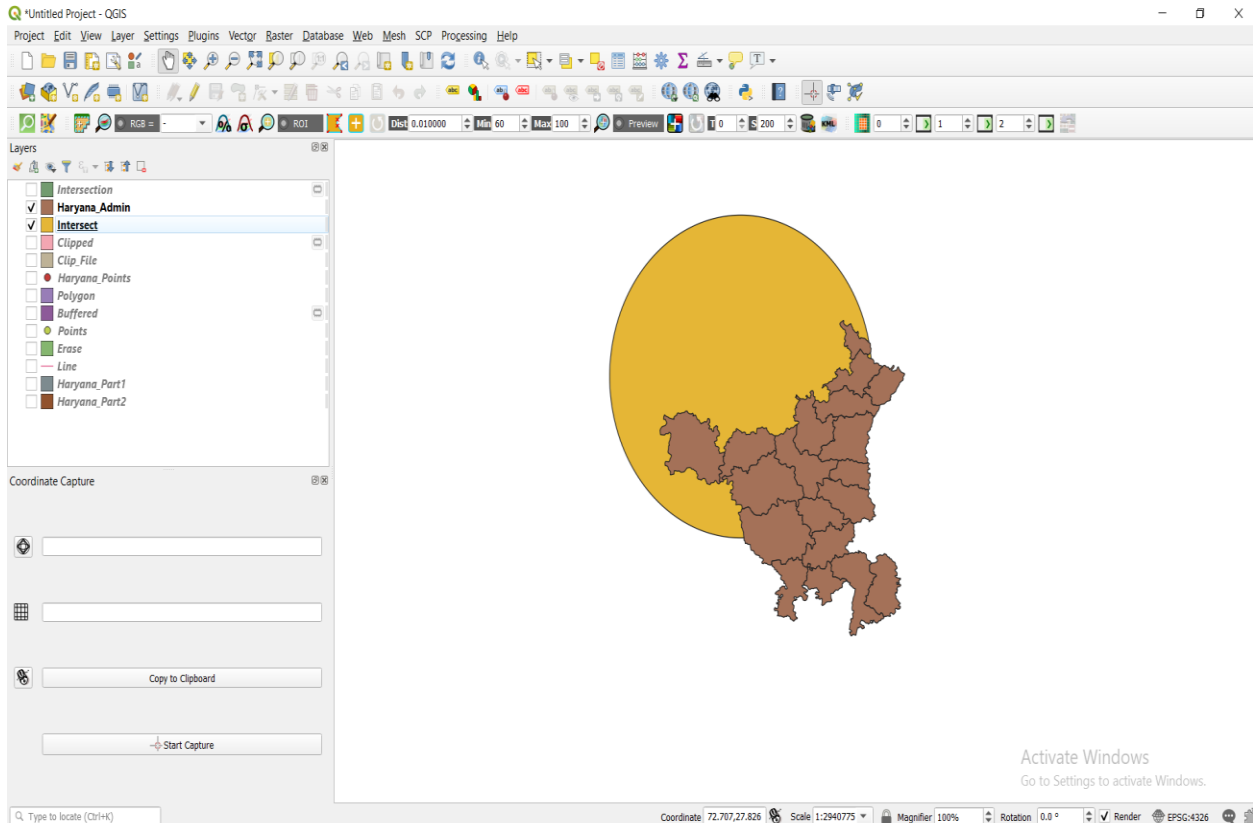
After running the clipping tool, we will get the clipped file.



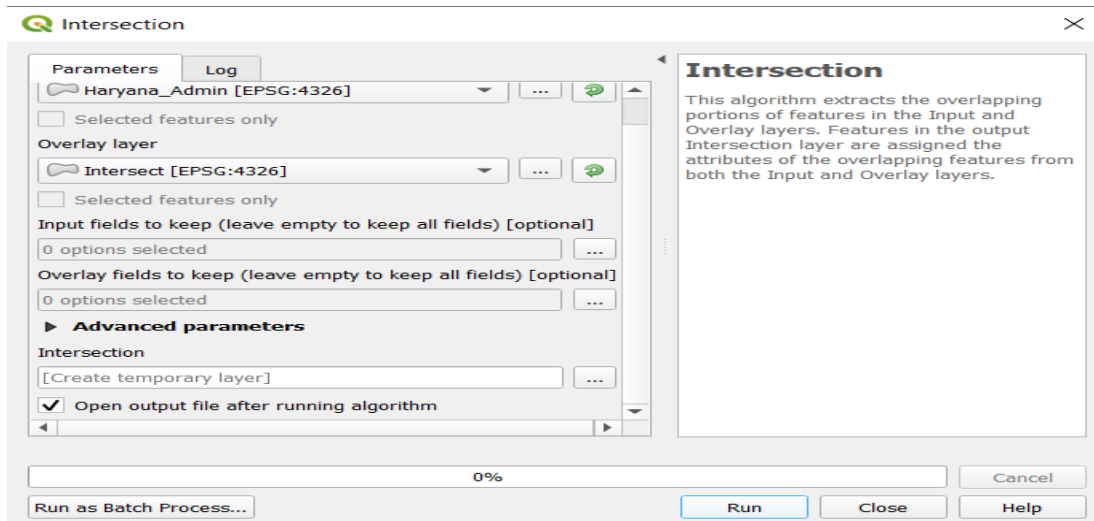
Intersection

Step 1: To intersect a shapefile, we need to have two shapefiles at the same time. Then choose Vector> Geoprocessing Tools> Intersection.

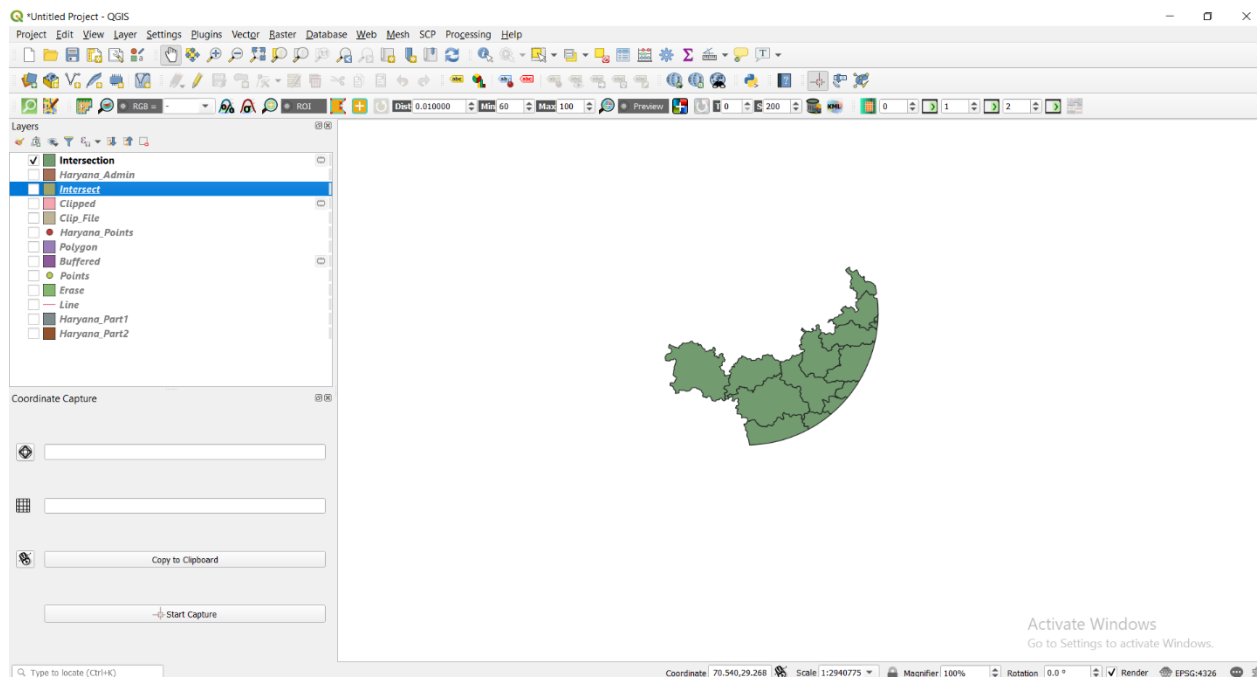
Two shapefile are used for the intersection which are “Haryana_Admin” shapefile and “Intersect” shapefile.



Step 2: Choose the parameters in the input layer and output layer and the click on “Run” option.



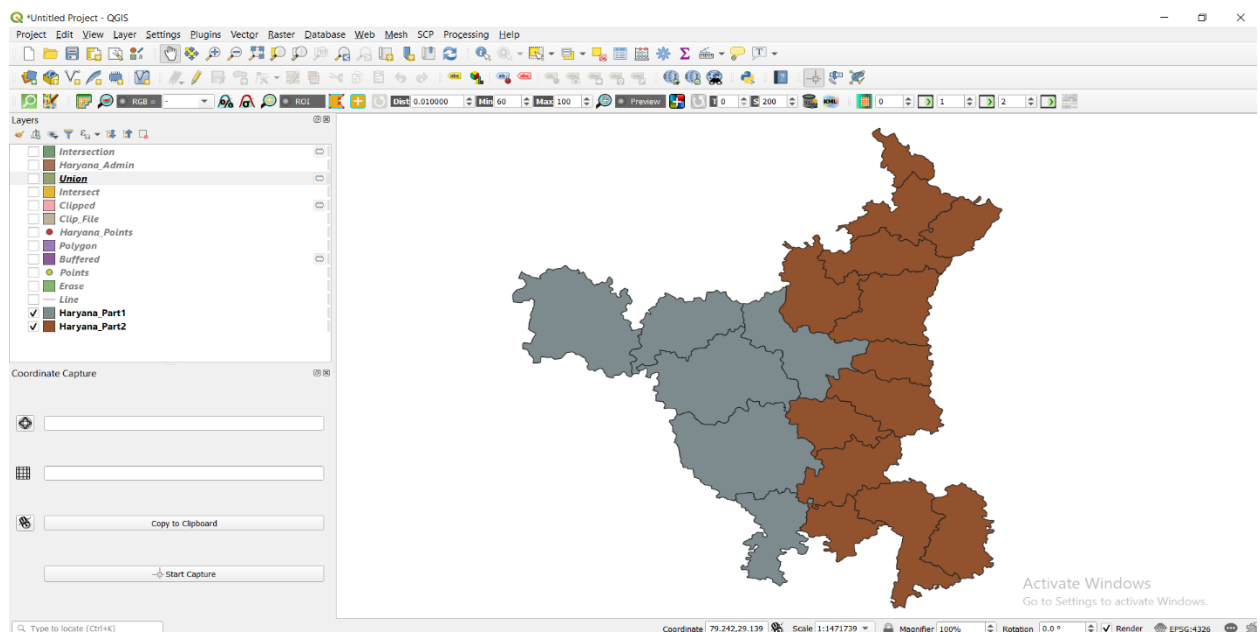
After running the intersection tool, we will get the intersected file.



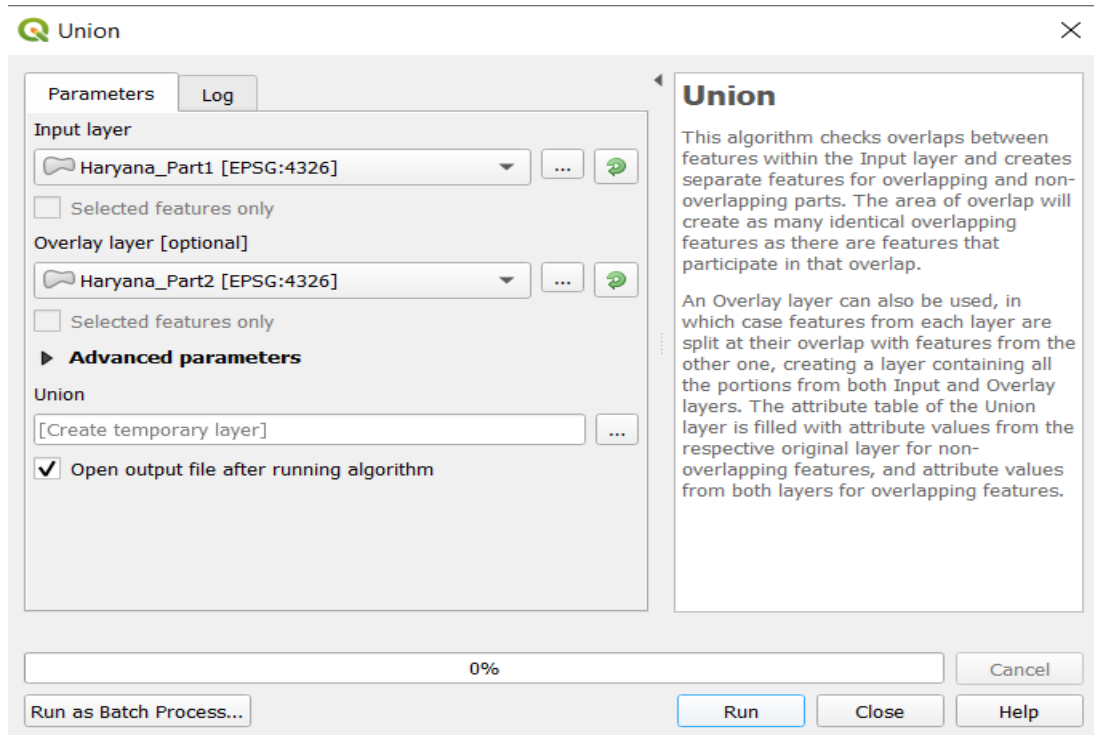
Union

Step 1: To intersect a shapefile, we need to have two shapefiles at the same time. Then choose Vector> Geoprocessing Tools> Union.

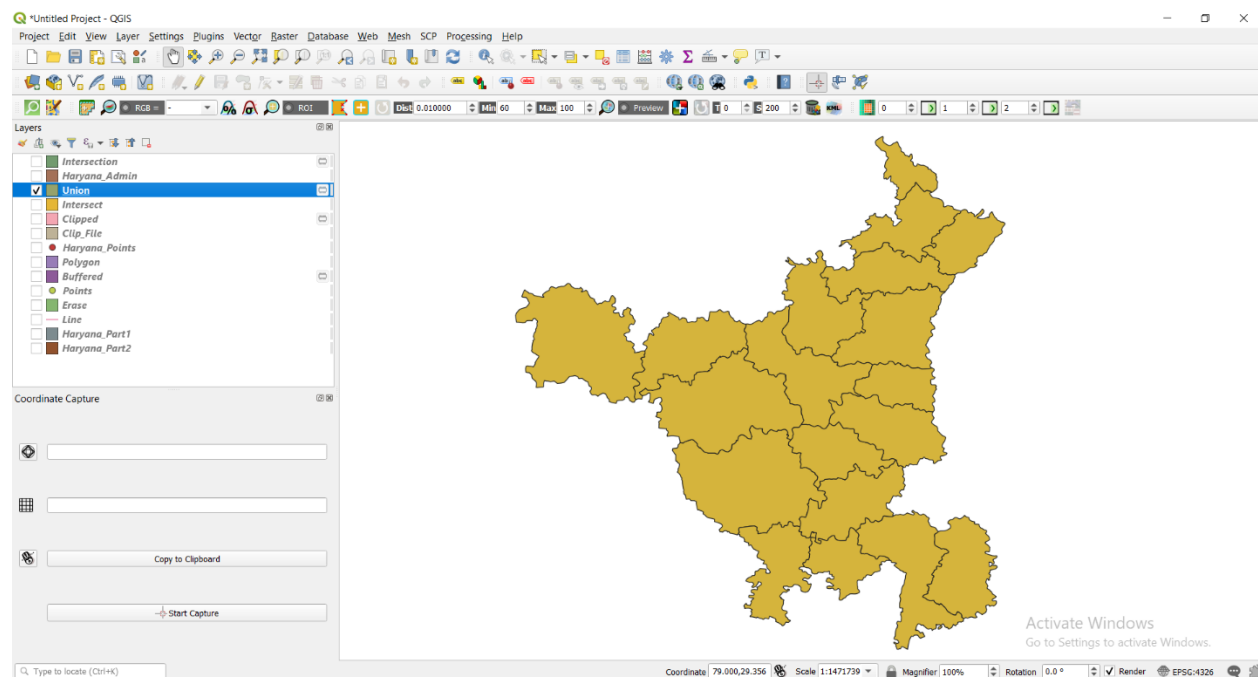
Like in this practical, we have two shapefiles i.e., Haryana_Part1 and Haryana_Part2.



Step 2: After clicking on “Union”, union window will pop-up. In this we will provide the all the necessary parameters which are needed. Then click on “Run”



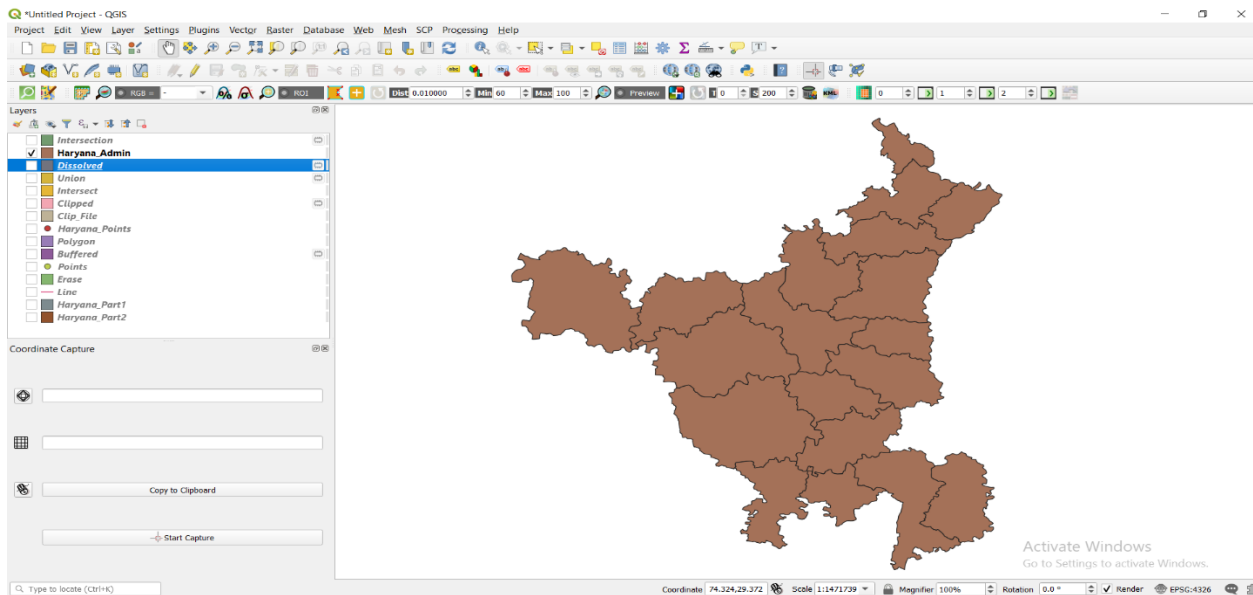
After running the union tool, two shapefile will get converted into one.



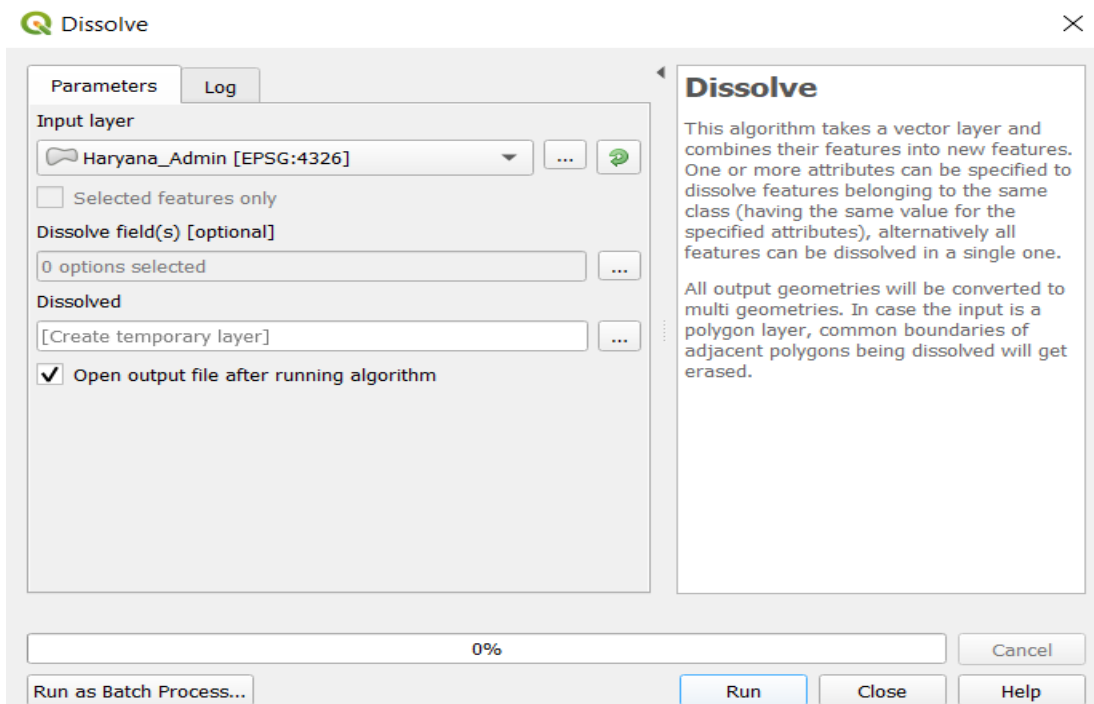
Dissolve

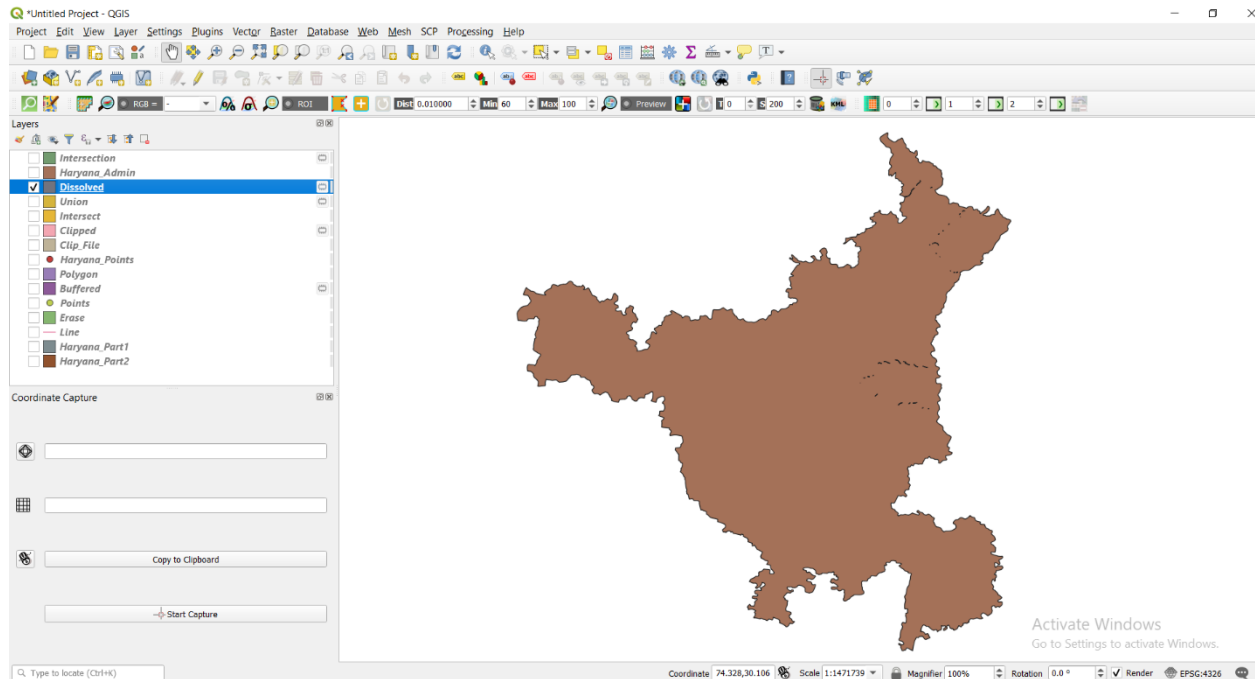
Step 1: To intersect a shapefile, we need to have two shapefiles at the same time. Then choose Vector> Geoprocessing Tools> Dissolve.

If we want to combine all the features present in the attribute table into one then we will use the dissolve tool.



Step 2: After clicking on “Dissolve”, dissolve window will pop-up. In this we will provide the all the necessary parameters which are needed. Then click on “Run”



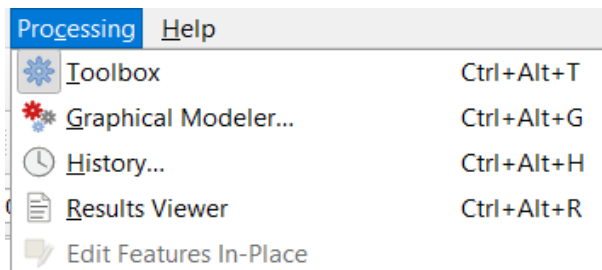


Spatial Interpolation of Vector Data

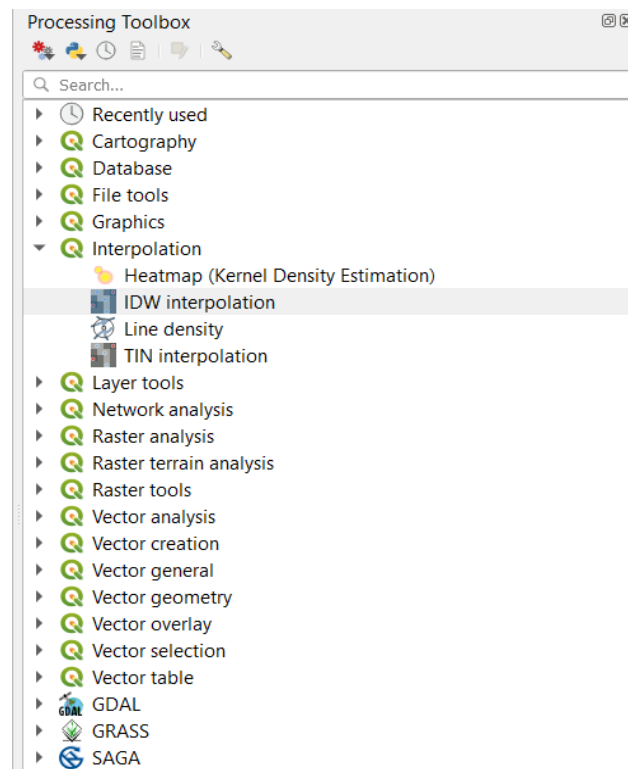
Interpolation predicts values for cells in a raster from a limited number of sample data points. There are several methods for performing spatial interpolation function in ArcGIS such as IDW, Kriging, Spline.

‘Inverse Distance Weighted (IDW)’ is a local interpolator that estimates the value at un-sampled location using a combination of samples weighted by an inverse function of distance from the point of interest to the sampled points. The samples near to the un-sampled location have more influence than the further. A power factor (k) is used to control the weights. If we increase the value of power factor that increases the influence of nearby points. IDW is a local exact interpolation technique.

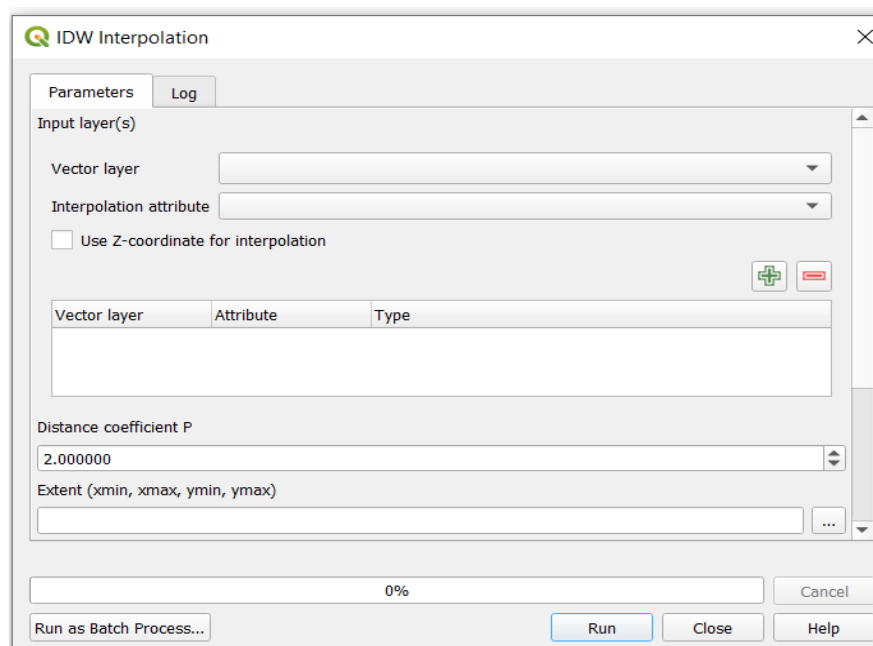
Step 1: Click on Processing> Toolbox



Step 2: Then choose the Interpolation> IDW Interpolation



Step 3: After providing the vector layer and choosing all the parameters as per the requirement the click on “Run”.



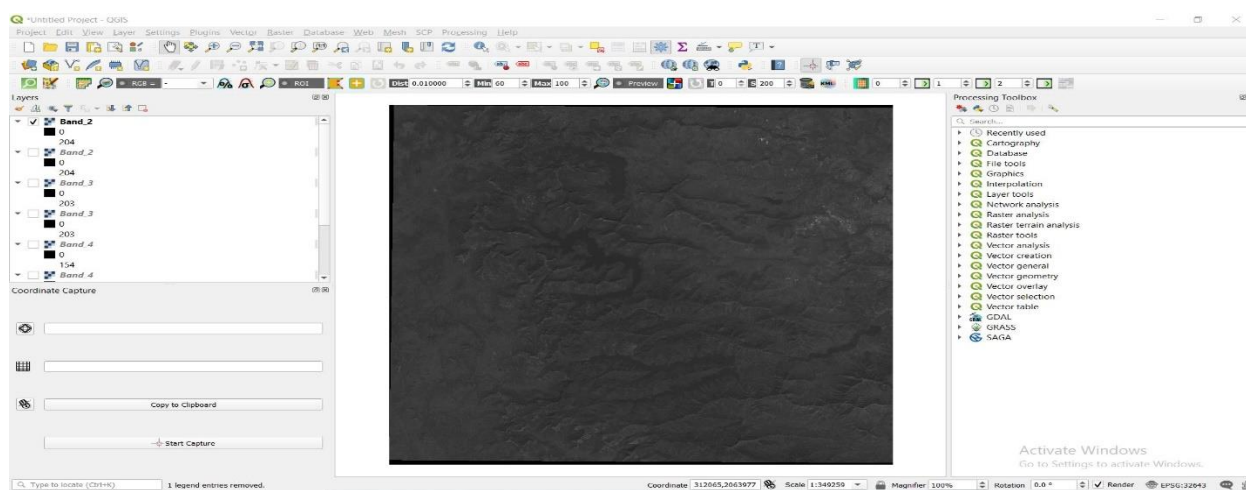
Practical 6: Image Mosaicking, PCA, Subset

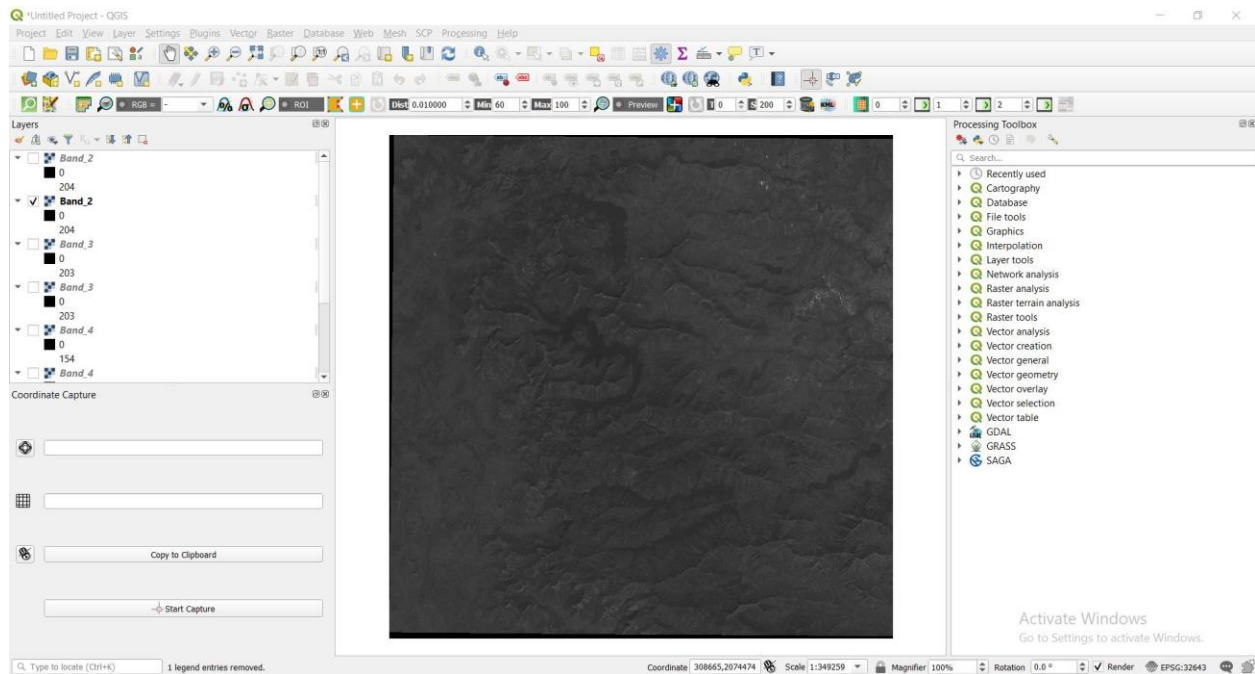
Satellite imageries come in varying swath widths, depending on their source of acquisition. For example, the LISS-3 imagery is approximately 140km swath width while the LandSat TM imagery covers 185km, and SPOT imagery is just 60km swath width. These images cover large swathes of land but in most of the cases the Area of Interest (AOI) does not cover by a single satellite image. We need to assemble the satellite images which cover the individual parts of AOI to form a single composite. This composition process is known as ‘Mosaicking’, it requires very accurate radiometric and geometric corrections to the constituent imageries (Rees, 1999).

Dataset used: <https://dst-iget.in/index.php/tutorialdetails/2/7>

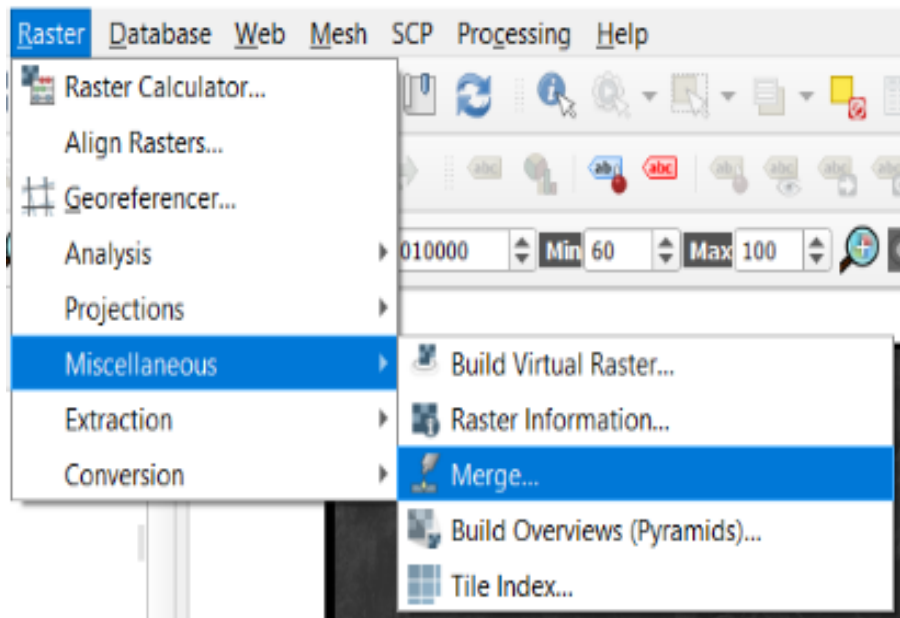
In this practical, we will use two satellite images of east and west of Pune metropolitan, east part belongs to Pune-Haveli Taluka and west part belongs to Mulshi Taluka and one South Pune Toposheet. The objective of this practical is to provide hands on guide to mosaic the satellite images.

Step 1: Add the LISS3 images in QGIS by clicking on the Layer> Add Layer> Add Raster Layer. In the window that pops up, navigate to the tutorial data’s Liss3 image folder ‘Left’. The layers may not be immediately visible, so change the dropdown menu selection (beside File name) from ‘All Recognised files’ to ‘All files’. Select all the *.tif files and click ‘Open’. Do the same for the layers in the folder ‘Right’.

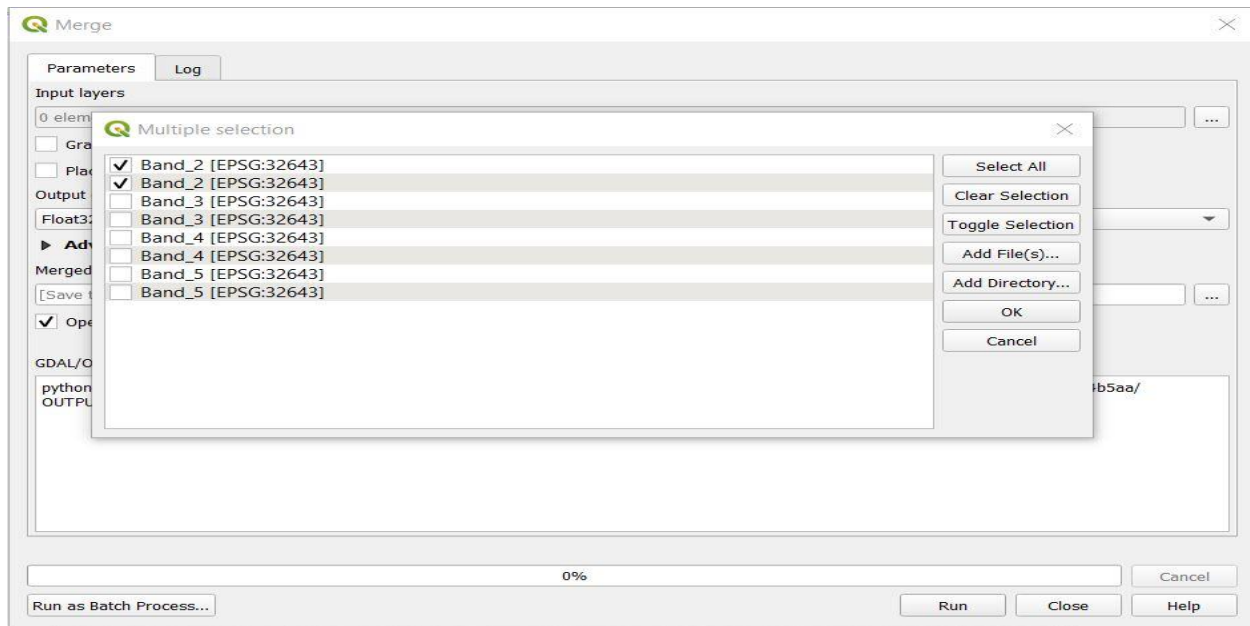




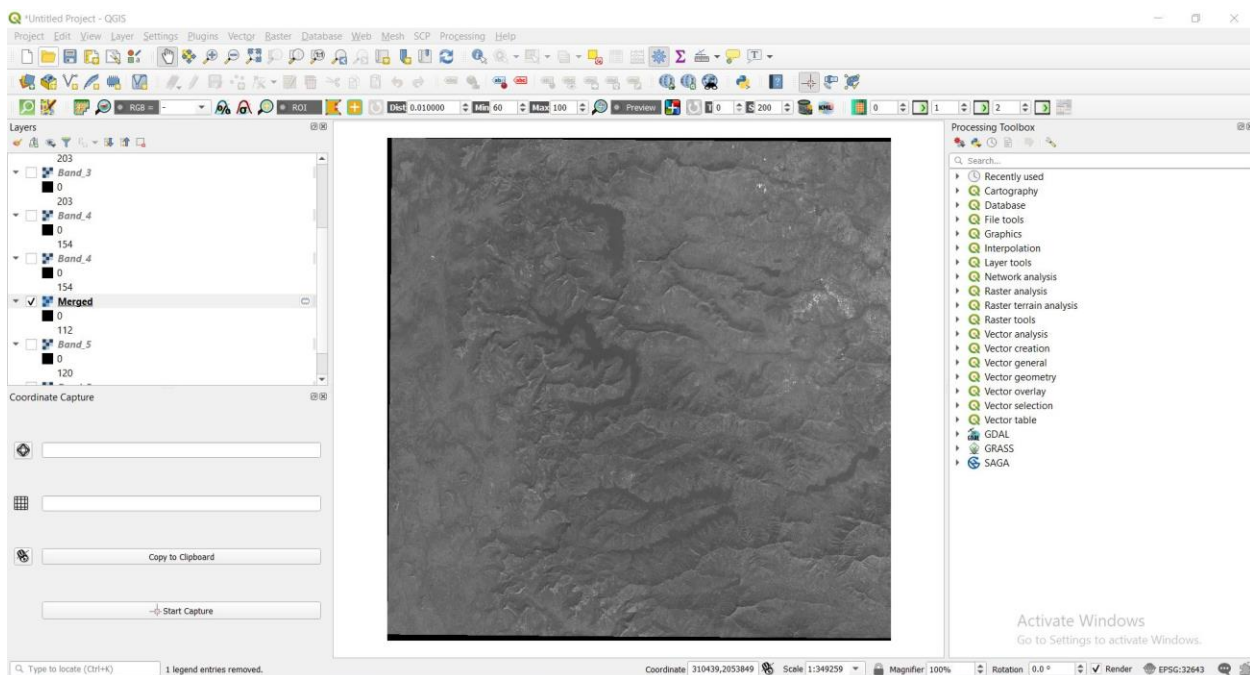
Step 2: After loading all the files, now click on Raster> Miscellaneous> Merge

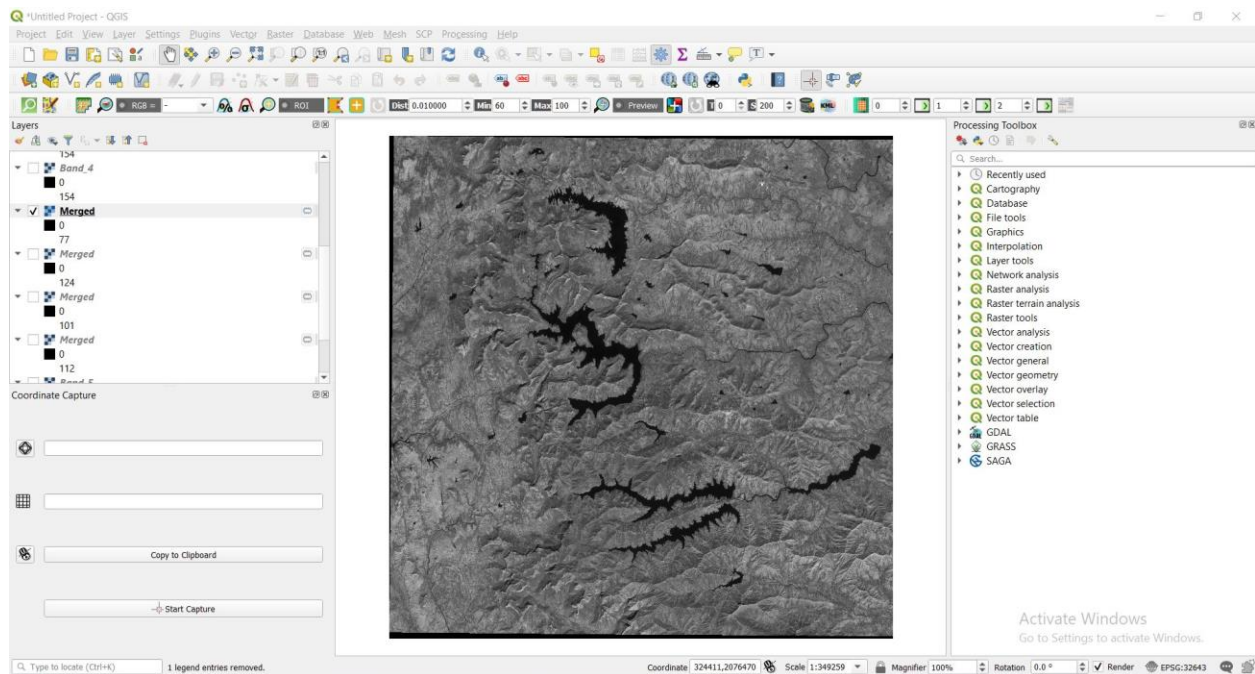


Step 3: Select the bands that you want to merge i.e. create the mosaic. Here, two Band_2 bands are selected one is of left and second is of right folder.



Step 4: After selecting the bands click on “Run”. The mosaicked image will automatically get added in Layers.





Repeat the above steps to mosaic the remaining bands.

PCA

Principal component analysis (PCA) is a technique for reducing the dimensionality of such datasets, increasing interpretability but at the same time minimizing information loss. It does so by creating new uncorrelated variables that successively maximize variance. Finding such new variables, the principal components, reduces to solving an eigenvalue/eigenvector problem, and the new variables are defined by the dataset at hand, not *a priori*, hence making PCA an adaptive data analysis technique.

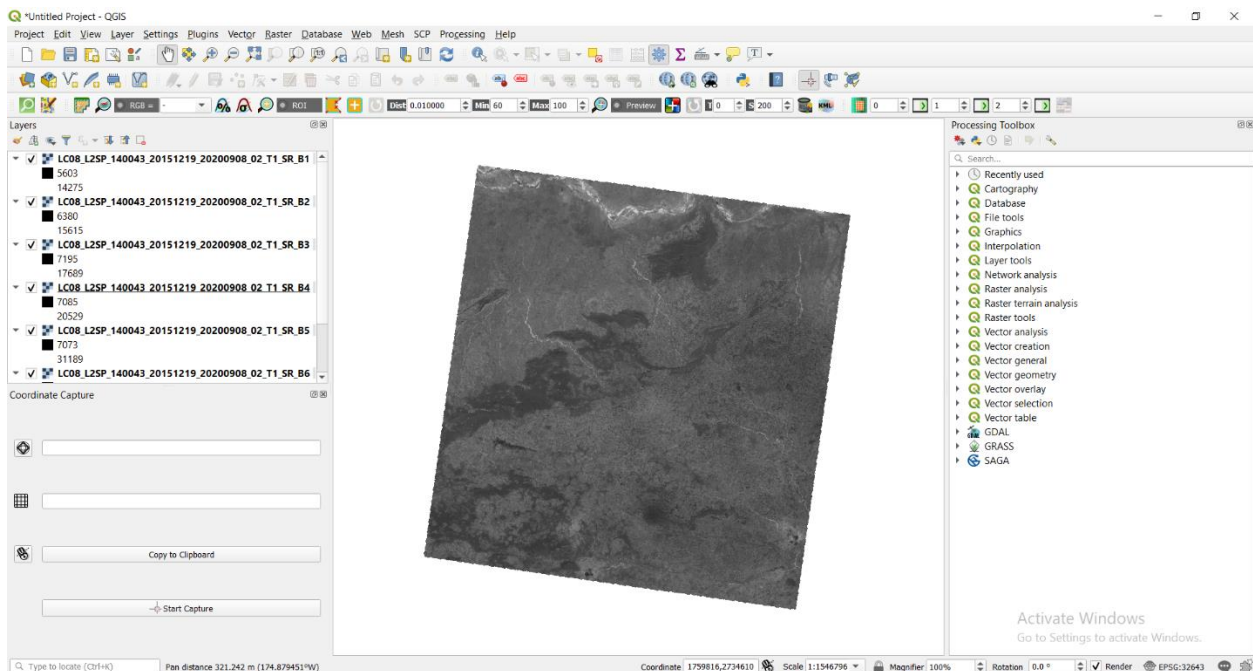
Dataset used: Landsat 8, Dhanbad District (10/9/2015)

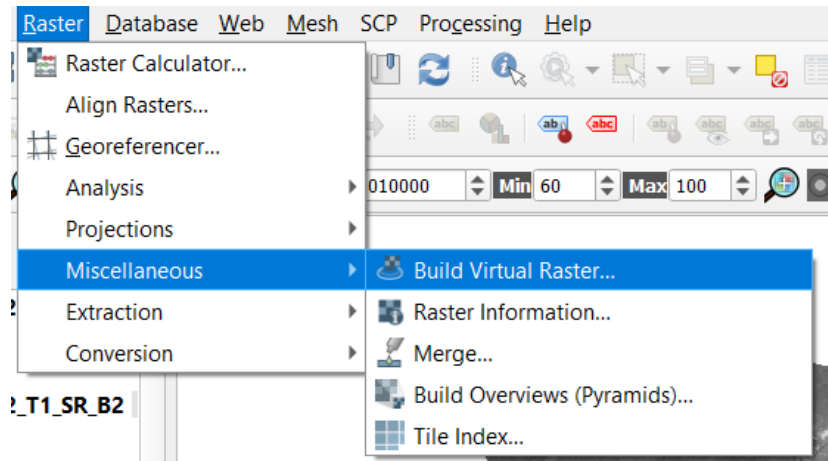
There are two ways to perform PCA in QGIS:

- i) By using the PCA for change detection plugin
- ii) By searching PCA in the Search bar

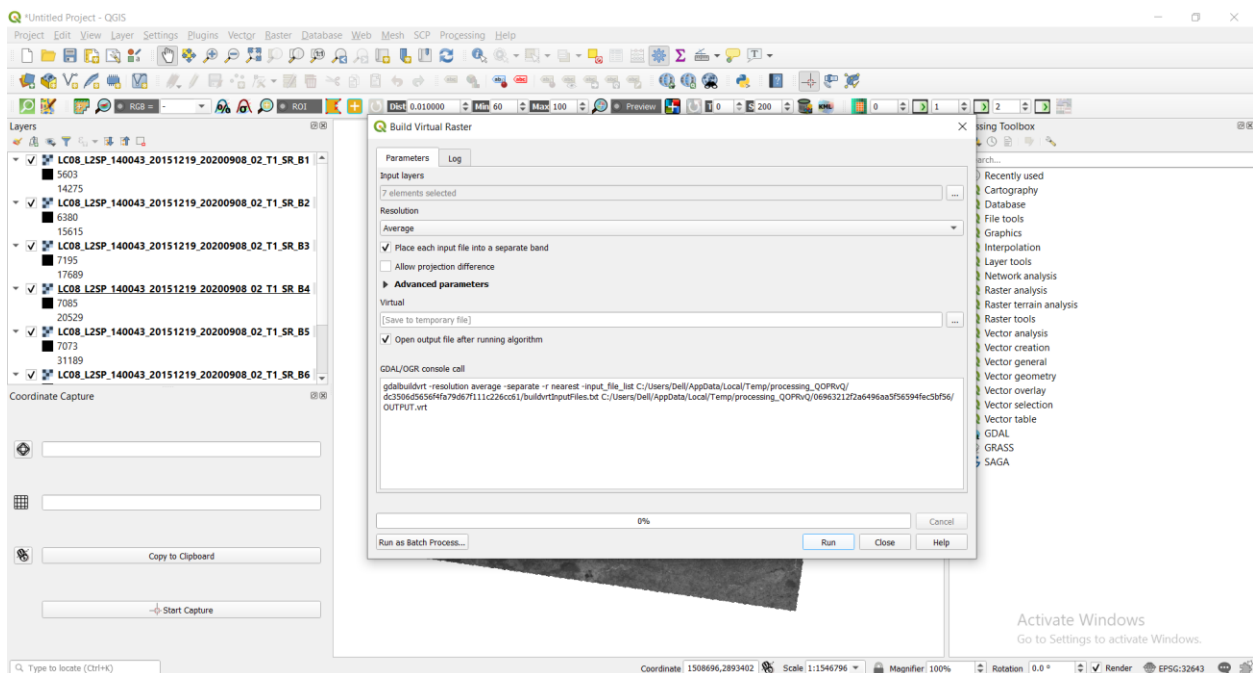
By using PCA plugin

Step 1: Load the dataset i.e. from Band 1 to Band 7. Then click on Raster> Miscellaneous> Build Virtual Raster to add all the 7 bands into 1.

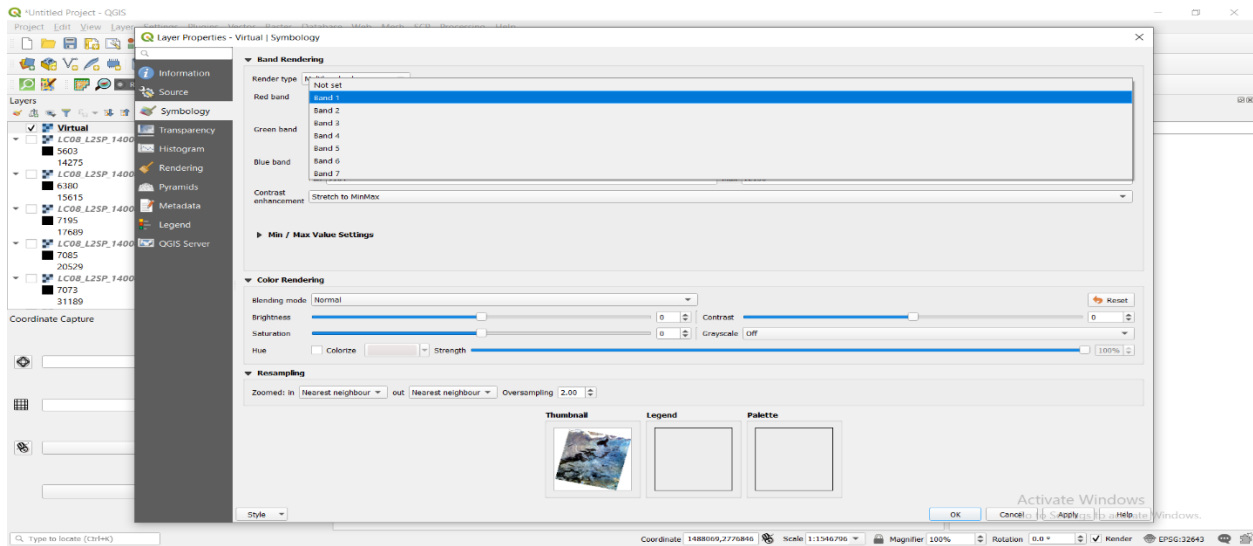




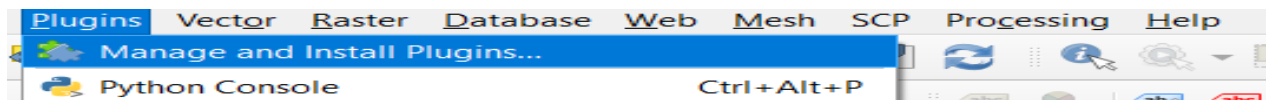
Step 3: Select all the bands to build virtual raster. Then click on “Run”.



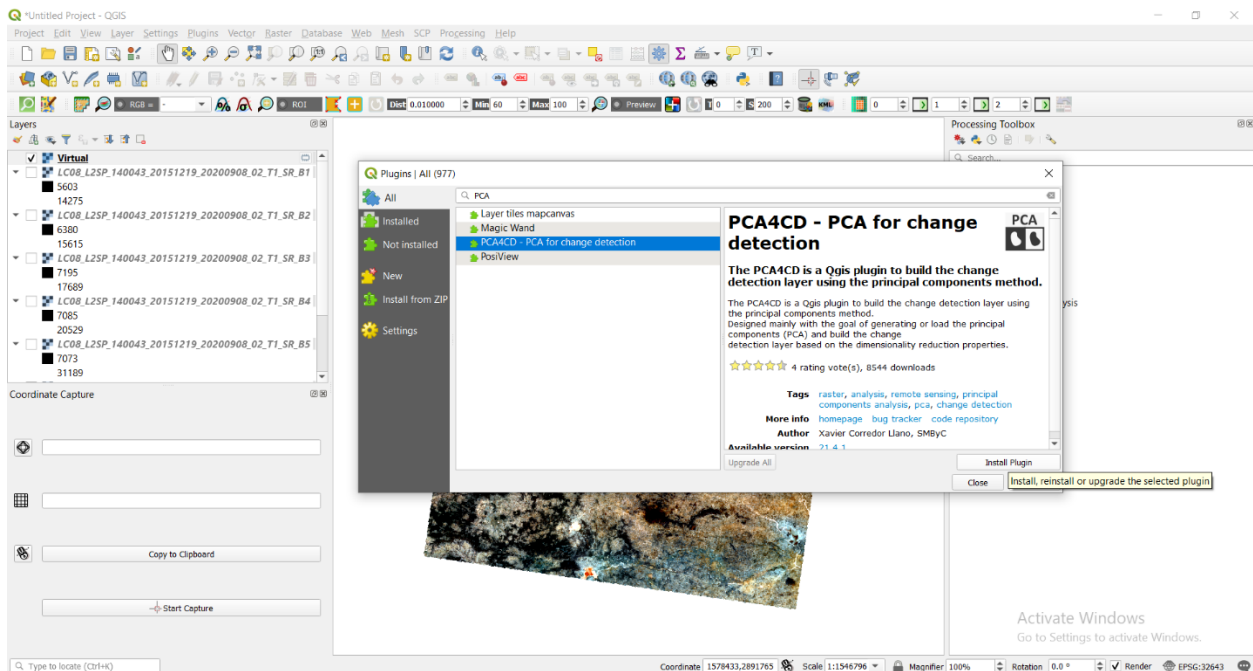
Now select the virtual file and click on properties option. In the symbology, we can see that all the bands are now present. Choose the appropriate bands to open the TCC.

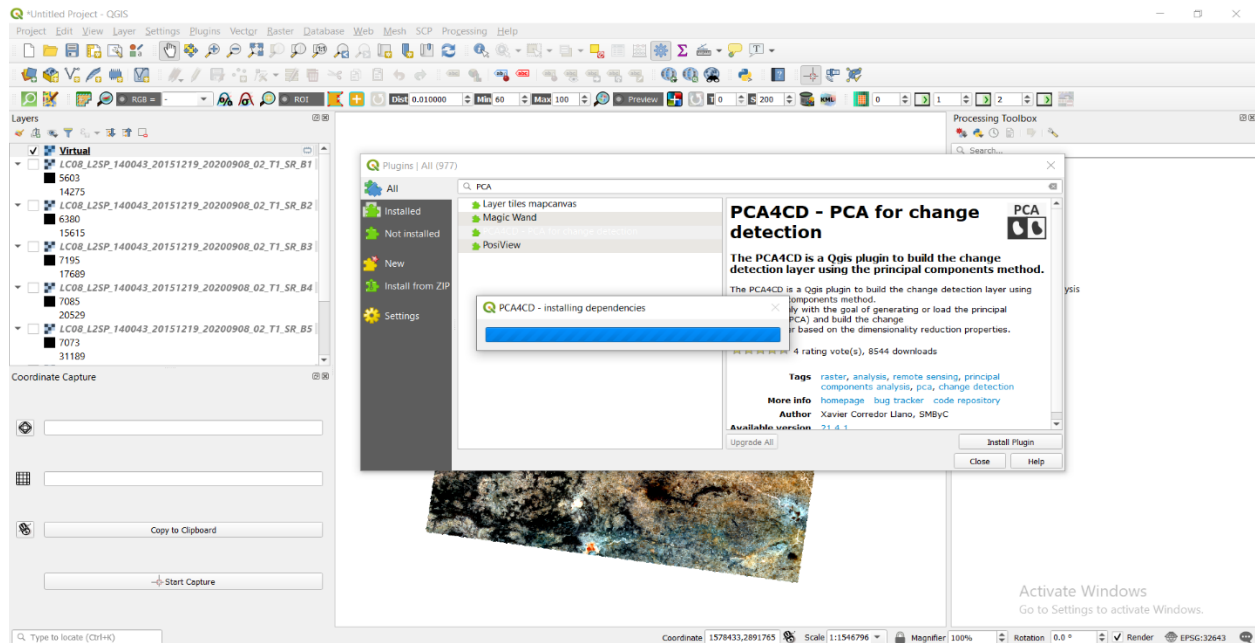


Step 4: Now download the “PCA for change detection” Plugin by clicking on Plugins> Manage and install plugins.

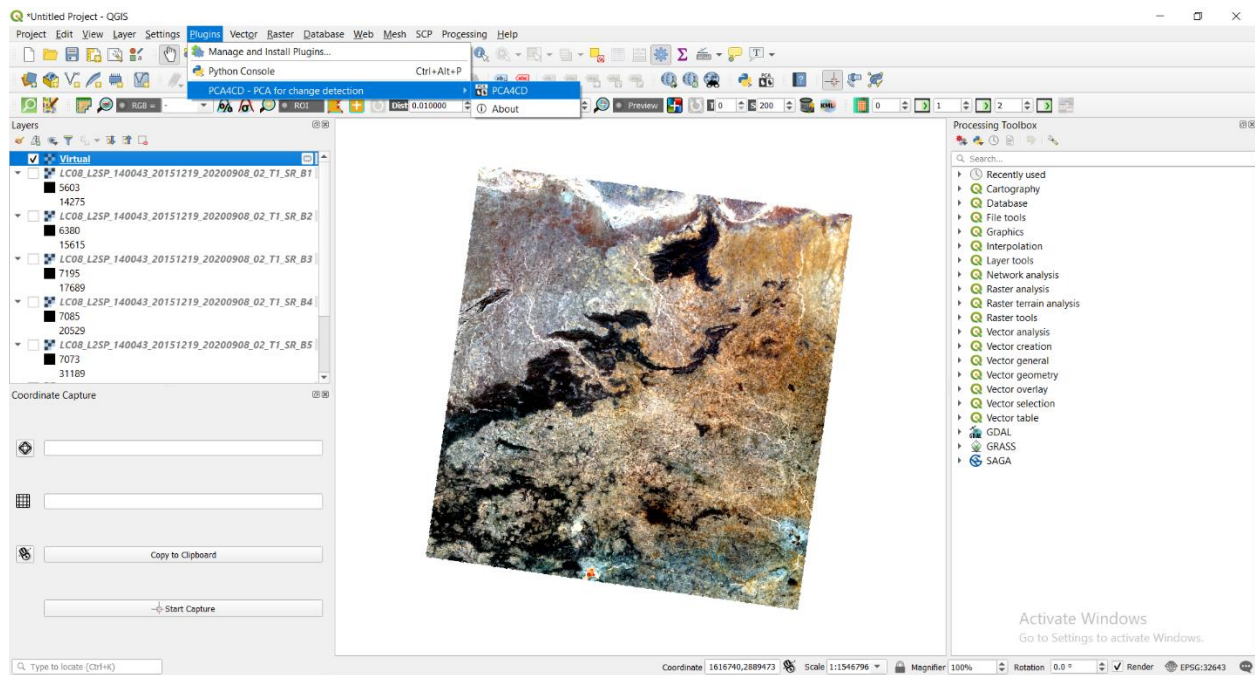


Search PCA in the search bar and install the plugin.





Step 5: Now again go to Plugins> PCC4CD.



The new PCA window will pop-up. In this choose the parameters like number of components, estimation matrix etc. Then click on “Compute Principal Components”.

PCA4CD - Compute or Load the PCA

Compute Principal Components

Compute the principal components based on one or two stacks.

Input Rasters Data

Select one or two (as reference and target) input layer. Each band in A (and B) is a variable for the PCA:

☒ A: Virtual ...

☐ B: ...

nodata value: 0.0

Principal Components

Number of components: 3

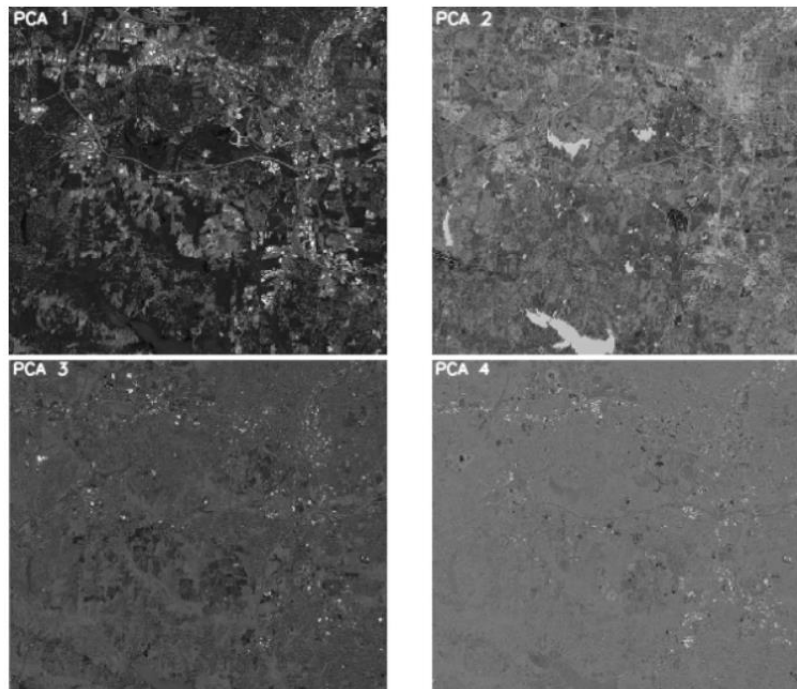
Estimator matrix: Correlation

☐ Process Settings

Compute Principal Components

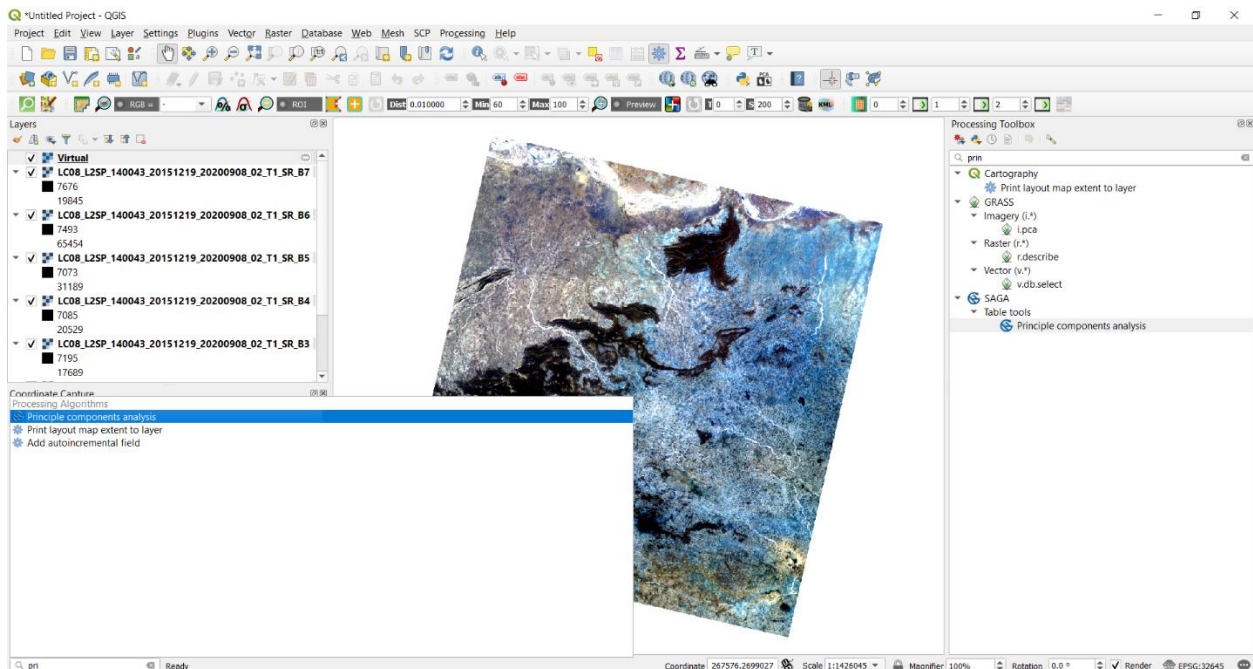
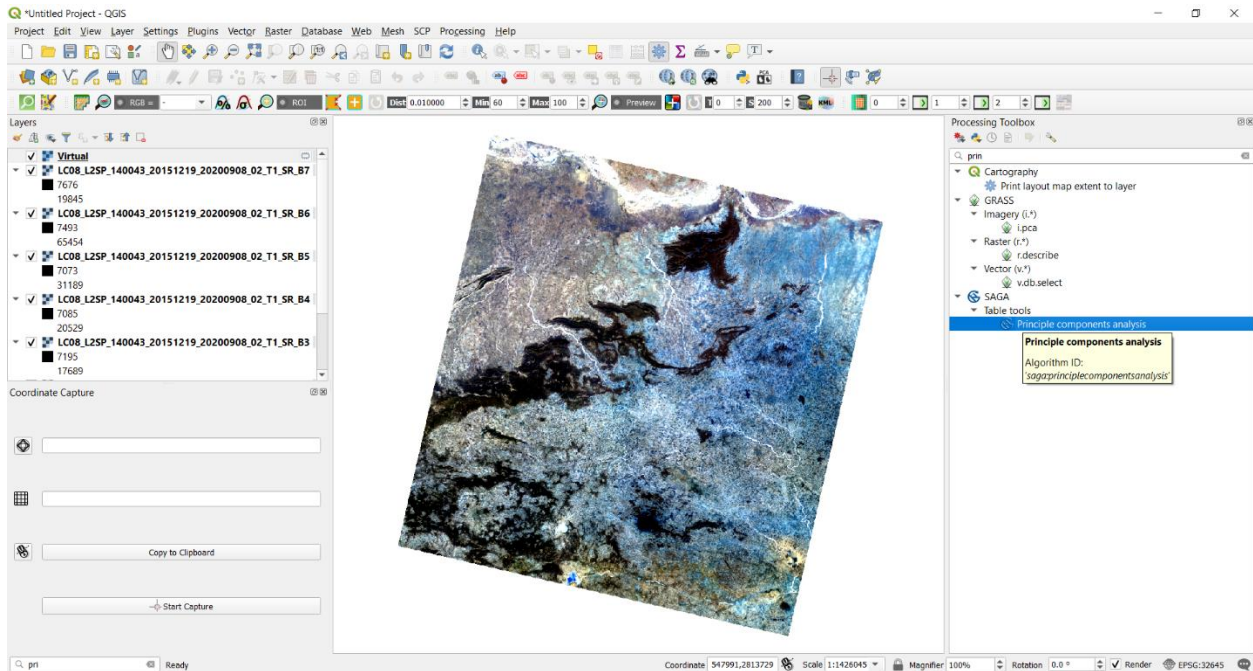
PCA v21.4.1 Docs Close

The PCA 1 to 4 will look like this.

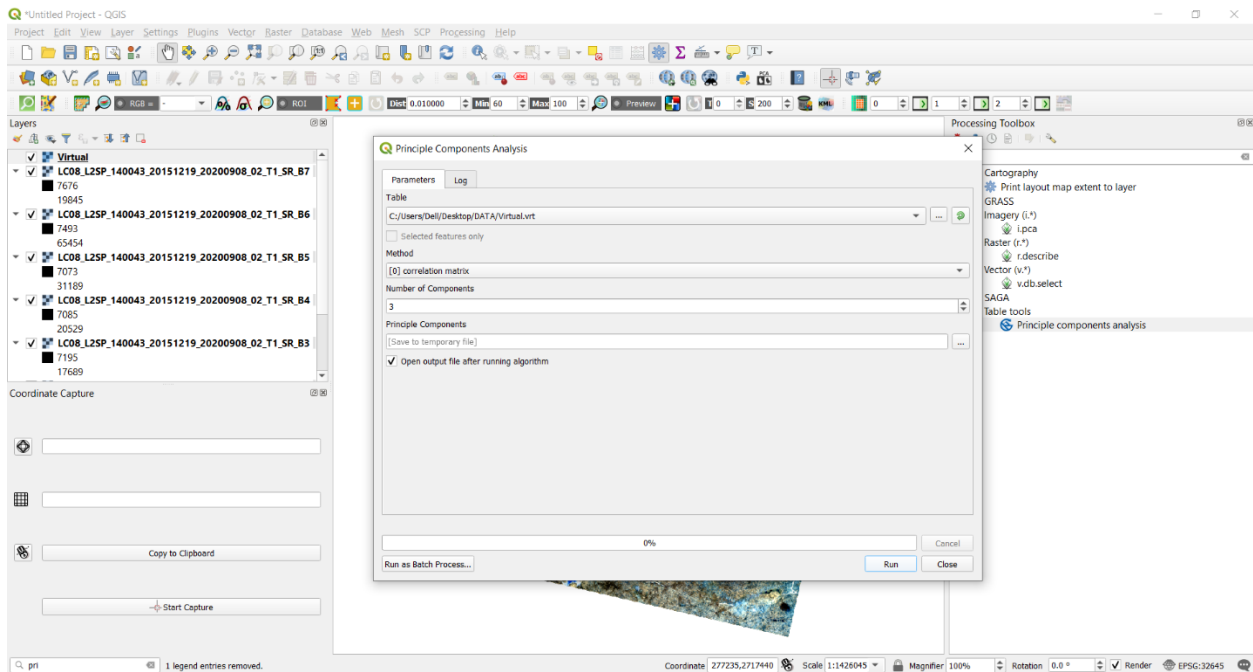


By searching PCA in the Search bar

Step 1: Perform the above steps from step 1 to step 3 to load the TCC of the image. Now click on Processing> Processing Toolbox and type PCA in the toolbox or you can directly search PCA in the search bar.



Step 2: A new PCA window will open. In this choose the input file, method and number of components you want and click on run.



Practical 7: Unsupervised Image Classification

To create a land use and land cover map of an area, we have to assign corresponding land use and land cover type to every pixel in the satellite imagery that exist at the time of acquisition. This is done based on the Digital Number (DN) values of the pixel which in turn represent the spectral properties of the ground surface. This assigning of classes to pixels in an image is called ‘Image classification’. More technically, it is an aspect of image processing in which quantitative decisions are made on the basis of the data present in the image, grouping pixels or regions of the image into classes representing different ground-cover types. The output of the classification stage may be regarded as a thematic map rather than an image (Rees, 1999).

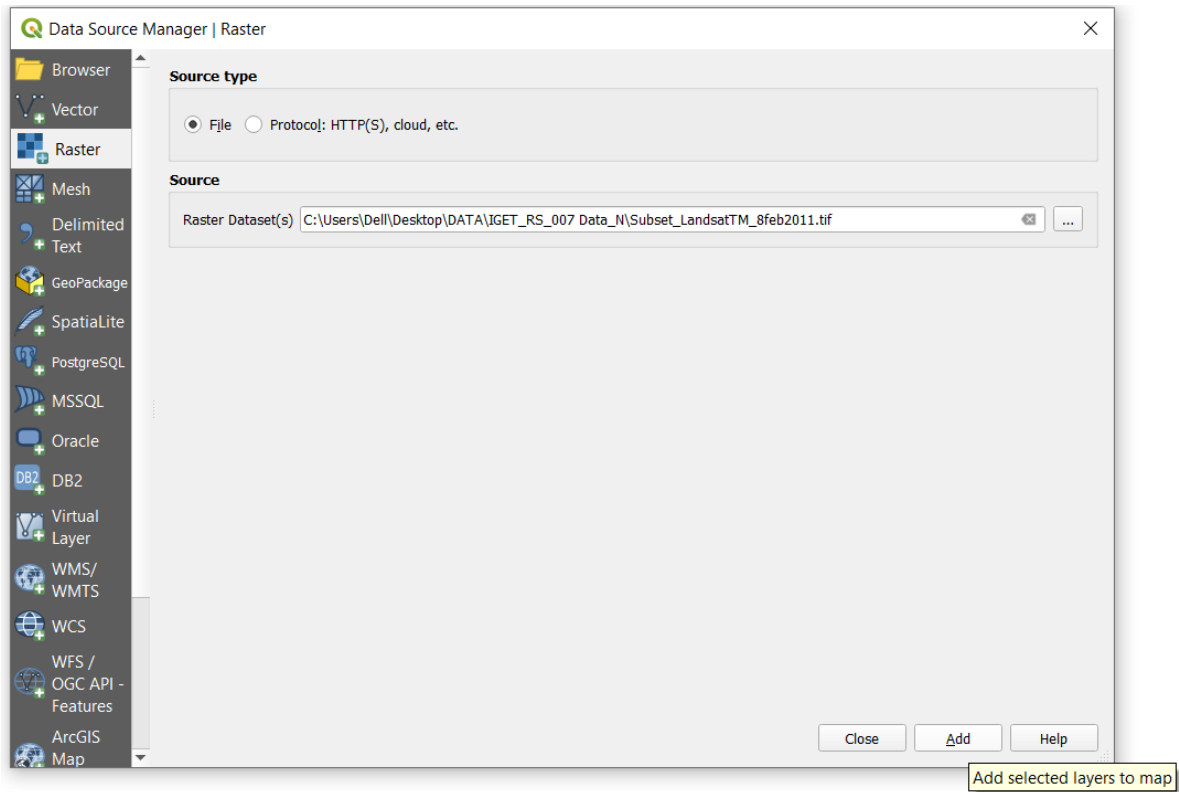
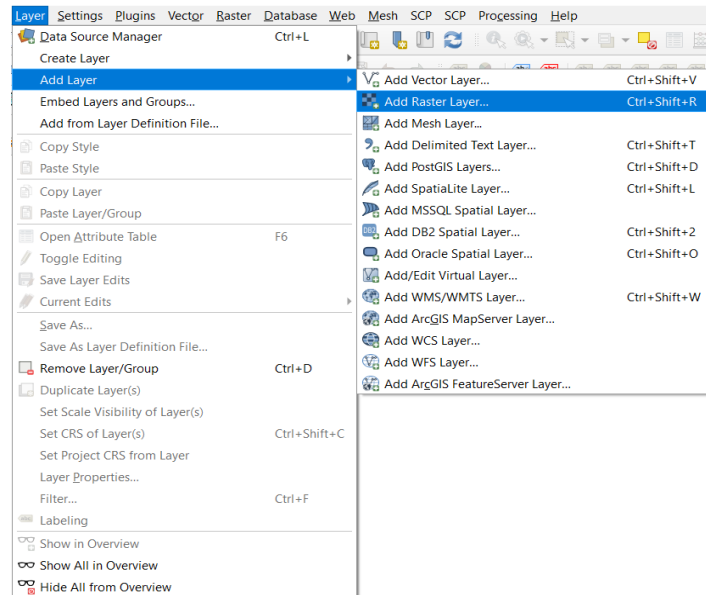
There are two broad types of image classification exists – ‘Supervised classification’ and ‘Unsupervised classification’.

In unsupervised classification, the algorithm analyzes all the bands of the image and pick out the clusters of pixels having similar values without the user intervention. The clusters are then assigned to their classes at the user’s discretion. Therefore, this method generally applied to the regions, where we don’t have any knowledge and information about land cover type.

In this practical, we will use a 7-band Landsat Thematic Mapper (TM) image to create a land cover map of Pune and its surrounding region. This Landsat TM imagery is downloaded from USGS earth explorer website: <http://earthexplorer.usgs.gov/>

Dataset Used: <https://dst-iget.in/index.php/tutorialdetails/2/9>

Step 1: Load the Landsat images into QGIS by clicking on the Layer> Add Layer> Add Raster Layer. Select the ‘Subset_LandsatTM_8feb2011.tif’ image and click on “Add”. This will import the image into QGIS.

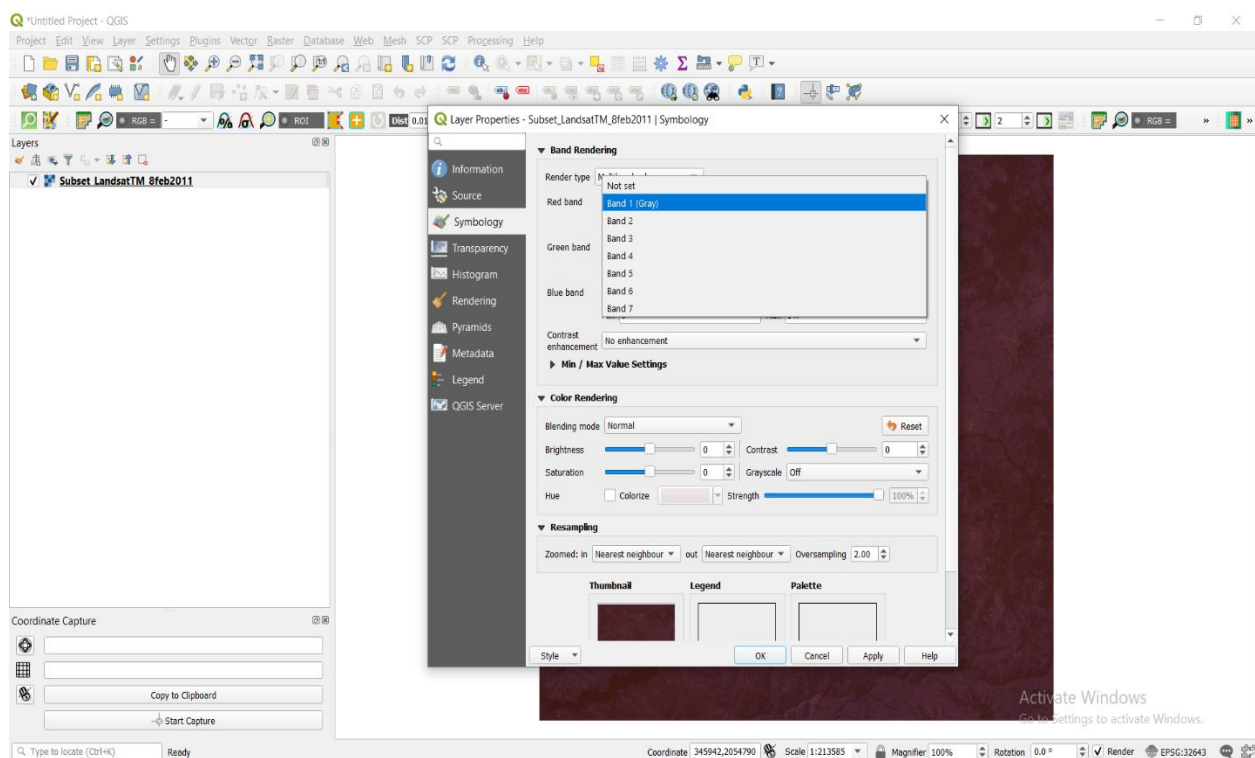


Step 2: The LandSat TM bands with their wavelengths and names are given below.

Band Number	Wavelength Range (nm)	Name
Band 1	0.45-0.52	Blue

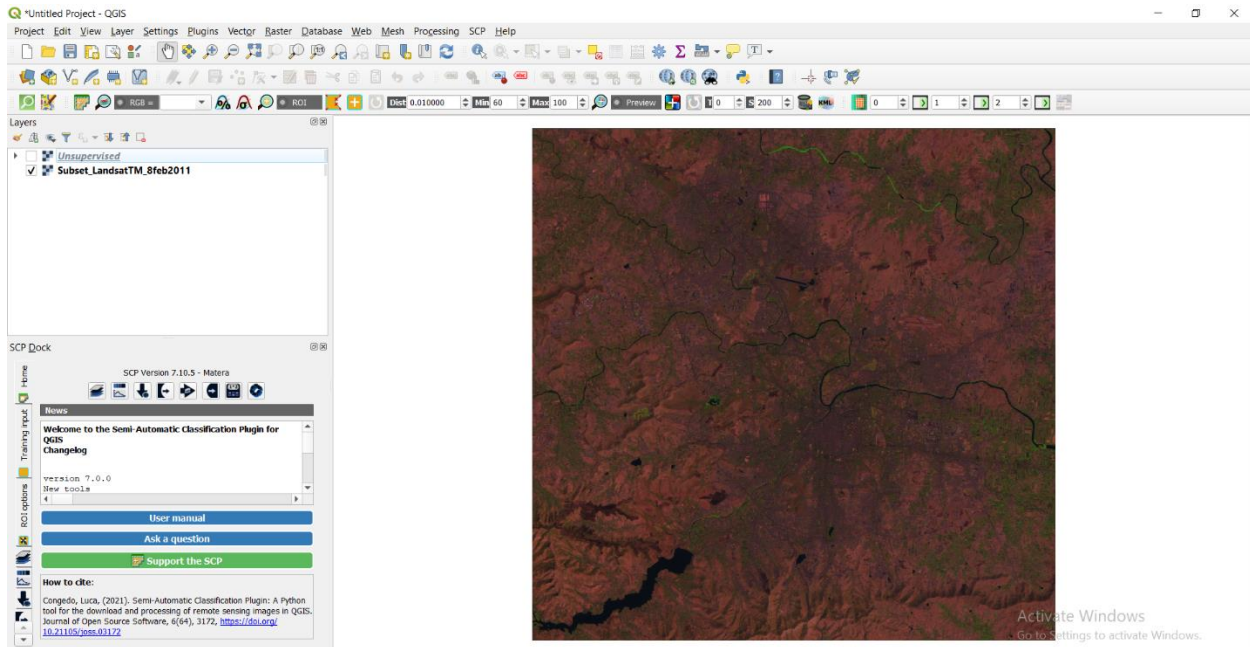
Band 2	0.52-0.60	Green
Band 3	0.63-0.69	Red
Band 4	0.76-0.90	Near Infra-Red
Band 5	1.55-1.75	Short Wave Infra-Red
Band 6	10.40-12.50	Thermal Infra-Red
Band 7	2.08-2.35	Mid Infra-Red

Step 3: We will change the composite band combination to True Color. Select “Subset_LandsatTM_8feb2011.tif” under Layers tab and right-click on it and choose ‘Properties’ option. In Layer Properties, navigate to ‘Symbology’ section, select the band combinations as shown in below figure and Click on ‘Apply’.

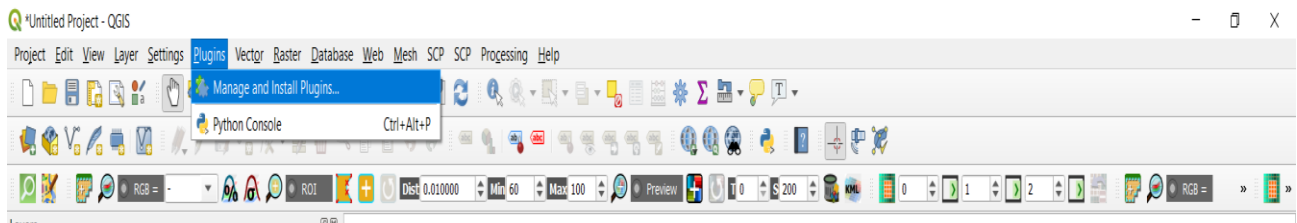


Step 4: The True Color Composite image would look like this.

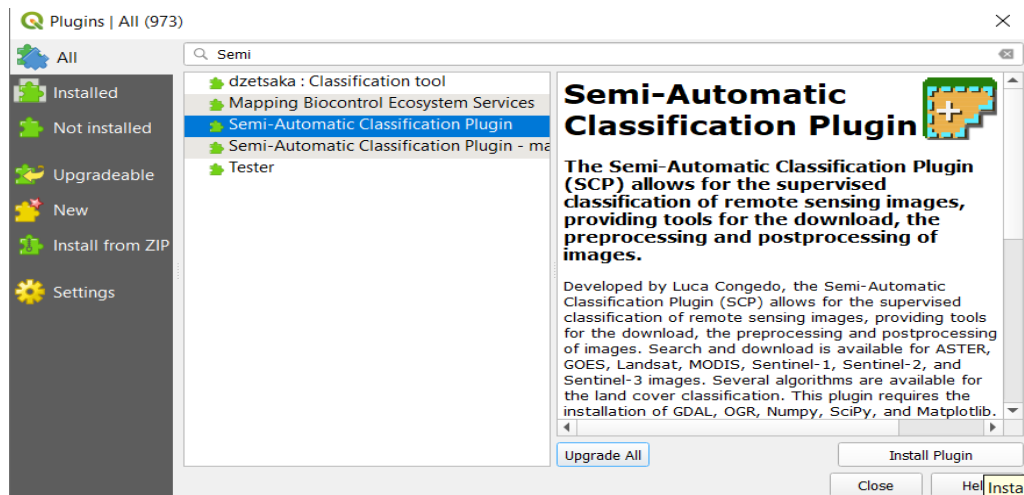
Similarly, we can visualize the study area by using False Color band combination and try to identify the land use and land cover in the false color composite by using visual interpretation elements.

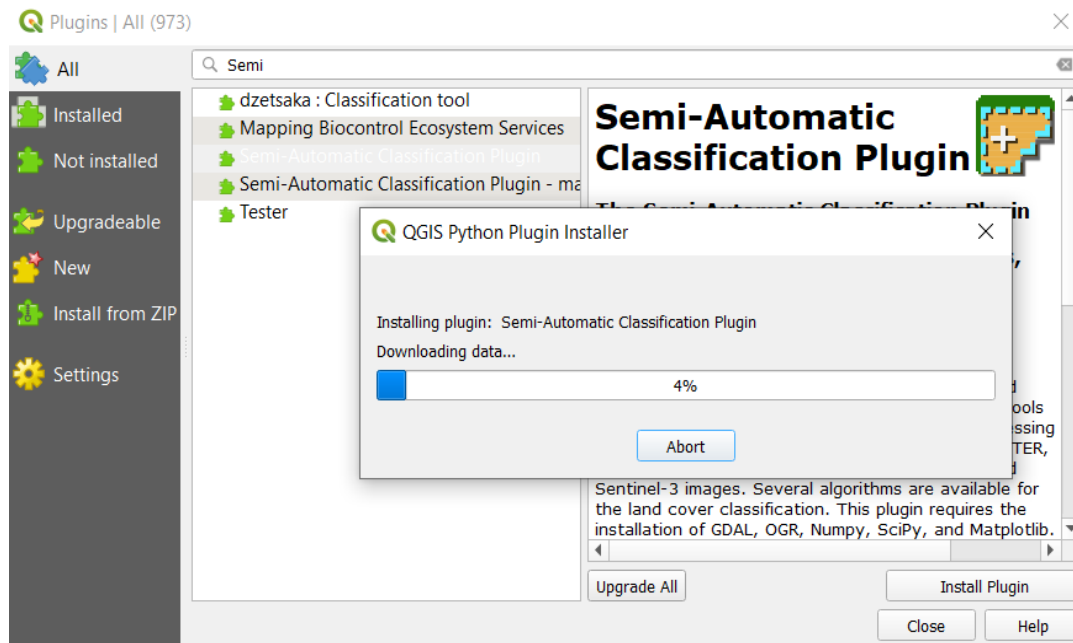


Step 5: For unsupervised classification, we will use “Semi- Automatic Classification Plugin”. To install the plugin click on Plugin> Manage and Install Plugin.

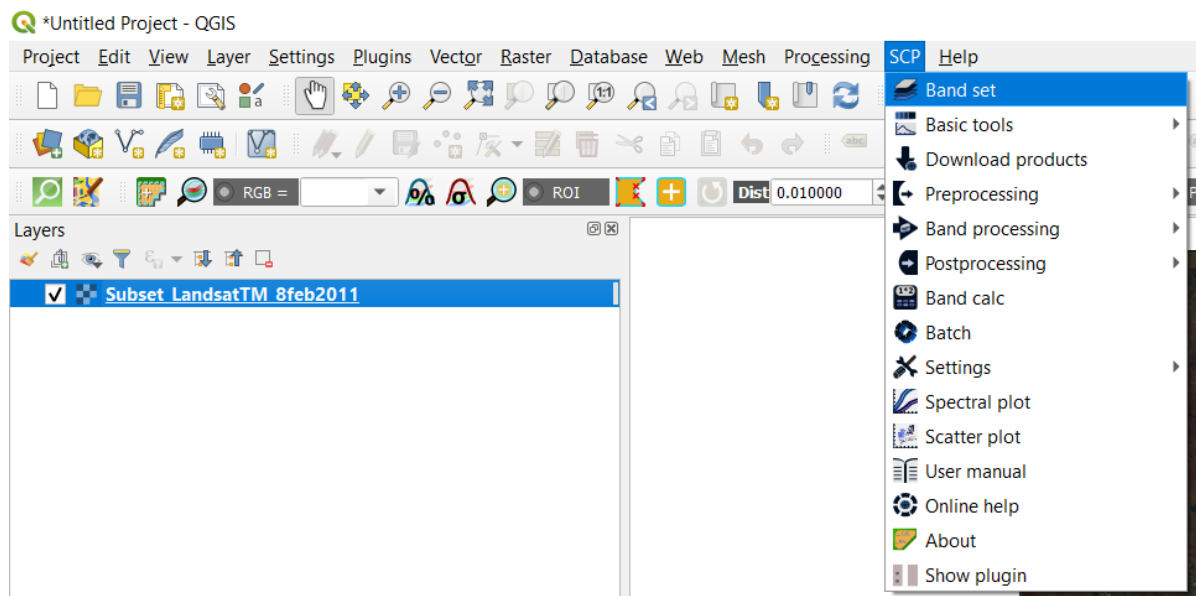


Step 6: The search for “Semi- Automatic Classification Plugin” and click on install.

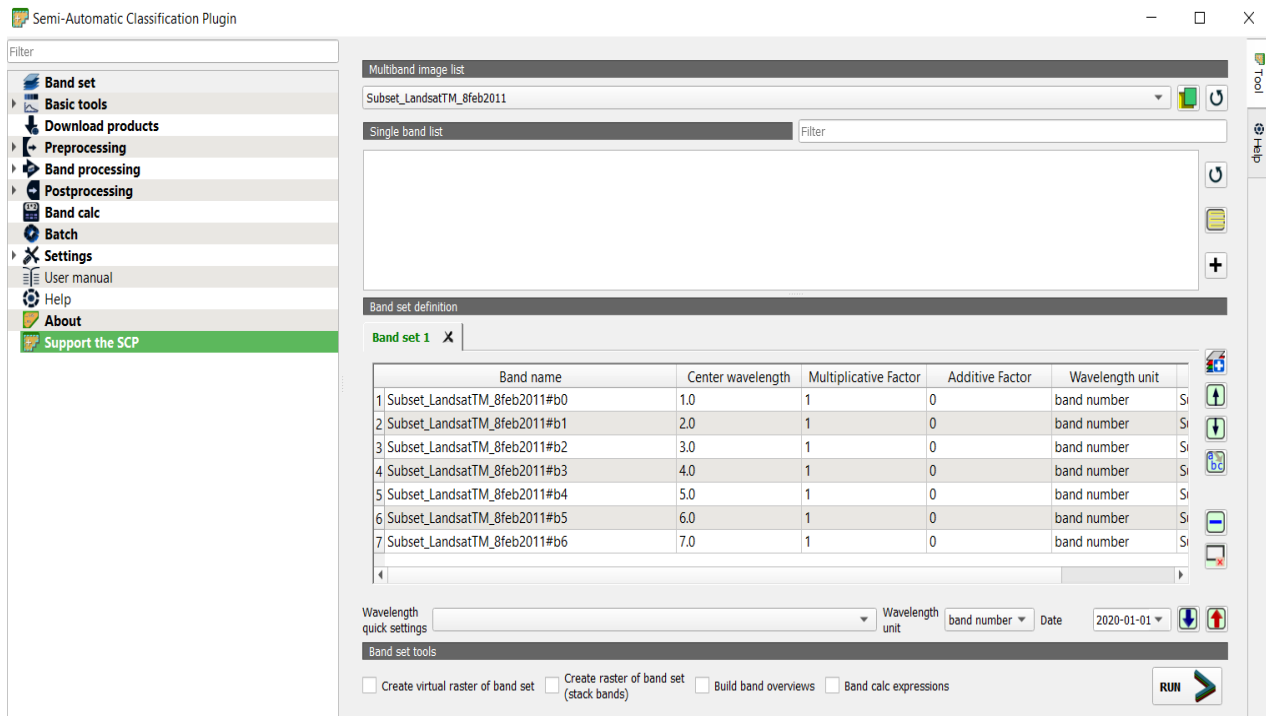




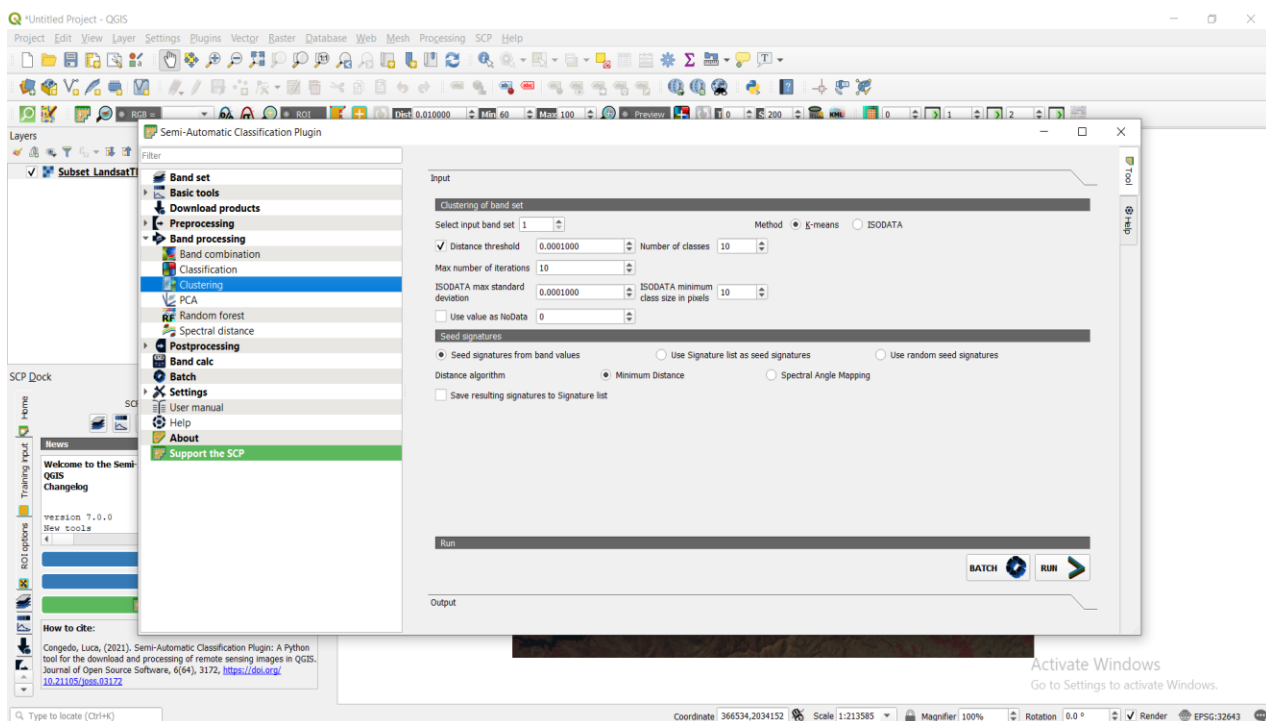
Step 7: From the toolbar click on SCP> Band set



Step 8: The Semi- Automatic Classification Plugin window will pop-up. In this choose the “Subset_LandsatTM_8feb2011” image in multiband image list.

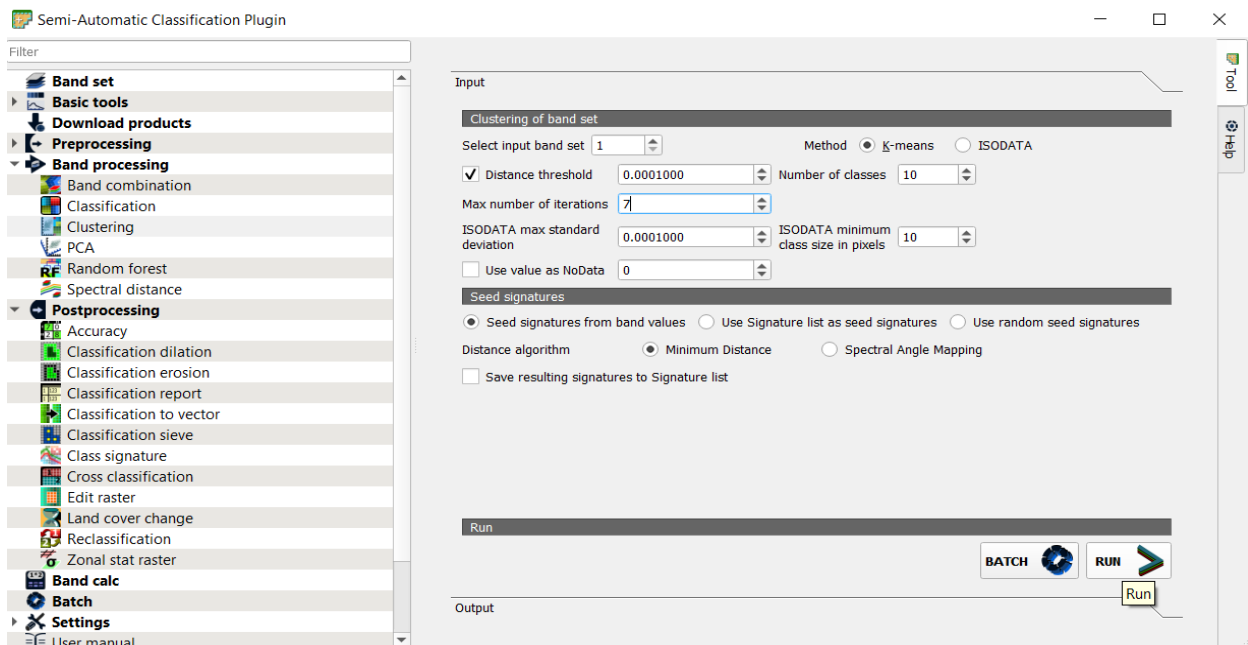


Step 9: Now click Band processing> Clustering

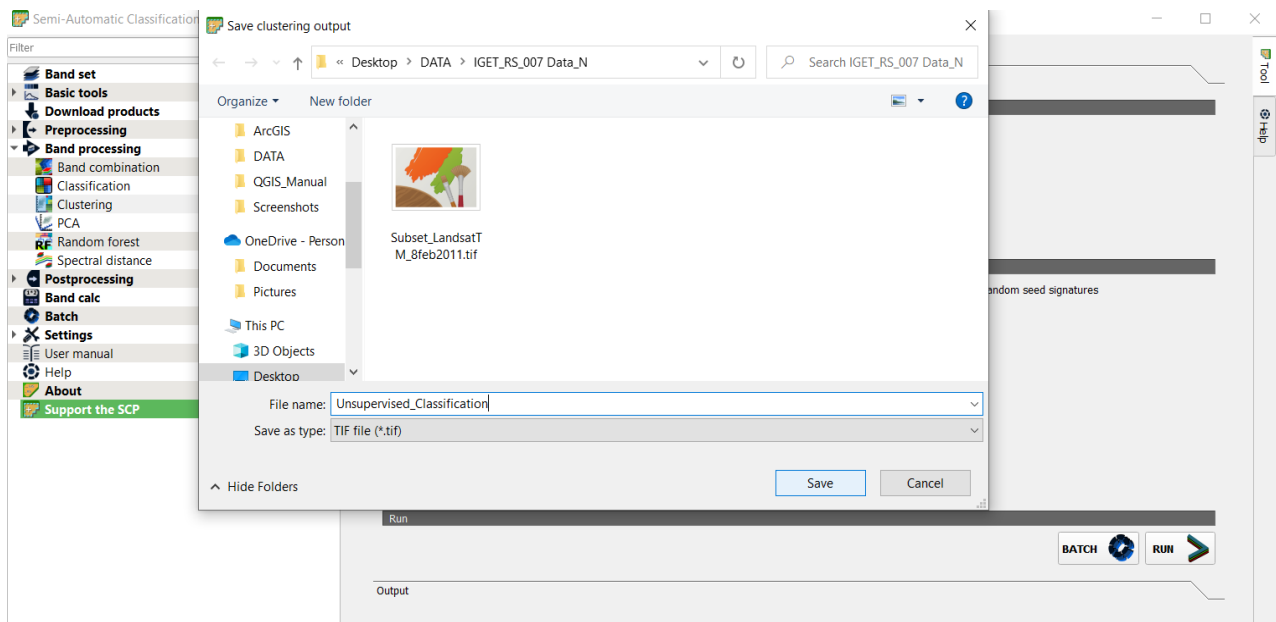


Step 10: There are two methods to perform unsupervised classification i.e. K-means and ISODATA. Here, we will use K-means technique.

In K-means, we need to choose the max. number of iterations and number of classes you want. After choosing these two things click on “Run”.



Step 11: Save the file where you wanted in .tiff format.



Semi-Automatic Classification Plugin

Filter

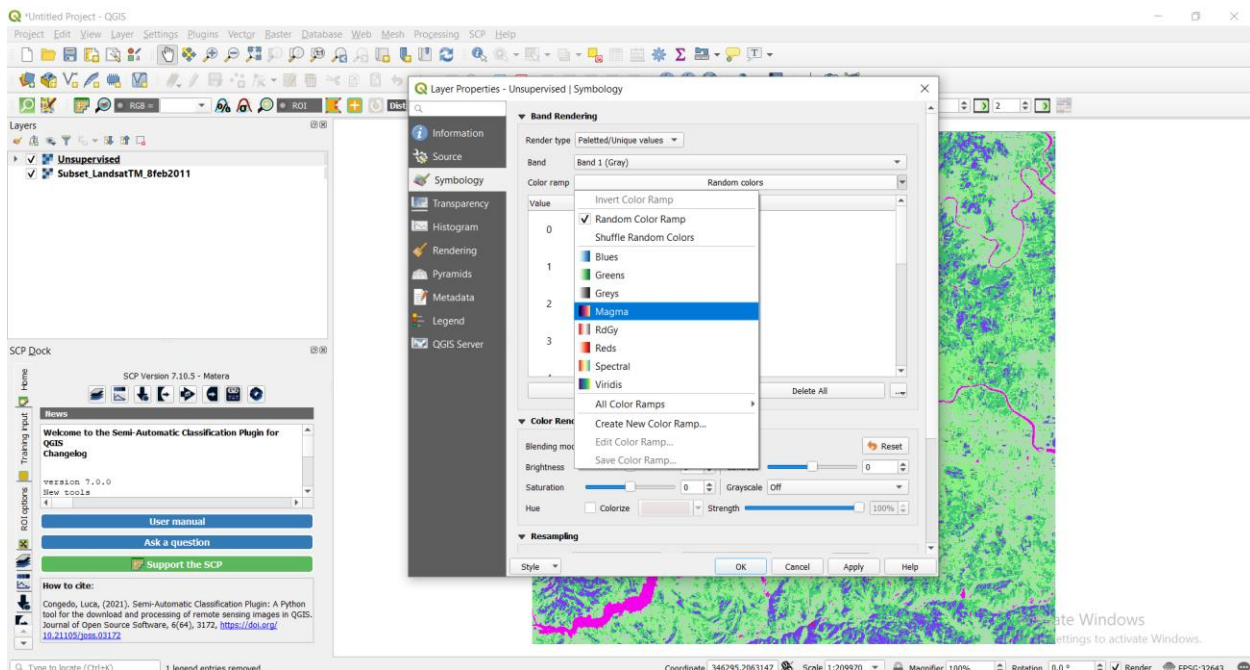
- Band set**
- Basic tools**
- Download products**
- Preprocessing**
 - Band processing**
 - Band combination
 - Classification
 - Clustering
 - PCA
 - Random forest
 - Spectral distance
 - Postprocessing**
 - Accuracy
 - Classification dilation
 - Classification erosion
 - Classification report
 - Classification to vector
 - Classification sieve
 - Class signature
 - Cross classification
 - Edit raster
 - Land cover change
 - Reclassification
 - Zonal stat raster
 - Band calc**
 - Batch**
 - Settings**
 - User manual

Input
Output

Clustering

Class	Signature	Distance
C_ID_1	0,0,0,0,0,0	0,0
C_ID_2	65.77799402238132,27.58514631264336,24.79665670396886,22.039758114964897,21.8358	
C_ID_3	71.28974100639627,32.308481532147745,33.7965231818686,45.54126150774577,59.99780	
C_ID_4	75.80823748696663,36.052318629957206,40.96192044315442,47.9484221078015,76.84553	
C_ID_5	77.24267855128748,38.037723713049814,46.134281176553074,53.00395984672741,95.929	
C_ID_6	78.43602574002574,39.7437323037323,50.74198198198198,59.042028314028315,114.7064	
C_ID_7	107.28232354778264,57.579637726420984,71.01124297314179,67.97876327295441,124.10	
C_ID_8	75.06060606060606,34.06060606060606,36.84848484848485,44.93939393939394,173.2424	
C_ID_9	0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0	0,0
C_ID_10	161.66666666666666,87.33333333333333,111.0,104.0,230.0,154.33333333333334,179.0	

Step 14: You can change the color by right-clicking on the unsupervised image in the Layers, then choose “Properties” option. In the Layer Properties choose “Symbology” option.



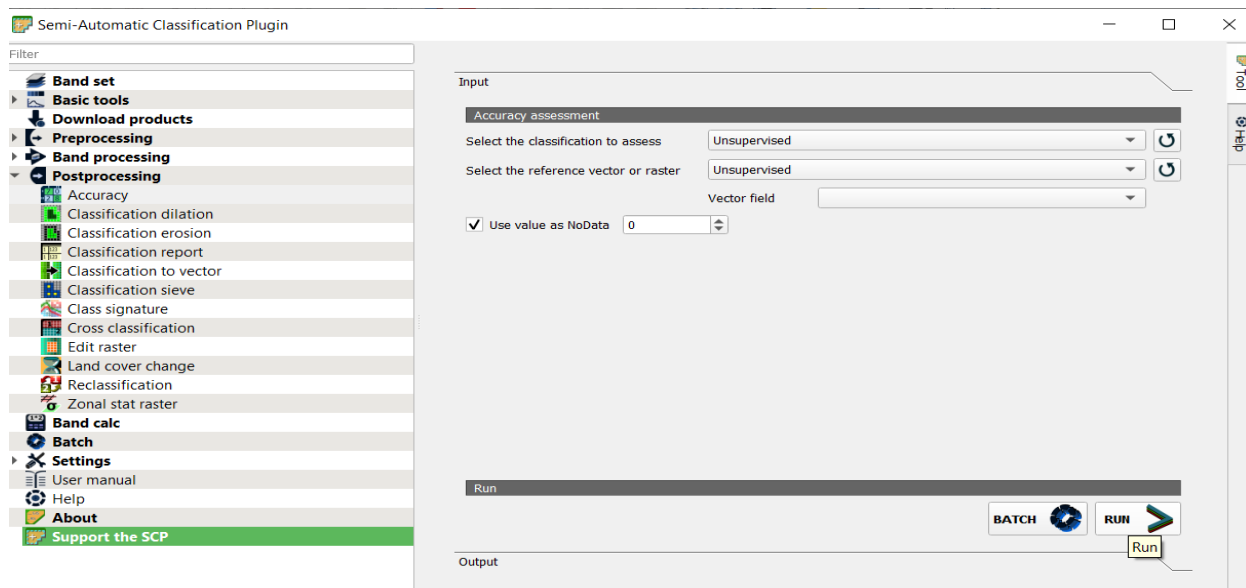
Accuracy Assessment

Thematic maps prepared by image classification will have some errors. The closely related classes are often misclassified. The process of accuracy assessment helps us to understand three most basic phenomenon: (i) The level of error in classification; (ii) Whether it is under acceptable percentage range & (iii) where the error is occurring. Based on these three decisions we can either reclassify the image or go on with the classification (if the error is under acceptable range).

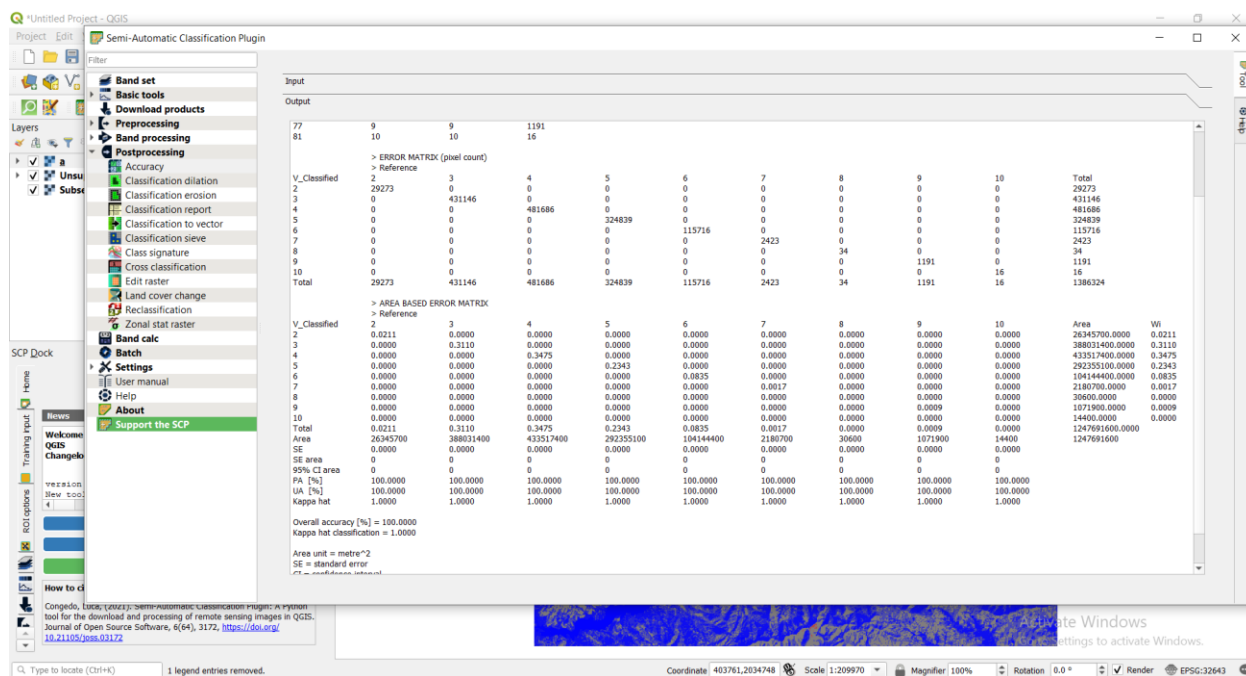
The process of accuracy assessment are as follows:

- Error Matrix preparation
- Calculation of Kappa coefficient; Overall Accuracy, Producers Accuracy, User Accuracy

Step 15: To get the confusion matrix and Khat (Kappa) index value, choose Postprocessing>Accuracy.



Step 16: Confusion matrix will then get drawn.



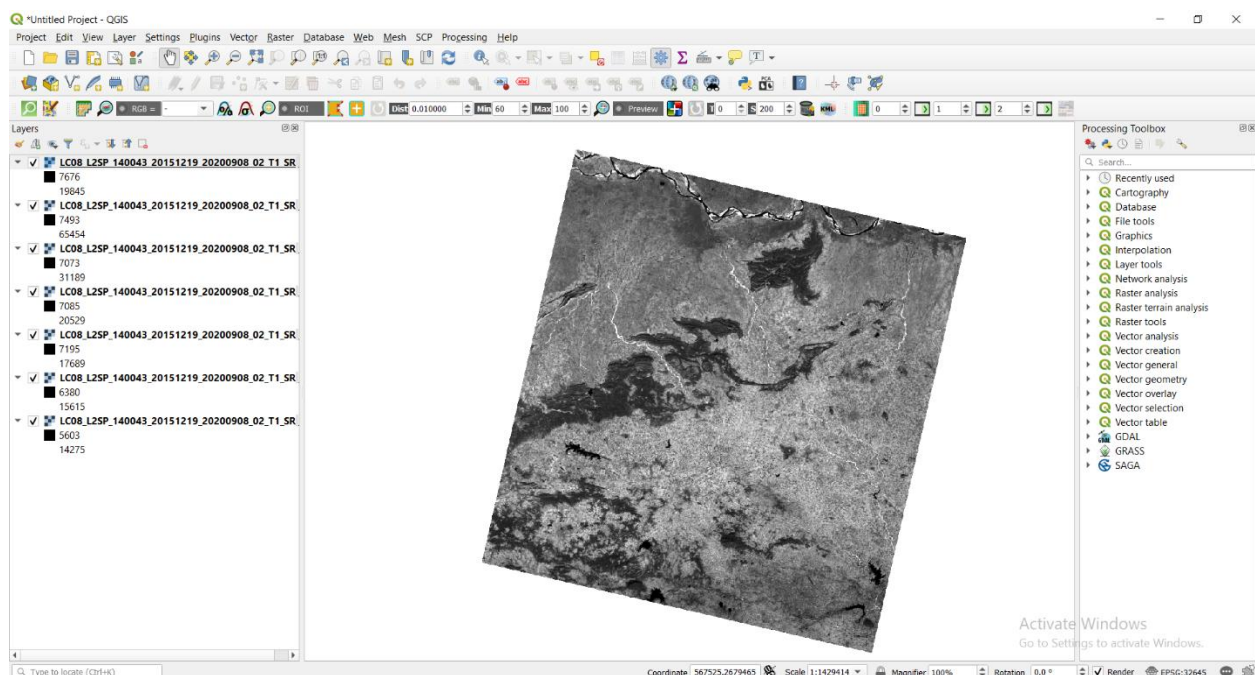
Practical 8: Supervised Image Classification, Accuracy Assessment and Change Detection

There are some limitations in using unsupervised classification method since we don't have full control over the computer's selection of pixel into clusters. In supervised classification, the user will select a group of pixels belongs to a particular land use / land cover known as training areas or training sites. Based on the pixel values in the training areas the software will create spectral signatures and the statistical information like range, mean, variance etc., of all classes in relation to all input bands. This information has been used to categorize each and every pixel in the image into corresponding land use and land cover class based on the classification algorithm used. Maximum Likelihood (ML), Minimum Distance to Mean (MDM) and Parallelepiped classification algorithms are most commonly used for supervised classification.

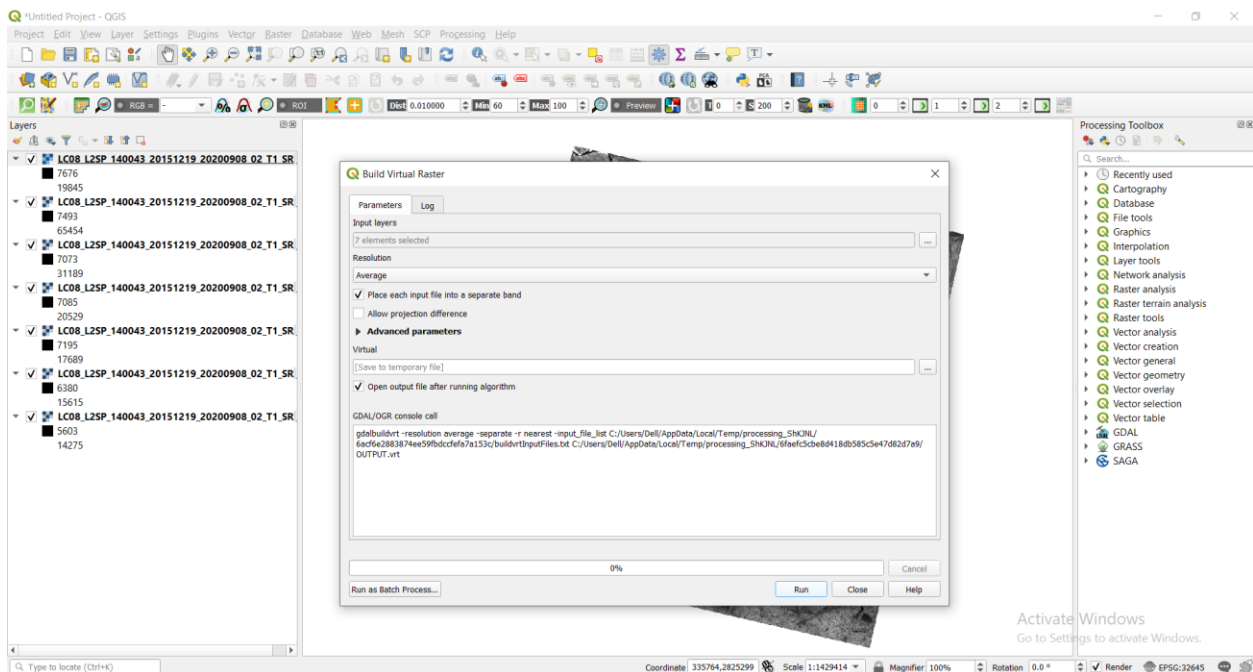
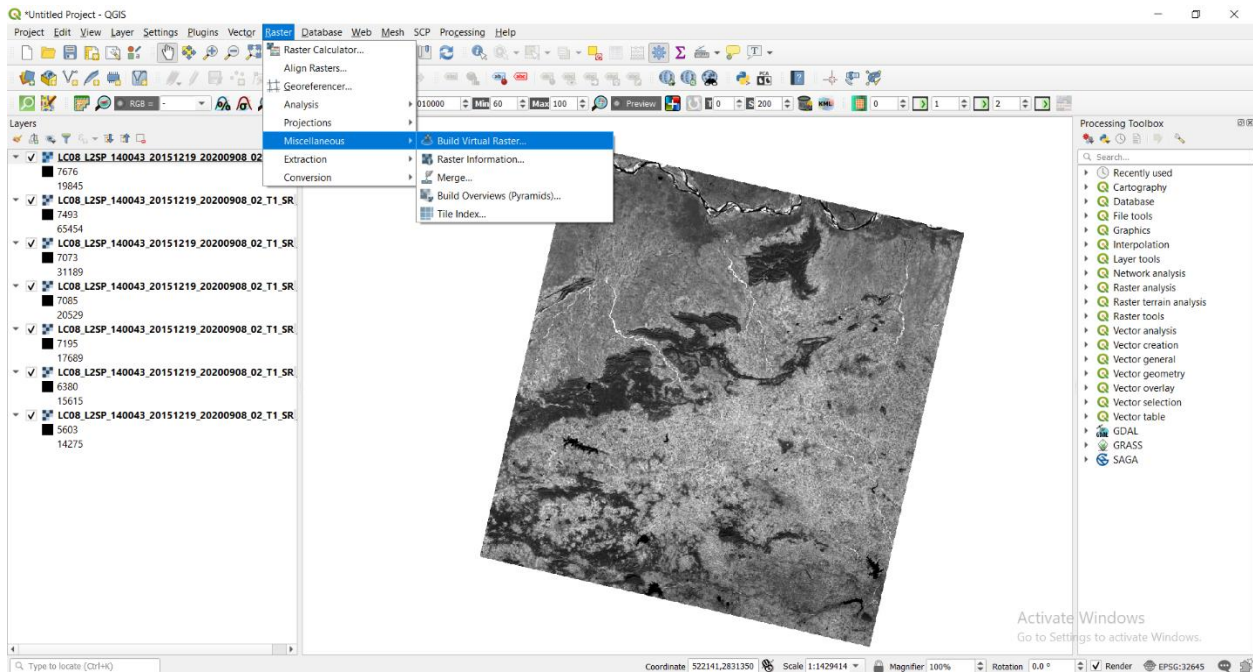
Since, the supervised classification method involves selection of training areas, we should have a good idea about different land cover classes existing in the study area. This knowledge can be acquired through field verification and other ancillary data.

Plugin needed to install: Semi-automatic classification plugin (SCP)

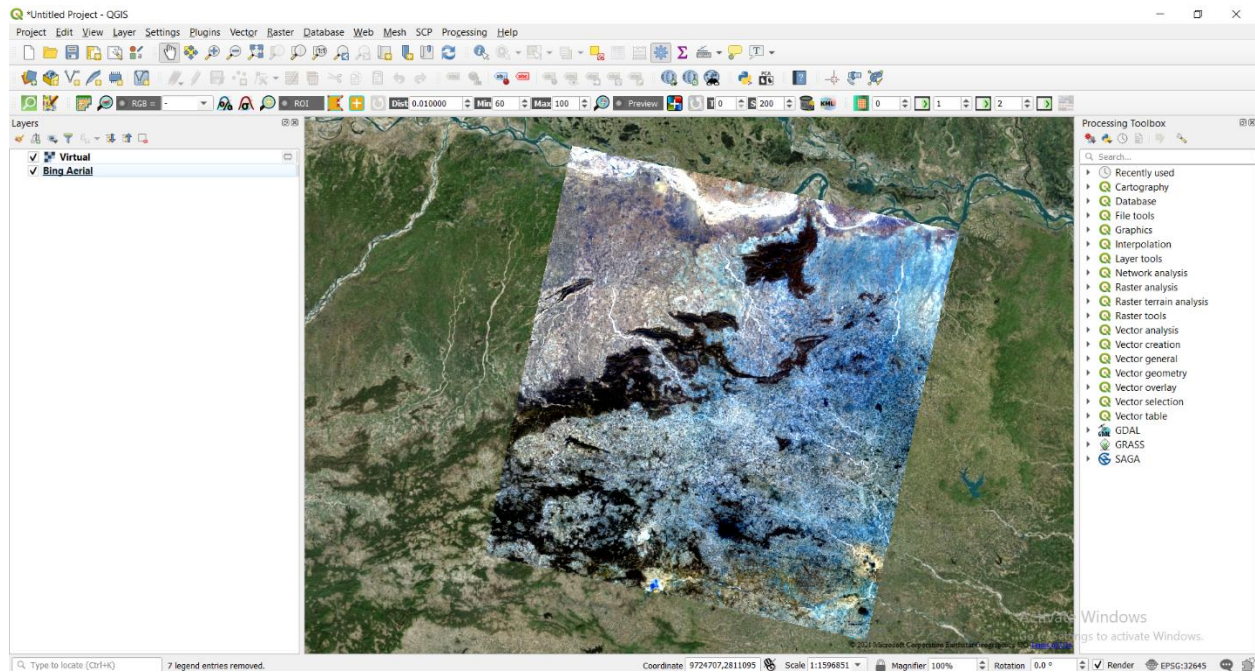
Step 1: Load the data by clicking on Layer> Add Layer> Add Raster Layer.



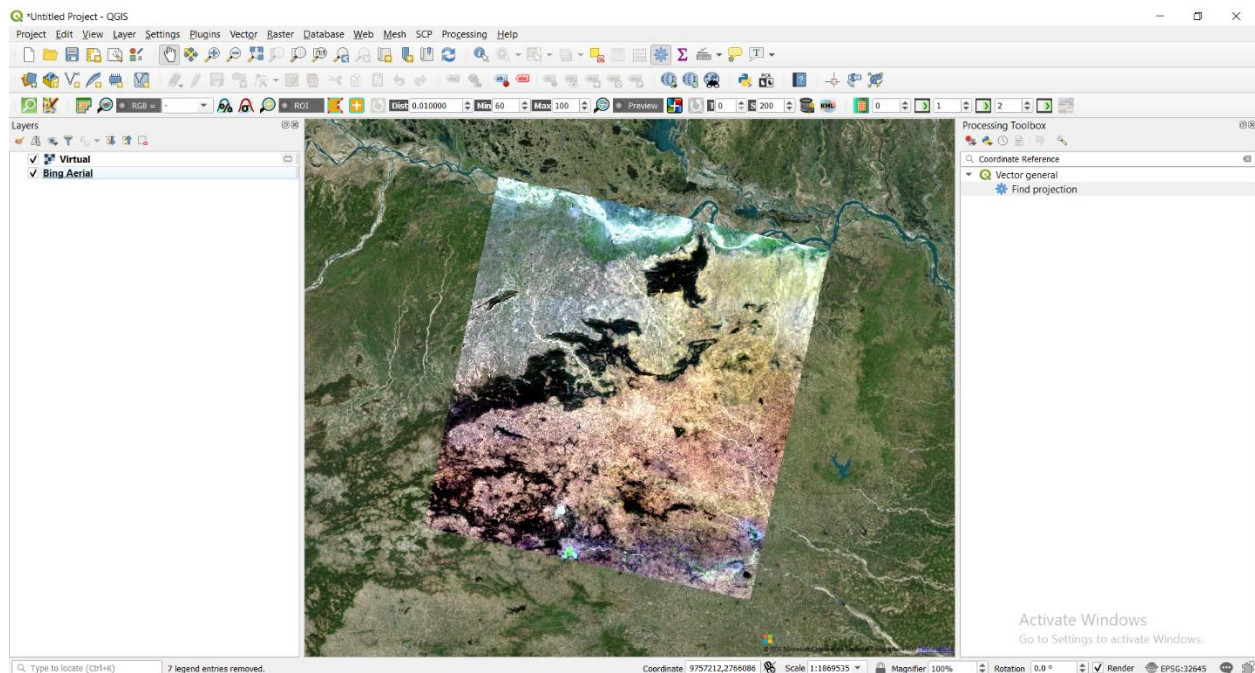
Step 2: To create a Virtual Raster, click on Raster> Miscellaneous> Build Virtual Raster. Select all the bands and click on “Run”.



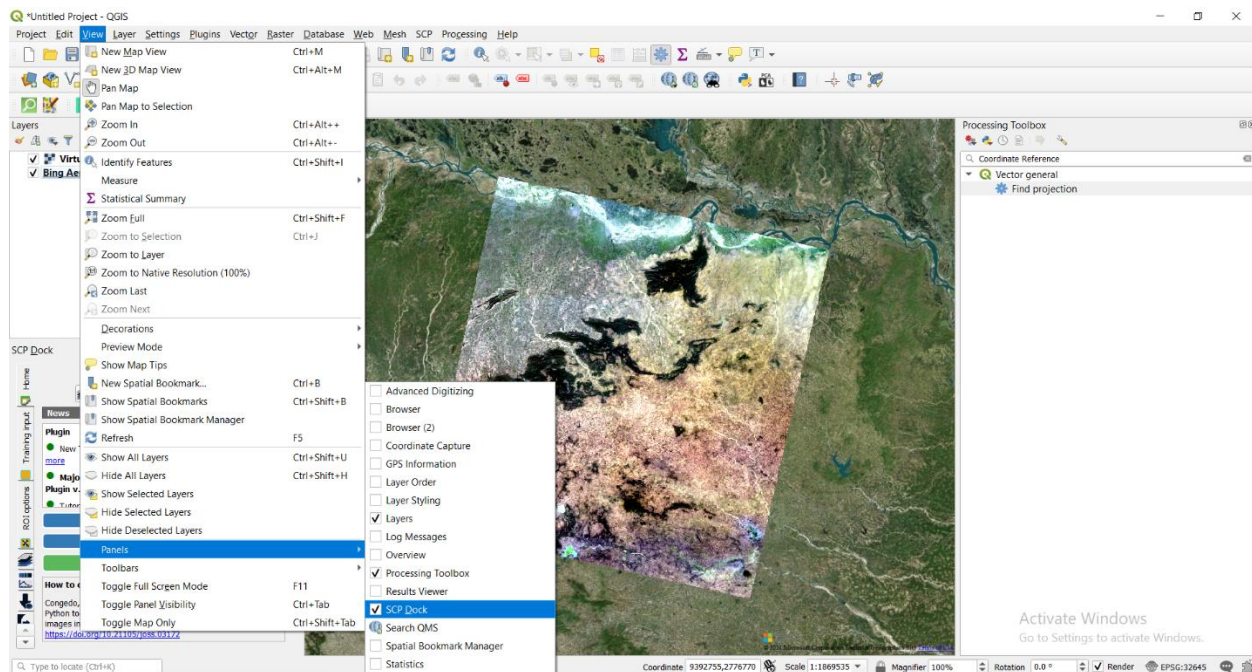
Step 3: Add the basemap to know about the classes more easily. We can add the basemap by clicking Web> OSM> Bing> Bing Aerial



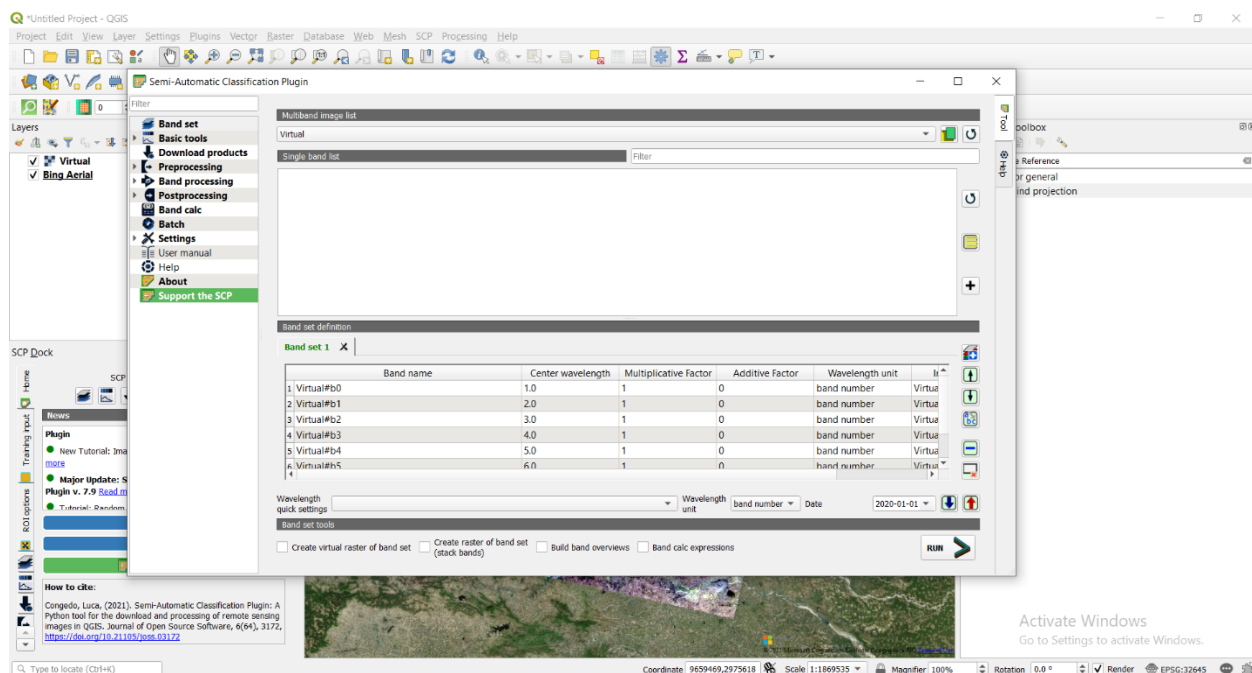
The FCC of the data is as follows.



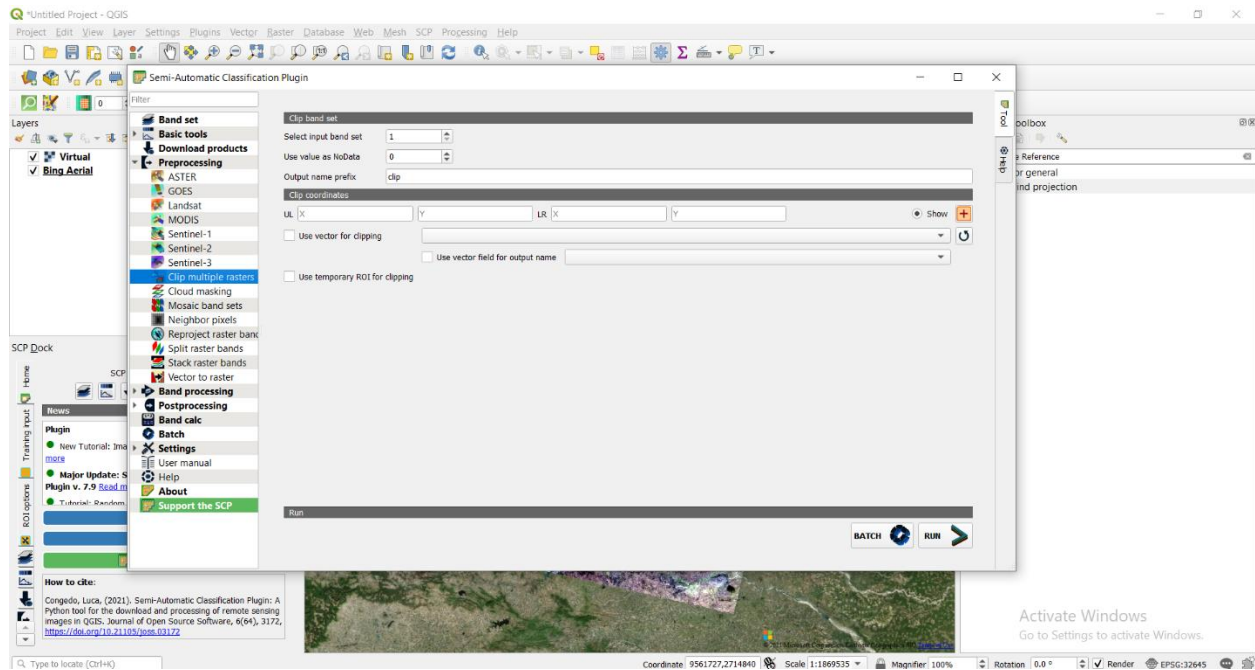
Step 4: Semi-automatic Classification Plugin needed to be added in the panels as well as in the toolbars. To add the SCP in the panel click on View> Panels> SCP Dock.



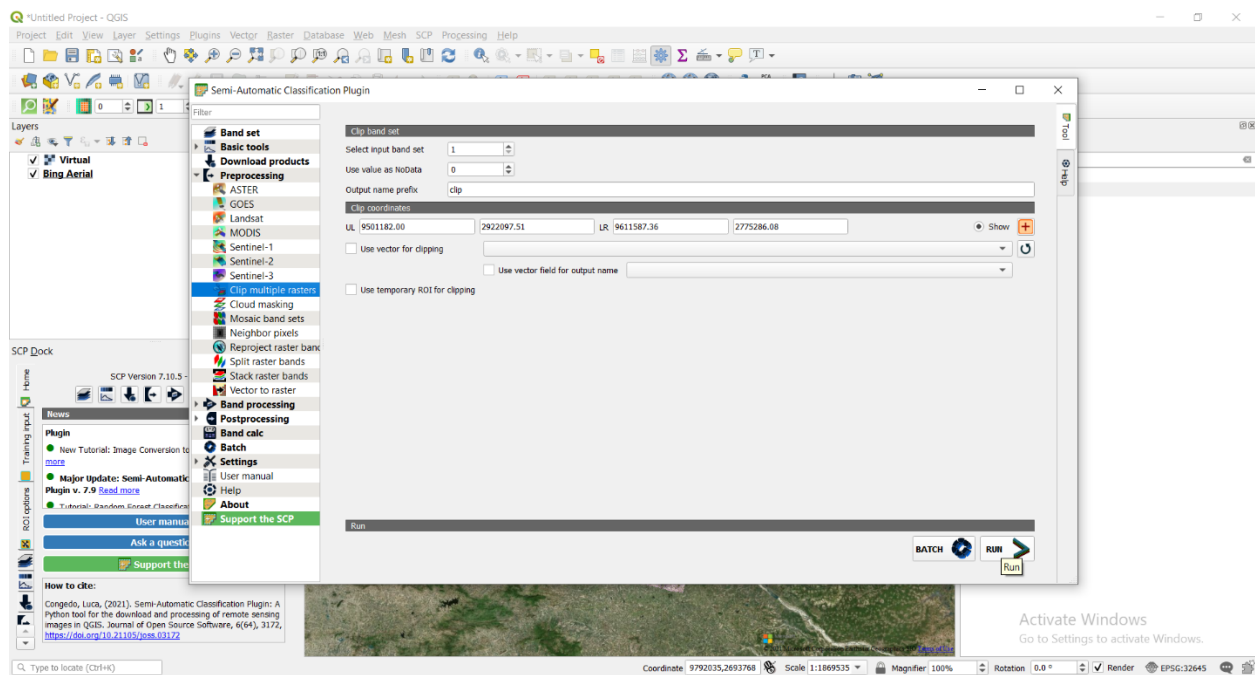
Then click on SCP> Band Set add the bands.



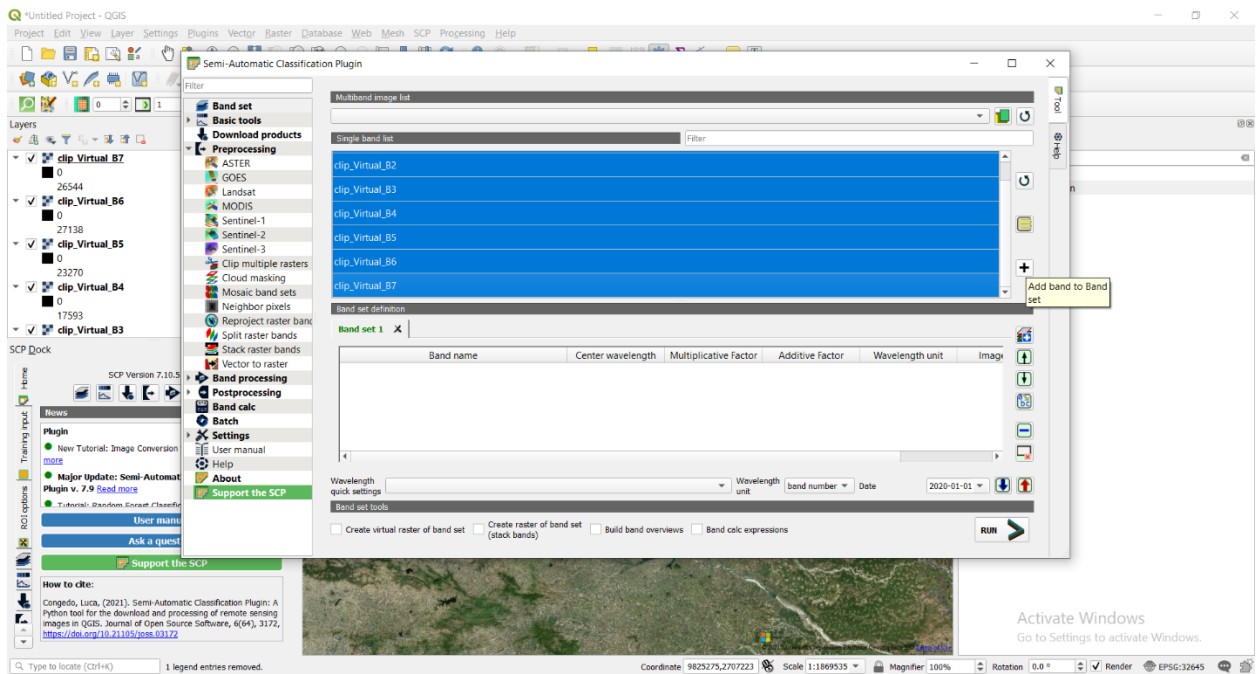
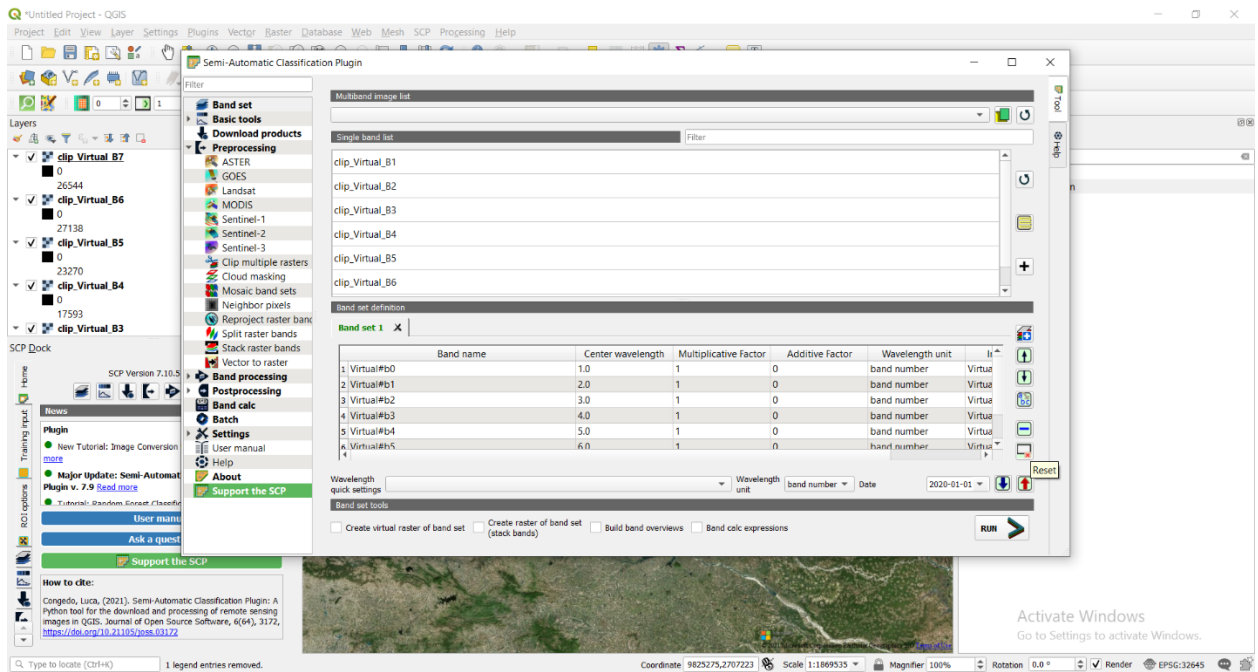
Step 5: To clip the area from the whole click on Preprocessing> Clip Multiple Raster. After choose the area you want to clip from the whole area.

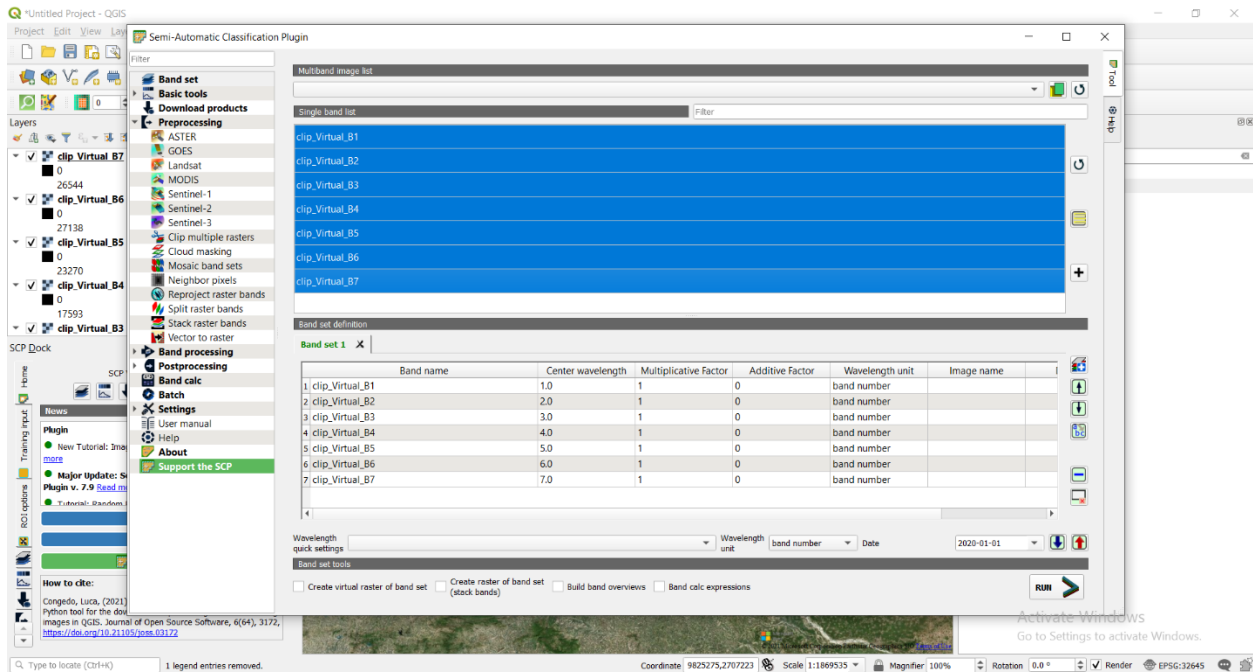


When we clip the area, the coordinates will automatically get added on the UL and LR. Then click on “Run”.

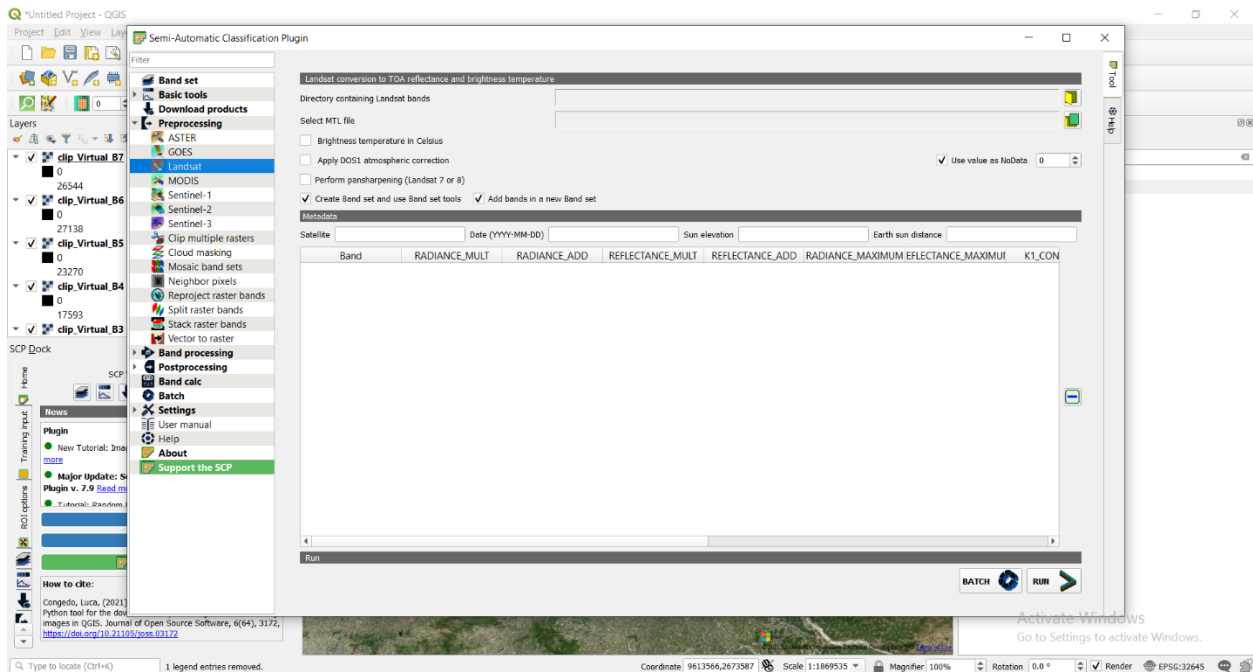


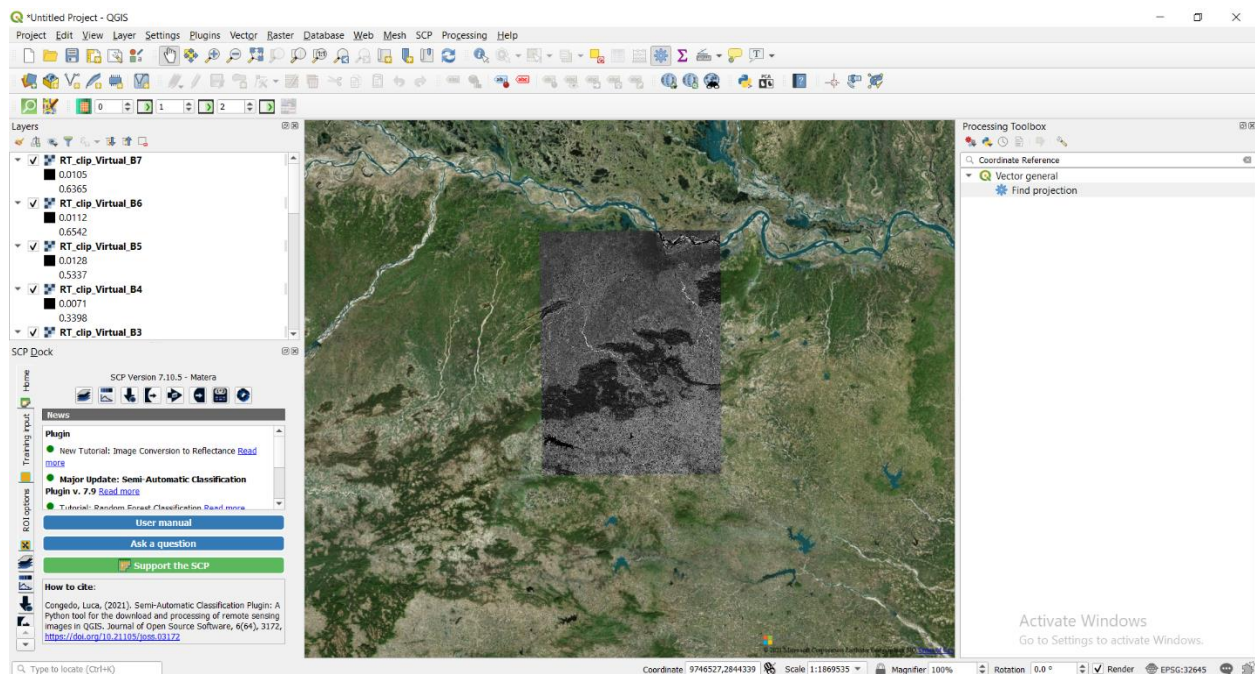
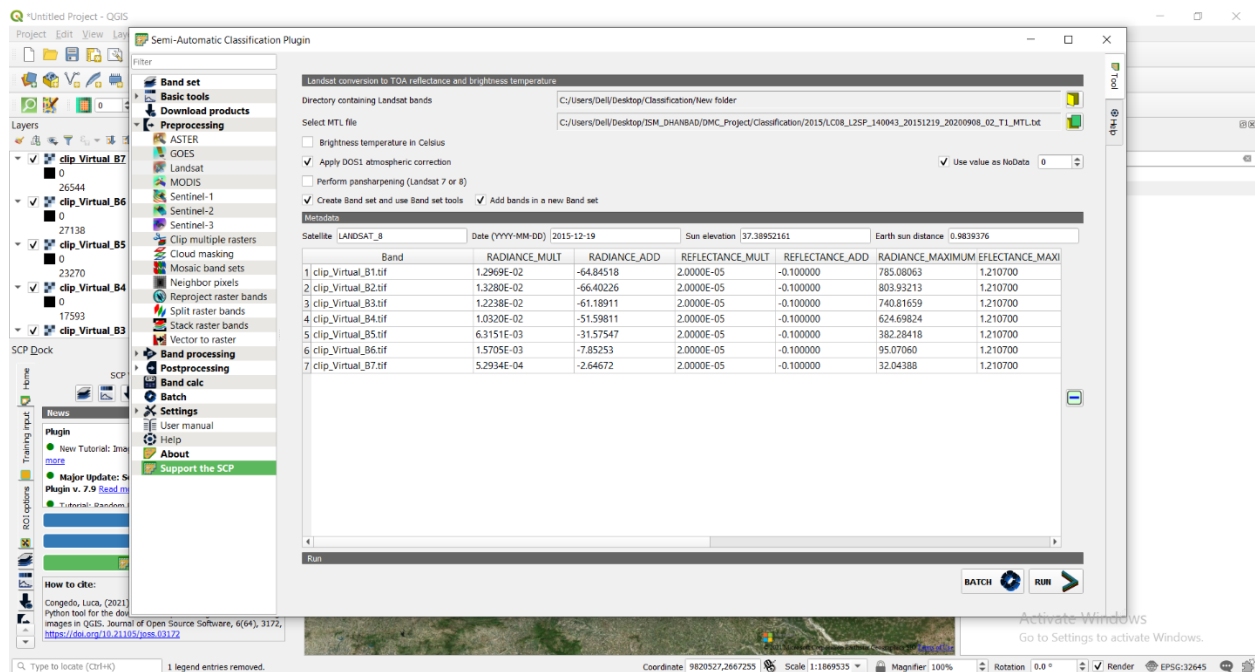
Step 6: Now the virtual bands which we have added earlier are of no use. We can remove that from the layers. Now we will add the clip_virtual bands and will work on that.



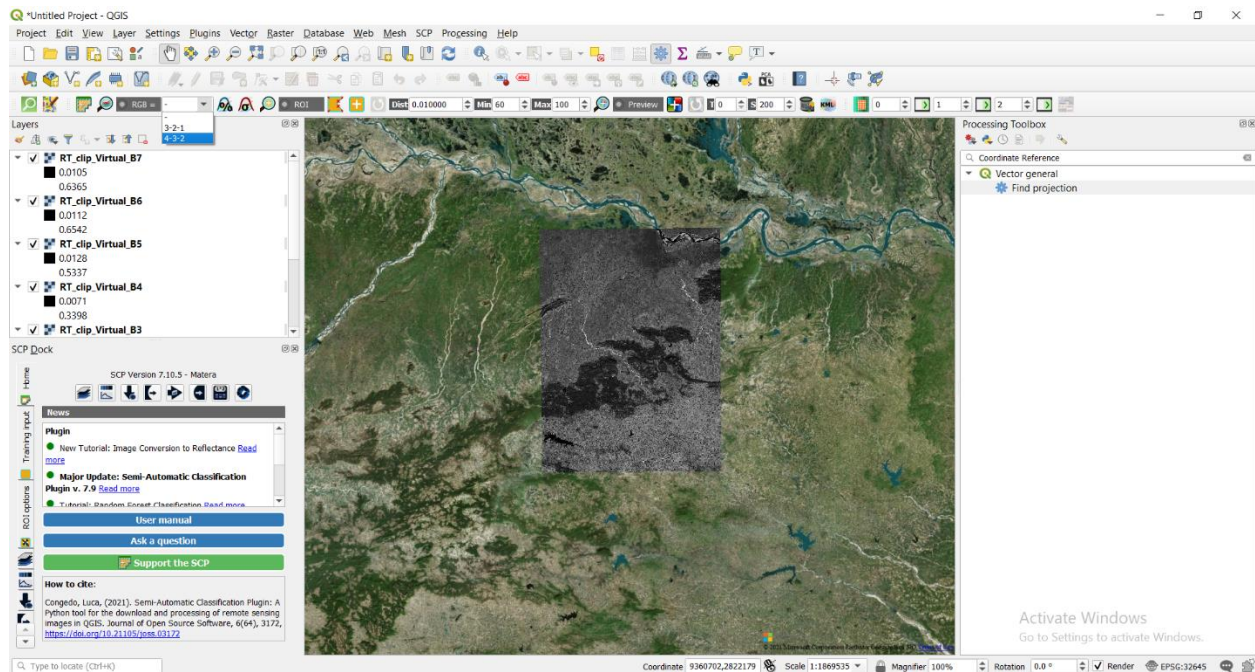
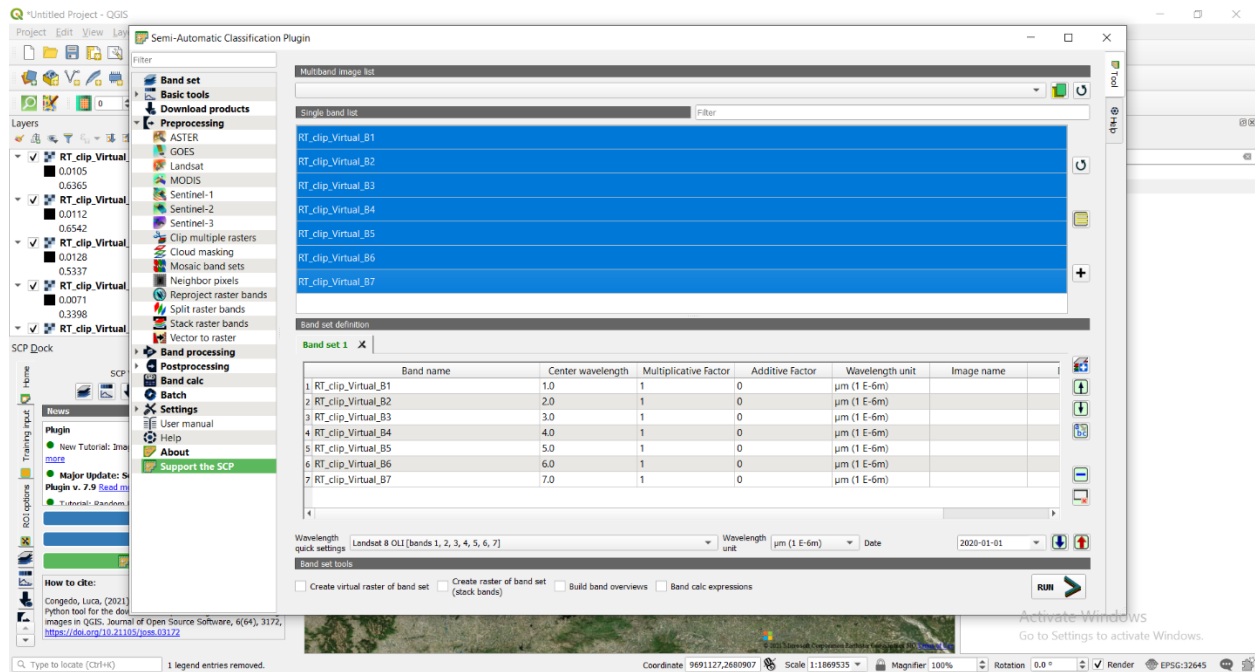


Step7: We need to preprocess the data i.e. atmospheric and geometric correction click on SCP> Preprocessing> Landsat and choose the clip bands and choose the atmospheric correction option then click on “Run”.

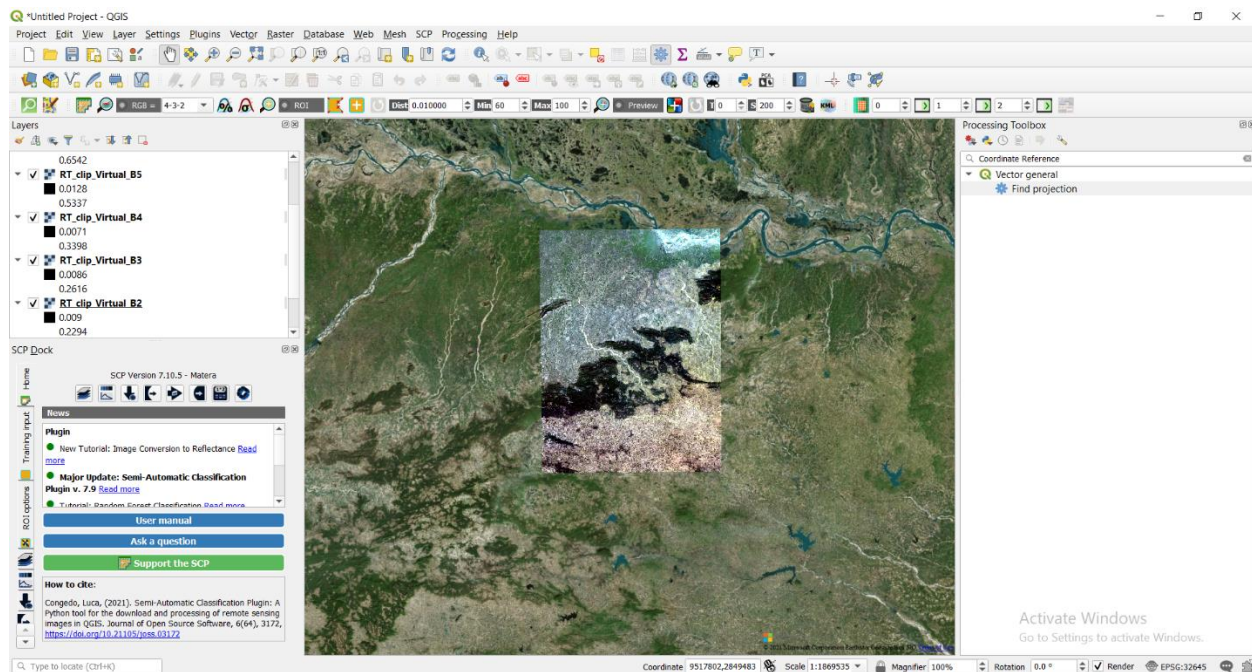




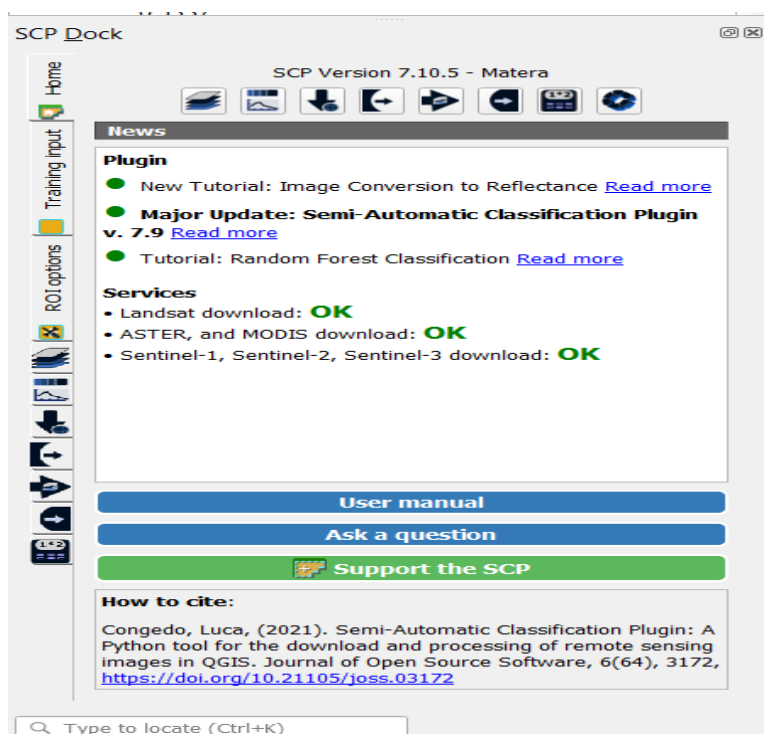
Step 8: Now again we will remove the clip_virtual bands from the bandset and add the RT_clip_virtual bands which are corrected bands.



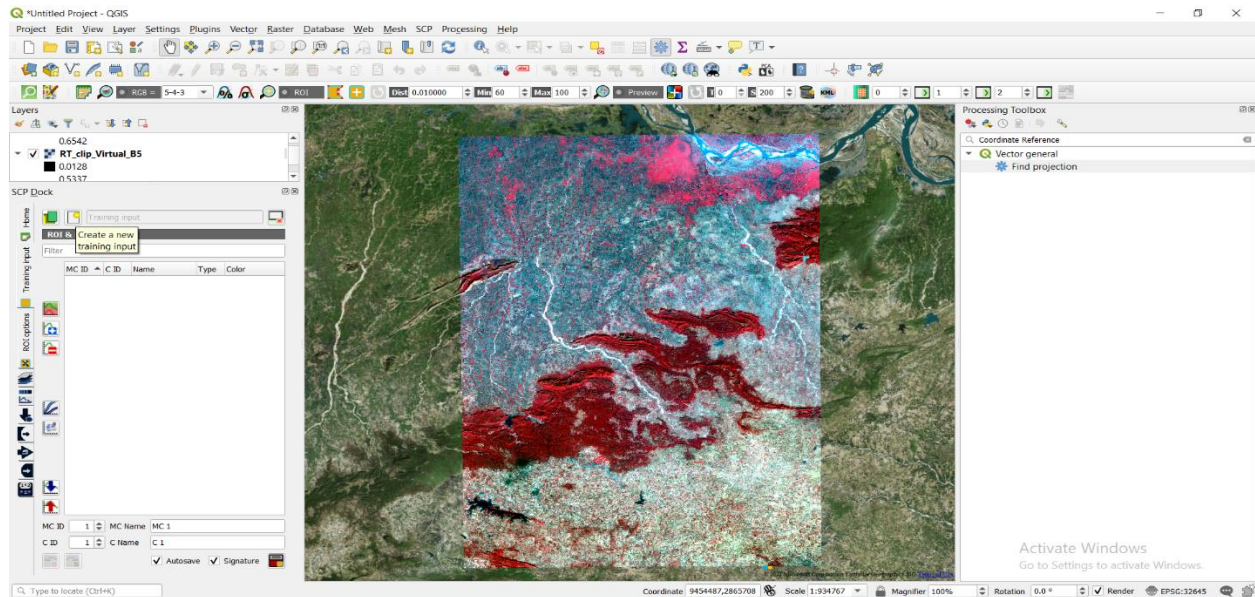
Then choose the appropriate bands to create the TCC and FCC.



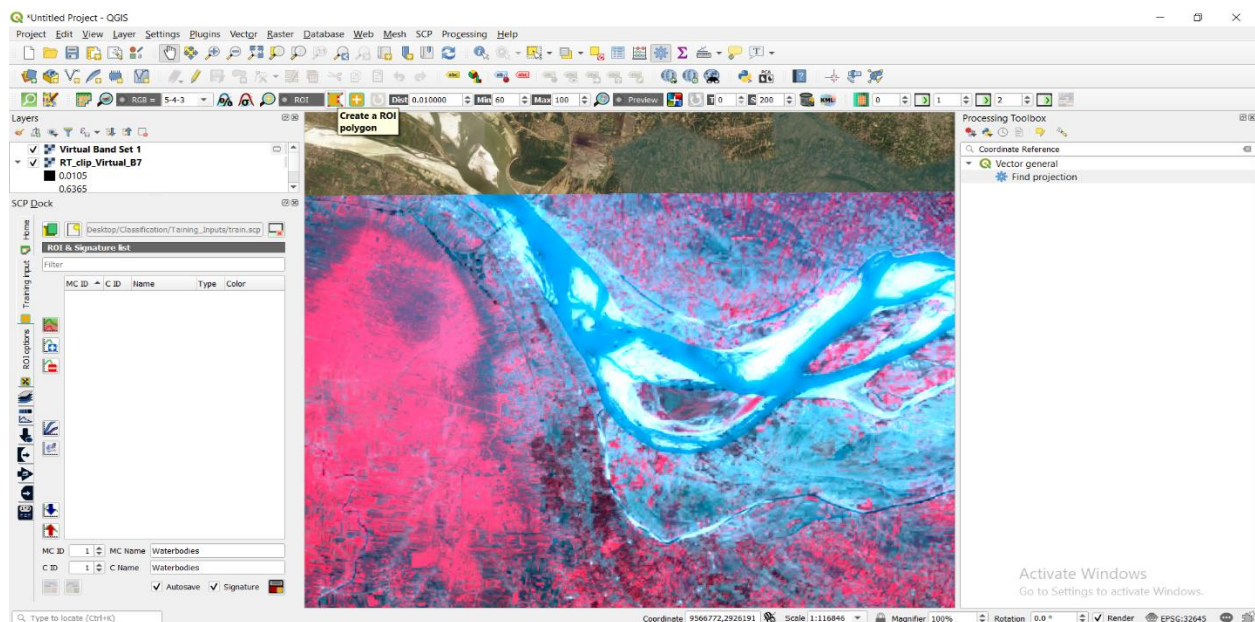
Step 9: In the SCP Dock that we have earlier in the panel click on Training Input.

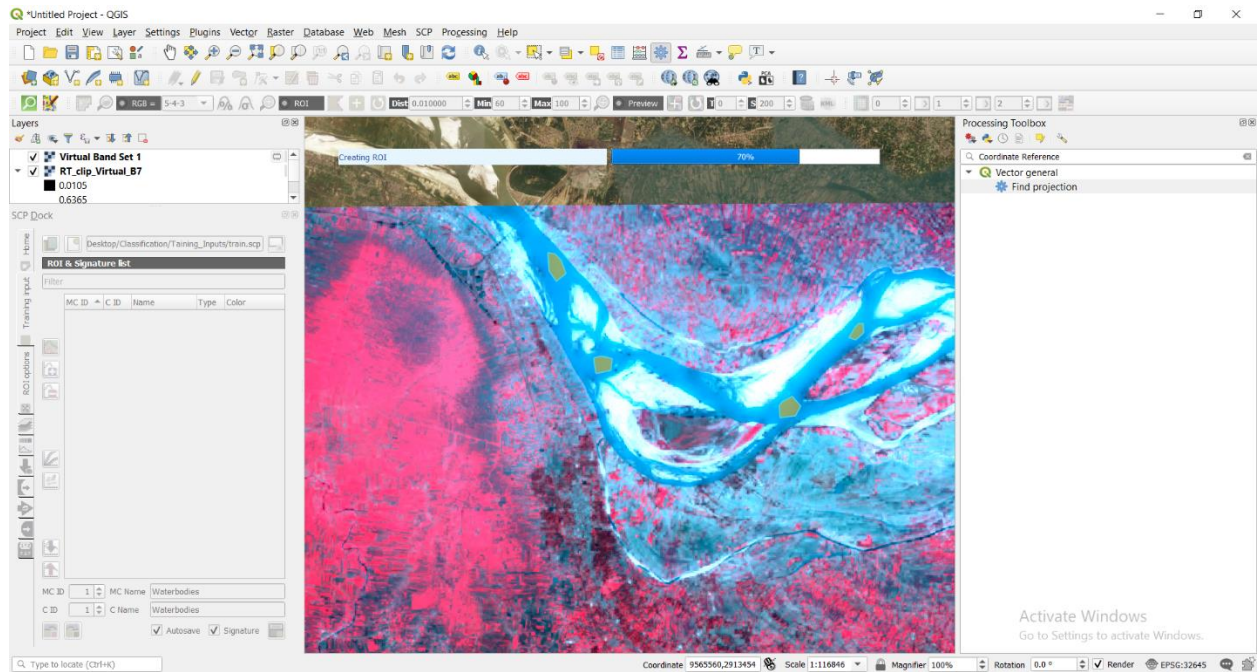


Then click on “Create a new training input” and create an training input folder which will automatically get added in the layer.

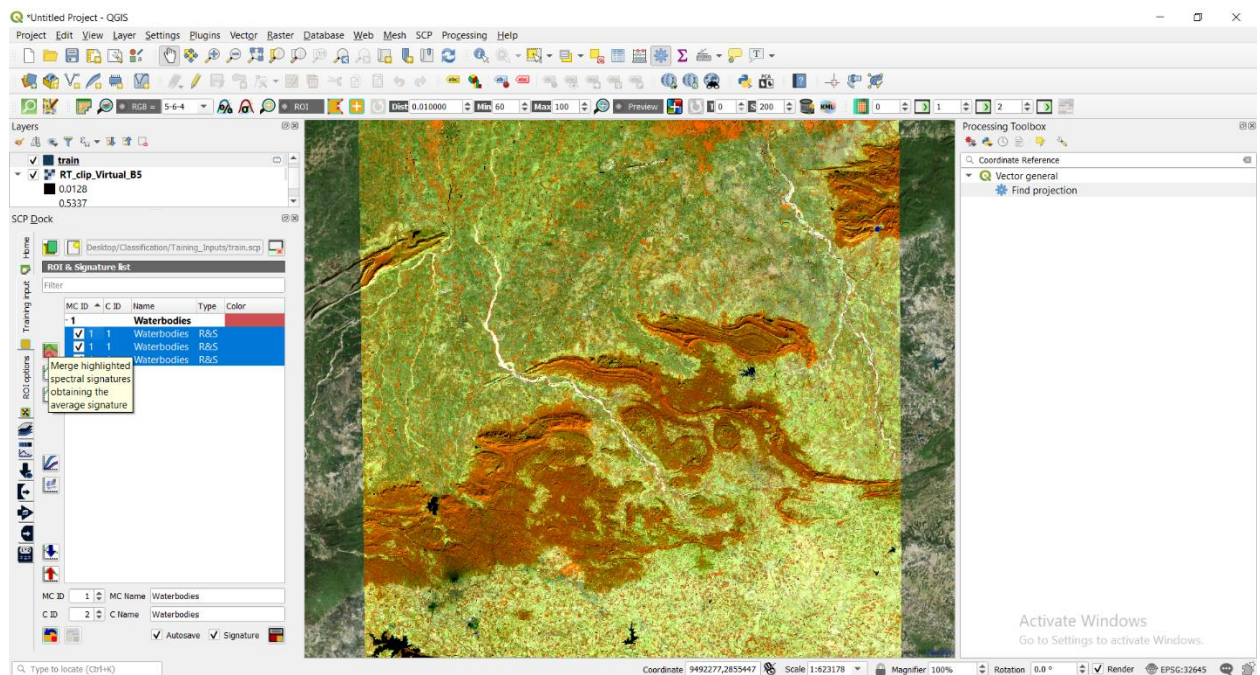


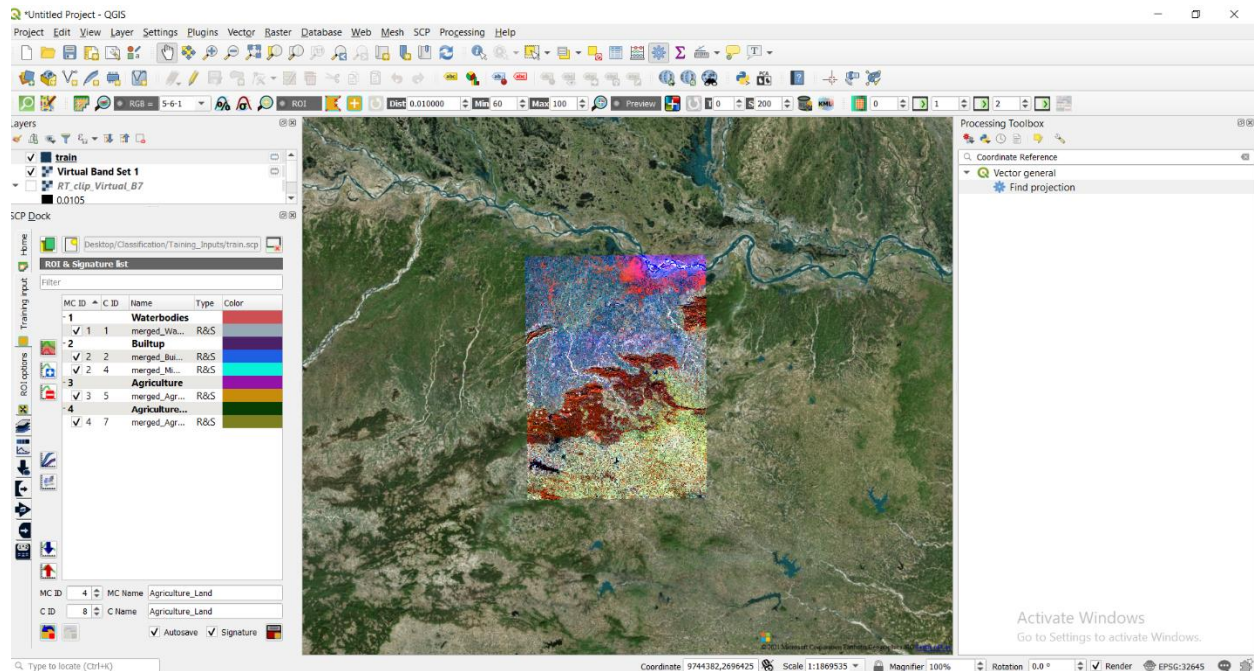
Step 10: In this plugin we have two classes one is a Macro Class (MC) and the other one is a Class (C). Macro class is the name given for the overall class which can consist of different type of subclasses for eg. water bodies can be a macro class and lake, rivers, ponds can be the different classes inside the macro class. But we will use the same class name in both the plugin classes for eg we will give the same in the i.e. waterbodies in both the plugin classes. Then click on “Create a ROI polygon” to create a polygon.



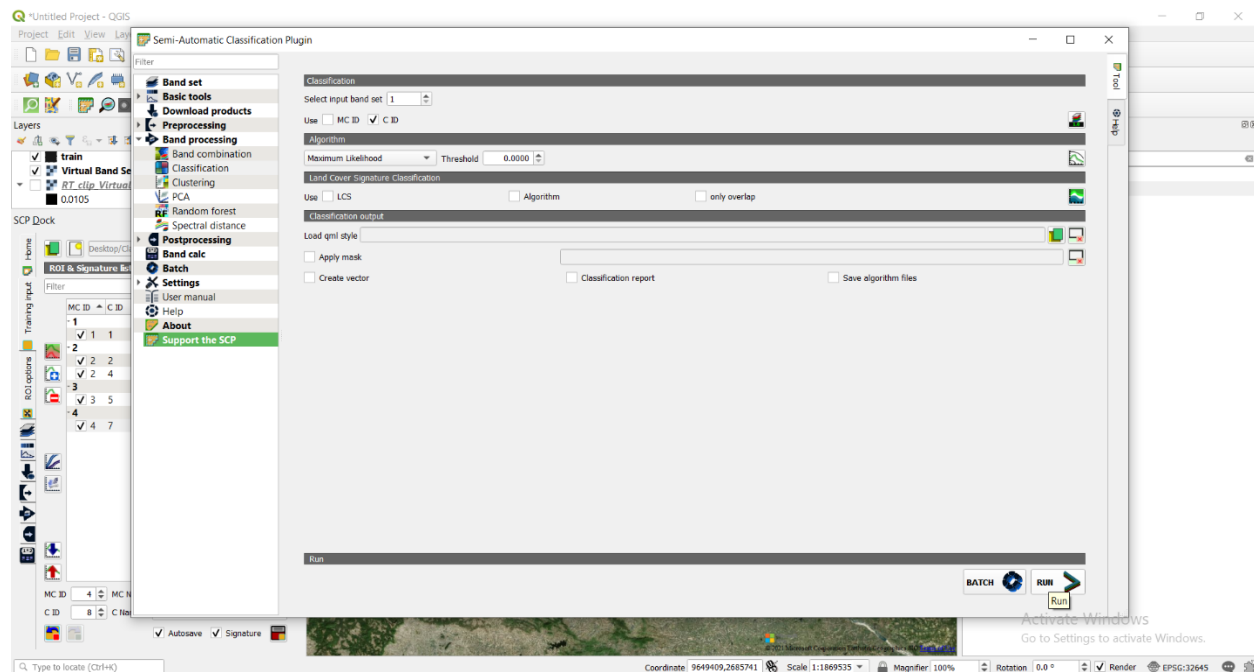


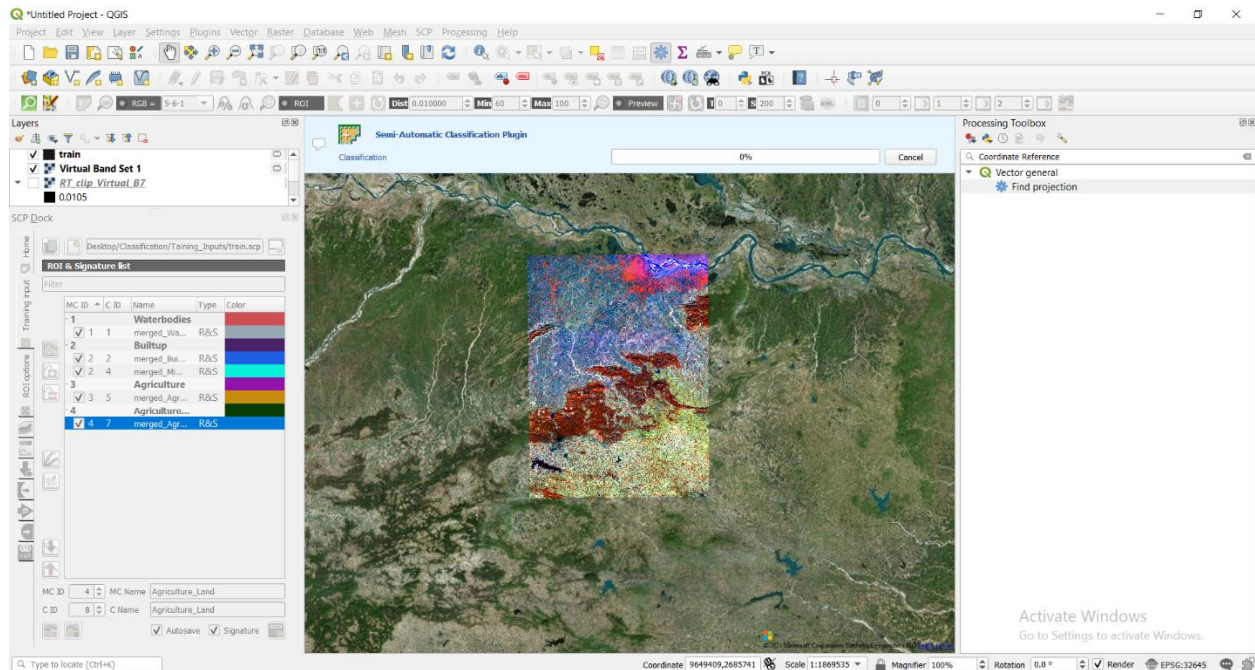
We create a multiple of ROI and then merge them into one. Repeat the same steps for the other classes i.e. mining area, agriculture, agricultural land.



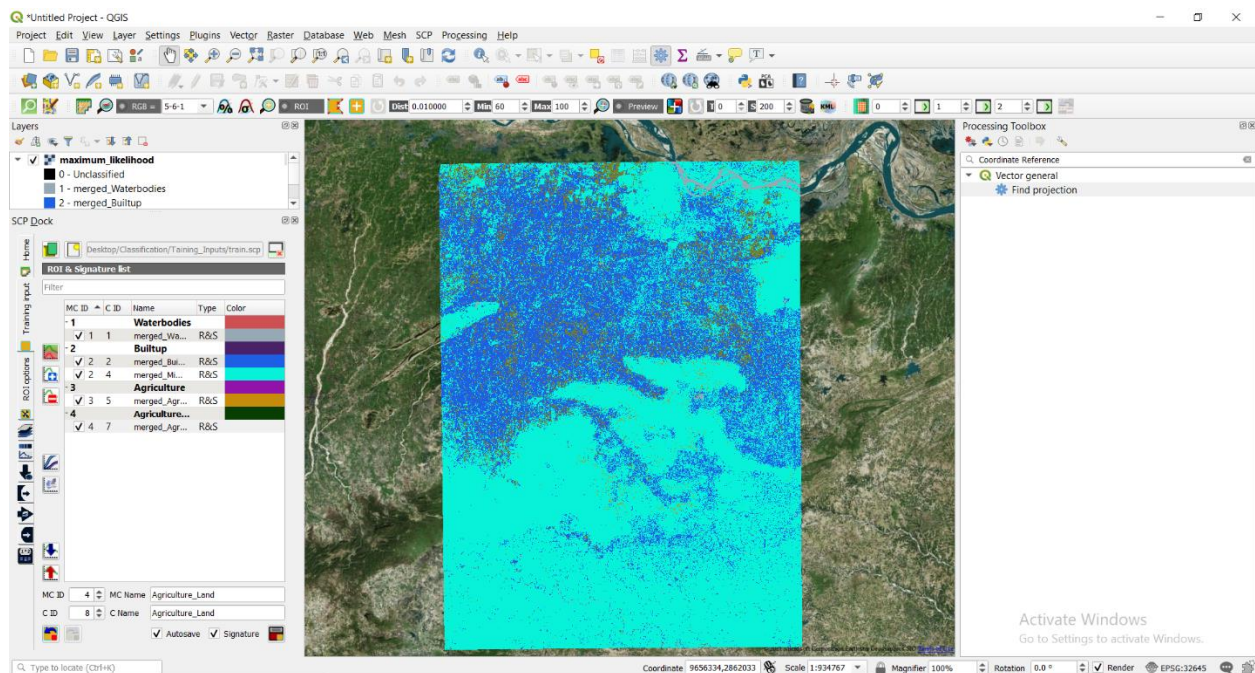


Step 11: The click on SCP> Band Processing> Classification. In this choose the C Id option to do the classification on the bases of classes rather than macro classes. Then choose the algorithm i.e minimum distance, maximum likelihood etc for the classification. Then click on “Run”.

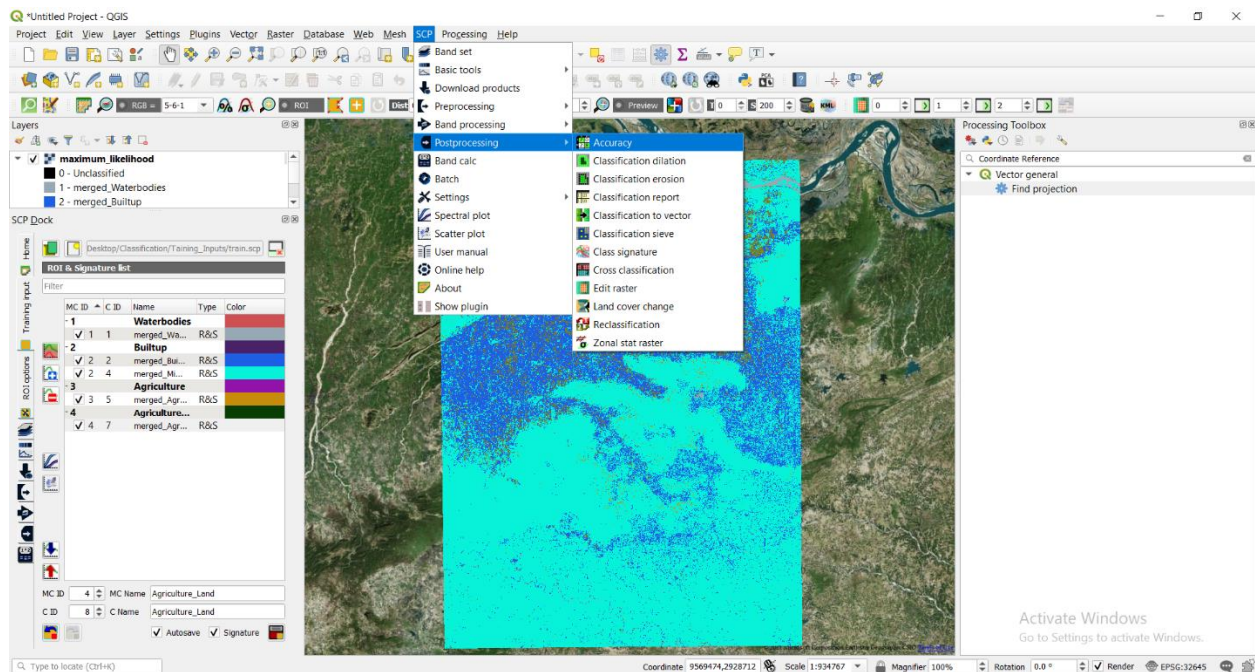




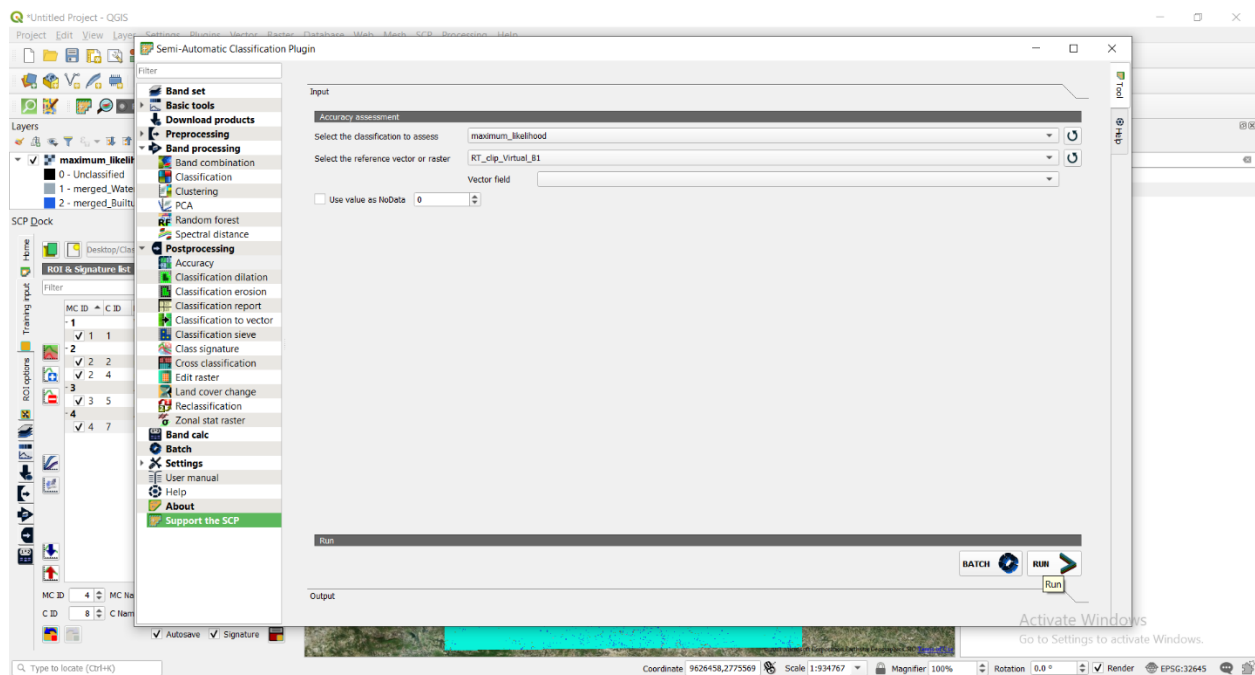
Click on “Preview” to load the classified image and we can also change the color of the different classes to identify the classified image more properly.

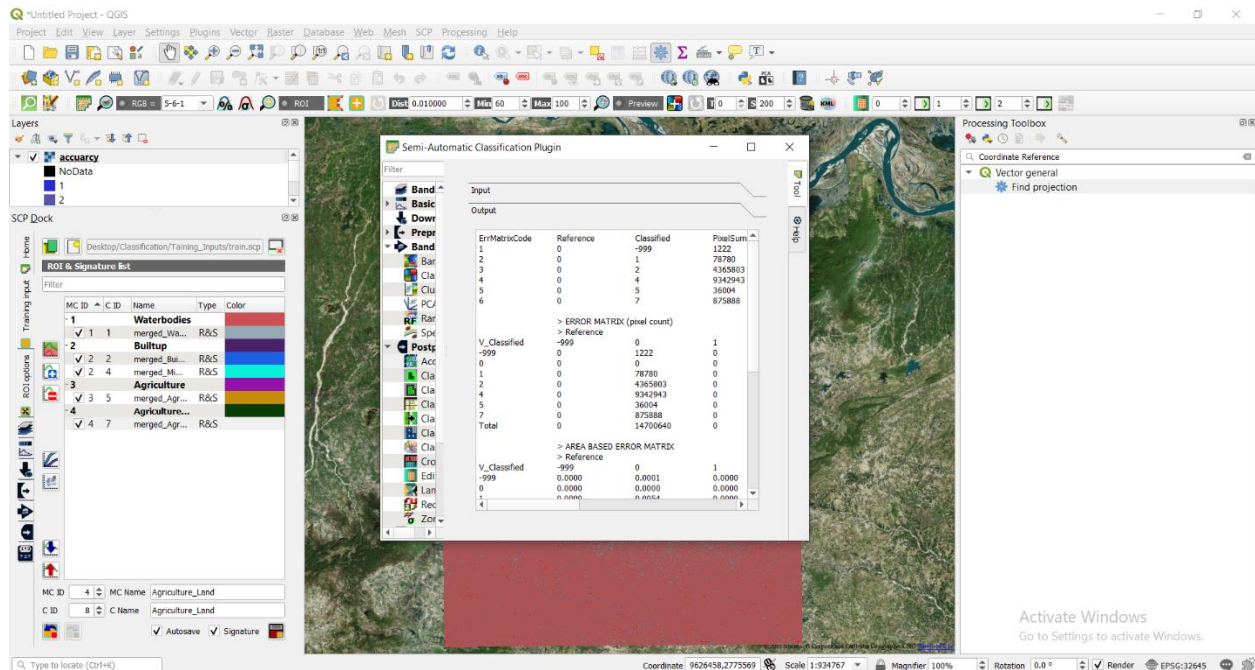


Step 12: To determine the accuracy of the classifier we have used and to draw the confusion matrix click on SCP> Postprocessing> Accuracy.



Then the reference raster and the classified raster and click on “Run”.





Change Detection

Change detection enables us to assess the gains and losses among various types of land use and land cover in a region over a period of time. Remote sensing data is widely used for change detection due its high temporal resolution, wide coverage and cost effectiveness over field surveys/inventories. Change detection can be used as diagnostic tool to understand the impact of anthropological effects on natural resources, which might further help us to prepare a sustainable planning measure to protect our environment. Change detection techniques are mainly of two types. The first one is spectral change detection, in this technique the unit of analysis is a pixel, a neighborhood, a multi temporal segment or a spectral class (Campbell & Wynne, 2011). One would require accurate radiometric, atmospheric and geometric corrections to be carried out before performing the spectral change detection. The second technique is post-classification change detection. It uses thematic maps (classified images) as inputs. It is a relatively easy and less accurate method compared to spectral change detection.

A single land-use map may not be enough to identify areas where change is taking place. To see what changes have taken place to the landscape, it becomes necessary to have at least two land cover maps of a region at two points in time. Therefore, the procedure of 'Change Detection'

utilizes two or more such thematic images belong to different periods to track the changes that have taken place in the study region over the length of that period. This allows us to assess which land classes are gained, losses and remained the same over the period of time.

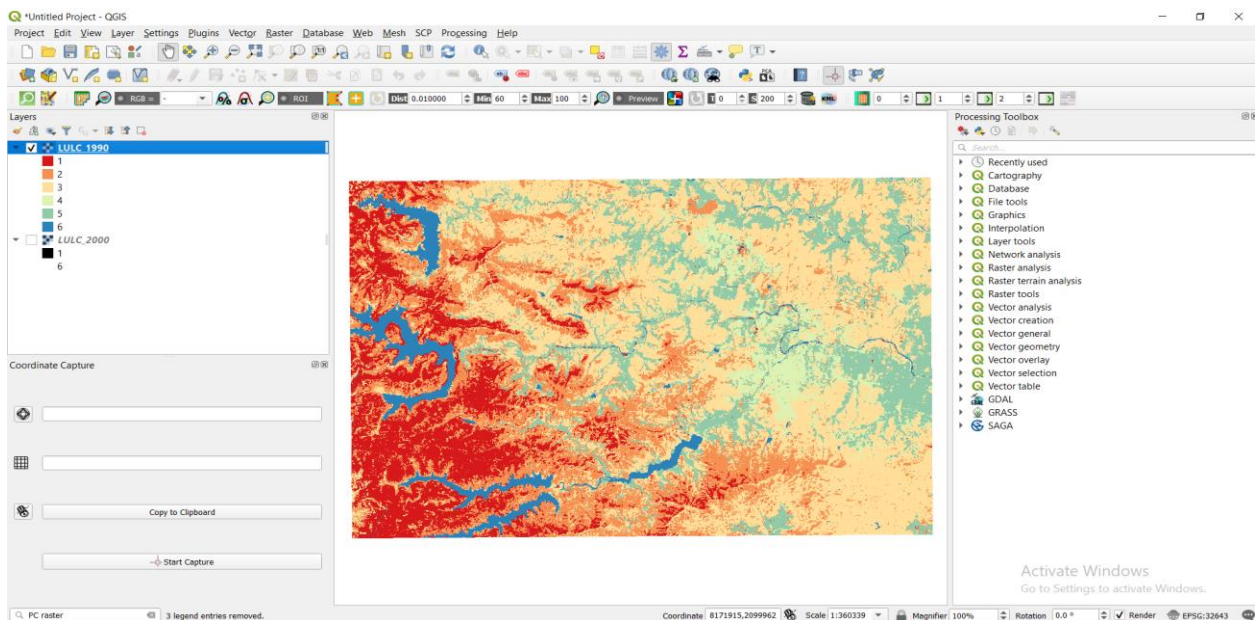
Dataset used: <https://dst-iget.in/index.php/tutorialdetails/2/12>

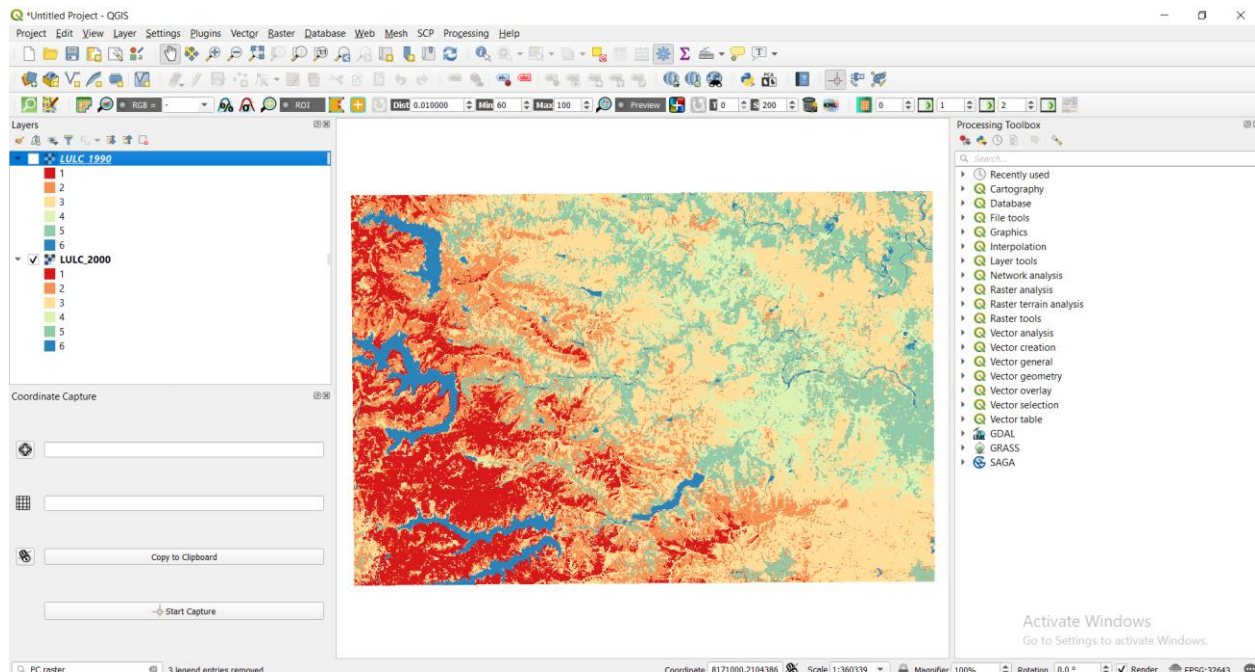
Step 1: Load the raster file i.e. LULC of 1990 and 2000 by clicking on Layer> Add Layer> Add Raster Layer. After choosing the file click on “Add”.

The LULC contains 6 classes i.e.

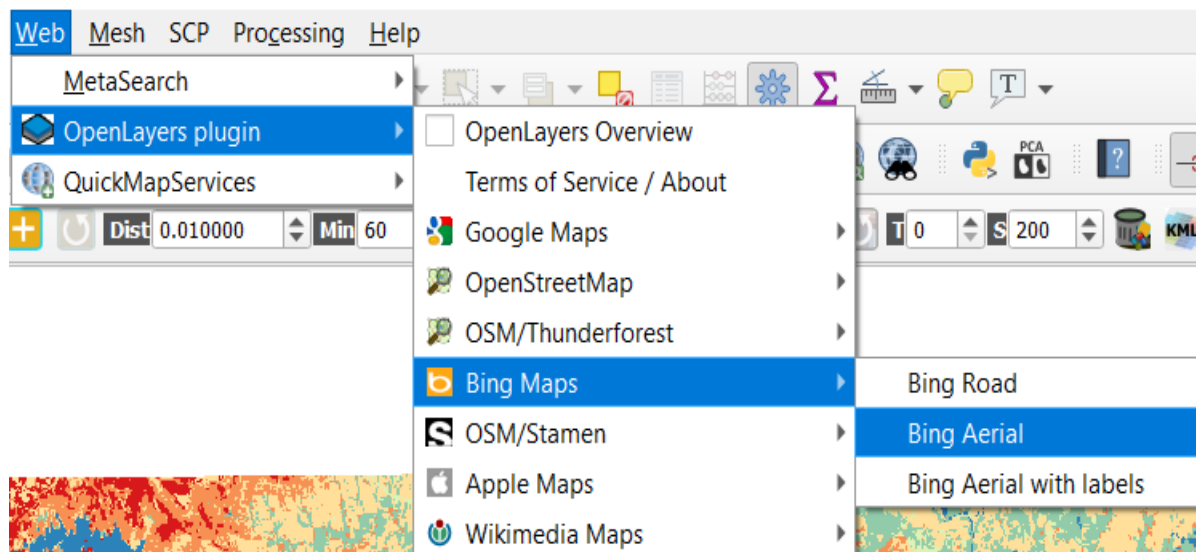
Color (Pixel)	Name	Description
27648	Forest	1
1556520	Shrubland	2
8454068	Grassland	3
2105599	Urban	4
4784127	Mixed Cropland	5
15365376	Water	6

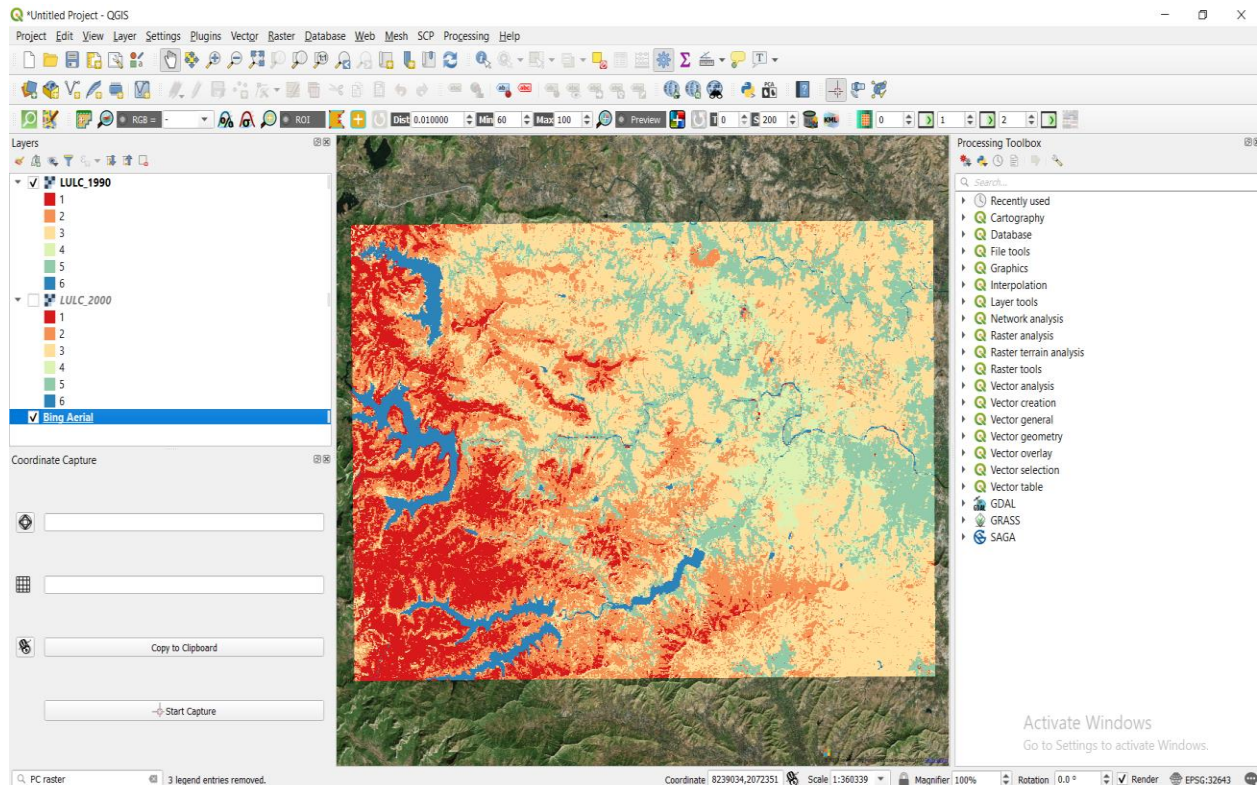
We can change the color composition as per our need by right clicking on LULC_1990> Properties> Symbology.



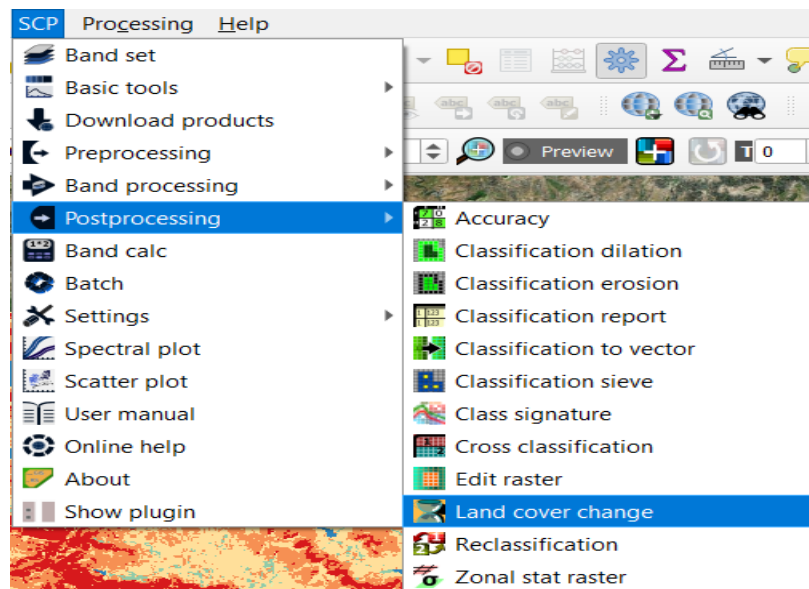


Step 2: We can also add the base map so that we can identify the classes more easily. To add the base map click on Web> OpenLayers plugin> Bing Maps> Bing Aerial.

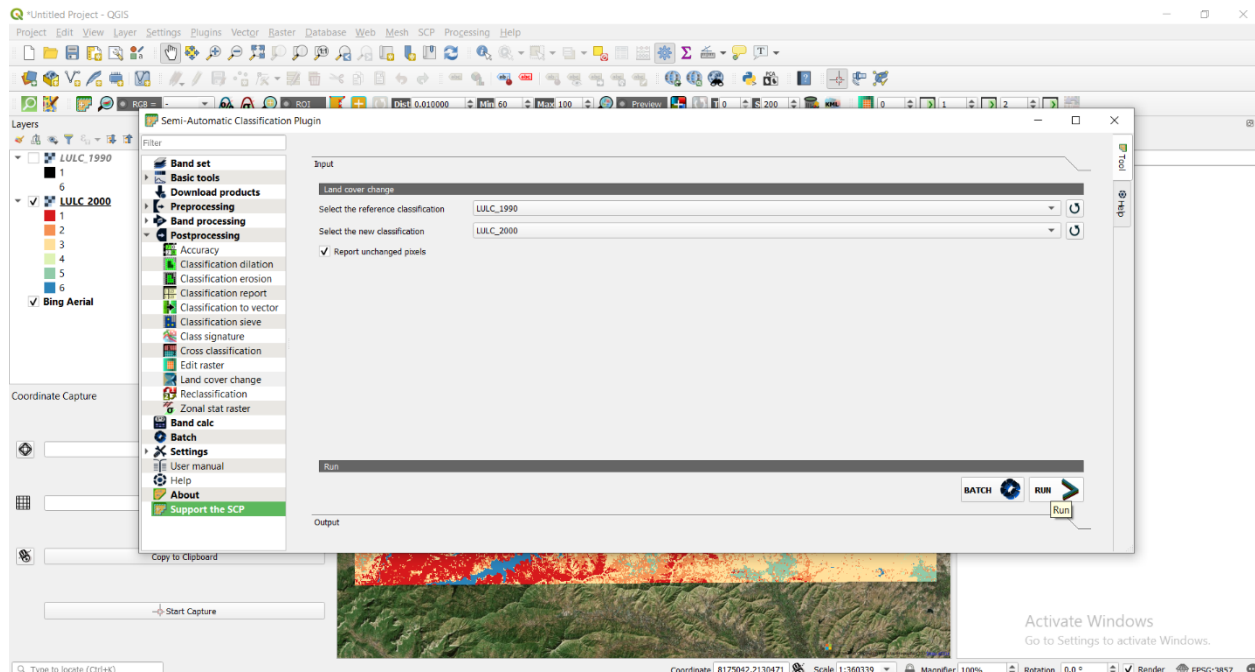




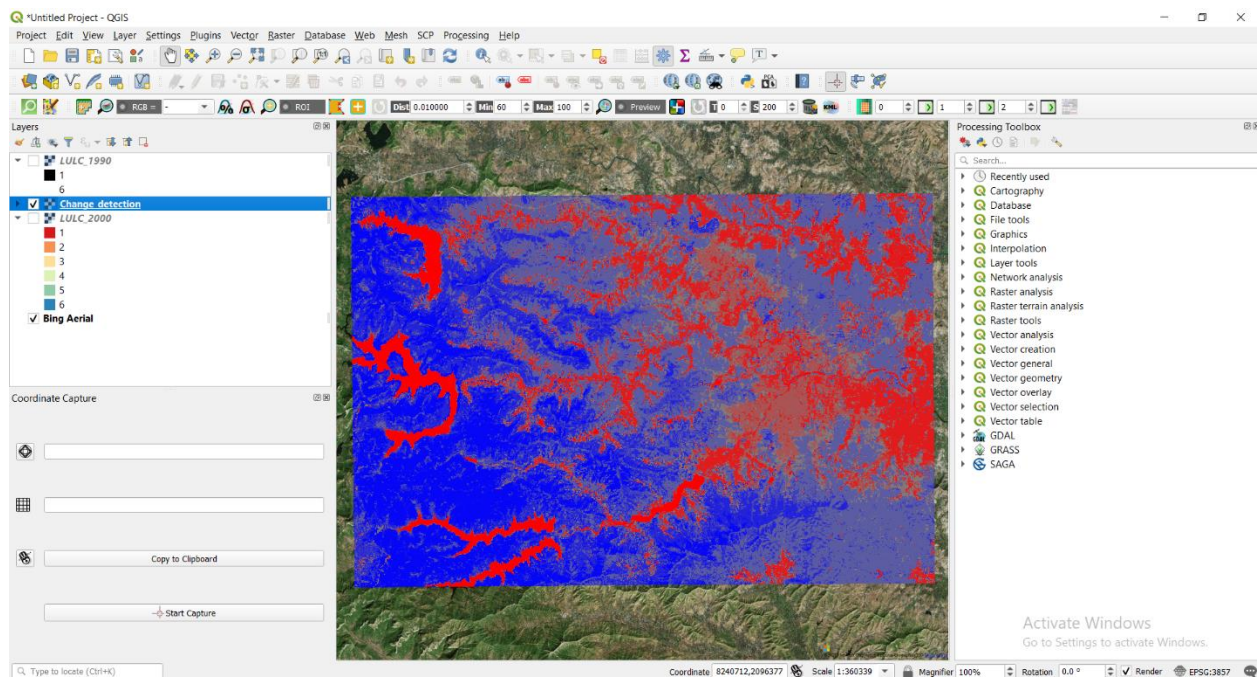
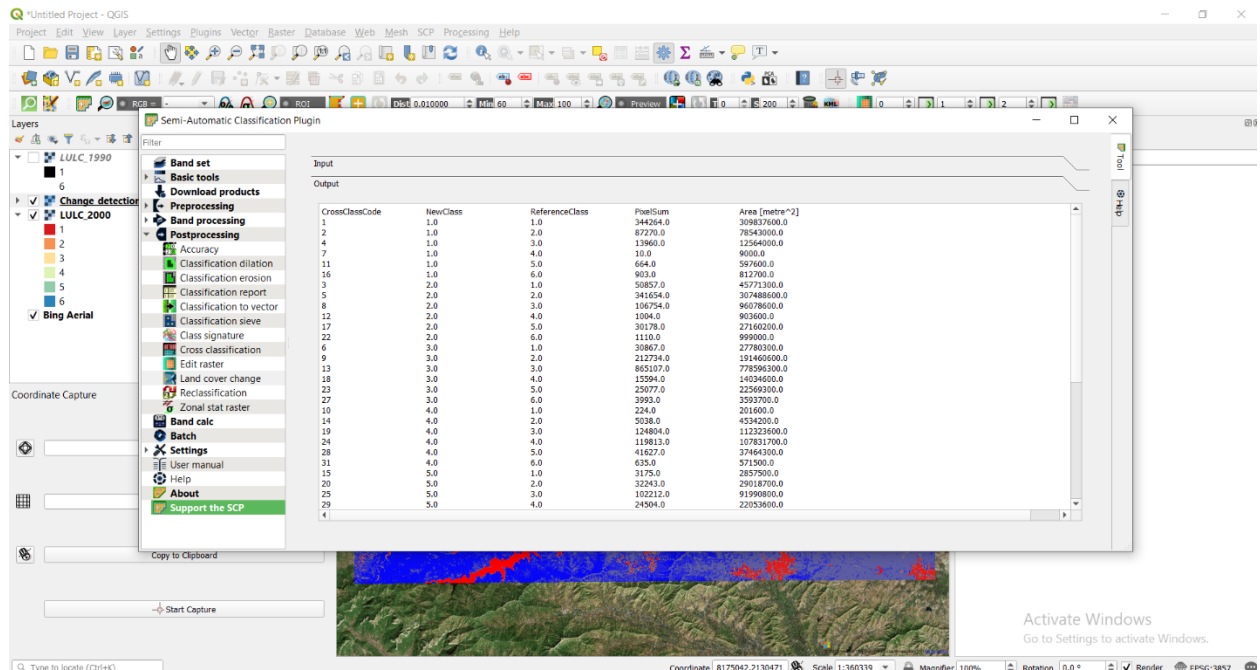
Step 3: To find the change that occurs in the classes which are defined earlier, click on SCP> Postprocessing> Land cover change.



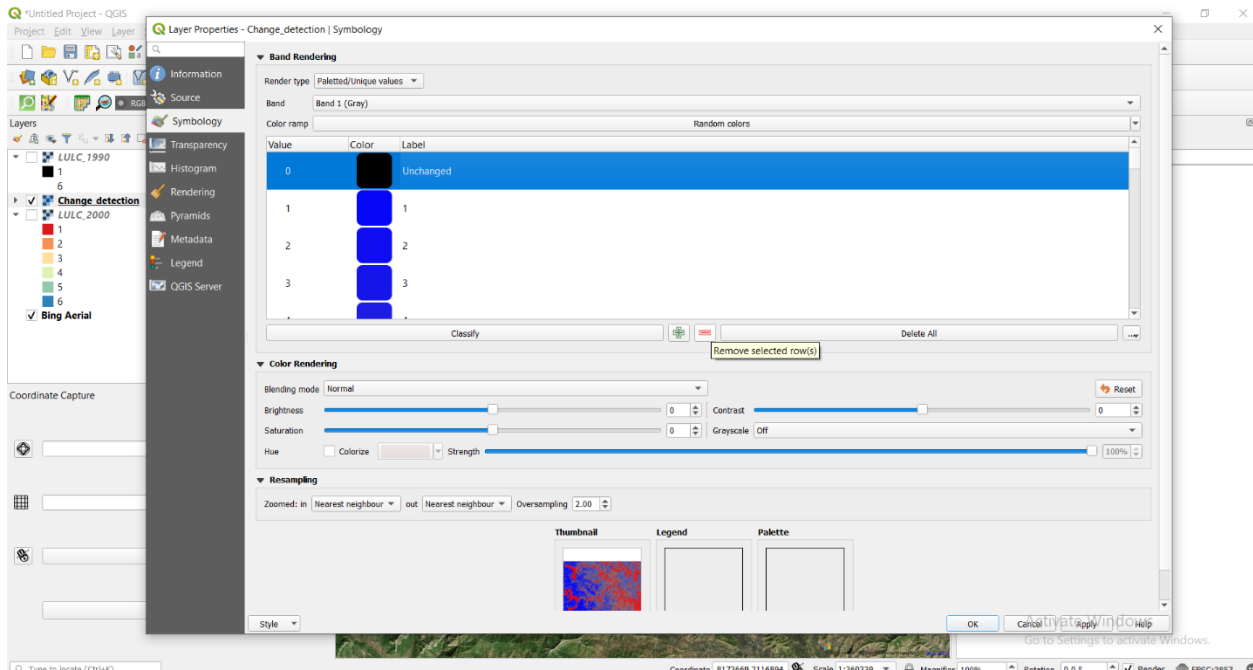
A new window will pop-up. In this, provide reference class and new class. In our case, LULC_1990 is the reference class and LULC_2000 is the new class. After providing the classes click on “Run”.



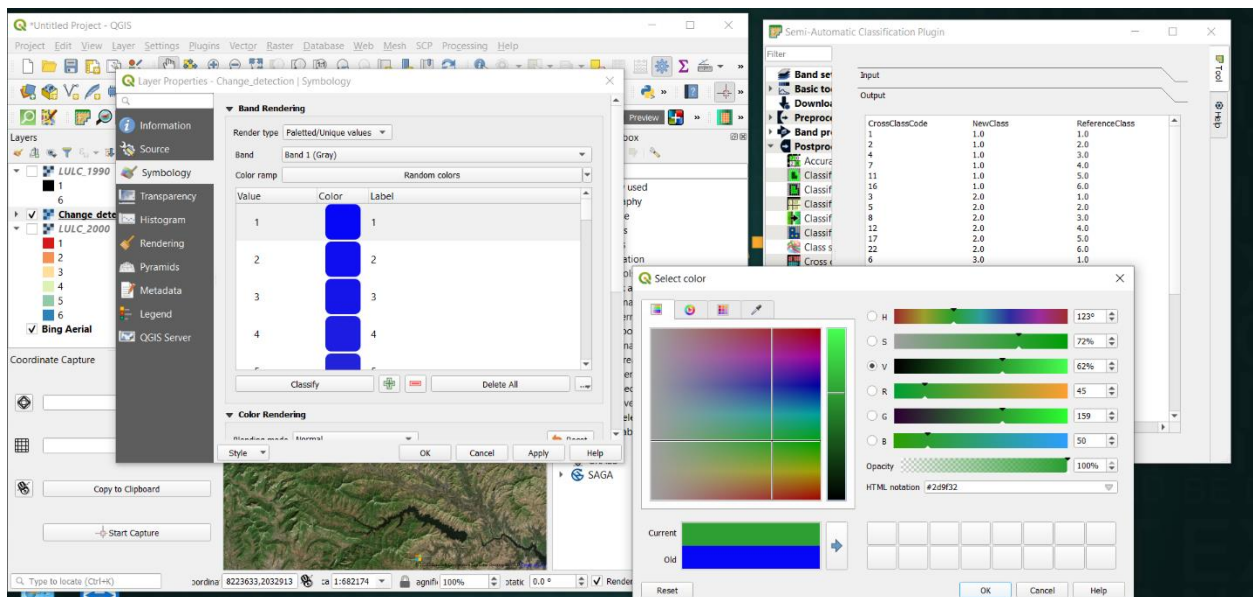
Step 4: When the process is completed a new raster file i.e. Change Detection will get added automatically in the Layers. The Cross Class Code contains values up to 36 this is because our dataset consist of 6 classes each. Reference class contains information of old class and the New Class contains the information of the changed classes.

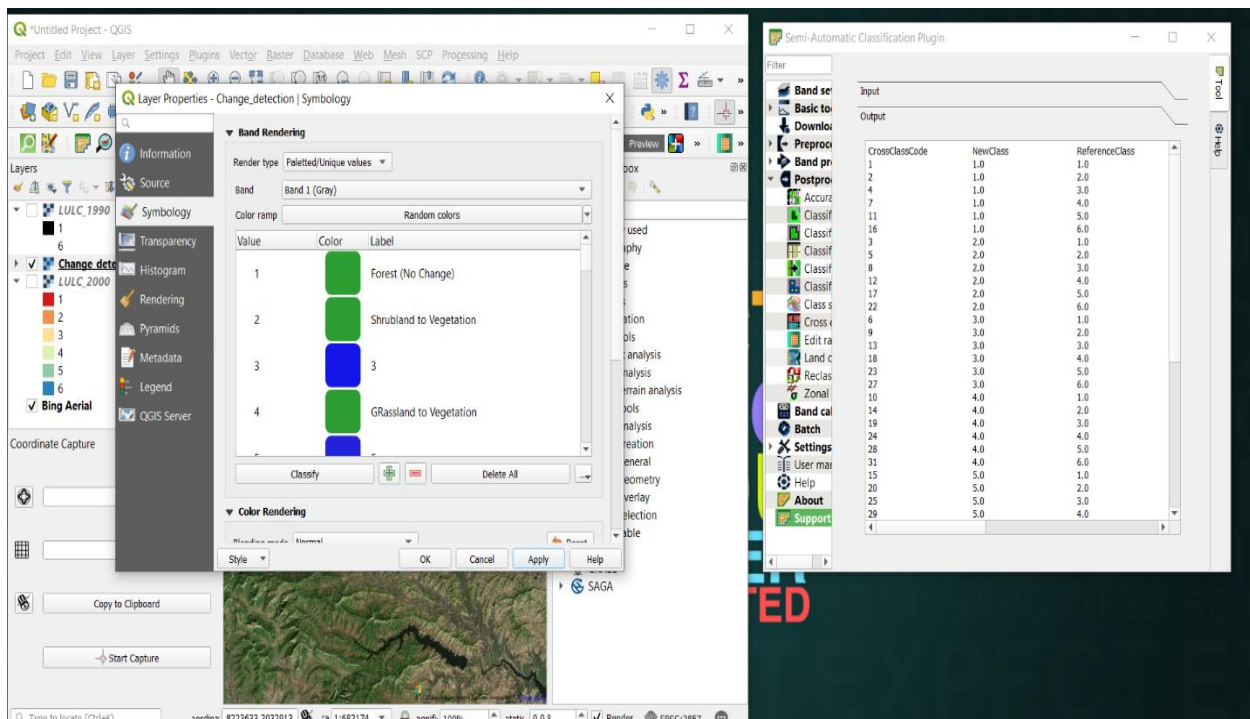
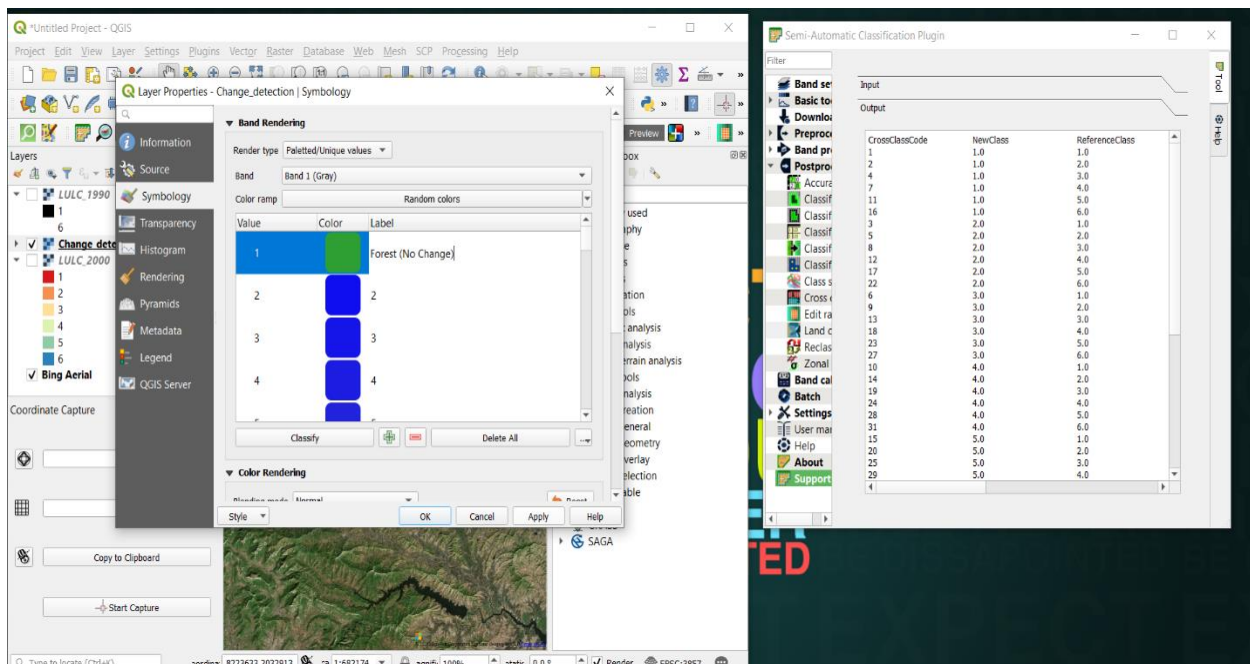


Step 5: Select the Change_Detection file, right click on it and click on Properties> Symbology. The unchanged does not contain any information we can remove it.

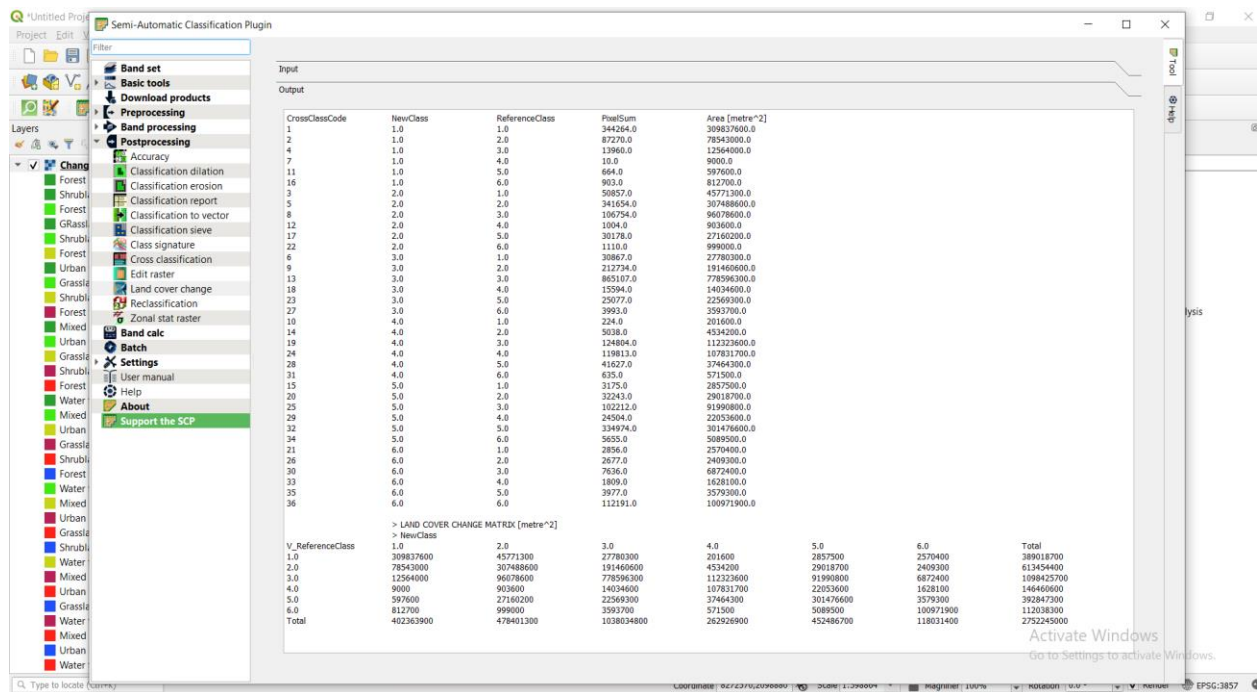
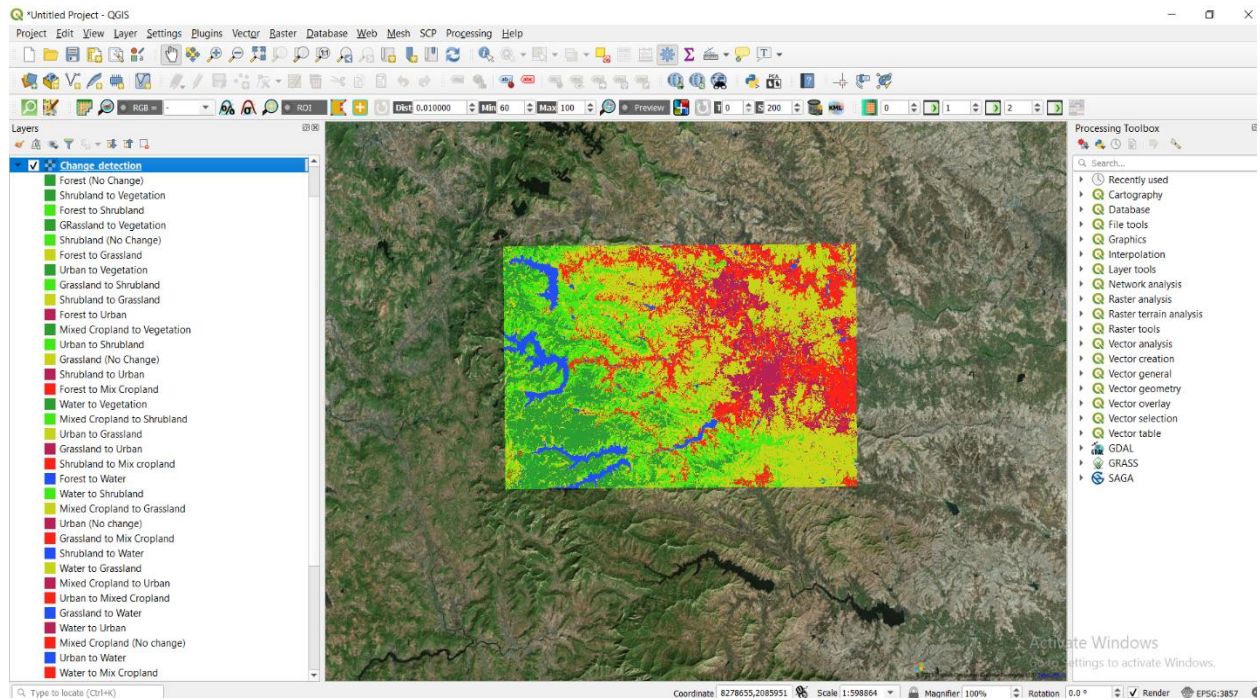


Now, open the window which contain the information of New Class and the Reference Class. If New and Reference class contain the same class it means that there is no change in that class in the past 10 years. For eg. New and Reference Class contain same class i.e. 1.0 which is a forest class. Then in the Band Rendering window we can edit the Label as “Forest (No Change)” and we can also change the color as per our choice. Then click on “Apply”.





The final change detection raster image will look like this.



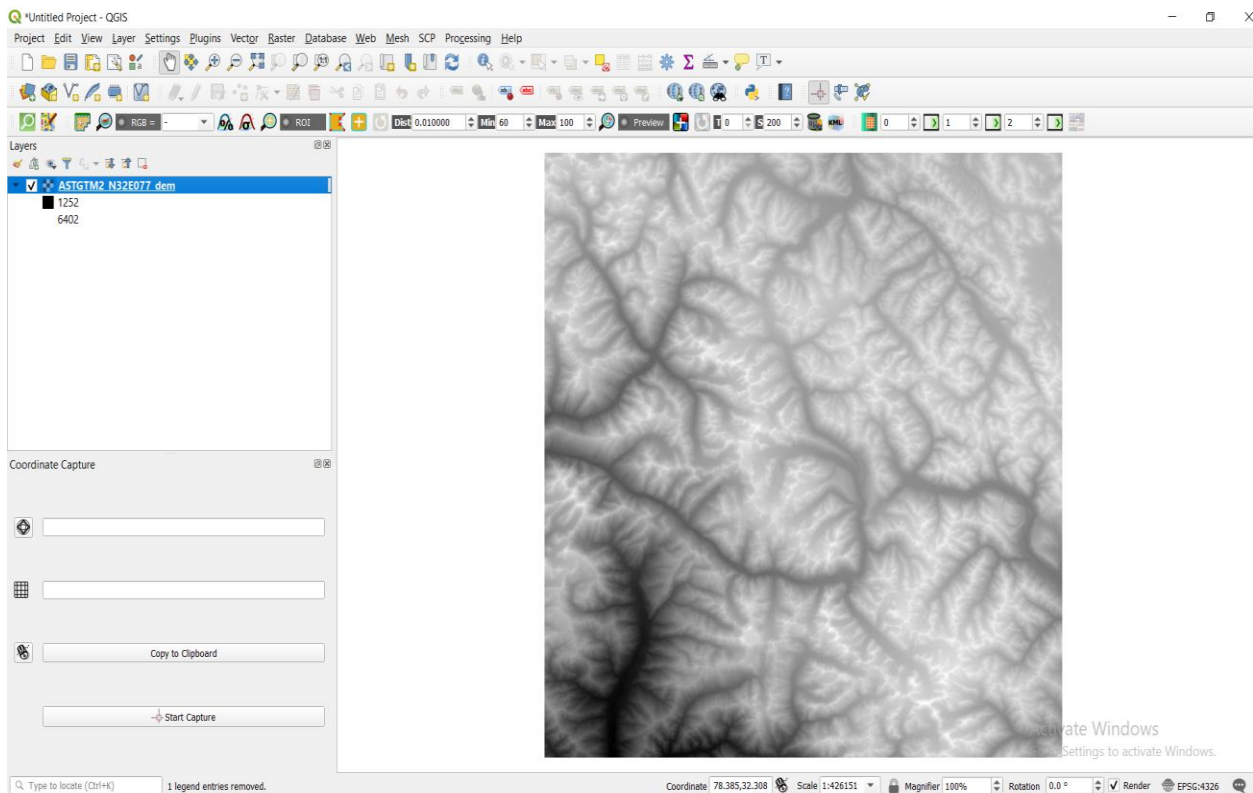
Practical 9: Introduction to Digital Elevation Model using QGIS

A Digital Elevation Model (DEM) is a representation of the bare ground (bare earth) topographic surface of the Earth excluding trees, buildings, and any other surface objects.

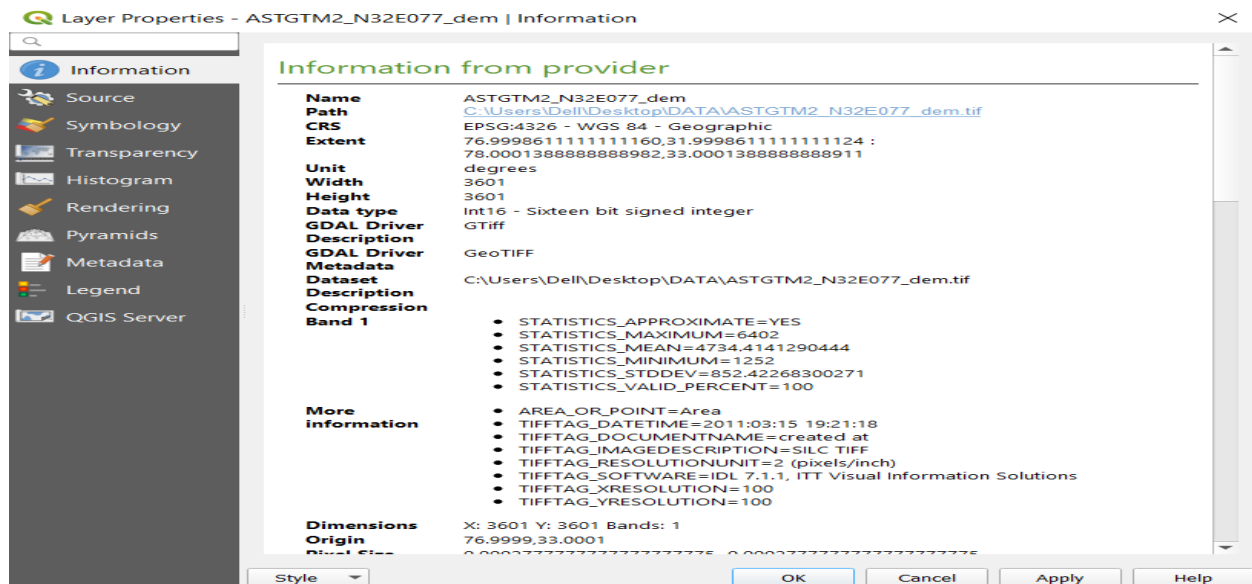
DEMs are created from a variety of sources. USGS DEMs used to be derived primarily from topographic maps. Those are being systematically replaced with DEMs derived from high-resolution LiDAR and IfSAR (Alaska only) data.

Open the DEM data and reproject the data

Step 1: Open the DEM data by clicking on Layer> Add Shapefile> Add Raster Shapefile. After selecting the DEM file click on “Add”.

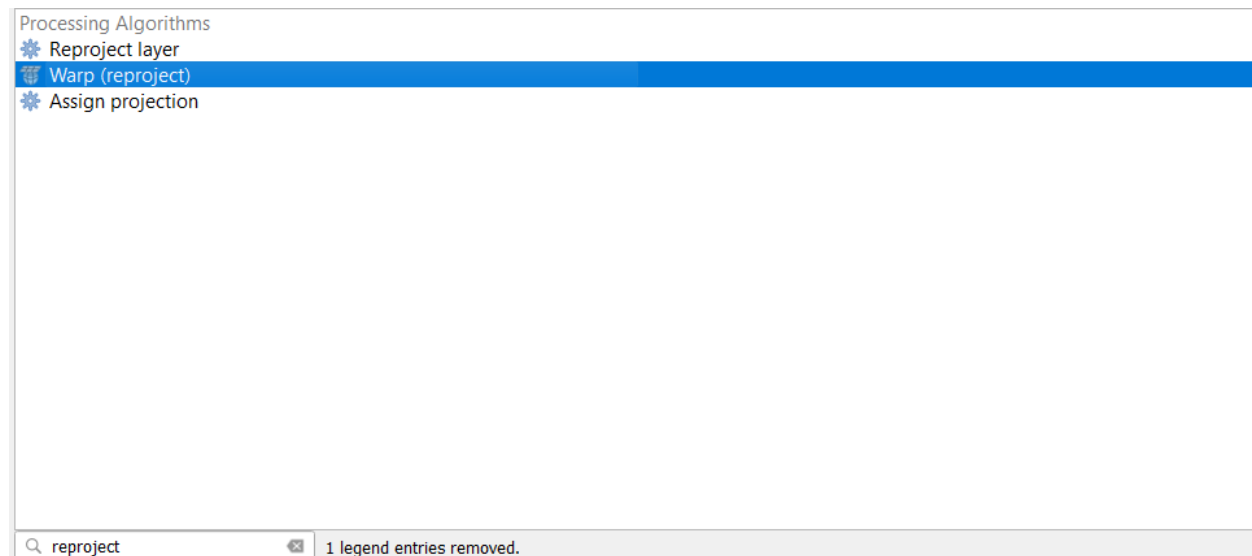


Step 2: After opening the DEM file in QGIS. Select the file name that you have opened and right click on the file in the Layers and then select the “Information” option.

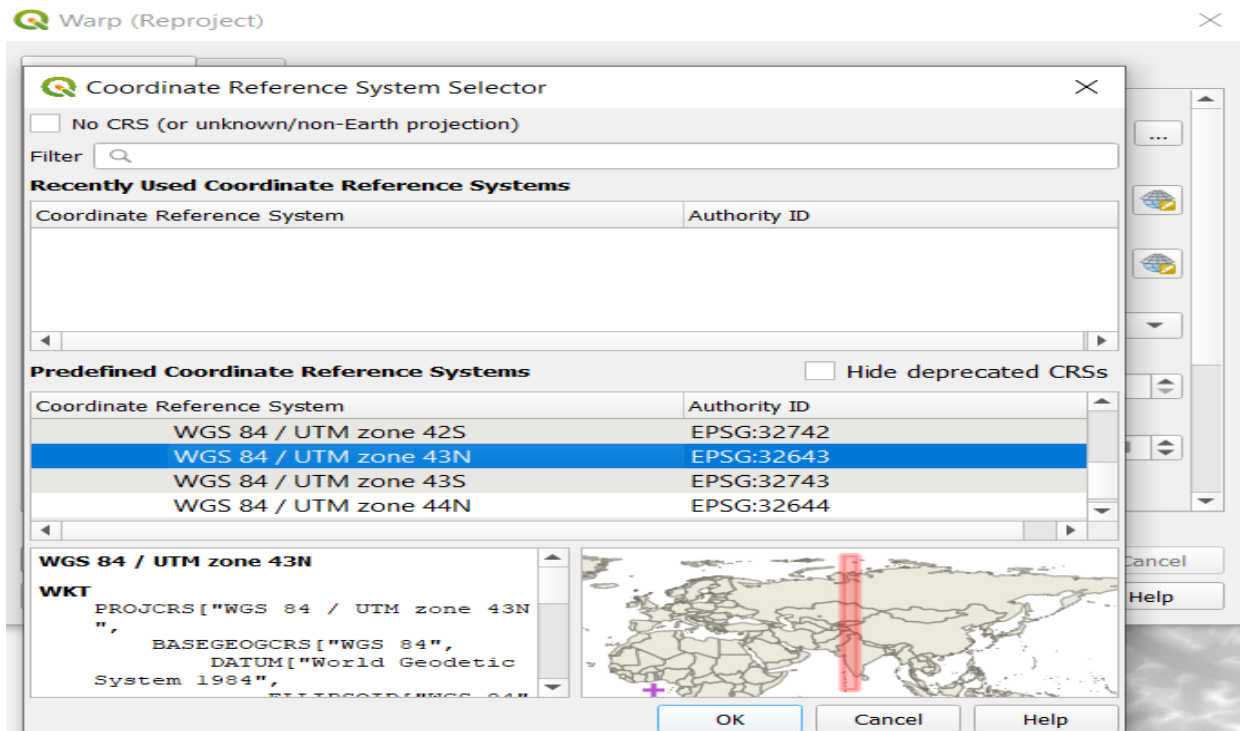


In the information window we can see that unit is in degrees and its coordinate reference system (CRS) is WGS 84. We need to convert degrees into meters and the CRS into the PRS i.e. UTM zone 43N. For this we have to re-project the DEM file.

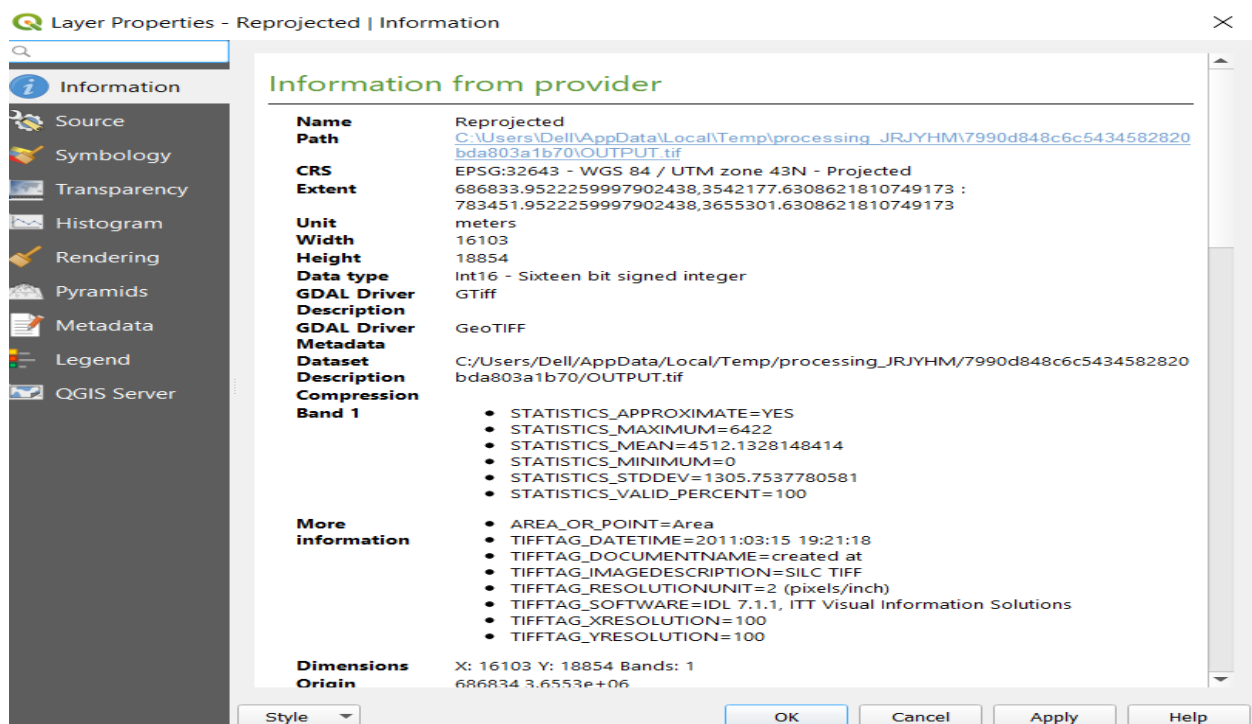
For re-projection, type “Re-project” in the search bar and choose the “Wrap (re-project)” option.



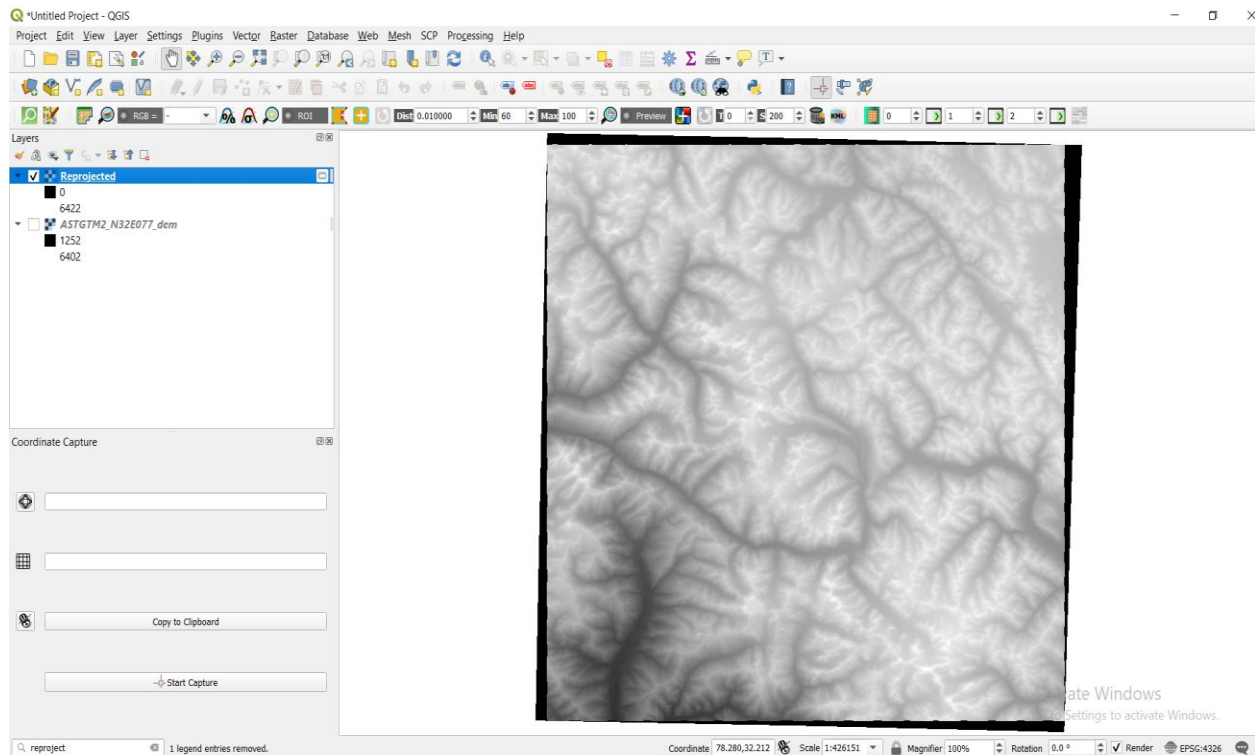
In the Warp (re-project) window choose the WGS 84/ UTM Zone 43N projection and click on “OK”.



Now again open the “Information” window of the re-projected DEM file. And then check the unit and the CRS.

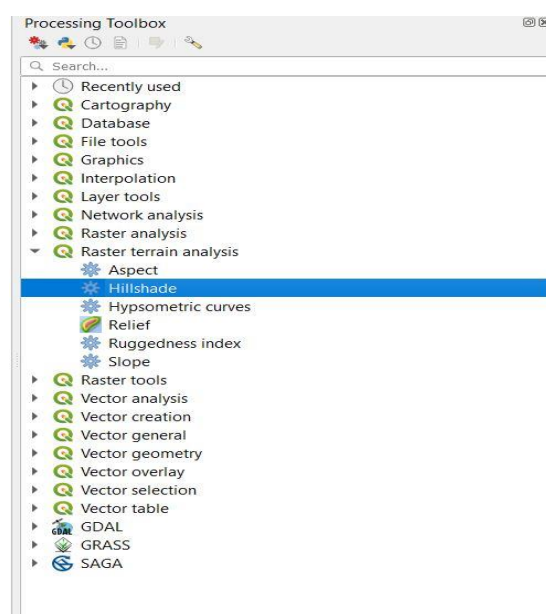
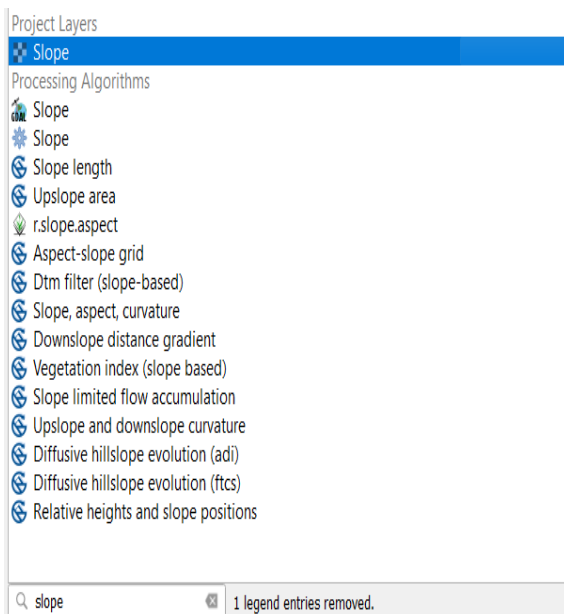


The re-projected DEM file will look like this,

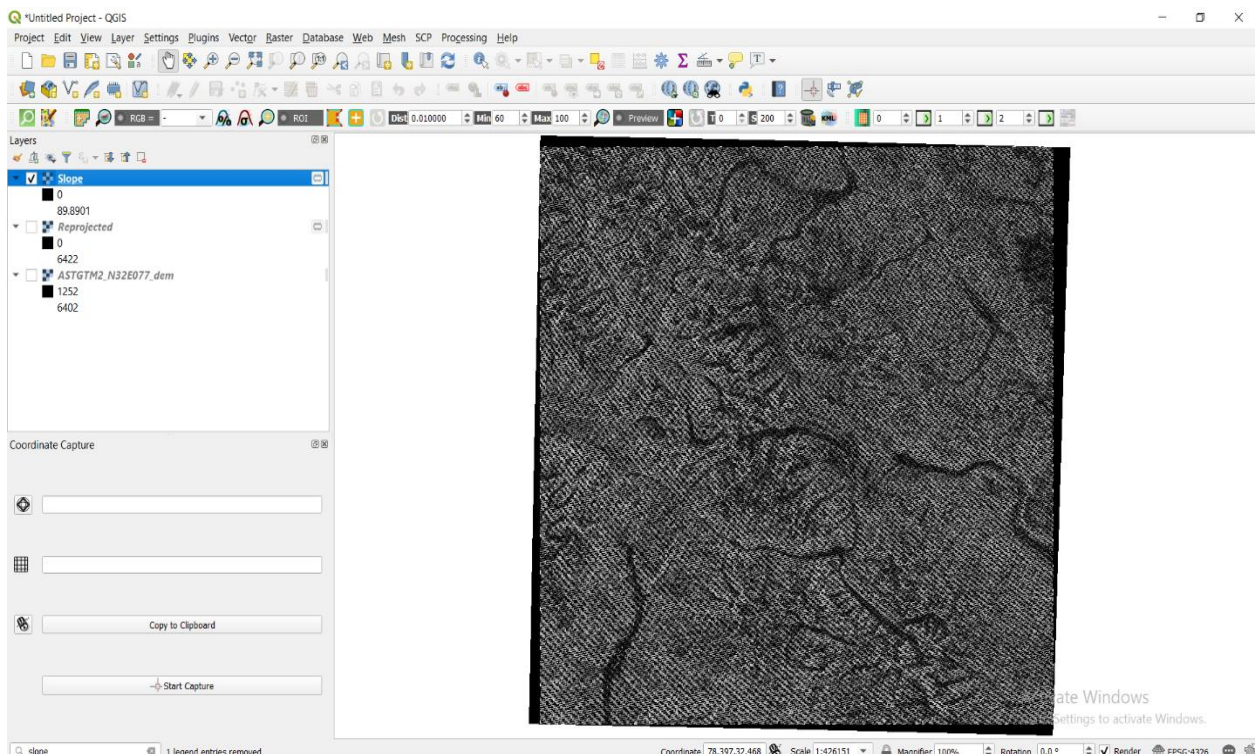
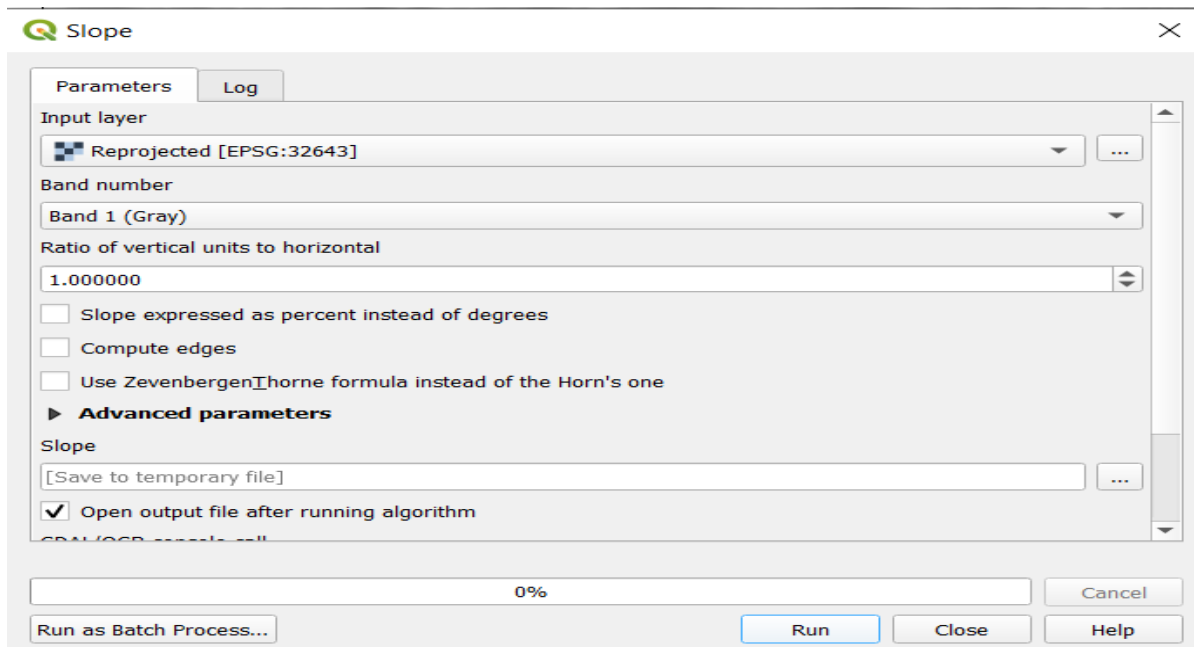


Slope

Step 3: To find the slope of DEM the simply type slope option in the search bar and choose the “Slope” option or else click on Processing> Toolbox> Raster terrain analysis> Slope

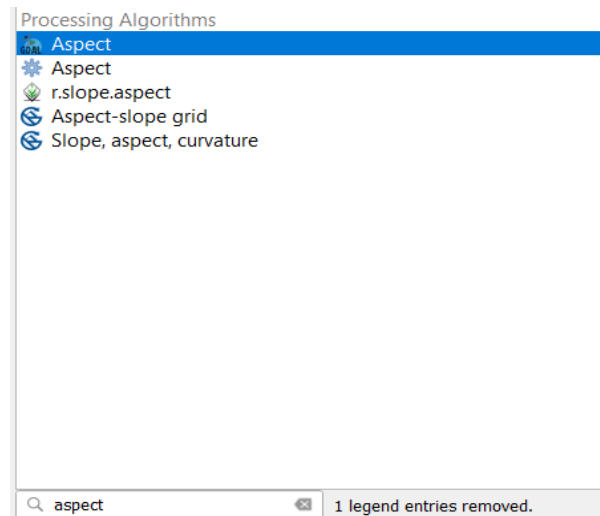


By clicking on the slope option a “Slope” window will pop-up. In this, choose the reprojected DEM file and choose the parameters as per the requirement and then click on “Run”.

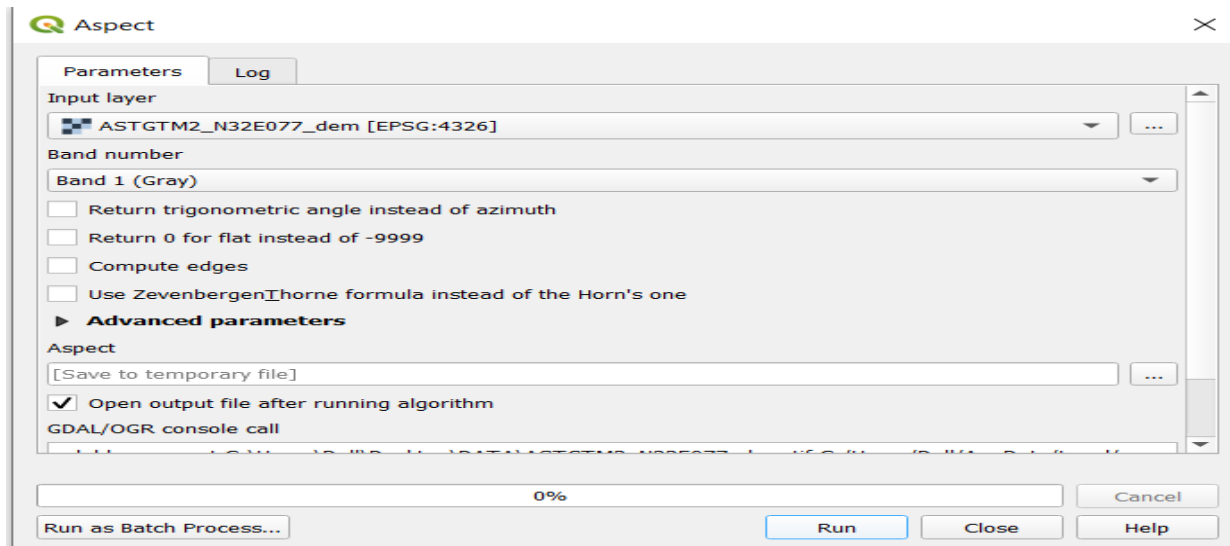


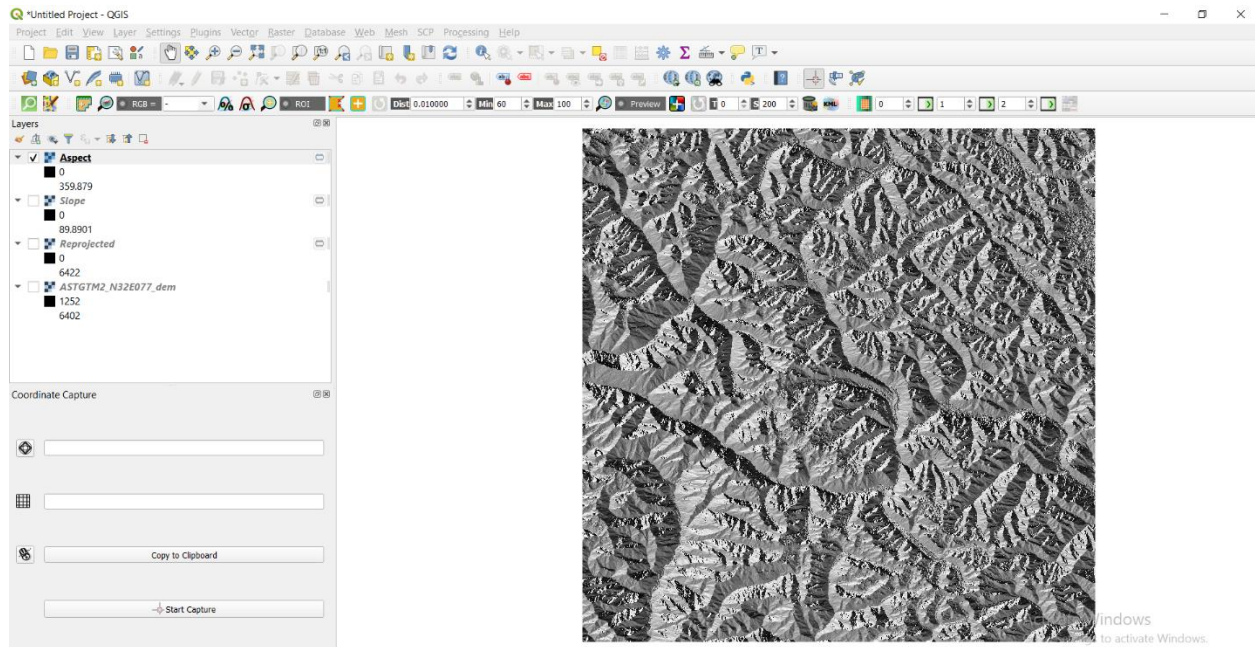
Aspect

Step 4: To find the aspect simply type slope option in the search bar and choose the “Aspect” option or else click on Processing> Toolbox> Raster terrain analysis> Aspect



By clicking on the aspect option a “Aspect” window will pop-up. In this, choose the reprojected DEM file and choose the parameters as per the requirement and then click on “Run”.

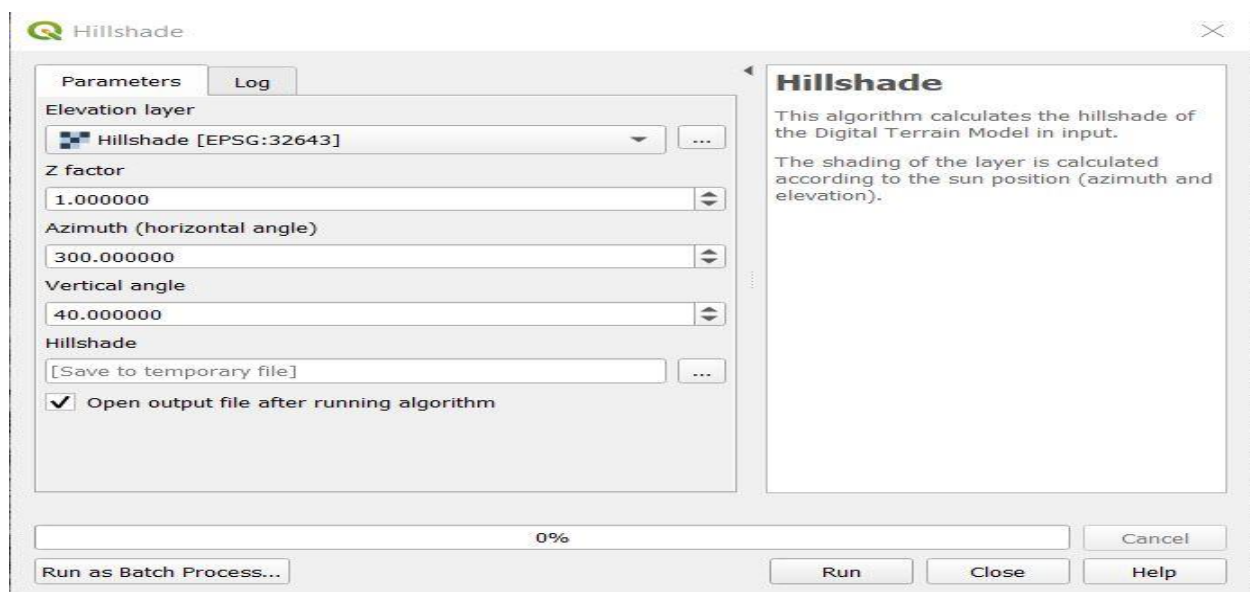


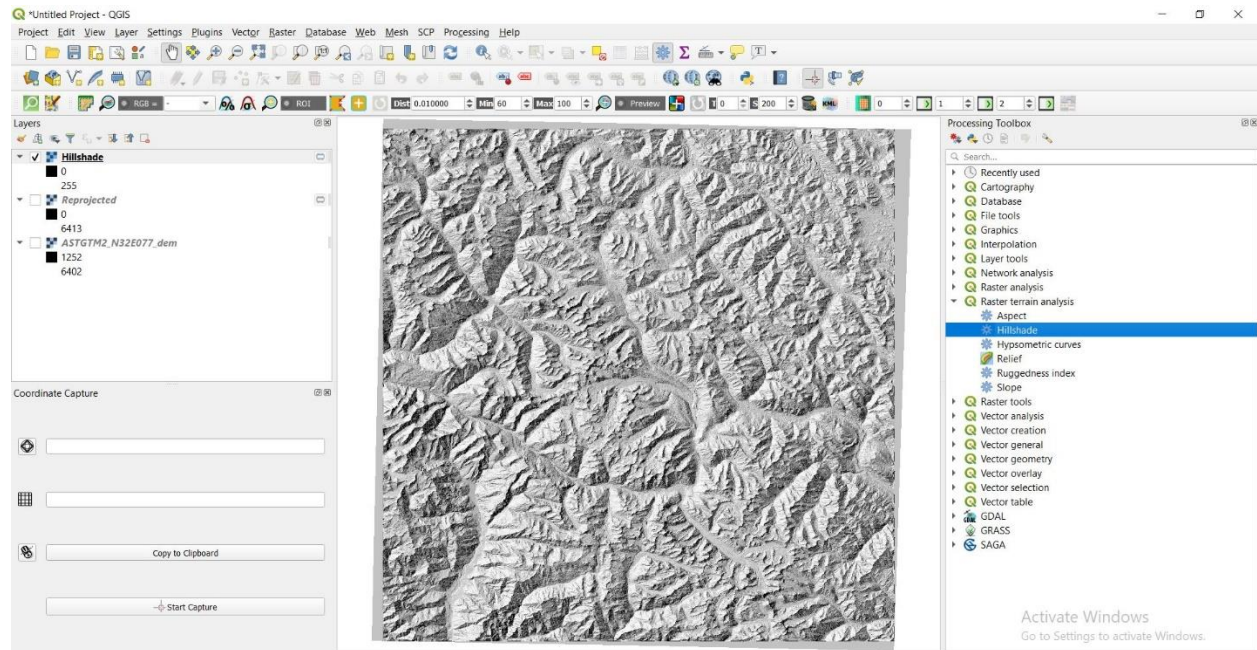


Hillshade

Step 5: To find the hillshade simply type slope option in the search bar and choose the “Hillshade” option or else click on Processing> Toolbox> Raster terrain analysis> Hillshade.

By clicking on the hillshade option a “Hillshade” window will pop-up. In this, choose the DEM file and choose the parameters as per the requirement and then click on “Run”.





Practical 10: Watershed Delineation

Watershed is the land area which contributes to drain water during rainstorm. The boundary of watershed consists of the line drawn across the contours joining the highest elevations surrounding the basin. Watershed delineation can be done by DEM data or topographic contour maps.

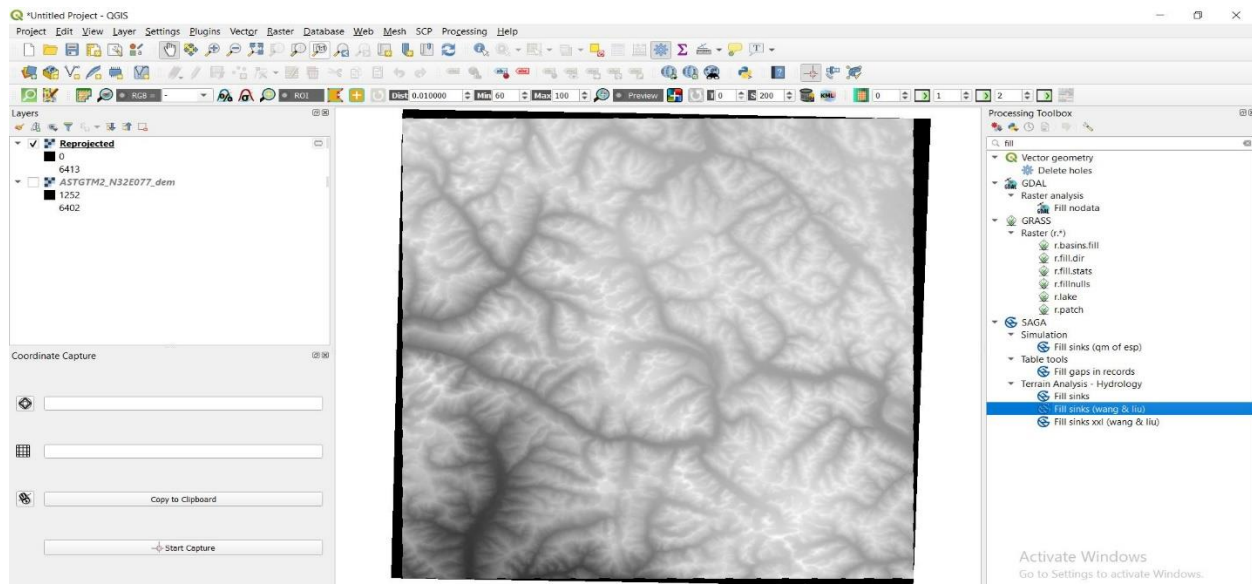


Step 1: Open the DEM data by clicking on Layer> Add Shapefile> Add Raster Shapefile. After selecting the DEM file click on “Add”. After opening the DEM file in QGIS. Select the file name that you have opened and right click on the file in the Layers and then select the “Information” option.

In the information window we can see that unit is in degrees and its coordinate reference system (CRS) is WGS 84. We need to convert degrees into meters and the CRS into the PRS i.e. UTM zone 43N. For this we have to re-project the DEM file.

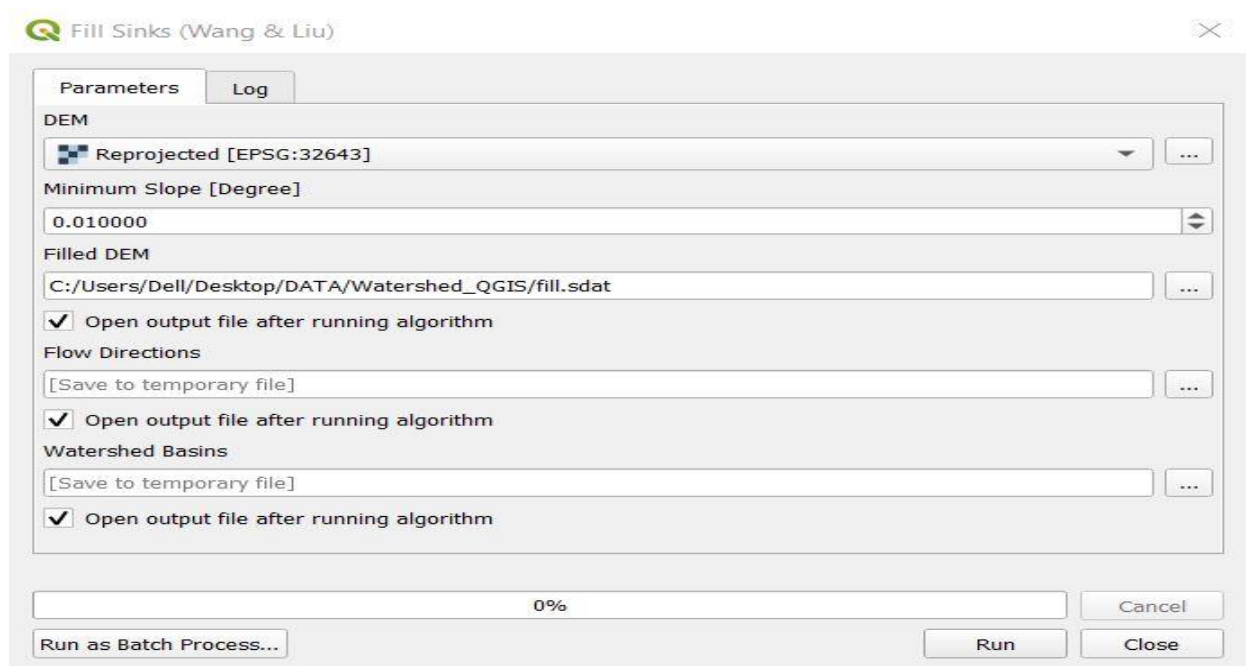
For re-projection, type “Re-project” in the search bar and choose the “Wrap (re-project)” option.

In the Warp (re-project) window choose the WGS 84/ UTM Zone 43N projection and click on “OK”. Then open the Reprojected DEM file.



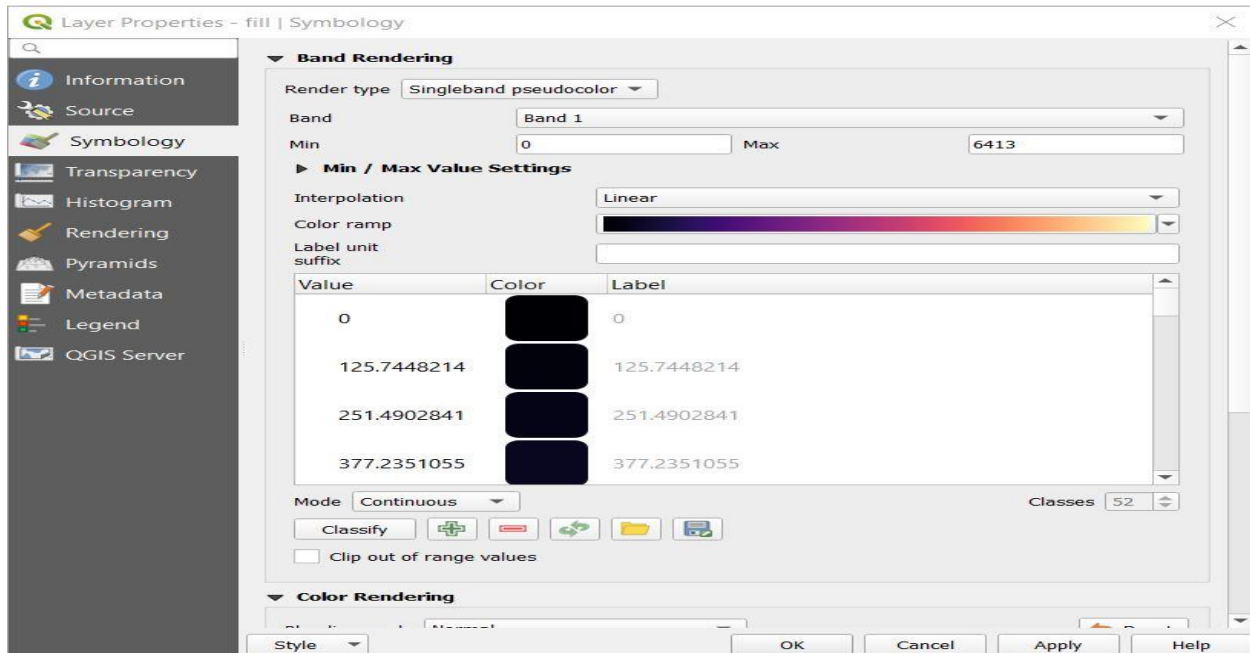
Fill

Step 2: To Fill DEM file click on Processing> Toolbox. When the Processing Toolbox gets opened then type the “Fill” in the search bar and select the “Fill sinks (wang& liu)” option. Then provide the reprojected file as input DEM file and click on “Run”.

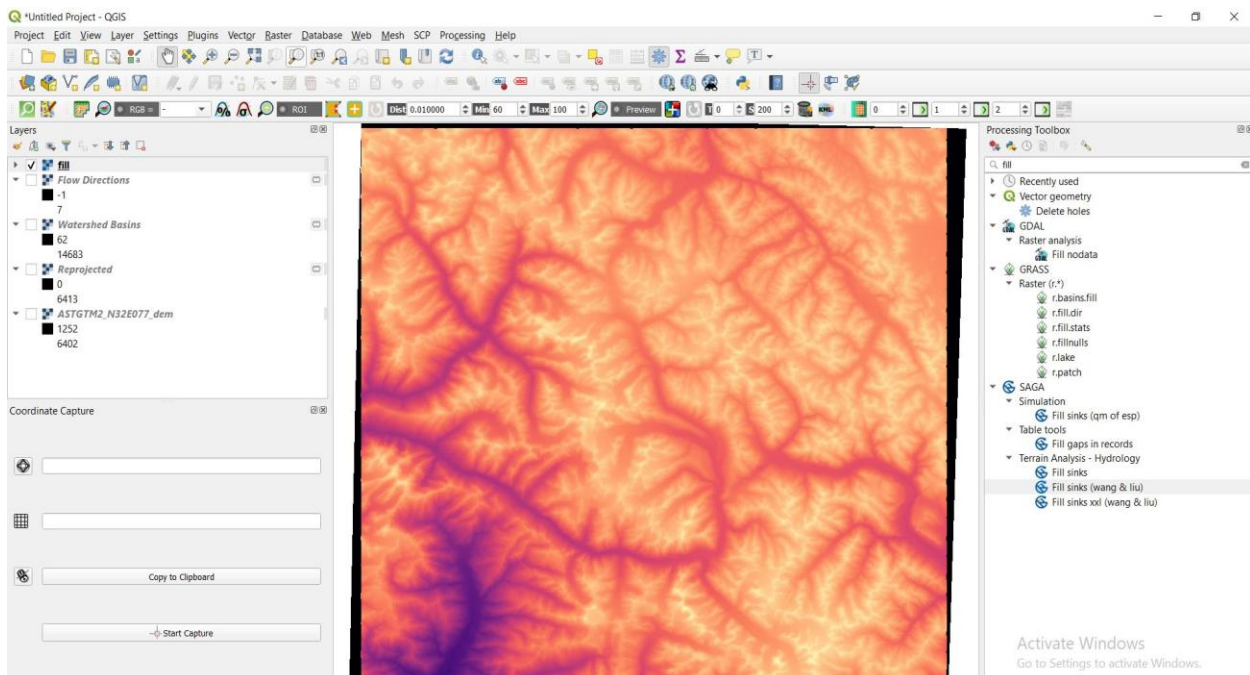


Along with “Fill” it will also provide the information regarding the “Flow Direction” and “Watershed Basin”. To enhance the “Fill”, choose the “Fill” file present in the Layers, right click

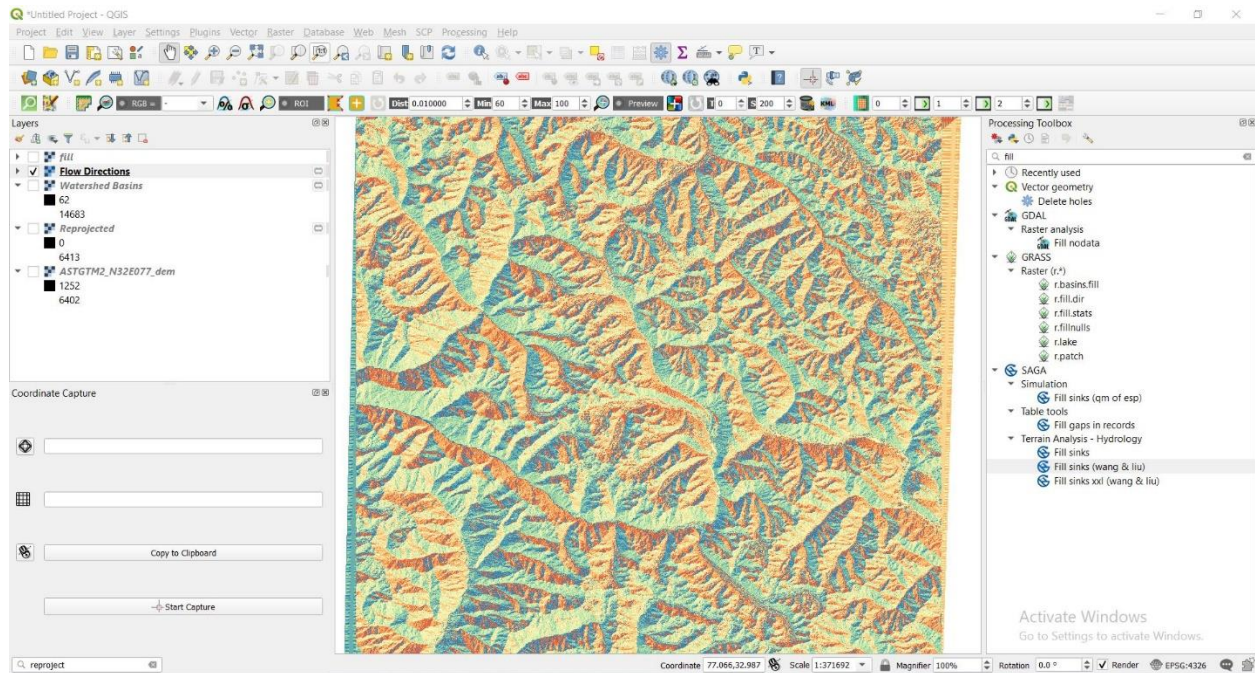
on it and click on properties. In the properties window, click on “Symbology”. Choose the style as per the need like in render type choose the “Singleband pseudocolor” and in color ramp choose the color as per the choice.



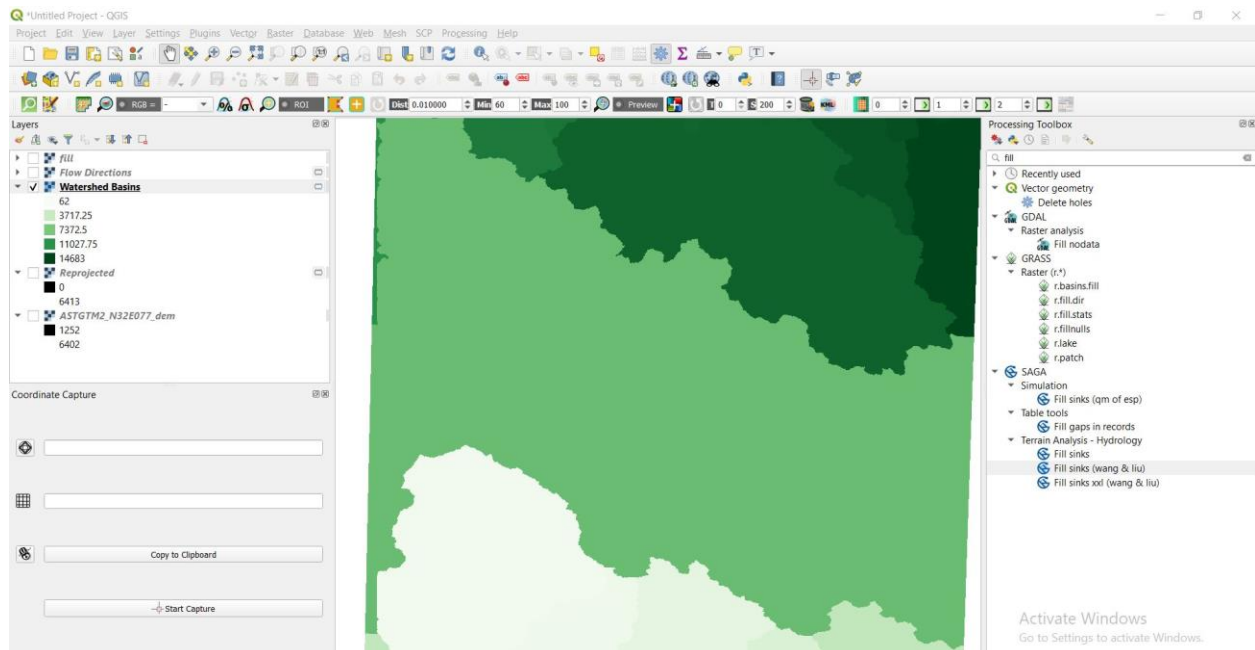
Then click on “Apply”.



Flow Direction

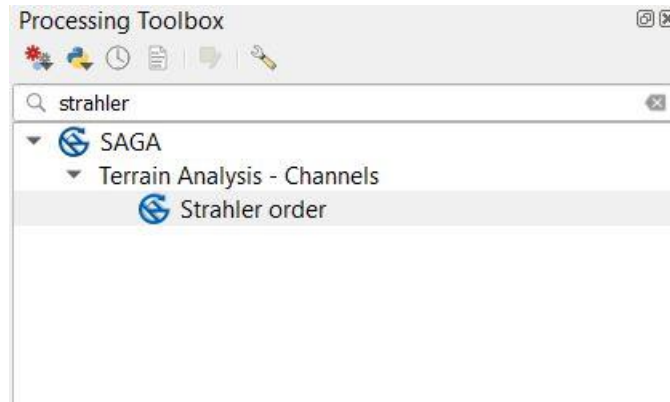


Watershed Basin

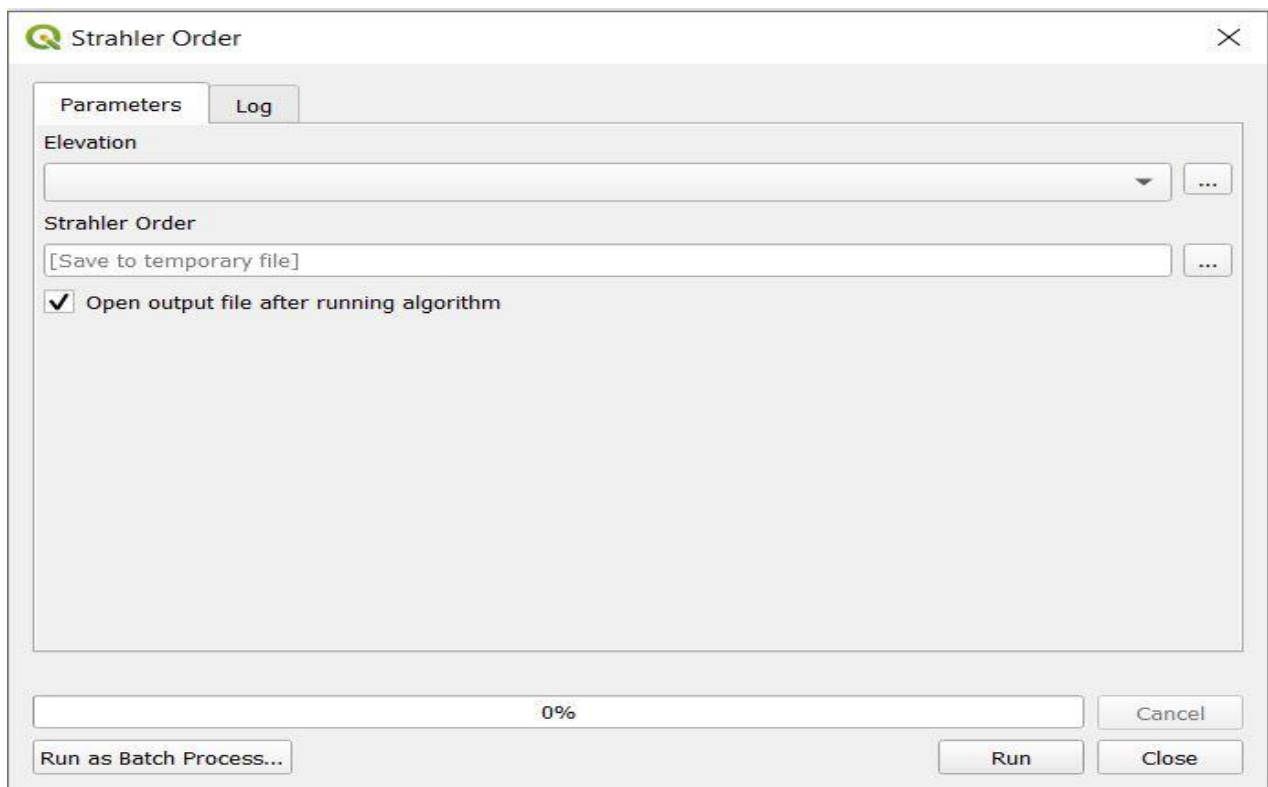


Stream Order

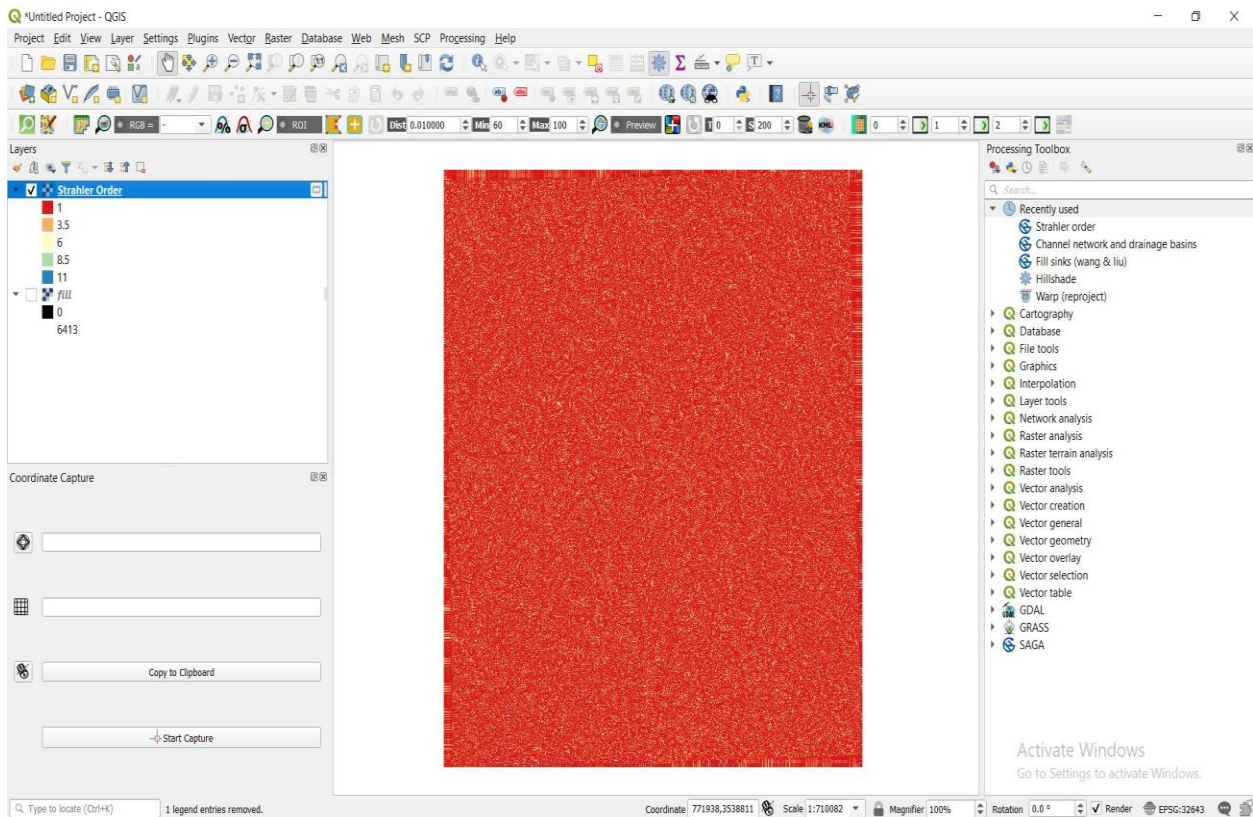
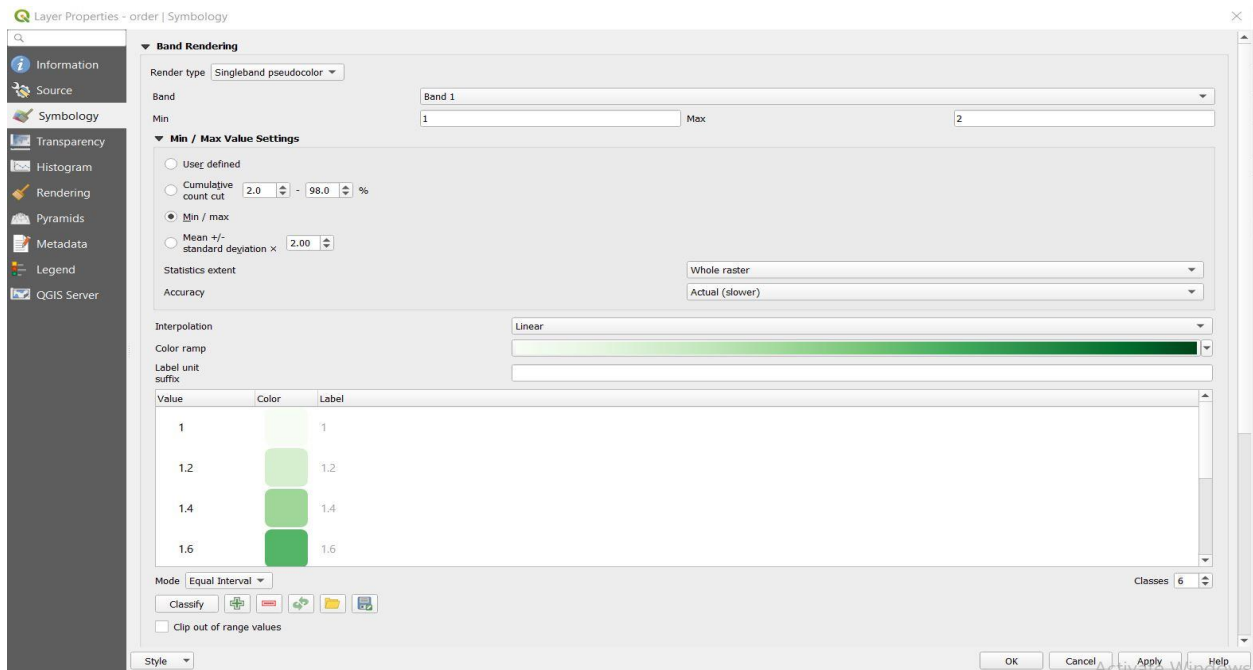
Step 3: To find the Strahler Order, type the “Strahler” in the search bar and select the “Strahler order” option.



Step 3: Provide the “Fill” DEM file as the input. Then click on “Run”.

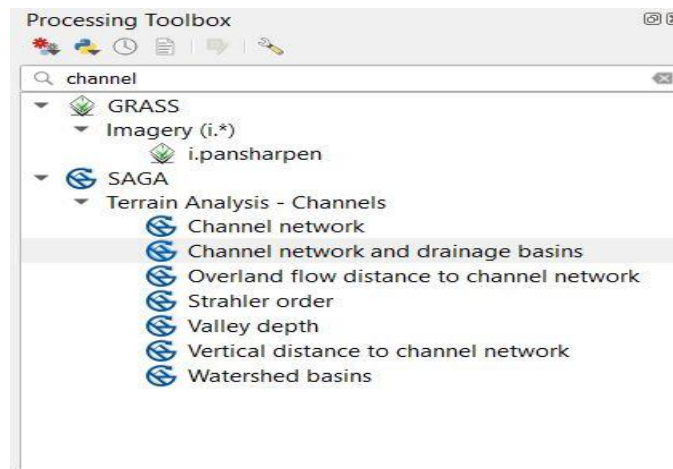


To enhance the “Strahler Order”, choose the “Order” file present in the Layers, right click on it and click on properties. In the properties window, click on “Symbology”. Choose the style as per the need like in render type choose the “Singleband pseudocolor” and in color ramp choose the color as per the choice, accuracy as “Actual” and mode as an “Equal Interval”.

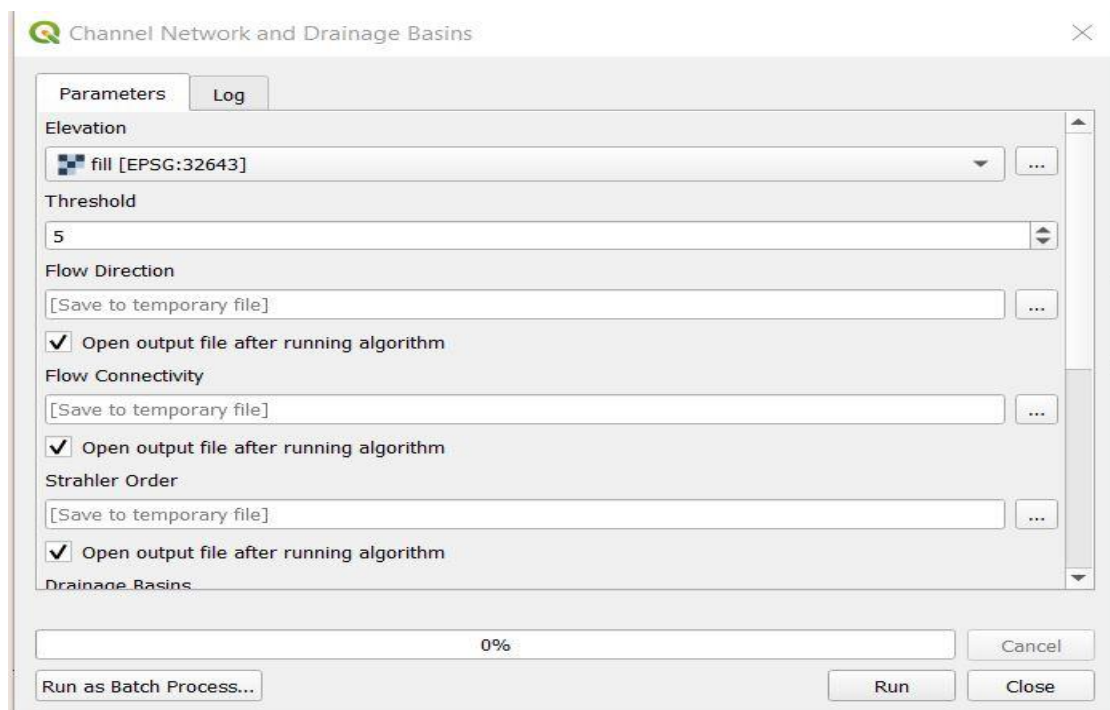


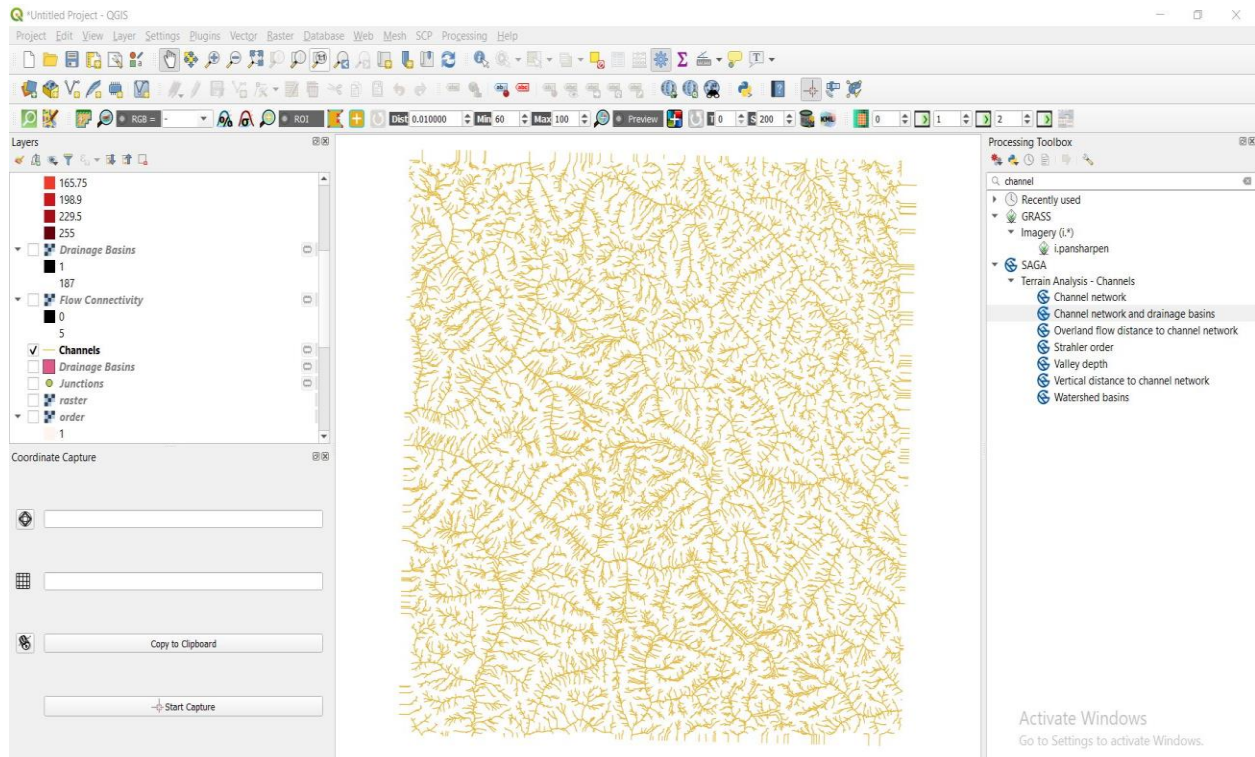
Channel network and drainage basins

Step 4: To find the channel network, type channel network on the processing toolbox and choose the “Channel network and drainage basins” option.

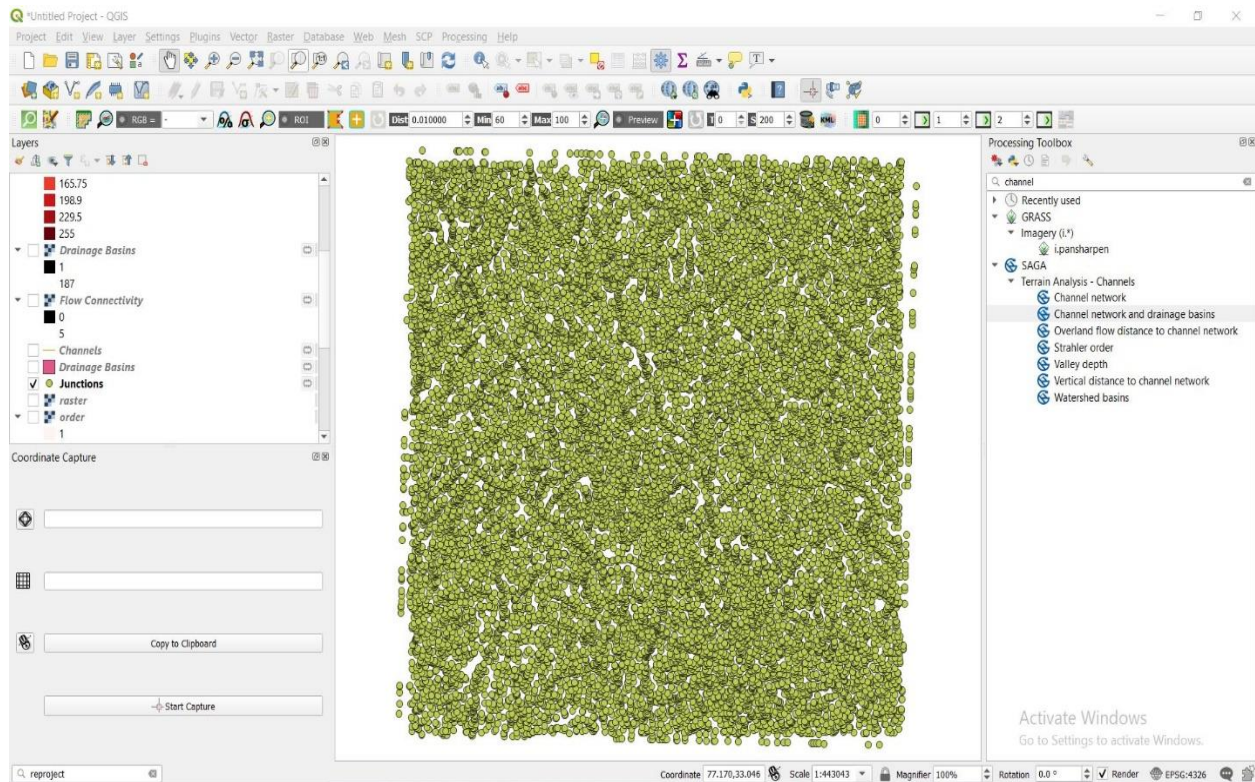


Choose the “Fill” DEM file as a the input file and click on”Run” option.

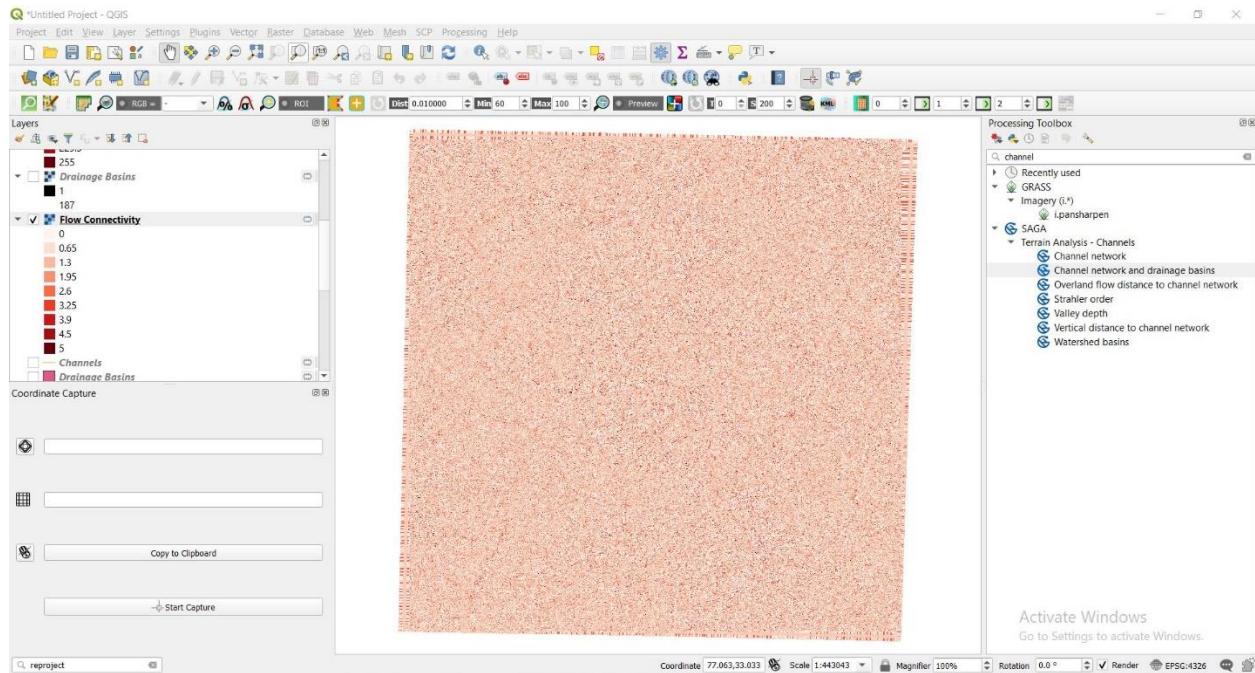




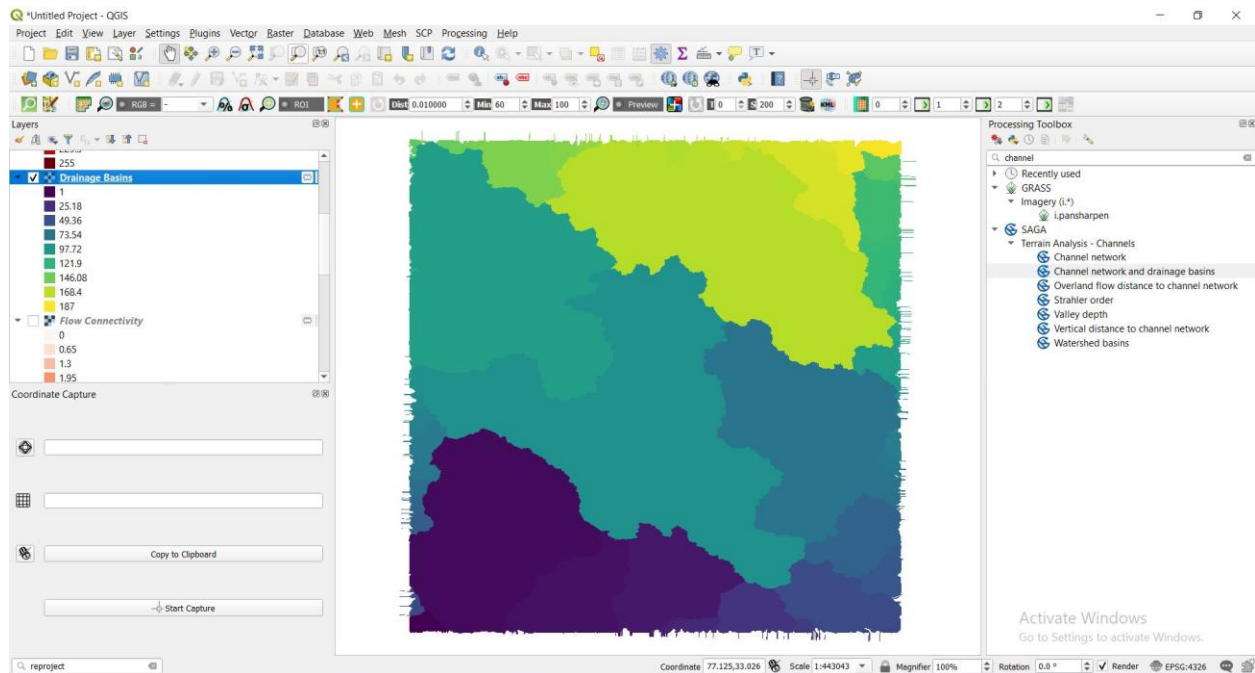
Junctions



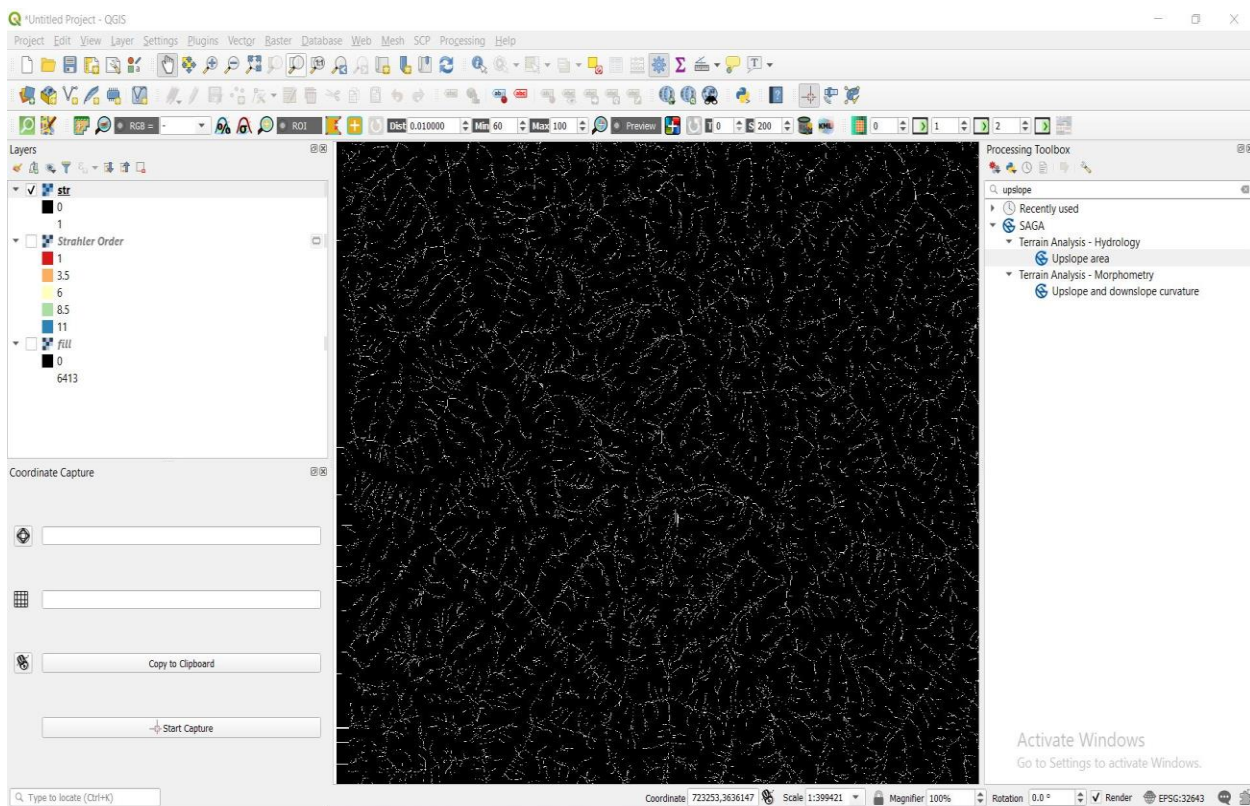
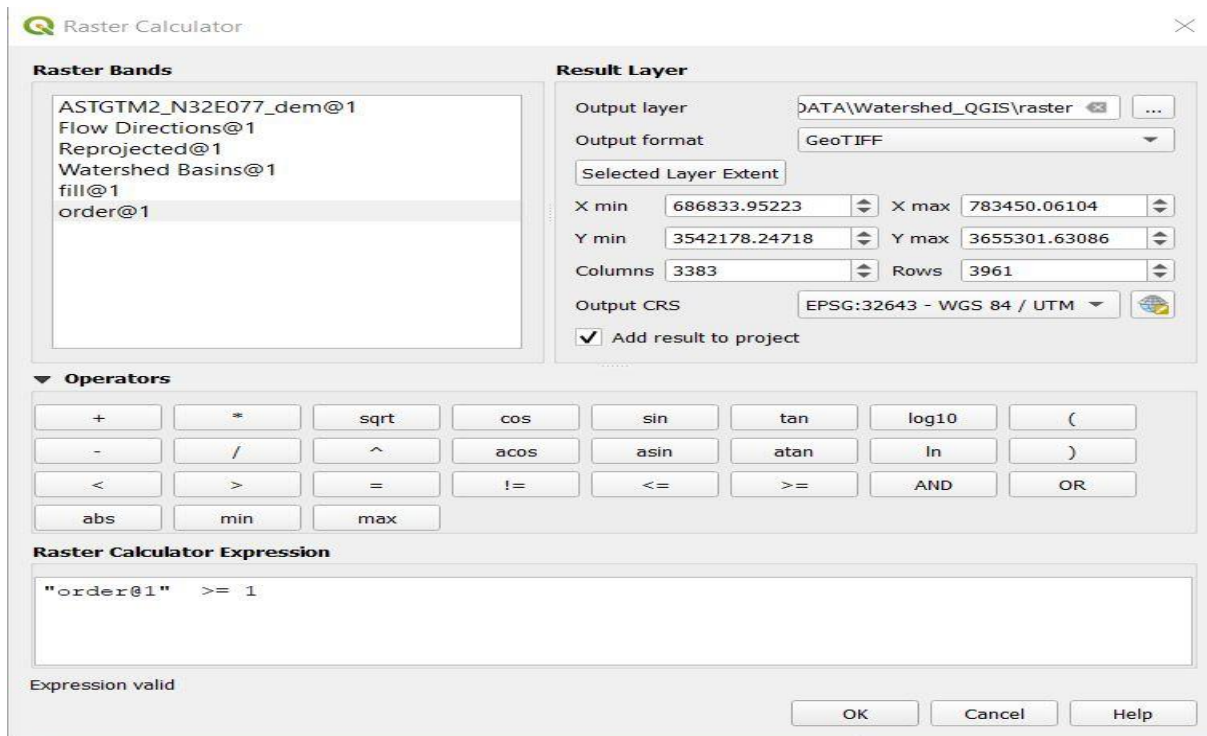
Flow Connectivity



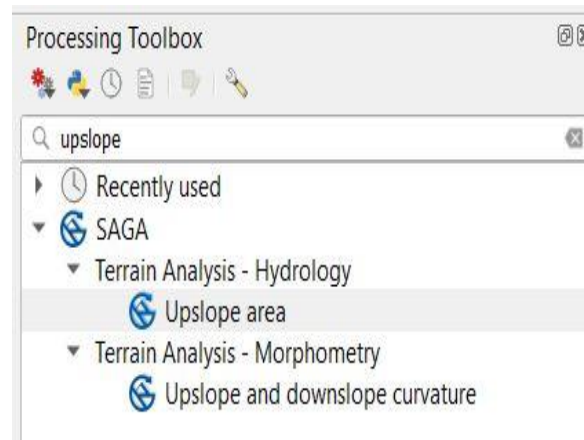
Drainage Basins



Step 5: To calculate the cutoff of stream order, click on Raster> Raster calculator and choose the order file. We can also provide the certain threshold values.



Step 6: To find the “Upslope area” in the DEM, type upslope area in the search bar of the processing toolbox and choose the “Upslope area” option.



In the Upslope window, provide the elevation file as “Fill” file and provide the x and y coordinates, choose the “Deterministic 8” method and the click on “Run”.



The watershed delineated but it is in raster. So we have convert the raster file into the vector file by Raster> Conversion> Raster to vector.