Chapter -III
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3:1 Preface:

Due to industrialization plenty of waste water is generated. This waste water pollutes fresh water resources like lakes, rivers, ponds, wells etc. Also man made activities like washing, bathing and wading of cattles may pollutes the waters. The analysis methodology can detect the strength of pollution of such water bodies. The analysis of physico-chemical, biological and bacteriological parameters of Radhanagari and Gaganbawada Tahsil was undertaken for present investigation.

3.2 Materials:

3.2.1 Sampling Sites:

1) Sampling station A:
The sampling station A is located near the wall side of the Tulshi dam.

2) Sampling Station B:
The sampling station B is located near the outlet of water of Laxmi dam. This is the forest Side.

3) Sampling Station C:
The Sampling station C is on south side of the Doodhganga dam. This is the forest side.

4) Sampling Station D:
The Sampling station D is near the outlet of water of Kumbhi dam. This is the forest Side.

3.2.2 Sampling schedule and Procedure:

A) Sampling to study Physical and chemical characters:
The water samples were collected in to five liter capacity plastic containers by gently wading the container in the upper layer of the water. The analysis of temperature and PH of water was made on site, as they are liable to change during transport to the laboratory. For the analysis of other parameters the samples were brought into laboratory and stored in refrigerator till the completion of analysis. The analysis was completed within 72 hours after
collection of water. For dissolved oxygen analysis sample were collected in 300 ml capacity B.O.D. bottles and oxygen was fixed on site & brought to laboratory for further analysis.

B) Sampling for Bacteriological Analysis BOD: -
For B.O.D study, the sample were collected in 100 ml sterilized bottles and brought in the laboratory and tests were carried out immediately after reaching the laboratory.

C) Sampling for Zooplankton study:-
With the help of zooplankton net zooplanktons were collected. At first approximately 50 liters of water sample is filtered and concentrated to 50 ml. This concentrated 50 ml samples were preserved in 4% formalin. These samples were used for microscopic analysis.

The phytoplankton sample should be immediately preserved in 4% formaline. The analysis of physical and chemical parameters are done with the help of standard methods of the examination of water (APHA 1985, Trivedi and Goel (1984).

3.3 Physical and chemical Parameters:-
The physical and chemical analysis of water sample of selected four Dams was carried out for different parameter as described in APHA (1989), Trivedi & Goel (1984) and Kodarkar (1992).

3.3.1 Temperature –
The temperature of the water bodies was measured at sampling sites by thermometer of 0 to 50°C range and 0.2°C least count. The water sample was taken in a plastic container and its temperature was recorded immediately by dipping the thermometer for about one minute. The temperature was recorded in degree Celsius.

3.3.2 pH:-
pH is the negative logarithm of hydrogen ion concentration, or hydrogen ion-activity. Portable digital pH meter was used for the measurement of pH values. Standard buffer solutions of pH 4.0 and 9.2 were used for calibration.

3.3.3 Electrical conductivity: -
The Pure water is a poor conductor of electricity. Acids, bases and salts present in water make it relatively good conductor of electricity and such substances are called electrolytes. The conductivity of water sample was measured with help of a
conductivity meter. Electrical conductivity was calculated using observed conductance, cell constant and temperature factor at 25°C (Trivedi and Goel 1984). The result was expressed as µmhos/cm.

3.3.4 Total Dissolved Solids (TDS):

The total dissolved solids were estimated by gravimetric method. (Trivedi and Goel, 1984). The result was expressed in mg/l.

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

Where,

- \(A\) = Final weight of the dish in gm
- \(B\) = Initial weight of the dish in gm
- \(V\) = Volume of sample taken in ml.

3.3.5 Total suspended Solids (TSS):

Determine total suspended solids as the difference between the total solids and total dissolved solids

\[
\text{TSS = TS – TDS}
\]

The total solids are determined as the residue left after evaporation of unfiltered sample

\[
\text{Total solid, gm/lit= } \frac{A - B}{V} \times 1000
\]

Where,

- \(A\) = Final weight of the dish in gm
- \(B\) = Initial weight of the dish in gm
- \(V\) = Volume of sample taken in ml.

The total dissolved solids are determined as the residue left after evaporation of the filtered sample

\[
\text{Total dissolved solid, gm/lit= } \frac{A - B}{V} \times 1000
\]

Where,

- \(A\) = Final weight of the dish in gm
B = Initial weight of the dish in gm
V = Volume of sample taken in ml.

3.3.6 Free Carbon Dioxide (CO₂):-

CO₂ was analyzed at the site by using Phenolphthalein indicator and sodium hydroxides titrate. The 50 ml of ample was taken in a conical flask and five drops of Phenolphthalein indicator I added to it. If the colour turned pink, free CO₂ was taken as absent, when it remained colourless, it was titrated with 0.02 N sodium hydroxide until pink colour appeared.

\[ \frac{X \times N \times 50 \times 1000}{Y} \]

Free CO₂ (mg/l) = \---------------------------

Where,
X = ml of titrate
Y = ml of sample
N = Normality of titrate

3.3.7 Carbonates :-

Carbonates can be estimated by titrating the sample with strong acid, first to pH 8.3 using phenolapthalein as an indicator and then further to pH between 4.2 to 5.4 with methyl orange indicator. In first case the value is called phenolapthalein alkalinity (PA) and in second case it is called total alkalinity (TA)

Values of carbonates can be computed from these two types of alkalinities.

3.3.8 Alkalinity:-

The two drops of Methyl orange indicator was added to the solution in which Phenolphthalein alkalinity was already determined. This was titrated with 0.1 N HCl to the end point, when the colour changed from yellow to pink.

Total alkalinity (mg/l) = \[ \frac{X \times N \times 50 \times 1000}{Y} \]

Where,
X = ml of titrate
Y = ml of sample
N = Normality of titrate.
3.3.9  **Dissolved Oxygen (DO):**

The dissolved oxygen was determined by modified Winkler’s method (Golterman *et. al* 1978, Trivedy and Goel 1984). The water sample was collected in 125ml glass Stoppard oxygen bottle. Then carefully, 1ml Magnous sulphate and 1ml of alkaline KI solution was placed at the bottom of the bottle to fix the dissolved oxygen. It was thoroughly mixed and then brown precipitate was allowed to settle. 2 ml of concentrated sulphuric acid was added along the sides of the bottle and the bottle was shaked well to dissolve the precipitate. 50 ml of the above solution was taken in a conical flask and titrated with 0.025 N sodium thiosulphate solution using starch as an indicator to a colorless end point.

\[
X \times N \times 8 \times 1000
\]

\[
\text{Dissolved Oxygen (mg/l)} = \frac{X \times N \times 8 \times 1000}{Y}
\]

Where,

- \(X\) = volume of sodium thiosulphate used (ml)
- \(Y\) = Volume of sample (ml)
- \(N\) = Normality of sodium thiosulphate.

3.3.10  **Total Phosphates** :-

Take 50 ml of sample in Erlenmeyer flask. Add 2ml of ammonium molybdate followed by 5 drops of Sncl$_2$ solution. A blue colour will appear. Take reading at 690 nm on a spectrophotometer using a distil water blank with same amount of the chemicals. Take the reading after 5 minutes but before 12 minutes of the addition of the last reagent. Find out the concentration with the help of the standard curve.

3.3.11  **Total Chloride** :-

The Chloride was determined by titrimetric method (Trivedi and Goel 1984). 2 ml of potassium chromate indicator was added to 50 ml of sample and titrated with 0.02 N silver nitrate until a persistant red tinge appear.

\[
\text{Chloride (mg/l)} = \frac{X \times N \times 35.5 \times 1000}{Y}
\]

Where,

- \(X\) = ml of titrate used.
\[ Y = \text{ml of sample} \]

### 3.3.12 Total Nitrogen (N₂):

The total nitrogen is calculated by Kjeldahl method.

\[ a - b \times 0.01 \times 1000 \times 14 \times d \]

\[ \text{N mg/l} = \frac{\text{ml sample distilled}}{a - b \times 0.01 \times 1000 \times 14 \times d} \]

Where,  
- \( a \) = ml of HCl used with sample
- \( b \) = ml of HCl used with blank
- \( d \) = dilution factor (2.5). The original volume (40ml) of sample has been made to 100 ml after digestion.

### 3.3.13 Biological Oxygen Demand (BOD):

B.O.D. of water sample was determined with the help of methods described in APHA (1989). The various samples were prepared for testing, two containing diluted sample and remaining two containing dilution water (Blank). First day Dissolved Oxygen was determined from one bottle & sample of one bottle of blank by Winkler Azide method (APHA, 1989). The remaining bottles were incubated at \( 20^\circ C \) for five days. After incubation period, the dissolved oxygen of these water samples were determined. The BOD was calculated as follows.

\[ \text{B.O.D. mg/lit} = \left( S_1 - S_5 \right) - \left( B_1 - B_5 \right) \times \text{dilution factor} \]

Where
- \( S_1 \) = D.O. of sample on first day
- \( S_5 \) = D.O. of sample after 5 day’s incubation
- \( B_1 \) = D.O. of blank water on first day
- \( B_5 \) = D.O. of blank water after 5 day’s incubation.

10 ml of water sample was inoculated aseptically in each tube of set 1. One ml of water sample was inoculated in each tube of set 2 and 0.1 ml of sample was inoculated each tube of set 3. All tubes were incubated at \( 37^\circ C \) for 48 hours. The tubes were examined for acid and gas production after 48 hrs. The number of positive tubes (acid & gas) from each tube were noted and computed on Mac Conkey's table given in APHA (1989) for estimation of most probable total number of Coliform organisms from sample per 100 ml.
BIOLOGICAL ANALYSIS:-

Physical and chemical characteristics of water bodies affect the abundance, species composition, stability, productivity and physiological condition of aquatic organism. Biological method is used for assessing water quality such as pollution which has chemically oriented and biological aspect has a subsidiary position because of number of complications in analysis and interpretation, collection of biological data. Biologically analysis of water includes collection, counting and identification of aquatic organisms.

Plankton Analysis:

The two hundred liters water samples were filtered through the net number 25 bolting silk. The samples collected were concentrated to a 50ml volume and preserved in 4% formalin. Each replicate of phyto and zooplankton samples was identified under research microscope using suitable keys, standard texts and monographs given by Pennak (1978), Tonapi (1980) and APHA (1985).