Chapter 4  
EXPERIMENTAL METHODS

4.1 Physicochemical Parameters

The following physicochemical parameters were analyzed. In general, the standard methods recommended by various standard organizations were adopted for determination of various parameters. The table below gives a brief detail of the proposed tests.

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<tr>
<td>25</td>
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<td>26</td>
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</tbody>
</table>

Table 4.1: PhysicoChemical parameters analysed

4.2 Physical characteristics
4.2.1 Colour

Colour is a physical parameter. coloured water is not acceptable for drinking (Aesthetic as well as toxicity reasons) In natural water, colour is due to the presence of humic acids, fulvic acids, metallic ions, suspended matter, plankton, weeds and industrial effluent

Definition
The term colour means true colour that is the colour of water from which turbidity has been removed. True colour of water is due to dissolved material. Apparent colour is due to suspended matter as well as due to substances on solution removed by filtrations.

Determination method
Colour is removed to make water suitable for general and industrial applications and is determined by visual comparison of the sample with distilled water.

Visual comparison
About 20ml of the sample and 20ml of distilled water were taken in two separate wide mouthed test tubes. The results were tabulated (as clear, greenish, greyish, brownish, blackish, etc) by comparing the colour of the sample with distilled water.

4.2.2 Temperature

Impinging solar radiation and atmospheric temperature brings about spatial and temporal changes in temperature, setting up convection currents and thermal stratification. Temperature plays a very important role in wetland dynamism affecting the various parameters such as alkalinity, salinity, dissolved oxygen, electrical conductivity etc. In an aquatic system, these parameters affect the chemical and biological reactions such as solubility of oxygen, carbon-di-oxide-carbonate-bicarbonate equilibrium, increase in
metabolic rate and physiological reactions of organisms, etc. Water temperature is important in relation to fish life. The temperature of drinking water has an influence on its taste.

**Apparatus required**
Thermometer- 0.1°C division.

**Procedure**
Surface water temperature was measured with the thermometer immersed directly in the water body, after a period of time sufficient to permit constant reading. Ground water temperature was measured by taking a portion of the water sample (about 1 litre) and immersing the thermometer into it for a sufficient period of time (till the reading stabilizes) and the reading was noted and expressed as, °C.

### 4.2.3 Turbidity

Turbidity is a measure of the collective optical properties of a water sample that cause light to be scattered and absorbed rather than transmitted in straight line. The suspended matter causing turbidity is expected to be clay, silt, non-living organic particulates, plankton, and other microscopic organisms, in addition to suspended organic or inorganic matter.

The domestic and industrial wastes may add great quantities of organic and some inorganic materials that contribute turbidity in river water. Organic materials reaching rivers serve as food for bacteria and resulting bacterial growth and other micro-organisms that feed upon the bacteria produce additional turbidity. Turbidity can also be caused by precipitated calcium carbonate in hard waters, aluminium hydrate in treated waters, and precipitated iron oxide in corrosive water.

**Principle**
It is based on comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard
reference suspension under the same conditions. Turbidity is expressed in the nephelometric turbidity unit (NTU) or Formazin turbidity units (FTU), depending on the method and the equipment used. The turbidity unit, NTU, is based on the amount of light scattered by particles of Formazin, a polymer used as a reference standard due to the reproducibility of the results. One mg/L of Formazin equals 1 NTU. Turbidity is measured by the nephelometric standard method.

Apparatus

- Sample Tubes - The sample tubes should be of clear and colourless glass.
- Turbidimeter- The turbidimeter shall consist of a nephelometer with a light source for illuminating the sample and one or more photo electric detectors with a readout device to indicate the intensity of light scattered at right angles to the path of the incident light. The turbidimeter should be so designed that little stray light reaches the detector in the absence of turbidity and should be free from significant drift after a short warm-u
- Lab-glass wares and
- Monopan balance

Procedure

Calibration

- First of all turbidimeter was calibrated by following the manufacturer’s operating instructions
- Rinse a clean, dry, scratch-free, index-marked cell with the turbidity standard selected at the NTU within the range of interest.
- Shake and pour standard into the sample cell to the fill mark and dry the cell exterior with a lint-free cloth.
- Follow manufacturer’s instructions for readout of turbidity value and record the NTU of the standard used and the turbidity value measured in the turbidimeter calibration log.
• Determine the required reading for the turbidity standard from the calibration curve for the instrument’s range and adjust the calibration to the required NTU reading.

Measurement of sample Turbidity less than 40 NTU

• Shake the sample to disperse the solids. Wait until air bubbles disappear. Pour sample into turbidimeter tube and read turbidity directly from the instrument scale.

Measurement of Turbidity above 40 NTU

• The samples were diluted with one or more volumes of turbidity free water until turbidity is less than 40 NTU after mixing and degassing. The turbidity of the original sample was calculated from the turbidity of the diluted sample and the dilution factor as shown below:

\[
\text{Turbidity (NTU)} = \frac{A \times (B+C)}{C}
\]

where,

\[A = \text{NTU found in diluted sample},\]
\[B = \text{volume of dilution water, in millilitres}, \]
\[C = \text{sample volume taken for dilution, in millilitres}.\]
4.2.4 Total Solids

Total solids is the term applied to the material residue left in the vessel after evaporation of the sample and its subsequent drying in an oven at a temperature of 103-105°C. Total solids include Total Suspended Solids (TSS) and Total Dissolved Solids (TDS).

**Principle**

A known volume (50 ml) of well-mixed sample is evaporated in a pre-weighed dish and dried to constant weight in an oven at 103-105°C. The increase in weight over that of the empty dish gives the total solids.

**Apparatus**

Evaporating dishes-100ml porcelain dish, steam bath, drying oven, desiccator, Monopan balance and measuring jars.

**Procedure**

A known volume of the well-mixed sample (50ml) is measured into a pre-weighed dish and evaporated to dryness at 103°C on a steam bath. The evaporated sample is dried in an oven for about an hour at 103-105°C and cooled in a desiccator and recorded for constant weight.

**Calculation**

\[
\text{Total solids} = \frac{(W_1-W_2) (1000)}{\text{Sample volume (ml)}}
\]

where,

- \(W_1\) = Weight of dried residue + dish
- \(W_2\) = Weight of empty dish
4. 3 Chemical Characteristics

4.3.1 pH

pH value is the logarithm of reciprocal of hydrogen ion activity in moles per litre. The pH scale value extends from 0 (very acidic) to 14 (very alkaline) with the middle value 7. In water solution, variations in pH value from 7 are mainly due to hydrolysis of salts of strong bases and weak acids or vice-versa. Dissolved gases such as carbon dioxide, hydrogen sulphide and ammonia also affect pH value of water. The overall pH value range of natural water is generally between 6 and 8. In case of alkaline thermal spring waters, pH value may be more than 9; while for acidic thermal spring waters the pH may be 4 or even less than 4. Industrial wastes may be strongly acidic or basic and their effect on pH value of receiving water depends on the buffering capacity of water. The pH value obtained in the laboratory may not be the same as that of water at the time of collection of samples due to loss-or absorption of gases, reactions with sediments, hydrolysis and oxidation or reduction taking place within the sample bottle. pH value should preferably be determined at the time of collection of sample. The pH value may be determined either electrometrically or calorimetrically.

Mathematically:

\[
pH = - \log[H^+]
\]

Principle

The pH value is determined by measurement of the electromotive force of a cell consisting of an indicator electrode (an electrode responsive to hydrogen ions such as a glass electrode) immersed in the test solution and a reference electrode (usually mercury/calomel electrode), contact between the test solution and the reference electrode is usually achieved by means of a liquid junction, which forms part of the reference electrode. The electromotive force is measured with a pH meter, that is, a high impedance voltmeter calibrated in terms of pH 2. Several types of electrodes have been suggested for electrometric determination of pH value. Although the hydrogen gas
electrode is recognized as primary standard the glass: electrode in combination with calomel electrode is generally used with reference potential provided by saturated calomel electrode. The glass electrode system is based on the fact that a change, of 1 pH unit produces an electrical change of 59.1 mV at 25°C. The active element of a glass electrode is a membrane of a special glass. The membrane forms a partition between two liquids of differing hydrogen ion concentration and a potential is produced between the two sides of the membrane which is proportional to the difference in pH between the liquids.

**Apparatus**

pH meter - With glass and reference electrode (saturated calomel), preferably with temperature compensation, Thermometer - With least Count of 0.5°C.

**Reagents**

- Buffer solution of 9.2 pH
- Buffer solution of 4.0 pH

**Procedure**

**Calibration**

- Each instrument/electrode system must be calibrated at a minimum of two points that bracket the expected pH of the samples and are approximately three pH units or more apart.
- Repeat adjustments on successive portions of the two buffer solutions until readings are within 0.05 pH units of the buffer solution value.
- Place the sample or buffer solution in a clean glass beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar. If field measurements are being made, the electrodes may be immersed directly into the sample stream to an adequate depth and moved in a manner to ensure sufficient sample movement.
across the electrode-sensing element as indicated by drift-free readings (< 0.1 pH).

- If the sample temperature differs by more than 2 EC from the buffer solution, the measured pH values must be corrected. Instruments are equipped with automatic or manual compensators that electronically adjust for temperature differences.
- Thoroughly rinse and gently wipe the electrodes prior to measuring pH of samples. Immerse the electrodes into the sample beaker or sample stream and gently stir at a constant rate to provide homogeneity and suspension of solids. Note and record sample pH and temperature. Repeat measurement on successive aliquots of sample until values differ by < 0.1 pH units. Two or three volume changes are usually sufficient.

### 4.3.2 Total Dissolved Solids

Dissolved solids are solids that are in dissolved state in solution. Total dissolved solids are made up primarily of inorganic salts with small concentrations of organic matter. Contributory ions are mainly carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium.

Waters with high dissolved solids generally are of inferior palatability and may induce an unfavourable physiological reaction in the transient consumer.

**Principle**

The difference in the weight of total solids and the total suspended solids expressed in the same units gives the total dissolved solids.

**Apparatus**

Glass-fiber filter disks, membrane filter funnel, filtration apparatus, suction flask and pump, drying oven and Grooch crucible.
**Procedure**

The difference in the weights of Total Solids ($W_1$) and Total Suspended Solids ($W_2$) expressed in the same units gives Total Dissolved Solids (TDS).

**Calculation**

\[
\text{Total Dissolved Solids} = \frac{(W_1 - W_2) \times 1000}{\text{Sample volume (ml)}}
\]

where,

- $W_1$ = Weight of total solids + dish
- $W_2$ = Weight of total suspended solids

**4.3.3 Total Suspended Solids**

Suspended solids are the portions of solids that are retained on a filter of standard specified size (generally 2.0 µ) under specific conditions. Water with high-suspended solids is unsatisfactory for bathing, industrial and other purposes.

**Principle**

A well-mixed sample is filtered through a weighed standard glass fibre filter and the residue that is retained on the filter is dried to a constant weight at 103-105 °C. The increase in the weight of the filter determines the total suspended solids.

**Apparatus**

Porcelain dish (100ml capacity), glass fiber filter disk, suction pump and flask, measuring jar, membrane filter funnel, oven and filtration apparatus.

**Procedure**

The known volume of vigorously shaken sample (50ml) is filtered into a pre-weighed glass fibre filter disk fitted to suction pump, and washed
successively with distilled water. The filter is carefully removed from the filtration apparatus and dried for an hour at 103-105 °C in an oven, cooled in dessicator and weighed for constant weight.

**Calculation**

\[
\text{Total Suspended Solids} = \frac{(W_1-W_2) (1000)}{\text{Sample volume (ml)}}
\]

where,

\[
W_1 = \text{Weight of dried glass fibre filter + residue}
\]

\[
W_2 = \text{Weight of glass fibre filter disk before filtering}
\]

**4.3.4 Electrical Conductivity**

Conductivity is a useful test in raw and finished water for quick determination of minerals.

Conductivity (specific conductance) is the numerical expression of the water's ability to conduct an electric current. It is measured in micro Siemens per cm and depends on the total concentration, mobility, valence and the temperature of the solution of ions. Therefore, presence of most inorganic acids, basis and salts increases the conductivity of water sample. on the other hand, organic compound molecules which do not dissociate, do not contribute to the conductivity *(Kudesia, 1990)*.

**Principle**

Electrolytes in a solution disassociate into positive (cations) and negative (anions) ions and impart conductivity. Most dissolved inorganic substances are in the ionised form in water and contribute to conductance. The conductance of the samples gives rapid and practical estimate of the variation in dissolved mineral content of the water supply. Conductance is defined as the reciprocal of the resistance involved and expressed as mho or Siemen (s). The unit of Electrical Conductivity is mmhos or µmhos or S.
\[ G = \frac{1}{R} \] where, \( G \) is Conductance (mho or Siemens) and \( R \) – Resistance

**Apparatus**

Conductivity meter

**Reagents**

- Conductivity water
- Standard KCl solution

**Procedure**

All the solutions used for electrical conductively measurement were prepared with conductively water with conductively less than 1 \( \mu \)mhos/cm and 0.01m, standard 140 potassium chloride solution with a conductivity of 1413 \( \mu \)mhos/cm at 25\(^\circ\)C. These were carefully diluted further to 0.001m and 0.005 m to give 141.3 and 717.8 \( \mu \)mhos/cm, at 25\(^\circ\)C. The cell constant of the conductively meter was adjusted by immersing the cell in a standard 0.001m KCl solution to a conductance of 147.0 \( \mu \)mhos/cm. The functioning of the meter was further checked by another standard 0.005m KCl solution which gave a conductively of 717.8 \( \mu \)mhos/cm. The cell was thoroughly washed twice or thrice with de-ionised water and then with sample. Thereupon, conductivity of the sample was measured and recorded.

4.3.5 **Total Hardness**

Hard water is generally considered that require considerable amount of soap to produce foam/lather and that produce scale in hot-water pipes, heating boilers and other units in which the temperature of water is increased materially. Surface raw water is softer than ground water (more rain less contact with soil minerals).

Hardness is predominantly caused by divalent cations such as calcium, magnesium; alkaline earth metal such as iron, manganese, strontium, etc. The total hardness is defined as the sum of calcium and magnesium concentrations, both expressed as CaCO\(_3\) in mg/L.
bicarbonates of calcium and magnesium cause temporary hardness. Sulphates and chlorides cause permanent hardness.

The hardness Chart (for drinking water) can be seen as below

<table>
<thead>
<tr>
<th>Hardness Level</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft</td>
<td>0 – 60</td>
</tr>
<tr>
<td>Medium</td>
<td>60 – 120</td>
</tr>
<tr>
<td>Hard</td>
<td>120 - 180</td>
</tr>
<tr>
<td>Very Hard</td>
<td>&gt; 180</td>
</tr>
</tbody>
</table>

**Principle**

In alkaline conditions EDTA (Ethylene-diamine tetra acetic acid) and its sodium salts react with cations forming a soluble chelated complex when added to a solution. If a small amount of dye such as Eriochrome black-T is added to an aqueous solution containing calcium and magnesium ions at alkaline pH of 10.0 ± 0.1, it forms wine red colour. When EDTA is added as a titrant, all the calcium and magnesium ions in the solution get complexed resulting in a sharp colour change from wine red to blue, marking the end point of the titration. Hardness of water prevents lather formation with soap rendering the water unsuitable for bathing and washing. It forms scales in boilers, making it unsuitable for industrial usage. At higher pH>12.0, Mg$^{++}$ ion precipitates with only Ca$^{++}$ in solution. At this pH, muroxide indicator forms a pink colour with Ca$^{++}$ ion. When EDTA is added Ca$^{++}$ gets complexed resulting in a change from pink to purple indicating end point of the reaction.

**Apparatus**

Lab glassware-burette, pipette, conical flask, beakers etc.

**Reagents**

- Buffer solution: 16.9 g of ammonium chloride and 1.25g of magnesium salt of EDTA is dissolved in 143ml of concentrated ammonium hydroxide and diluted to 250ml with distilled water.
• Eriochrome black-T indicator: 0.5 g of Eriochrome black-T indicator is dissolved in 100g of triethanolamine.

• Standard EDTA titrant: 0.01M or Ng AR grade EDTA is dissolved in distilled water and diluted to 1000ml and is standardised against standard calcium solution, 1ml = 1mg CaCO₃.

• Standard Calcium Solution: 1.0g of AR grade CaCO₃ is weighed into a 250ml conical flask, to which 1+1 HCl is added till all CaCO₃ is dissolved completely.200ml of distilled water is added and boiled to expel carbon-di-oxide, and diluted to 1000ml.1ml = 1mg CaCO₃.
**Procedure**

A known volume (50ml) of the sample is pipetted into a clean conical flask, to which 1ml of sodium hydroxide and 1ml of iso-propyl alcohol is added. A pinch of murexide indicator is added to this mixture and titrated against EDTA until the pink colour turns purple.

**Calculation:**

\[
\text{Calcium as Ca} = \frac{T \times 400.5 \times 1.05}{\text{Sample taken, ml}}
\]

*where,*

\[T = \text{volume of titrant, ml}\]

**4.3.6 Calcium Hardness**

The presence of calcium (fifth most abundant) in water results from its passage through or over deposits of limestone, dolomite, gypsum and other such rocks bearing calcium. Calcium contributes to the total hardness of water and is an important micro-nutrient in aquatic environment and is especially needed in large quantities by molluscs and vertebrates. It is measured by EDTA titrimetric method. Small concentration of calcium carbonate prevents corrosion of metal pipes by laying down a protective coating. But increased amount of calcium precipitates on heating to form harmful scales in boilers, pipes and utensils.

**Principle**

When EDTA (Ethylene-diamine tetra acetic acid) is added to the water 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 iso-propyl 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.

**Apparatus**

Burettes, pipette, conical flask, beakers and droppers.

**Reagents**

- Sodium hydroxide (8%): 8g of sodium hydroxide is dissolved in 100ml of distilled water.
- Murexide indicator (ammonium purpurate): 0.2 g of murexide is ground well with 100g of sodium chloride thoroughly.
- Standard EDTA titrant, 0.01M: 3.723 g of EDTA (disodium salt) is dissolved in distilled water and made up to 100ml with the same.

**Procedure**

A known volume (50ml) of the sample is pipetted into a clean conical flask, to which 1ml of sodium hydroxide and 1ml of iso-propyl alcohol is added. A pinch of murexide indicator is added to this mixture and titrated against EDTA until the pink colour turns purple.

**Calculation:**

\[
\text{Calcium as Ca} = \frac{T \times 400.5 \times 1.05}{\text{Sample taken, ml}}
\]

where,

\[
T = \text{volume of titrant, ml}
\]

\[
\text{Calcium hardness} = \frac{T \times 1000 \times 1.05}{\text{Sample taken, ml}}
\]

4.3.7 **Magnesium Hardness**
Magnesium is a relatively abundant element in the earth's crust, ranking eighth in abundance among the elements. It is found in all natural waters and its source lies in rocks, generally present in lower concentration than calcium. It is also an important element contributing to hardness and a necessary constituent of chlorophyll. Its concentration greater than 125 mg/L can influence cathartic and diuretic actions.

**Principle**

Magnesium hardness can be calculated from the determined total hardness and calcium hardness.

**Calculation**

\[
\text{Magnesium (as mg/L)} = (T - C) \times 0.243
\]

where,

\[
T = \text{Total hardness mg/L (as CaCO}_3\text{)}
\]

\[
C = \text{Calcium hardness mg/L (as CaCO}_3\text{)}
\]

High concentration of magnesium proves to be diuretic and laxative, and reduces the utility of water for domestic use while a concentration above 500 mg/L imparts an unpleasant taste to water and renders it unfit for drinking. Chemical softening, reverse osmosis and electro dialysis or ion exchange reduces the magnesium hardness to acceptable levels.

**4.3.8 Total Alkalinity**

Alkalinity of water is its capacity to neutralize acidity. Carbonates, bicarbonates, and hydroxides are the most common forms of alkalinity in natural waters. These chemicals are mostly compounds of calcium and magnesium coming from mineral deposits such as limestone and dolomite. Industrial discharges can also cause alkalinity. Large amount of alkalinity imparts bitter taste in water

**Principle**
The alkalinity of water can be determined by titrating the water sample with Sulphuric acid of known values of pH, volume and concentrations. Based on stoichiometry of the reaction and number of moles of Sulphuric acid needed to reach the end point, the concentration of alkalinity in water is calculated.

When a water sample that has a pH of greater than 4.5 is titrated with acid to a pH 4.5 end point, all OH⁻, CO₃²⁻ and HCO₃⁻ will be neutralized. For the pH more than 8.3, add phenolphthalein indicator, the colour changes to pink colour. This pink colour is due to presence of hydroxyl ions. If sulphuric acid is added to it, the pink colour disappears i.e. OH⁻ ions are neutralized. Then add mixed indicator, the presence of CO₃²⁻ and HCO₃⁻ ions in the solution changes the colour to blue. While adding sulphuric acid, the colour changes, this colour change indicates that all the CO₃²⁻ and HCO₃⁻ ions has been neutralized. This is the end point.

Alkalinity can be measured as Phenolphthalein Alkalinity and Total Alkalinity. The Phenolphthalein Alkalinity is determined by neutralizing the sample to a pH of 8.3 using a dilute sulphuric acid solution and phenolphthalein indicator. This process converts hydroxide ions to water, and carbonate ions to bicarbonate ions:

The following reactions are occurring during titration;

pH range above 8.3

\[
\text{OH}^- + \text{H}^+ \rightarrow \text{H}_2\text{O} \\
\text{CO}_3^{2-} + \text{H}^+ \rightarrow \text{HCO}_3^- 
\]

pH range between 8.3 and 4.5

\[
\text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{CO}_3 
\]

**Apparatus**

pH Meter, Burette - 50-ml capacity, Magnetic Stirrer Assembly

**Reagents**

- Distilled Water
- Standard Solution of Sulphuric Acid - 0.02 N.
• Phenolphthalein Indicator
• Mixed indicator Solution - Dissolve 0.02 g methyl red and 0.01gm bromocresol green in 100 ml, 35 %, ethyl or isopropyl alcohol.

Procedure

20 ml of sample was pipetted out into a 100-ml beaker. If the pH of the sample is over 8.3, then add 2 to 3 drops of phenolphthalein indicator and titrate with standard sulphuric acid solution till the pink colour observed by indicator just disappears (equivalence of pH 8.3). Record the volume of standard sulphuric acid solution used. Add 2 to 3 drops of mixed indicator to the solution in which the phenolphthalein alkalinity has been determined. Titrate with the standard acid to light pink colour (equivalence of pH 3-7). Record the volume of standard acid used after phenolphthalein alkalinity.

Calculation

Total alkalinity (as mg/l CaCO3) = \( \frac{(A + B) \times N \times 5000}{V} \)

where,

\begin{align*}
A &= \text{ml of standard sulphuric acid used to titrate to pH 8.3,} \\
B &= \text{ml of standard sulphuric acid used to titrate from pH 8.3 to pH 3.7,} \\
N &= \text{normality of acid used, and} \\
V &= \text{volume in ml of sample taken for test.}
\end{align*}
4.3.9 Chlorides

The presence of chlorides in natural waters can mainly be attributed to dissolution of salt deposits in the form of ions (Cl⁻). Otherwise, high concentrations may indicate pollution by sewage, industrial wastes, intrusion of seawater or other saline water. It is the major form of inorganic anions in water for aquatic life. Upland and mountain supplies are quite low in chlorides, whereas river and groundwater usually have a considerable amount. High chloride content has a deleterious effect on metallic pipes and structures, as well as agricultural plants. They are calculated by Argentometric method.

Principle

When AgNO₃ is added to the sample of water it reacts with chloride ions of water sample to form AgCl precipitate. When all the chloride ions gets precipitated to AgCl at the end point the titrant or Ag ions react with chromate ions to form red brown colour of Ag₂CrO₄ when potassium chromate is added as an indicator.

Apparatus

Lab glassware

Reagents

- Potassium chromate indicator solution: 50g of potassium chromate is dissolved in minimum amount of distilled water and silver nitrate is added drop wise till a red precipitate is formed. The mixture is allowed to stand for about 12 hours and diluted to 1000ml with distilled water.
- Silver nitrate solution (0.014N): 2.395g of silver nitrate is dissolved in distilled water and made up to 1000ml.
Procedure:

To a 20.0 ml of standard sodium chloride solution 1.0ml potassium chromate indicator solution was added and diluted to 100ml with distilled water. It was titrated with silver nitrate solution to be standardised with continuous stirring until the red precipitate of silver chromate was just observed. The same procedure was carried out for reagent blank. The concentration of silver nitrate was calculated using the titre values.

Then a known volume of filtered sample (50ml) was taken in a conical flask, to which about 0.5ml of potassium chromate indicator was added and was titrated against standardised silver nitrate solution with continuous stirring till the formation of red silver chromate precipitate was just observed. The amount of chloride in the sample was calculated using the titre values.

Calculation

\[
\text{Chlorides (Cl)} = \frac{(A-B) \times (N) \times (35.45)}{\text{Sample taken in ml}}
\]

where,

- \(A\) = Volume of silver nitrate consumed by the sample
- \(B\) = Volume of silver nitrate consumed by the blank
- \(N\) = Normality of silver nitrate

4.3.10 Sodium

Sodium is one of the most abundant elements and is a common constituent of natural waters. The sodium concentration of water is of concern primarily when considering their solubility for agricultural uses or boiler feed water. The concentration ranges from very low in the surface waters and relatively high in deep ground waters and highest in the marine waters. It is calculated by flame photometric method.

Principle of Flame photometer
The emission of characteristic radiations by alkali and alkaline earth metals and the correlation of the emission intensity with the concentration of the element form the basis of flame photometry. The principle of the flame photometer depends on the "Emission Spectroscopy" in which the electrons of the metals after absorbing energy get excited from ground state to higher energy level and return back to the ground state with emission of light. The sample under test is introduced into flame in solution by means of atomizer. The radiation from the flame enters a dispersing device and isolates it (radiation) from the flame to the desired region of the spectrum. The phototube measures the intensity of isolated radiation, which is proportional to the concentration of the element present in the sample.

**Apparatus**

Flame photometer, lab glassware and Whatman filter paper

**Reagents**

- Deionised water
- Stock sodium solution: 2.542g of Sodium chloride of dried (at 140° C) was dissolved in 1000ml distilled water to give 1ml = 1mg of sodium.
- Working sodium solution: Working standards of suitable strengths were prepared from the stock solution.

**Procedure**

The filter of the flame photometer was set to 589nm (marked for Sodium, Na). By feeding distilled water the scale was set to zero and maximum using the standard of highest value. A standard curve between concentration and emission was prepared by feeding the standard solutions. The sample was filtered through filter paper and fed into the flame photometer and the concentration was found from graph or by direct readings.

**4.3.11 Potassium**
Potassium ranks seventh among the elements in order of abundance, behaves similar to sodium and remains low. Though found in small quantities (<20mg/L) it plays a vital role in the metabolism.

**Principle**

Trace amount of potassium can be determined by direct reading of flame photometer at a specific wavelength of 766.5nm by spraying the sample into the flame. The desired spectral lines are then isolated by the use of interference filters or suitable slit arrangements. The intensity of light is measured by the phototube.

Working principle of Flame photometer: The emission of characteristic radiations by alkali and alkaline earth metals and the correlation of the emission intensity with the concentration of the element form the basis of flame photometry. The principle of the flame photometer depends on the "Emission Spectroscopy" in which the electrons of the metals after absorbing energy get excited from ground state to higher energy level and return back to the ground state with emission of light. The sample under test is introduced into flame in solution by means of atomizer. The radiation from the flame enters a dispersing device and isolates it (radiation) from the flame to the desired region of the spectrum. The phototube measures the intensity of isolated radiation, which is proportional to the concentration of the element present in the sample.

**Apparatus**

Flame photometer, lab glassware and Whatman filter paper.

**Reagents**

- Deionised distilled water.
- Stock potassium solution: 1.907g of dried Potassium chloride, is dissolved in 1000ml of distilled water, to give 1ml = 1mg of potassium
- Working Potassium solution: Working standards of suitable strengths are prepared from the stock solution.
Procedure

The filter of the flame photometer is set at 766.5nm (marked for Potassium, K) the flame is adjusted for blue colour. The scale is set to zero and maximum using the highest standard value. A standard curve of different concentration is prepared by feeding the standard solutions. The sample is filtered through the filter paper and fed into the flame photometer. The concentration is found from the standard curve or as direct reading.

4.3.12 Dissolved Oxygen

The term Dissolved Oxygen is used to describe the amount of oxygen dissolved in a unit volume of water. Dissolved oxygen (DO) is essential for the maintenance of healthy lakes and rivers. Dissolved oxygen (DO) is one of the most important indicators of the quality of water for aquatic life. It is essential for all plants and animals. Oxygen dissolved in water is a very important parameter in water analysis as it serves as an indicator of the physical, chemical and biological activities of the water body. Hence, analysis of dissolved oxygen is an important step in water pollution control and wastewater treatment process control.

The two main sources of dissolved oxygen are diffusion of oxygen from the air and photosynthetic activity. Diffusion of oxygen from the air into water depends on the solubility of oxygen, and is influenced by many other factors like water movement, temperature, salinity, etc. because rates of biological oxidation increases with temperature and oxygen demand increases accordingly. Photosynthesis, a biological phenomenon carried out by the autotrophs, depends on the plankton population, light condition, gases, etc. Oxygen is also dissolved in water through diffusion and surface turbulence. Oxygen is poorly soluble in water, roughly 10 parts per million (ppm) at 0-2°C compared to almost 1700 ppm for carbon dioxide at the same temperature. When oxygen levels in the water fall below 3-5 ppm, most fish and marine organisms are stressed and cannot survive.
The solubility of oxygen is less in salt containing water than it is in clean water. For this reason the solubility for a given temperature decreases as one progress from fresh water to estuary water to the ocean.

In general, oxygen levels during mid-day at the surface are near saturation (the maximum level sustained at the temperature) and drop as the water depth increases. Dissolved oxygen levels are an indicator of water quality. Oxygen levels may be reduced because of warm water temperatures and poor flushing. Run-off from farms or lawns containing fertilizers and other nutrients can over-fertilize aquatic plants. At first, aquatic vegetation will flourish and raise the dissolved oxygen levels found in the water. As the plants begin to die, the process of decomposition will deplete the oxygen content of the water.

**Determination Method**

The concentration of dissolved oxygen can be readily, and accurately, measured by the method originally developed by Winkler in 1888 (*Ber.Deutsch Chem.Gos.,* 21, 2843).
Winkler’s method

Principle

Oxygen present in the sample oxidizes the dispersed divalent manganous hydroxide to the higher valency to precipitate as a brown hydrated oxide after addition of potassium iodide and sodium hydroxide. Upon acidification, manganese reverts to its divalent state and liberates iodine from potassium iodide, equivalent to the original dissolved oxygen content of the sample. The liberated iodine is titrated against N/80 sodium thiosulphate using fresh iodine as an indicator. Iodine is reduced to iodide and the thiosulphate is oxidized to tetrathionate. The stoichiometric equations for the reactions described above are:

During the determination of the dissolved oxygen in water samples by the Winkler method the following chemical reactions occur:

Fixation in the alkaline solution:

\[
\begin{align*}
\text{MnCl}_2 + 2\text{NaOH} & \rightarrow \text{Mn(OH)}_2 + 2\text{NaCl} \\
2\text{Mn(OH)}_2 + \text{O}_2 & \rightarrow 2\text{Mn(OH)}_3 + 2\text{H}^+ (2\text{H}^+ + 2\text{OH}^- \text{ (excess)} = 2\text{H}_2\text{O})
\end{align*}
\]

After acidification:

\[
\begin{align*}
2\text{Mn(OH)}_3 + 6\text{HCl} & \rightarrow 2\text{MnCl}_3 + 6\text{H}_2\text{O} \\
2\text{MnCl}_3 + 2\text{KI} & \rightarrow 2\text{MnCl}_2 + 2\text{KCl} + \text{I}_2
\end{align*}
\]

During the titration:

\[
2\text{Na}_2\text{S}_2\text{O}_3 + \text{I}_2 \rightarrow \text{Na}_2\text{S}_4\text{O}_6 + 2\text{NaI}
\]

From this it can be seen that one oxygen atom releases one iodine atom and this then oxidizes one thiosulphate molecule. Nitrite and Fe (III) ions could possibly interfere. Nitrite is made «harmless» with Sodium Azide, Fe (III) with phosphoric acid.
**Apparatus required**

BOD bottles - 300ml capacity, sampling devices, lab glassware - measuring cylinder, conical flasks, etc... and Bunsen burner.

**Reagents**

- Manganese sulphate: 480g of manganous sulphate tetra hydrate is dissolved and made up to 1000ml with distilled water (Discarded if it changes colour with starch).
- Alkaline iodide-azide reagent: 500g of sodium hydroxide and 150g of potassium iodide along with 10g of Sodium Azide (NaN₃) is dissolved and made up to 1000ml with distilled water.
- Conc. sulphuric acid
- Starch indicator: 0.5g of starch is dissolved in distilled water and boiled for few minutes.
- Stock sodium thiosulphate: 24.82g of sodium thiosulphate penta hydrate (Na₂S₂O₃.5H₂O) is dissolved in distilled water and made up to 1000ml.
- Standard sodium thiosulphate (0.025N): 250ml of the stock sodium thiosulphate penta hydrate is made up to 1000ml with distilled water to give 0.025N.

**Procedure**

The samples are collected in BOD bottles, to which 2ml of manganous sulphate and 2ml of potassium iodide are added and sealed. This is mixed well and the precipitate allowed to settle down. At this stage 2ml of concentrated sulphuric acid is added, and mixed well until all the precipitate dissolves.203ml of the sample is measured into the conical flask and titrated against 0.025N sodium thiosulphate using starch as an indicator. The end point is the change of colour from blue to colourless.
Calculation

203ml because \( (200) \frac{(300)}{(200-4)} = 203 \text{ml}. \)

1ml of 0.025N Sodium thiosulphate = 0.2mg of Oxygen

\[
\text{Dissolved Oxygen (as mg/L)} = \frac{(0.2) \, (1000 \text{ ml of Sodium thiosulphate})}{200}
\]

4.3.13 Biological Oxygen Demand

Biological Oxygen Demand (BOD) is the amount of oxygen, expressed in mg/L or parts per million (ppm), required by microorganisms for stabilizing biologically decomposable organic matter (carbonaceous) in water under aerobic conditions. The carbohydrates (cellulose, starch, sugars), proteins, petroleum hydrocarbons and other materials that comprise organic matter get into water from natural sources and from pollution. They may be dissolved, like sugar, or suspended as particulate matter, like solids in sewage. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste. In this case, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. Nitrates and phosphates in a body of water can contribute to high BOD levels. When BOD levels are high, dissolved oxygen (DO) levels decrease because the oxygen that is available in the water is being consumed by the bacteria. Since less dissolved oxygen is available in the water, fish and other aquatic organisms may not survive.

Determination Method

The BOD test is used to determine the pollution load of wastewater, the degree of pollution and the efficiency of wastewater treatment methods. 5-Day BOD test being a bioassay procedure (involving measurement of oxygen consumed by bacteria for degrading the organic matter under aerobic conditions) requires the addition of nutrients and maintaining the standard conditions of pH and temperature and absence of microbial growth inhibiting substances.
**Principle**

The method consists of filling the samples in airtight bottles of specified size and incubating them at specified temperature (20 °C) for 5 days. The difference in the dissolved oxygen measured initially and after incubation gives the BOD of the sample.

**Apparatus**

BOD bottles - 300ml capacity, air incubator - to be controlled at 20°C ±1°C, oximeter and magnetic stirrer.

**Reagents**

- Phosphate buffer solution: Dissolve 8.5 g KH$_2$PO$_4$, 21.75 g K$_2$HPO$_4$, 33.4g Na$_2$HPO$_4$-7H$_2$O and 1.7g NH$_4$Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard if any sign of biological growth in the stock bottle.
- Magnesium Sulphate solution: Dissolve 22.5 g MgSO$_4$-7H$_2$O in distilled water and dilute to 1L.
- Calcium Chloride solution: Dissolve 27.5 g CaCl$_2$ in distilled water and dilute to 1L.
- Ferric chloride solution: Dissolve 0.25 g FeCl$_3$-6H$_2$O in distilled water and dilute to 1L.
- Glucose-glutamic acid solution: Dry reagent-grade glucose (or dextrose) and reagent-grade glutamic acid at 103 °C for 1 hr. Add 150 mg each to distilled water an dilute to 1 L. Prepare fresh immediately before use.

**Preparation of Dilution Water**
• To 1000ml of water, 1ml each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solution is added, before bringing it to 20°C and aerating it thoroughly.

**Calculation**

\[
\text{BOD} = \frac{(D_1 - D_2) - (B_1 - B_2) \times f}{p}
\]

*(in mg/L)*

where,

- \(D_1\) = 1\textsuperscript{st} day D.O of diluted sample
- \(D_2\) = 5\textsuperscript{th} day D.O of diluted sample
- \(P\) = decimal volumetric fraction of sample used.
- \(B_1\) = 1\textsuperscript{st} day D.O of control
- \(B_2\) = 5\textsuperscript{th} day D.O of control

**4.3.14 Chemical Oxygen Demand**

The chemical oxygen demand (COD) is an indirect measure of the quantity of organic material in water. The organic material in water is oxidized with a strong chemical oxidant (dichromate). The quantity of oxidant used in the reaction is measured colourimetrically and expressed as mg of O2 per L of water (similar to the convention of expressing eq/L in terms of mg/L of CaCO₃).

**Apparatus**

Reflux apparatus, Nessler’s tube, Erlenmeyer flasks, hot plate and lab glassware.

**Reagents**

- Standard potassium dichromate solution (0.250M): 12.25g of potassium dichromate dried at 103 °C for about 2 hours is dissolved in distilled water and made up to 1000ml.
- Standard ferrous ammonium sulphate (FAS) 0.25N: 98g of FAS is dissolved in minimum distilled water to which 20ml of conc. sulphuric
acid is added and made up to 1000ml using distilled water to give 0.25N of ferrous ammonium sulphate.

- Ferroin indicator: 1.485g of 1,10-Phenanthroline monohydrate and 695mg of ferrous sulphate is dissolved in 100ml of distilled water.
- Conc. sulphuric acid
- Silver sulphate crystals
- Mercuric sulphate crystals

Procedure

- Standardize the ferrous ammonium sulfate. Calculate the normality of the ferrous ammonium sulfate using the following formula

\[
\text{Normality of Ferrous Ammonium Sulphate } N = \frac{(\text{Vol. of Potassium Dichromate, mL}) \times 0.250}{\text{Vol. of Ferrous Ammonium Sulphate, mL}}
\]

- Prepare the sample water
- Prepare a blank.
- Reflux both the sample flask and the blank flask.
- Titrate both the sample flask and the blank flask.

Calculation

\[
\text{COD } = \frac{(A - B) \times M \times 8000}{\text{Sample taken, ml}}
\]

where,

\[
A = \text{mL of titrant used for sample}
B = \text{mL of titrant used for blank}
M = \text{normality of ferrous ammonium sulfate}
\]

4. 4 Trace Metals

4.4.1 Iron (Fe)

Source

Iron is chemically very reactive and corrodes rapidly, particularly in moist air, but it is commonly found in combination with carbon. In surface water
supplies, the presence of iron is almost exclusively due to corrosion of pipes and storage tanks. In ground water supplies, in addition to corrosion problems of the distribution system, high content of iron can be encountered due to the frequency of elevated iron level in the earth strata related to the feeding aquifers.

**Effects**

It is known fact that iron in trace amounts is essential for nutrition. The objection to iron in the distribution system is not due to health reasons but to staining of laundry and plumbing fixtures and appearance, colour and taste of water may also get affected.

### 4.4.2 Zinc (Zn)

**Source**

Many zinc salts are highly soluble in water; others are not (carbonate, oxide, Sulphide). It is likely that the presence of zinc can be detected only in traces in natural waters; but industrial pollution, wastes from pharmaceuticals, galvanizing, paint, pigments, several insecticides, cosmetics etc… increases its concentration in appreciable amounts in the water.
Effects

Zinc at a low level (under 20 mg/L) has no adverse physiological effects upon man but its higher concentration is aesthetically objectionable in drinking water due to a milky appearance and a greasy film in boiling and it also causes undesirable taste to the water.

4.4.3 Copper (Cu)

Source

Copper is very commonly found on the earth’s crust as sulphides, oxides, and rarely as metal. It is generally found in surface water at concentrations below 20 µg/L but higher values may be detected from corrosion of brass and copper pipes and also from wastes of industries by smelting, refining, copper wire mills, electroplating, tanning, engraving, photography, insecticides, fungicides etc… In ground water also it can be detected as an industrial pollutant.

Effects

Trace amounts of copper are necessary for normal body metabolism and its absence is known to cause nutritional anemia in children. Large oral doses of copper can cause emesis and may eventually result in liver damage. Individuals with Wilson’s disease (disorder of copper metabolism) are at additional risk from the toxic effects of copper. Copper at a concentration higher than 1.0 mg/L reacts with soap and causes green stains on clothes.
4.4.4 Cadmium (Cd)

Source

Since cadmium is found in low concentrations in rocks, coal, and petroleum, it is found in ground water more than in surface water as a natural occurrence. Therefore, it may enter the water supply from mining, industrial operation and leachates from landfill. Also cadmium may enter the distribution system from corrosion of galvanized pipes.

Effects

Cadmium is highly toxic when taken orally or inhaled and has been implicated in some cases of food poisoning. Cadmium causes death results from pulmonary oedema, shortness of breath. It affects liver, kidney, and pancreas.

4.4.5 Chromium (Cr)

Source

Chromium occurs in higher concentration in the wastes from electroplating paints, dyes, chrome tanning explosives, ceramics and paper industries etc... It is also used as a corrosion inhibitor in the textile, glass and photographic industries.

Effects

The harmful effects in human are due to hexavalent chromium whereas trivalent is considered non-toxic. Hexavalent chromium has a deleterious effect on the liver, kidney and respiratory organs with haemorrhagic effects, dermatitis and ulceration of the skin for chronic and sub-chronic exposure.

4.4.6 Lead (Pb)

Source
Lead is also a toxic element and it increases in water due to the discharge of industrial waste water, such as from printing, dyeing, batteries and oil remarries etc.

**Effects**

Lead is not considered an essential nutritional element but a serious poison tends to accumulate in the bone structure. Accumulation of significant amount of lead may result in sever permanent brain damages and death. It may also cause anaemia, gastrointestinal disturbance, tenderness and gradual paralysis in the muscles with possible cases of lethargy and moroseness.

### 4.4.7 Mercury (Hg)

**Source**

Mercury is a highly poisonous substance and increases in natural water by industrial wastes. It is one of the least abundant elements in the earth’s crust. This metal is used in electrical equipment and some water pumps. It usually gets into water as a result of improper waste disposal.

**Effects**

Elemental mercury primarily causes health effects when it is breathed as a vapour where it can be absorbed through the lungs. These exposures can occur when elemental mercury is introduced into the air, particularly in warm or poorly ventilated indoor spaces. Symptoms from mercury exposure include tremors; emotional changes (e.g., mood swings, irritability, nervousness, and excessive shyness); insomnia; neuromuscular changes (such as weakness, muscle atrophy, twitching); headaches; disturbances in sensations; changes in nerve responses; and performance deficits on tests of cognitive function. At higher exposures there may be kidney effects, respiratory failure and death.
4.4.8 Manganese (Mn)

Source
Manganese is leached out of rocks and minerals as well as man-made materials, such as iron and steel pipes. Groundwater supplies having been in contact with rocks for long periods of time generally have much higher levels of manganese than surface water sources. Sometimes discharge of acidic industrial wastes or mine drainage can increase manganese problems in affected surface water sources. Manganese can also be found in many food items, including grains and cereals as well as being quite high in tea.

Effects
Manganese (Mn) is an element that can impart undesirable taste and odour in drinking water. Manganese also contributes to discoloured water that may result in dirty or stained clothes during washing. There have been evidences of neurological, cognitive, and neuropsychological effects of manganese exposure from drinking water. Manganese at high levels can cause damage to the brain, liver, kidneys, and the developing foetus.
4.4.9 Nickel (Ni)

Source

Nickel is an abundant element on Earth, and most notably in the planet's iron/nickel core. It is used in the manufacture of many alloys and products such as stainless steel, ceramic paint, jewellery, kitchen ware, batteries, textiles and coins. Nickel is released into the environment by power plants, metal factories and waste incinerators. It is also used in fertilizers and enters groundwater from farm runoff. Nickel can be up 60% recyclable. The human body contains about 10 mg of Nickel.

Effects

Though Nickel is a useful and a necessary element in many organism's diets but can become toxic and carcinogenic when taken in high doses. Feminine gender is more commonly allergic to nickel exposure than masculine. Exposure to skin can cause dermatitis upon contact. When ingested through water, in small amounts, it is harmless to humans and in fact necessary in our diet. Inhalation of nickel is the greatest risk of developing health problems, as it becomes highly carcinogenic.

4.4.10 Arsenic (As)

Source

Though there is no specific industrial process that creates arsenic pollution, the amount of arsenic that leaches from the earth’s crust into groundwater systems can be exasperated by human activity. Groundwater systems are the only source of water for local communities, and this water is extracted from
deep in the earth’s surface using pump wells. Water from these wells is used for a variety of purposes, including irrigation for agricultural fields. Over pumping of water for agriculture causes higher concentrations of natural arsenic into groundwater systems, which contaminates entire water sources.

**Effects**

Arsenic is known to be a dangerous toxin that can lead to death when large amounts are ingested. Small amounts of arsenic exposure over long periods of time can also lead to numerous health problems, including abnormal heart beat, damage to blood vessels and a decrease of red and white blood cells, nausea and vomiting, and clearly visible irritations of the skin. A common effect of arsenicosis, or arsenic poisoning, is dark patches of skin, corns, or warts on the body. Arsenic is also a documented human carcinogen, and exposure over long periods has been found to cause cancer of the bladder, skin, lungs, kidney, and liver.