CHAPTER II

MATERIALS AND METHODS
Water pollution studies depend entirely upon the planning made prior to collecting the samples. The plan includes the location of sampling sites, parameters to be analysed, time schedule to be followed, methods of data collection and also the handling of samples. Sampling points should be such that they provide an accurate understanding of the existing water quality.¹

**Sample collection:**

All the samples were collected in three litre capacity polythene bottles having double stopper. Prior to the collection, the well cleaned sample bottles were rinsed thoroughly with the sample water having being collected. After collecting the samples, the bottles were immediately closed tightly. In case of open wells, the samples were collected from a depth of about one meter from the upper surface of water. All bottles containing water samples were sealed and brought to the laboratory as soon as possible after protecting them from direct sunlight during transportation. Temperatures were recorded on the spot itself. Each sample bottle was clearly labelled with a glass marker and relevant details were recorded.
All the samples were analysed for pH, hardness, calcium, magnesium, chloride, sulphate, phosphate, etc. according to the standard methods prescribed in the literature\(^2\).

Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were analysed as per standard procedure.

pH, ORP, conductivity and TDS were first determined. Different samples were collected from various sources of water including rivers, different types of wells in and around the area of investigation at an interval of three to four months, for three successive seasons and analysed.

The samples so collected were kept at a low temperature in the dark, before analysis in order to retard the changes if any caused by the growth of organism. All the precautions were taken while selecting the sites and collecting the water samples, keeping in view the analytical procedures and precision along with the other prescribed standard procedure. Efforts were made to exclude external contaminants such as atmospheric air and dust.
Expression of Results:

Results have been expressed in parts per million (ppm). These units have been prescribed by American Chemical Society, Indian Standard Institution (IS 3025/1964)^3, International Standard for Drinking Water, World Health Organisation 1984 Geneva^4 and Indian Council of Medical Research (ICMR)^5.

The pH has been expressed in its usual term. Temperature is expressed in Celsius. Conductivity has been expressed in micromhos/Cm at 25°C.

Materials used:

Chemicals and reagents of standard quality were used. Most of these were of Anal R, GR and E Merck grades.

For Physico-chemical determination of pH, ORP, TDS and conductivity, Portable water analysis kit (Century) was used for data collection.

A colourimeter was used for the determination of phosphate, sulphate and . Before analysing the samples, instruments were standardised with standard solutions and calibrated as far as practicable.

Glassware of Borosil and Pyrex makes were utilised throughout the experimental works.
Methods adopted for the determination of the various parameters are described below:

**Conductivity:**

Electrical conductivity was measured by conductivity cell with cell constant 1.15. The results have been expressed as umhos/cm at 25°C. The conductivity was measured at room temperature and then converted to values at 25°C. Usually electrical conductivity increases with temperature at a rate of approximately 2 percent per degree centigrade.°

Conductivity = Observed conductance X Cell constant X temperature factor at 25°C.

**Oxidation Reduction Potential (ORP):**

The measurement of ORP of all the samples was done immediately after the collection of the samples by pH electrode (probe) of water analysis kit. The mode-switch of the instrument was adjusted at M3-position. Then, the electrode was dipped in buffer solution of pH = 4.0. M3-Switch of instrument was then adjusted at mV position. The instrument was calibrated with Asym-knob at the sample's temperature. After adjustment, the electrode was then placed in a water sample to find out the ORP of the solution. It is expressed in mV.
Temperature:

Temperature has been measured by using temperature probe of Portable water analysis kit (century) and the results are expressed as °C.

pH:

The measurement of pH of the all samples were carried out immediately after the collection of the samples by a pH electrode (probe) of Portable water analysis kit as per the instructions available with the instrument.

Inorganic Phosphorus:

A coulorometric method was used for determining inorganic phosphorous content in the water samples. The phosphates in water with ammonium molybdate and form complex heteropolyacid (molybdosphoric acid) which gets reduced to a complex of blue colour in the presence of SnCl₂. The absorption of light by this blue colour was measured at 690 nm to calculate the concentration of phosphates and then comparing it with a standard curve. It is expressed in ppm.¹

Total Dissolved Solids (TDS):

TDS was measured by conductivity cell (with cell constant 1.15) of the water analysis kit. The conductance values obtained in μhmos/cm was converted into ppm by taking help of the relation:¹

$$2 \text{ } \mu \text{hmos/cm} = 1 \text{ } \text{ppm}.$$
Dissolved Oxygen (DO):

Winkler's Iodometric Method was followed to determine dissolved Oxygen.

The manganous sulphate reacts with alkali (NaOH or KOH) to form a white precipitate of manganous hydroxide, which in the presence of oxygen gets oxidised to a brown coloured compound. In the strong acidic medium manganic ions are reduced by iodide ions which get converted into iodine equivalent to the original concentration of oxygen in the sample. The liberated iodine was titrated against sodium thiosulphate solution (0.025 N) and by using starch as indicator and the calculation was done by the following working formula:

\[
\text{Dissolved Oxygen (mg/l)} = \frac{(ml \times N) \text{ of sodium thiosulphate} \times 8 \times 1000}{V_2 \left( \frac{V_1 - V}{V_1} \right)}
\]

Where \(V_1\) = Volume of the sample after placing the stopper (250 ml)

\(V_2\) = Volume of the part of the content titrated (50 ml)

\(V\) = Volume of MnSO₄ and KI added.
Biochemical Oxygen Demand (BOD):

The method consists of placing a sample in a full two 250 ml BOD bottles. One used for initial DO determination and the other BOD bottle containing sample for DO determination after 5 days incubation at 20°C.

Same reagents were added to both BOD bottles before DO determination like phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride. Then the other BOD bottle was kept for incubation.

The BOD was computed from the difference between initial DO and DO after incubation.

\[ \text{BOD (mg/L)} = (D_0 - D_5) \times \text{diluted factor} \]

Where \( D_0 \) = initial DO in the sample

\( D_5 \) = DO after 5 days.

Chemical Oxygen Demand (COD):

The water sample was well stirred and then 250 ml of it was taken in 500 ml bottle and to it 10 ml of 1:4 \( \text{H}_2\text{SO}_4: \text{H}_2\text{O} \) mixture solution was added, followed by accurate measured volume of N/80 KMnO\(_4\) and then mixed with gentle rotation. It was then placed in a water bath at 27°C for 3 to 4 hours. The same procedure was adopted to determine blank, for blank, double distilled water was used in place of sample.
COD (mg/L) = \frac{(b-a) \times N \times KMno_4 \times 1000}{80} \times \frac{4}{ml \ of \ sample}

Where b = Standard (black reading)

b = Sample reading.

**Sulphate:**

A Turbidimetric method was used for determining the sulphate content in the water samples. Sulphate ion is precipitated in the form of barium sulphate by adding barium chloride in hydrochloric acid medium. The concentration of the sulphate can be determined from the absorbance of the light by barium sulphate at 420 nm of colourimeter and then comparing it with a standard curve, it is expressed in ppm.

**Chloride:**

The estimation of chloride of all the samples have been made by the Argentometric method by titrating samples against standard silver nitrate solution using potassium chromate solution.

Silver nitrate reacts with chloride to form very slightly soluble white precipitate of AgCl. At the end point when all the chlorides gets precipitated free silver ions react with chromate to form silver chromate of reddish brown colour.
The results have been expressed as 'Cl' in ppm.

\[
\text{Chloride (mg/l) = } \frac{\text{(ml} \times N) \times \text{AgNO}_3 \times 1000 \times 35.5}{\text{ml sample}}
\]

**Alkalinity:**

The alkalinity in water is generally imparted by the salts of carbonates, bicarbonates, phosphates, nitrates borates, silicates, etc., together with hydroxyl ions in free state. However, most of the waters are rich in carbonates and bicarbonates with a little concentration of other alkalinity imparting ions. Hence the alkalinity of all the samples have been determined by titrating the samples with standard hydrochloric acid. Phenolphthalein and methyl orange were used as indicators. In the case of the use of phenolphthalein as an indicator, the value is called as phenolphthalein alkalinity (PA) and in case of methyl orange it is called as total alkalinity (TA). From the neutral point obtained by the two indicators, alkalinity expressed as \( \text{CaCO}_3 \) and as \( \text{CO}_3 \) and \( \text{HCO}_3^- \) was calculated. The results have been expressed in ppm and as individual ion in mg/L. PA and TA have been calculated as follows:

\[
\text{PA as } \text{CaCO}_3, \text{ mg/L} = \frac{(Ax \text{ Normality}) \times \text{HCl} \times 100 \times 50}{\text{ml of sample}}
\]
TA as CaCO₃ (mg/L) = \frac{(B \times \text{Normality}) \text{ of HCl} \times 1000 \times 50}{\text{ml of sample}}

Where

A = \text{ml of HCl with only phenolphthalein}

B = \text{ml of total HCl used with phenolphthalein and methyl orange.}

PA = \text{Phenolphthalein alkalinity}

TA = \text{Total alkalinity}

**Hardness:**

Total hardness of all the samples has been determined by titrating the samples with standard EDTA solution using Eriochrome Black T, a dye, as indicator at pH 11.2 in presence of ammonia - ammonium chloride buffer solution. The results have been expressed as calcium carbonate in mg/L.

\[
\text{Hardness (EDTA) as CaCO₃ (mg/L)} \;= \; \frac{\text{ml EDTA used} \times 1000}{\text{ml sample}}
\]

**Calcium:**

Calcium of the samples has been also determined by titrating the samples with standard solution of EDTA in a strongly alkaline medium using sodium hydroxide solution as buffer at pH 12.0 Murexide (ammonium perpureate) was used as indicator.
The results are expressed in mg/L

$$\text{Calcium (mg/L)} = \frac{V \times 400.8}{\text{ml of sample}}$$

Where $V =$ Volume of EDTA used.

**Magnesium:**

Magnesium ion (Mg$^{++}$) can be calculated adopting the following relationship:

$$\text{Magnesium (mg/L)} = \frac{(y - x) \times 400.8}{\text{Volume of sample} \times 1.645}$$

Where $y =$ EDTA used in hardness determination.

$x =$ EDTA used in calcium determination for the same volume of the sample.
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