4. MATERIALS AND METHODS

The water and sediment samples collected from the 10 stations were analysed for various physico-chemical parameters. Study period was from June 2010 – June 2012. Monthly sampling was done during the first year i.e. from June 2010 – June 2011 and as there was no significant variation in the results obtained, sampling was done quarterly basis and analysed for the same parameters as done in the previous year. Because of heavy rain and flood, the local authority imposed restriction to collect the samples and hence sampling was not done in the month of November 2010.

Statistical analysis was carried out using software PAST 2.16. Correlation analysis measures the closeness of the chosen parameters. Statistical analysis of the data were interpreted using single factor analysis of variance (one way – ANOVA, p<0.05 and p<0.001) to measure the significant differences between sampling stations. This was also applied to sediment parameters.

Water samples from the ten stations were collected with 4L polyethylene bottles. The bottles were rinsed with river water before collection. Temperature and pH of the water samples were measured immediately after collection. Water samples for DO and BOD measurements were collected with 300 mL BOD bottles. Water samples in the bottles were acidified with 1.5 mL concentrated HNO₃/L of sample (APHA, 1995, A17) after pH, conductivity and temperature measurements. Sediment samples were also collected at each station in thick quality polyethylene bags and immediately transported to the laboratory. They were shade dried (except for the analysis of moisture content) and stored. The dried samples were ground using pestle and mortar and sieved through a 2-mm mesh sieve.
Physico-chemical parameters for monthly samples were analysed according to the standard methods provided by Allison et al (A11), American Public Health Association (A12) and National Environmental Engineering Research Institute NEERI (N8). Physico-chemical parameters of water and sediment and its respective method of analysis were given in the tables 4.1 and 4.2.

Table 4.1 Water quality parameters and its method of analysis

<table>
<thead>
<tr>
<th>Physical Parameter</th>
<th>Method of Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Mercury Thermometer</td>
</tr>
<tr>
<td>Total Dissolved Solids (ppm)</td>
<td>Gravimetric</td>
</tr>
<tr>
<td>Total Suspended Solids (ppm)</td>
<td>Gravimetric</td>
</tr>
<tr>
<td><strong>Chemical Parameter</strong></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>Electrometric</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>Electrometric</td>
</tr>
<tr>
<td>Alkalinity (ppm)</td>
<td>Titrimetric (acid-base)</td>
</tr>
<tr>
<td>Chloride (ppm)</td>
<td>Argentometric</td>
</tr>
<tr>
<td>Total Hardness (ppm)</td>
<td>Titrimetric (EDTA method)</td>
</tr>
<tr>
<td>Calcium hardness (ppm)</td>
<td>Titrimetric (EDTA method)</td>
</tr>
<tr>
<td>Magnesium hardness (ppm)</td>
<td>Titrimetric (EDTA method)</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>Titrimetric (Winkler’s method)</td>
</tr>
<tr>
<td>Biological Oxygen Demand (mg/L)</td>
<td>Titrimetric (BOD₅ test)</td>
</tr>
<tr>
<td>Sodium (ppm)</td>
<td>Flame Photometer</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>Flame Photometer</td>
</tr>
<tr>
<td>Sulphates (ppm)</td>
<td>Gravimetric</td>
</tr>
<tr>
<td>Nitrates (ppm)</td>
<td>Spectrophotometer (Brucine method)</td>
</tr>
<tr>
<td>Phosphates (ppm)</td>
<td>Ammonium molybdate</td>
</tr>
</tbody>
</table>
Table 4.2 Soil quality parameters and its method of analysis

<table>
<thead>
<tr>
<th>Physical Parameter</th>
<th>Method of Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content (%)</td>
<td>Gravimetric</td>
</tr>
<tr>
<td>Soil texture analysis</td>
<td>Sedimentation</td>
</tr>
</tbody>
</table>

**Chemical Parameter**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method of Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Electrometric</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>Electrometric</td>
</tr>
<tr>
<td>Chloride (mg/g)</td>
<td>Argentometric</td>
</tr>
<tr>
<td>Potassium (mg/g)</td>
<td>Flame Photometer</td>
</tr>
<tr>
<td>Sulphates (mg/g)</td>
<td>Gravimetric</td>
</tr>
<tr>
<td>Nitrates (mg/g)</td>
<td>Spectrophotometer</td>
</tr>
<tr>
<td>Phosphates (mg/g)</td>
<td>Ammonium molybdate</td>
</tr>
<tr>
<td>Soil Organic Carbon (%)</td>
<td>Wet Oxidation method</td>
</tr>
</tbody>
</table>

4.1 Water parameters

4.1.1 Temperature

Temperature of the water samples were measured with calibrated mercury filled Celsius thermometer with an accuracy of 0.1°C.

4.1.2 pH

At a given temperature, the intensity of acidic or basic character of a solution is indicated by pH or hydrogen ion concentration.

**Determination**

pH of the water samples is determined using pH meter with glass-calomel electrode.
**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.

**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.

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 - The glass electrode consists 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.

**Reagents and standards**

pH 4 buffer solution - 10.12g potassium hydrogen phthalate (KHC_8H_4O_8) is dissolved in distilled water and diluted to 1L.
pH 7 buffer solution - 1.361g anhydrous potassium dihydrogen phosphate (KH$_2$PO$_4$) and 1.42g anhydrous disodium hydrogen phosphate (Na$_2$HPO$_4$) is dissolved in distilled water and diluted to 1L.

pH 9.2 buffer solution - 3.81gm borax (Na$_2$B$_4$O$_7$.10H$_2$O) is dissolved in distilled water and diluted to 1L.

**Calibration**

First the electrodes are rinsed with distilled water. The electrodes are dried by gentle wiping with a soft tissue and are calibrated against freshly prepared standard buffer solution of known pH.

**Procedure**

50ml of water sample is taken in a 100ml beaker. The calibrated electrodes are immersed in the beaker. The pH value of the sample water is obtained directly from the pH meter.

4.1.3 Electrical Conductance

Conductivity is the ability of a material to conduct electric current. Since the charge on ions in solution facilitates the conductance of electrical current, the conductivity of a solution is proportional to its ion concentration. The basic unit of conductance is the siemen (S), formerly called the mho. It is expressed in µS/cm or mS/cm.

**Apparatus and reagents**

Conductivity meter with conductivity cell

Conductivity Water - The conductivity of the water should be less than 1 mmho/cm.
Standard potassium chloride (0.01M) - 745.6mg of anhydrous KCl is dissolved in conductivity water and made up to 1,000mL at 25°C.

**Procedure**

Conductivity meter is first calibrated with standard potassium chloride solution. 50ml of water sample is taken in a 100ml beaker. The conductivity cell connected to the conductivity meter is kept immersed in the beaker. The conductance value is directly obtained from the conductivity meter.

**4.1.4 Total Hardness**

Hardness is the property of water which prevents the lather formation with soap. It mainly depends on the amount of calcium or magnesium salts or both.

**Determination**

Hardness of water can be determined by EDTA method. This method makes use of complexometric titration of water sample with EDTA using EBT indicator.

**Principle**

The indicator EBT, which is a blue coloured dye, forms an unstable complex with Ca/Mg ions in water at a pH of 8-10. The complex is wine red in colour.

\[
\text{Ca}^{2+}/\text{Mg}^{2+} + \text{EBT} \longrightarrow [\text{Ca}^{2+}/\text{Mg}^{2+}-\text{EBT}]
\]

When this solution is titrated against EDTA, the free Ca\(^{2+}/\text{Mg}^{2+}\) ions in water forms a stable metal ion-EDTA complex.

\[
\text{Ca}^{2+}/\text{Mg}^{2+} + \text{EDTA} \longrightarrow [\text{Ca}^{2+}/\text{Mg}^{2+}-\text{EDTA}]
\]

Once the free metal ions are complexed, the EDTA replaces Ca and Mg ions from the unstable indicator complex, with the result the indicator is set free. The end point is the appearance of blue colour.


\[ \text{[Ca}^{2+}/ \text{Mg}^{2+}-\text{EBT}] + \text{EDTA} \rightarrow \text{[Ca}^{2+}/ \text{Mg}^{2+}-\text{EDTA}] \]

Thus the amount of EDTA used corresponds to the hardness of water.

**Solutions required**

Standard CaCO\(_3\) solution (0.05M) - 1g of CaCO\(_3\) is dissolved in minimum amount of HCl and then dissolved in 500ml of distilled water. Each ml of this solution contains 1mg of CaCO\(_3\) equivalent hardness.

EDTA solution (0.05M) - 4.65gms of disodium salt of EDTA is dissolved in 500ml of distilled water.

Ammoniacal buffer solution - Basic buffer solution is prepared by dissolving 12.5gms of NH\(_4\)Cl and 150ml of NH\(_4\)OH in 500ml of distilled water.

EBT solution (indicator) - 0.5g of EBT is dissolved in 100ml alcohol.

**Procedure**

50ml of the water sample is pipetted out in to a 100ml beaker. 12.5ml of NH\(_4\)Cl-NH\(_4\)OH buffer solution is added to maintain the pH of 8-10. Few drops of EBT indicator is added. It is then titrated slowly with EDTA solution taken in the burette. The colour change from wine red to steel blue is the end point.

**Calculation**

Hardness in milligrams of CaCO\(_3\) per litre = \( V_2 \times A \times 1000 / V_1 \)

\( V_1 \) - volume of the water sample taken (ml)

\( V_2 \) - volume of EDTA consumed (ml)

\( A \) - Milligrams of CaCO\(_3\) equivalent to 1ml of EDTA titrant.
4.1.5 Alkalinity

Alkalinity is a measure of the ability of water to neutralise the acids. Alkalinity of water is due to the presence of bicarbonates, carbonates and hydroxides of sodium, potassium, calcium and magnesium.

Principle

Determination of alkalinity due to different ions is based on the titration of the water sample against a standard acid making selective use of indicators. The indicators used are phenolphthalein and methyl orange. The reactions taking place is as follows:

\[
\begin{align*}
\text{OH}^- + \text{H}^+ & \rightarrow \text{H}_2\text{O} \\
\text{CO}_3^{2-} + \text{H}^+ & \rightarrow \text{HCO}_3^- \\
\text{HCO}_3^- + \text{H}^+ & \rightarrow \text{H}_2\text{CO}_3
\end{align*}
\]

Volume of the acid used up to phenolphthalein end point corresponds to the reaction (i) and (ii) i.e. complete neutralisation of OH\(^-\) and neutralisation of CO\(_3^{2-}\) ions up to HCO\(_3^-\) stage (half neutralisation of CO\(_3^{2-}\) ions).

Volume of acid used up to methyl orange end point corresponds to the reaction (i), (ii) and (iii) i.e. complete neutralisation of OH\(^-\), CO\(_3^{2-}\) and HCO\(_3^-\) ions.

Reagents

HCl (0.05N)

Phenolphthalein indicator

Methyl Orange indicator.
**Procedure**

50ml of the water sample is taken in a beaker. Few drops of phenolphthalein indicator is added. It is titrated with HCl until it become colourless. Then to the same solution few drops of methyl orange indicator is added. The titration is continued till a pale pink colour is obtained. If the titration due to phenolphthalein end point is zero, then the alkalinity is due to bicarbonate alone.

If the titration is due to methyl orange end point, then the alkalinity is due to hydroxides.

If phenolphthalein end point is half the total titration, then carbonate alkalinity is present.

If phenolphthalein end point is greater than half the total titration, then carbonate and hydroxide alkalinity is present.

If phenolphthalein end point is lesser than half the total titration, then carbonate and bicarbonate alkalinity is present.

**Calculation**

Alkalinity in milligrams of CaCO₃ per litre = \( V_2 \times 1000 / V_1 \)

\( V_1 \) - volume of the water sample taken (ml)

\( V_2 \) - volume of acid consumed (ml)

**4.1.6 Chloride**

The salty taste produced by chloride depends on the chemical composition of the water.

**Determination**

Chloride ions can be determined by Argentometric titration i.e. titration of the water sample against a standard solution of Silver nitrate using potassium chromate as indicator.
Principle

When AgNO₃ is added to the water sample in presence of K₂CrO₄, the chlorides present in it are precipitated as AgCl. As soon as all the chlorides are precipitated out, even a drop of AgNO₃ added in excess gives a reddish brown precipitate of Silver Chromate (Ag₂CrO₄), which indicates the end point.

\[
\text{AgNO}_3 + \text{NaCl} \rightarrow \text{AgCl} + \text{NaNO}_3
\]

\[
2\text{AgNO}_3 + \text{K}_2\text{CrO}_4 \rightarrow \text{Ag}_2\text{CrO}_4 \downarrow + 2\text{KNO}_3
\]

Reagents

Silver Nitrate solution (0.05N) - 0.85g of AgNO₃ is dissolved in distilled water and diluted to 100ml.

Sodium Chloride solution - 0.146g of NaCl is dissolved in distilled water and diluted to 50ml. This is used to standardise AgNO₃ solution.

Potassium Chromate solution - 0.5g of K₂CrO₄ is dissolved in 50ml distilled water.

Procedure

50ml of water sample is taken in a beaker and 5ml of K₂CrO₄ indicator is added. It is then titrated against AgNO₃ taken in the burette. The end point is the appearance of reddish brown colour.

Calculation

Chloride ion (mg/l) = 35.46 x N x V₂ x 1000 / V₁

\[V_1 \text{ – Volume of water sample taken (ml)}\]

\[V_2 \text{ – Volume of AgNO}_3 \text{ consumed (ml)}\]

\[N \text{ – Normality of AgNO}_3\]
4.1.7 Total Dissolved Solids

Water contains a variety of solid materials ranging from rags to colloidal materials. A known volume of well mixed water sample is filtered through a standard glass fibre filter. The filtrate when evaporated to dryness at 180 ± 2°C gives the amount of TDS. Higher temperature is maintained here to remove all the mechanically occluded water. If the organic matter is very low, losses due to higher drying temperature is negligible.

Apparatus

Evaporating dishes

Desiccator (with anhydrous Calcium Chloride)

Hot-air oven

Whatman No.41 filter paper.

Procedure

100ml of the water sample is filtered through Whatman No.41 filter paper. The filtrate is collected in an already weighed evaporating dish. It is evaporated in a hot-air oven at 180 ± 2°C. After the whole water is evaporated, the evaporating dish is cooled in the desiccator and then weighed.

Calculation

\[
\text{TDS (mg/l)} = \frac{(w_2 - w_1) \times 1000}{V}
\]

\(w_1\) – Initial weight of the evaporating dish (g)

\(w_2\) – Final weight of the evaporating dish (g)

\(V\) – Volume of the water sample (ml)
4.1.8 Total Suspended Solids

A known volume of well mixed water sample is filtered through a standard glass fibre filter. The residue retained on the filter paper when dried to 103-105°C gives the weight of TSS.

Procedure

100ml of the water sample is filtered through whatmann No.41 filter paper. The residue is washed with small portions of distilled water. After all the traces of water is removed, the residue is then dried in an oven at 103-105°C for 1 hour. It is then cooled in a desiccator and weighed.

Calculation

\[
\text{TSS (mg/l) = } \frac{(w_2 - w_1) \times 1000}{V}
\]

\[
w_1 - \text{Weight of filter (g)}
\]

\[
w_2 - \text{Weight of filter + residue (g)}
\]

\[
V - \text{Volume of the water sample (ml)}
\]

4.1.9 Dissolved Oxygen (DO)

The oxygen dissolved in water called dissolved oxygen is important for aquatic life.

Determination

DO is determined by Winkler’s method or Iodometric titration. It is a titrimetric procedure based on the oxidising property of water.

Principle

Iodometric titration is based on the fact that DO in water oxidises KI and an equivalent amount of iodine is liberated. It is then titrated against standard hypo solution.
using starch as indicator. Since DO is in molecular state and is not capable of reacting with KI, an oxygen carrier such as manganese hydroxide is used. Manganese hydroxide is prepared by the reaction between manganese sulphate and potassium hydroxide.

\[
\begin{align*}
\text{MnSO}_4 + 2\text{KOH} & \rightarrow \text{Mn(OH)}_2 + \text{K}_2\text{SO}_4 \\
\text{Mn(OH)}_2 + \text{O}_2 & \rightarrow 2\text{MnO(OH)}_2 \\
2\text{MnO(OH)}_2 + \text{H}_2\text{SO}_4 & \rightarrow \text{MnSO}_4 + 2\text{H}_2\text{O} + [\text{O}] \\
2\text{KI} + \text{H}_2\text{SO}_4 + [\text{O}] & \rightarrow \text{K}_2\text{SO}_4 + \text{H}_2\text{O} + \text{I}_2
\end{align*}
\]

Some water sources contain nitrates and sulphates which can oxidise KI and hence their presence gives incorrect results. Thus before the addition of KI these have to be eliminated and sodium azide is commonly used for this purpose.

**Reagents**

Manganese sulphate solution - 0.24g of manganous sulphate monohydrate is dissolved in 50ml of distilled water.

Alkali-iodide solution - 22.5g of KOH and 6.75g of KI is dissolved in distilled water. 1g of sodium azide is added to this and diluted to 50ml.

Sodium thiosulphate solution (0.05N) - 6.2g of Sodium thiosulphate is dissolved in distilled water and diluted to 50ml.

Starch indicator - 1g of soluble starch is dissolved in 100ml of boiling water with stirring.

Conc. $\text{H}_2\text{SO}_4$ - 98% w/w

**Procedure**

The water sample is collected in a DO bottle of 250ml capacity without any air bubbles. 1ml of manganous sulphate solution followed by 1ml of alkali-iodide solution is
added. The tip of the pipette should be below the liquid level while adding these reagents. The stopper is placed carefully to exclude air bubbles and mixed by inverting the bottle repeatedly for at least 15 minutes. The brown precipitate formed is allowed to settle. The stopper is removed carefully and 1ml of conc.\( \text{H}_2\text{SO}_4 \) is added. The bottle is closed and mixed by inverting until the precipitate is completely dissolved. 50ml of the solution is pipette out from the bottle and titrated with Sodium thiosulphate solution, when it becomes straw yellow starch indicator is added. At the end point the blue colour turns to colourless.

**Calculation**

\[
\text{DO (mg/l)} = \frac{(5.6 \times N \times V \times (b/b-2) \times 1000)}{a}
\]

- \( N \) – Normality of Sodium thiosulphate solution
- \( V \) – Volume of Sodium thiosulphate solution (ml)
- \( b \) – Volume of sample bottle (ml)
- \( (b-2) \) – Volume of sample bottle-2 (ml)
- \( a \) – Volume of water sample titrated (ml)

**4.1.10 Biological Oxygen Demand**

BOD is the amount of oxygen required by a definite volume of water for oxidising the organic matter in it by micro-organisms under specific conditions.

**Determination**

It is not practical to determine the ultimate oxygen demand till the oxidation gets completed. It has become a standard practice to measure oxygen over a shorter period of 5 days. This is known as 5 day BOD or BOD₅ test. BOD₅ is the total amount of oxygen consumed by micro-organisms during the first 5 days of bio-degradation.
Procedure

The water samples are collected simultaneously during the collection of samples for DO. BOD determination is made in a known volume of bottle which is incubated for 5 days at 25°C without adding any reagents. After 5 days the samples are allowed to retain the room temperature and DO is analysed as per the standard procedure. BOD value is calculated by subtracting the final DO from the initial DO.

Calculation

\[
\text{BOD (mg/l)} = \frac{[\text{Initial DO} – \text{Final DO}] \times 5.6 \times N \times (b/b-2) \times 1000}{a}
\]

- \(b\) – Volume of sample bottle (ml)
- \((b-2)\) – Volume of sample bottle-2 (ml)
- \(a\) – Volume of water sample titrated (ml)

4.1.11 Sodium

Sodium levels in water may vary from less than 1 mg Na/L to more than 500 mg Na/L.

Determination

Sodium is determined by flame emission photometric method.

Principle

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 conditions. The desired spectral line is isolated by the use of interference filters or by a suitable slit arrangement in light-dispersing devices such as prisms or gratings. The intensity of light is measured by a phototube potentiometer. The intensity of light at 589
nm is approximately proportional to the concentration of the element. The calibration curve gives the concentration of sodium in the sample.

**Apparatus and reagents**

Flame photometer

Stock sodium solution: Dissolve 2.542 g NaCl is dissolved in distilled water and diluted to 1000mL (1 mL = 1 mg Na).

Intermediate sodium solution: 10 mL of the stock sodium solution is diluted with to 100mL (1 mL = 100 µg Na). This intermediate solution is used to prepare calibration curve in sodium range of 1 to 10 mg/L.

Standard sodium solution: 10 mL of the intermediate sodium solution is diluted with water to 100 mL (1 mL = 10 µg Na). This solution is used to prepare calibration curve in sodium range of 0.1 to 1 mg/L.

**Procedure**

A series of standard solutions are prepared by diluting the standard sodium solution. The intensity of light emitted for the blank solution, standard solutions and also for the water sample is measured at 589 nm using flame photometer. Calibration graph is drawn between intensity of light emitted and concentration of sodium. From this graph the concentration of sodium in the water sample can be determined.

**4.1.12 Potassium**

Potassium is an essential element in both plant and human nutrition and occurs in groundwater as a result of mineral dissolution.

**Determination**

Potassium is determined by flame emission photometric method.
**Principle**

Potassium can be determined by flame emission photometry at the wavelength of 766.5 nm. The sample is sprayed into a gas flame and excitation is carried out under carefully controlled conditions. The desired spectral line is isolated by the use of interference filters or by a suitable slit arrangement in light-dispersing devices such as prisms or gratings. The intensity of light is measured by a phototube potentiometer. The intensity of light at 766.5 nm is approximately proportional to the concentration of the element. The calibration curve gives the concentration of potassium in the sample.

**Apparatus and reagents**

Flame photometer

Stock potassium solution: 1.907 g of KCl is dissolved in distilled water and diluted to 1000mL (1 mL = 1 mg K).

Intermediate potassium solution: 10 mL of the stock potassium solution is diluted with to 100mL (1 mL = 100μg K). This intermediate solution is used to prepare calibration curve in potassium range of 1 to 10 mg/L.

Standard potassium solution: 10 mL of the intermediate sodium solution is diluted with water to 100 mL (1 mL = 10μg K). This solution is used to prepare calibration curve in potassium range of 0.1 to 1 mg/L.

**Procedure**

A series of standard solutions are prepared by diluting the standard potassium solution. The intensity of light emitted for the blank solution, standard solutions and also for the water sample is measured at 766.5 nm using flame photometer. Calibration graph is drawn between intensity of light emitted and concentration of potassium. From this graph the concentration of potassium in the water sample can be determined.
4.1.13 Nitrate

Nitrate is the most highly oxidised form of nitrogen compounds commonly present in natural waters.

Determination

Nitrate in water sample is determined by brucine method. The reaction between nitrate and brucine produces an intense yellow colour. The intensity of the colour is measured at 410nm. This method is recommended for the concentration range of 0.1-2mg of NO$_3^-$ N/L.

Apparatus and reagents

Spectrophotometer (410nm)

Distilled water (nitrate-free water)

Stock nitrate solution - 0.7218g of potassium nitrate is dissolved in distilled water and diluted to 1000ml (1ml = 100 µg NO$_3^-$ (N)).

Standard nitrate solution - The stock solution of nitrate is diluted to 1000ml with distilled water (1ml = 1 µg NO$_3^-$ (N)).

Sodium arsenite solution - 5g of sodium arsenite is dissolved in distilled water and diluted to 1000ml.

Brucine – sulphanilic acid solution - 1g of brucine and 0.1g of sulphanilic acid is dissolved in 70ml hot water. 3ml of Conc. HCl is added to it and made up to 100ml.

Sulphuric acid - 50ml of conc.H$_2$SO$_4$ is added to 125ml of water.

Sodium Chloride solution - 300g of NaCl is dissolved in water and diluted to 1000ml.
**Procedure**

A series of standard solution of nitrate is prepared by diluting 1, 2, 4, 7 and 10ml of standard nitrate solution to 10ml with distilled water. 10ml of water sample is taken. 2ml of NaCl solution is added to the series of standard solutions, blank solution and water sample. It is mixed well and 10ml of H\textsubscript{2}SO\textsubscript{4} is added. It is mixed thoroughly and allowed to cool. 0.5ml of brucine-sulphanilic acid reagent is added. It is then placed in a stirred boiling water to maintain a temperature of 95\textdegree C. After 20 minutes the samples are taken and kept in cold water bath. When the room temperature is reached, the absorbance is measured using spectrophotometer at 410nm. Calibration graph is drawn and from this the concentration of nitrate in the water sample is determined.

**4.1.14 Phosphate**

Phosphorus is an essential nutrient for the growth of organisms and helps for the primary productivity of a water body.

**Determination**

Phosphate is determined by ammonium molybdate spectrophotometric method.

**Principle**

Ammonium molybdate reacts with orthophosphate under acidic condition to form molybdophosphoric acid. It is reduced by stannous chloride to a blue colour complex. The intensity of the blue colour is measured at 690nm, which gives the concentration of phosphate in the sample.

**Reagents**

Stock phosphate solution - 0.7111g of anhydrous potassium dihydrogen phosphate is dissolved in distilled water and diluted to 1000ml (1ml = 0.5mg PO\textsubscript{4}^{3-}).
Standard phosphate solution - 100ml of the stock solution is diluted to 1000ml (1ml = 0.05mg PO$_4^{3-}$).

Ammonium molybdate solution - 2.5g of ammonium molybdate is dissolved in 17.5ml of distilled water. 2.8ml of conc. H$_2$SO$_4$ is added to 40ml of distilled water. It is cooled and molybdate solution is added and diluted to 100ml.

Stannous chloride solution- 2.5g of stannous chloride dehydrate is dissolved in 100ml glycerol. It is then heated in a water bath with stirring.

**Procedure**

A series of standard phosphate solution of concentration 0.1,0.2,0.3,0.5mg/l is prepared by diluting the standard solution. 4ml of molybdate reagent and 0.5ml of stannous chloride is added. After 10 minutes the blue colour developed is measured using uv-visible spectrophotometer at 690nm.

The absorbance for the blank solution (distilled water) and the water sample are measured by the above same procedure. The concentration of phosphate is determined from the standard curve.

**4.1.15 Sulphate**

Sulphate is widely distributed in water ranging from few mg/l to several thousand mg/l.

**Determination**

Sulphate in water samples is determined by barium sulphate gravimetric method.

**Principle**

Sulphate is precipitated in HCl solution as barium sulphate by adding barium chloride solution. The precipitation is carried out near the boiling temperature and after a
period of digestion the precipitate is filtered, washed with water (till it is free of Cl ions), dried and weighed as barium sulphate.

**Apparatus**

Drying oven, desiccator, filter paper (pore size 0.45 µm)

**Reagents**

Barium Chloride solution (0.1N) and HCl

**Procedure**

50ml of water sample is taken in a 200ml beaker. pH (4.5-5) of the water is adjusted with few drops of HCl using pH meter. 1-2ml of HCl is added in excess. It is heated to boiling and warm BaCl$_2$ solution is added slowly with gentle stirring, till the precipitation is completed. Then 2ml is added in excess. The precipitate is digested at 80-90$^0$ C for more than 2 hours. A small amount of ash less filter paper pulp is added and filtered. The precipitate is washed with warm water to remove chloride ions. It is dried in an oven and then weighed.

**Calculation**

Sulphate (mg/l) = \( \frac{W \times 411.5}{V} \)

\[ W – \text{Weight of BaSO}_4^{2-} \]

\[ V – \text{Volume of water sample (ml)} \]

**4.1.16 Calcium**

Calcium contributes to the total hardness of water and is an important micronutrient in aquatic environment and is especially needed in large quantities by molluscs and vertebrates. It is determined by EDTA titrimetric method.
Principle

When EDTA is added to the water sample containing calcium and magnesium, it combines first with calcium. Calcium can be determined directly with EDTA when pH is made sufficiently high such that the magnesium is largely precipitated as hydroxyl compound (by adding NaOH and isopropyl alcohol). When murexide indicator is added to the solution containing calcium, all the calcium gets complexed by the EDTA at pH 12-13. The end point is indicated from a colour change from pink to purple.

Reagents

Sodium hydroxide (1N)

Murexide indicator (ammonium purpurate): 0.2 g of murexide is ground well with 100 g of sodium chloride.

Standard EDTA solution (0.01M)

Procedure

50ml of water sample is taken in a conical flask and 2ml of 1N NaOH solution is added (to produce pH of 12-13). A pinch of murexide indicator is added and titrated against EDTA solution until the pink colour changes to purple.

Calculation

\[
\text{Ca (mg/L)} = \frac{A \times 400.8 \times B}{V}
\]

A= volume of titrant (mL)
V= volume of sample taken (mL)
B= mg of CaCO3 equivalent to 1mL of EDTA titrant
4.1.17 Magnesium

Magnesium is an important element contributing to hardness and a necessary constituent of chlorophyll. Magnesium hardness can be calculated from the determined total hardness and calcium hardness.

**Calculation**

\[
Mg \text{ (mg/L)} = (TH \text{ as mg CaCO}_3/\text{L} - \text{Calcium Hardness as mg CaCO}_3/\text{L}) \times 0.243
\]

TH – Total Hardness (mg CaCO\(_3\)/L)

4.2 Sediment Parameters

4.2.1 pH

The pH of soil is an important physico-chemical property that influences the availability of nutrients, microbial activity and physical property of soil. It also shows the bottom decomposition condition of aquatic ecosystem.

pH of the sediment samples are determined using pH meter with glass-calomel electrode. 10g of air dried sediment sample is taken in a 50ml beaker. 20ml of distilled water is added and stirred well with a glass rod. It is then allowed to stand for 30minutes with intermittent stirring. The electrodes are immersed in the beaker containing soil-water suspension. The pH value of the sample is obtained directly from the pH meter.

4.2.2 Electrical Conductivity

Electrical Conductivity (EC) of sediment samples are measured with a conductivity meter. 20ml of distilled water is added to 10g of air dried sediment taken in a 100ml beaker. It is stirred well with a glass rod and allowed to stand for 30minutes with intermittent stirring. The conductivity cell connected to the conductivity meter is
immersed in the beaker containing soil-water suspension. The conductance value of the sample is obtained directly from the conductivity meter.

### 4.2.3 Moisture content

Moisture content or water content is the mass of water which can be removed from a soil, usually by heating at 60-80\(^0\)C (soils containing organic matter or gypsum should be dried at low temperature because higher temperature may burn the organic matter). It is expressed as the percentage of dry mass.

\[
W = \frac{M_w}{M_s} \times 100(\%)
\]

- \(M_w\) – mass of water
- \(M_s\) – mass of dry soil

#### Determination

The moisture content in sediment is determined by Oven drying method.

#### Apparatus

- Oven (60-80\(^0\)C)
- Weighing bottle with lid
- Desiccator with active desiccant (anhydrous Calcium sulphate).

#### Procedure

The weighing bottle with lid is cleaned, dried and weighed (\(M_1\)). 10 grams of the sediment sample is weighed in the bottle (\(M_2\)). The lid is removed and the bottle is kept in the oven and dried at 60-80\(^0\)C for 16-24 hours. After drying the bottle is cooled in the desiccator. The lid is replaced and weighed (\(M_3\)).

#### Calculation

Moisture content, \(W = \frac{M_w}{M_s} \times 100(\%) = \frac{M_2 - M_3}{M_3 - M_1} \times 100\) (%)  
\(M_1\) – weight of the bottle
$M_2$ – weight of wet sample

$M_3$ – weight of the sample after drying

### 4.2.4 Soil Organic Carbon

The resistant or stable fraction of soil organic matter contributes mainly to nutrient holding capacity (cation exchange capacity) and soil colour. It also acts as a source and sink of nutrients (Nitrogen, Phosphorus and Sulphur).

#### Determination

Soil Organic Carbon is determined by wet oxidation method of El-Wakeel and Riley.

#### Principle

Chromic acid in combination with $H_2SO_4$ favours the oxidation of organic matter in the sample. During oxidation, potassium dichromate reacts with $H_2SO_4$ to give nascent oxygen, which ultimately reacts with carbon to form $CO_2$. Excess of chromic acid used is determined by back titration with ferrous ammonium sulphate using diphenylamine indicator.

\[
2K_2Cr_2O_7 + 8H_2SO_4 \rightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 + 6[O] + 8H_2O
\]

\[
3C + 6[O] \rightarrow 3CO_2
\]

#### Reagents

Potassium dichromate (1N) – 49.04g of potassium dichromate (dried at 105 °C) is dissolved in distilled water and made up to 1 litre.

FAS (Mohr's Salt) (0.5N) – Dissolve 196g of Ferrous Ammonium Sulphate is dissolved in distilled water, 50mL conc. H2SO4 is added and made up to 1000 mL.
Conc. H₂SO₄ 98% w/w

O-Phosphoric acid – 85% (specific gravity 0.171)

Diphenylamine indicator – 0.5g of diphenylamine is dissolved in required amount of H₂SO₄ and made up to 100ml with distilled water.

Procedure

5g of air-dried sediment was taken in a 250ml conical flask. 10ml of 1N K₂Cr₂O₇ and 20ml of conc H₂SO₄ were added. The mixture is mixed gently by swirling and allowed to stand for 30 minutes. It was then made up to 200ml with distilled water. 10ml of o-phosphoric acid and 1ml of diphenylamine indicator were added. It was then titrated with ferrous sulphate solution, where the dark blue colour changes to dark green colour. Blank titrations (without sediment) were also carried out in the same way.

Calculation

From the equation:

\[ 2\text{Cr}_2\text{O}_7^{2-} + 3\text{C} + 16\text{H}^+ \rightarrow 4\text{Cr}^{3+} + 8\text{H}_2\text{O} + 3\text{CO}_2 \uparrow \]

1 mL of 1 N Dichromate solution is equivalent to 3 mg of carbon.

\[
\text{Organic Carbon (\%)} = \frac{0.003g \times N \times 10ml \times (1-T/S) \times 100}{W}
\]

\[
= \frac{3 (1-T/S)}{W}
\]

N - Normality of K₂Cr₂O₇ solution
T - Volume of FeSO₄ used in sample titration (mL)
S - Volume of FeSO₄ used in blank titration (mL)
W - weight of sample (g)
4.2.5 Nitrate

Nitrogen exists in soil in organic and inorganic forms. However, most of the nitrogen in soil water is in inorganic form like $\text{NH}_4^+$, $\text{NO}_3^-$ and $\text{NO}_2^-$.

**Determination**

Nitrate in sediment samples is determined by Phenol Disulphonic Acid Method. This method of estimation of nitrates depends on the nitration of 2,4-disulphonic acid in fuming sulphuric acid.

\[
\text{C}_6\text{H}_5\text{OH} (\text{HSO}_3)_2 + 2\text{HNO}_3 \rightarrow \text{C}_6\text{H}_2(\text{OH})(\text{NO}_2)_3 + 2\text{H}_2\text{SO}_4 + \text{H}_2\text{O}
\]

The product trinitrophenol behaves as a nitrophenolic type indicator and is colourless in acid medium and yellow when neutralised or in alkaline solution. Hydroxide such as KOH or NH$_4$OH is employed to shift the pH to the yellow colour range.

\[
\text{C}_6\text{H}_2(\text{OH})(\text{NO}_2)_3 + 2 \text{KOH} \rightarrow \text{C}_6\text{H}_2(\text{OK})(\text{NO}_2)_3 + \text{H}_2\text{O}
\]

The nitrate solution is dried prior to determination, as the reaction is effected in the presence of water.

**Apparatus and reagents**

Spectrophotometer (420nm)

Extractant solution - 20mL of 1N CuSO$_4$ is mixed with 100mL of 0.6% Ag$_2$SO$_4$ solution and diluted to 1 L.

Phenol 2,4 disulphonic acid - 25g of pure phenol is dissolved in 150 mL of conc.H$_2$SO$_4$. Then 85ml of conc. H$_2$SO$_4$ is added, stirred well and heated for 2 hours on water bath.

6N NH$_4$OH solution - One part of strong ammonia is mixed in one part of water.
Standard nitrate solution - 0.1629 g of pure KNO$_3$ is dissolved in water and diluted to 1L to give 100 ppm of NO$_3$. It is also further diluted to get standard NO$_3$ solution of 10 ppm concentration.

Extraction of NO$_3$ from soil - 50g of sediment is taken in a 500 mL conical flask and 250 mL of extraction reagent is added and shaken for 10 minutes followed by addition of 0.5 g of Ca (OH)$_2$ and 1 g of MgCO$_3$. The filtrate is collected by filtering through a dry Whatman No. 42 filter paper.

**Estimation**

- 20 mL of aliquot is taken in a porcelain dish and evaporated to dryness.
- It is cooled and 3 mL of Phenol 2,4 disulphonic acid is added directly into the centre of the basin.
- The content is swirled so that all the residual salts come in contact with acid and is kept for 10 minutes.
- 15 mL of cold water is added and the solution is stirred with a glass rod.
- 6N NH$_4$OH added slowly till the solution turns yellow (by alkaline condition) and 3 mL is added in excess (total volume should not exceed 100 mL).
- All the contents are transferred to 100 mL volumetric flask.
- Using spectrophotometer, the intensity of yellow colour at 420 nm is measured.
- The absorbance for the standard solutions (1, 2, 3, 4 and 5 ppm) are also measured
- Calibration graph is drawn between absorbance and concentration. From this graph the concentration of nitrate in the samples can be determined.

**Calculation:**

\[
\text{NO}_3^- (N) = \frac{X \times \text{Vol. made up} \times \text{Vol. of extractant} \times 2}{\text{Aliquot taken} \times \text{Weight of soil}}
\]

\[X= \text{nitrate content of soil extract (ppm)}\]
4.2.6 Phosphate

The inorganic phosphorus content in soil is higher than organic P content.

Determination

Phosphate in sediment samples is determined by ammonium molybdate Spectrophotometric method.

Apparatus and reagents

Spectrophotometer (690nm)

Stock phosphate solution - It is prepared by dissolving 4.388 g of dried anhydrous potassium hydrogen phosphate in distilled water to make 1000 mL. Now 10 mL of this solution is taken and diluted to 1 litre using distilled water (1 mg P/L).

Standard phosphate solution - Series of standard solutions are prepared from the stock solution (0.5 mL for 0.01 ppm, 1.0 mL for 0.02 ppm, 1.5 mL for 0.03 ppm, 2.0 mL for 0.04 ppm etc).

Ammonium molybdate solution - 31.4 g of ammonium molybdate is dissolved in 200 mL distilled water. 250 mL of conc. H₂SO₄ is added carefully to 400 mL distilled water. Cooled and added 3mL conc. HNO₃. To this ammonium molybdate solution is added and diluted to 1000 mL.

Sulphuric acid (0.002N) - 16.66 mL of concentrated sulphuric acid is diluted to 100mL to get the solution of strength 2N. Further, it is diluted by taking 1mL to 1000 mL to prepare solution of strength 0.002N.
**Procedure**

Take 1 g of air-dried sediment sample is taken in 500mL flask and 200mL H$_2$SO$_4$ is added. It is shaked for about half an hour and the suspension is filtered through the filter paper (Whatman No.50). Now 10 mL of this suspension is taken and 1 mL ammonium molybdate solution is added followed by 3 drops of stannous chloride to get a blue colour. Absorbance is measured using a spectrophotometer. At the same time the absorption for the standard solution is also estimated.

**Calculation**

\[ \text{PO}_4^{3-} (P) \text{ (mg/g)} = \frac{X \times V}{1000 \times Y} \]

- \(X\) - \text{PO}_4^{3-} (P) estimated in the suspension (mg/L)
- \(V\) - Total volume of suspension (mL)
- \(Y\) - Weight of air dry sediment taken.

**4.2.7 Sulphate**

Sulphur exists in soil and soil water or sediment as SO$_4^{2-}$ ions in combination with the cations Ca, Mg, K, Na or NH$_4^+$.

**Determination**

Sulphate in sediment samples is determined using Spectrophotometer at wavelength of 420 nm.

**Apparatus and reagents:**

- Spectrophotometer (420nm)
- Conditioning reagent - 50 mL glycerol is mixed with a solution containing 30 mL concentrated HCl, 300 mL distilled water, 100 mL 95% ethyl alcohol or isopropyl alcohol and 75 g NaCl.
Barium Chloride crystals

Standard sulphate solution - 147.9 mg of anhydrous Na$_2$SO$_4$ is dissolved in distilled water and diluted to 1000 mL (1mL = 100mg SO$_4$).

**Procedure**

10g of sediment is weighed and 100 mL of distilled water is added and the suspension is filtered using filter paper Whatman No. 44. 20mL of this suspension is taken in a conical flask and 2 mL of conditioning reagent is added followed by pinch of BaCl$_2$. Now, the readings are taken on the spectrophotometer at wavelength 420 nm. At the same time the reading of standard solution of sulphate (1 mL for 2 ppm, 2 mL for 4 ppm, 3mL for 6 ppm, 4 mL for 8 ppm) are also taken. Calibration graph is drawn between intensity of light absorbed and concentration of sulphate. From this graph the concentration of sulphate in the samples can be determined.

**Calculation**

\[
\text{Sulphate (mg/g)} = \frac{X \times V}{W \times 1000}
\]

- \(X\) = Sulphate content of soil extract (mg/L)
- \(V\) = total volume of soil extract (mL)
- \(W\) = weight of air-dried sediment.

**4.2.8 Potassium**

The concentration of K$^+$ ions in water is relatively low if compared with their content in soil. In soil and sediment K$^+$ ions exist mostly as exchangeable cations.
**Determination**

Potassium content of the sediment samples are determined by flame photometric method.

**Apparatus and reagents:**

Flame photometer (420nm)

Stock potassium solution - 1.907 g of potassium chloride is dissolved in 1000 mL of distilled water to get (1 mL = 1 mg K).

Standard potassium solution - Dilute 10 mL of the stock solution is diluted to 1 litre (1mL = 0.10 mg K).

Soil Extract - 10 g of sediment sample is weighed and 100 mL ammonium acetate solution is added to get a suspension of 1:10 w/v ratio. The solution is then filtered using the filter paper Whatman no.44.

**Procedure**

The flame photometer is calibrated using the standard solution of sodium and potassium. A series of standard solutions are prepared by diluting the standard solutions. The intensity of light emitted for the blank solution, standard solutions and also for the sediment sample is measured using flame photometer. Calibration graph is drawn between intensity of light emitted and concentration of sodium and potassium. From this graph the concentration of sodium and potassium in the samples can be determined.

**Calculation:**

\[
\text{Potassium (mg/g)} = \frac{X \times V}{W \times 10000}
\]

- \( X \) = potassium content of soil extract (mg/L)
- \( V \) = total volume of soil extract (mL)
- \( W \) = weight of air-dried sediment.
**4.2.9 Chloride**

Most of the chlorides in the sediment are soluble in water and determined directly in soil solution or suspension by argentometric method.

20ml of distilled water is added to 10g of air dried sediment taken in a 100ml beaker. It is stirred well with a glass rod and allowed to stand for 30minutes with intermittent stirring. 10ml of the solution is taken in a conical flask and 1ml of potassium chromate is added. It is then titrated against silver nitrate solution which gives the end point of colour change from yellow to brick red.

**Calculation:**

\[
\text{Chlorides (mg/g)} = \frac{A \times V}{10 \times W}
\]

- \(A\) = chloride estimated in filtrate (mg/g)
- \(V\) = total volume of suspension (mL)
- \(W\) = weight of soil or sediment used in suspension (g)