MATERIAL AND METHODS

For the analysis of water standard methods were used as described in APHA(1992); Trivedi and Goel (1986); Maiti (2004); Rozar (2002). Following parameters were studied for water quality assessment.

1) Temperature: -

The temperature of water was measured by using ordinary **thermometer.** It is expressed in degrees Celsius (^{0}C).

2) pH: -

pH, is a measure of the concentration of hydrogen ions in the water. The pH of water was measured by using **pH meter** (Elico LI 120 and Control Dynamics APX 175 E).

3) Electrical Conductivity: -

The EC of sample was measured by using **Systronic Electronic conductivity meter.**

4) Total Solids:- (Trivedi and Goel , 1986)

A porcelain evaporating dish was washed, cleaned, dried at 103 -105^{0}C and weighed (W1). Fifty ml of unfiltered sample was taken in it. The sample was evaporated on a water bath up to dryness and after cooling the dish it was again weighed (W2).

A total solid was calculated by using following formula and it is expressed in (mg/L).

$$TS\ (mg/l) = \frac{(W2 - W1)\ X\ 1000\ X\ 1000}{V}$$

W1 - Initial weight of dish.

W2 - Final weight of dish.

V - Volume of sample taken.

5) Total Dissolved Solid: - (Trivedi and Goel , 1986)

A porcelain evaporating dish was washed, cleaned, dried at 103 -105^{0}C with distilled water and weighed (W1). The initial weight was taken and recorded it as W1. Hundred ml. of sample was filtered through Whatman filter paper no. 41.

The filtered sample was taken in evaporating dish and it is kept on water bath for evaporation. After complete evaporation, final weight of dish was taken and noted as W2.

Total dissolved solids were calculated with following formula and expressed in (mg/L).

$$TDS\ (mg/l) = \frac{(W2 - W1) \times 1000 \times 1000}{V}$$

W1 – Initial weight of evaporating dish.

W2 – Final weight of evaporating dish.

V - Volume of sample.

6) Total suspended solids (TSS) : - (Trivedi and Goel, 1986)

Total suspended solids were calculated using following formula.

TSS (mg/l) = TS – TDS

TS – Total solids

TDS – Total dissolved solids.

7) Hardness: - (Trivedi and Goel, 1986)

Hardness of water was determined by following **EDTA method.**

Fifty ml of sample was taken in a conical flask and one ml of ammonium buffer solution was added. Hundred to two hundred mg or a pinch of Eriochrome Black T indicator was added, the solutions turned wine red. It was titrated against EDTA solution. Wine red to blue was the end point.

The hardness of water was calculated by using following formula.

$$Hardness\ as\ mg/L\ CaCO3 = \frac{ml\ EDTA\ used \times 1000}{ml\ of\ sample}$$

8)Total Alkalinity: - (Trivedi and Goel 1986)

Hundred ml of sample was taken in a conical flask and 2 drops of phenolphthalein indicator was added. When the solution remained colourless, PA was 0, and total alkalinity

was determined. The solution remained colourless then two to three drops of methyl orange was added to the same and continued the titration further, until the yellow colour changed to pink (end point) This was the Total Alkalinity calculated as follows.

$$\text{TA as CaCO3 (mg/L)} = \frac{\text{ml of sample (B X Normality) of HCL X 1000 X 50}}{\text{ml of sample}}$$

9) Free CO_2: - (Trivedi and Goel 1986)

Hundred ml of sample was taken in a conical flask and a few drops of phenolphthalein indicator was added. When the colour turned pink, free CO_2 was considered as zero, when the sample remained colourless it was titrated against 0.05 N NaOH. End point was appearance of pink colour.

The freeCO_2 can be calculated by following formula.

$$\text{FREE } CO_2 \text{ (mg/L)} = \frac{\text{(ml X N) of NaOH X 1000 X 44}}{\text{ml of sample}}$$

10) Dissolved Oxygen: - (Trivedi and Goel, 1986)

The Dissolved Oxygen (DO) was measured by modified **Winkler's Iodometric method**.

Water was sampled out in 300ml capacity BOD bottles without bubbling. Two ml of alkaline potassium iodide and manganous sulphate was added to the bottle. Contents were mixed thoroughly by inverting bottle. The brown coloured precipitation was formed. Two ml of concentrated sulphuric acid was added to the bottle to dissolve the precipitate. Then the solution from bottle was titrated against 0.025N Sodium thiosulphate. For the titration starch was used as an indicator. The end point of titration was disappearance of dark blue colour.

The DO was calculated by following formula.

$$\text{DO (mg/l)} = \frac{\text{V1 X N X 8 X 1000}}{\text{V2 - V3}}$$

V1 – Volume of titrant

V2 – Volume of sample bottle after placing the stopper.

V3 - Volume of manganous sulphate and potassium iodide

N - Normality of sodium thiosulphate.

Chemical Oxygen Demand: - (Trivedi and Goel 1986)

For the testing of COD **Potassium dichromate reflux method** was followed.

Twenty ml sample is taken in COD flask of 300 ml capacity. Ten ml of Potassium dichromate solution was added to the flask. Pinch of silver sulphate and mercury sulphate was then added to it. Thirty ml concentrated sulphuric acid was added to COD flask and refluxed on hot plate for a period of two hours. After cooling of the flask contents were diluted to 150 ml and titrated against ferrous ammonium solution, ferronin was used as indicator. End point of this titration was change of blue green colour to wine red. The blank reading was taken by following the same procedure by using distilled water except the sample.

The COD was calculated by following formula.

$$\text{COD (mg/L)} = \frac{\text{(b -a) X N of } Fe(NH_4)_2(SO_4)_2 \text{ X 1000 X 8}}{\text{ml of sample}}$$

a - ml. of titrant with sample.

b - ml. of titrant with blank.

12) Biological Oxygen Demand:- (Trivedi and Goel, 1986)

Dilution water was made in a glass container by bubbling compressed air in distilled water for about 30 minutes. One ml of phosphate buffer, magnesium sulphate, calcium chloride, and ferric chloride solutions were added for each liter of dilution water and mixed thoroughly. Sample was neutralized to pH 7.0 by using 1N NaOH or H_2SO_4. Since DO in the sample was likely to be exhausted, it was prepared to suitable dilution of the sample according to the expected BOD range. Prepared dilution was taken in a bucket, content was mixed thoroughly. Two sets of BOD bottles were filled. One set of the was kept in BOD incubator at 20^0 C for 5 days and DO content in another set was determined immediately. DO in sample bottle was determined after completion of 5 days. The method was modified slightly and when zero reading was obtained after 5 days, incubation was done for 3 days only.

BOD was calculated by using following formula.

BOD (mg/l) = ($D_0 - D_5$) X dilution factor.

D_0 - Initial DO in the sample.

D_5 - DO after 5 days.

13) Chlorides:- (Trivedi and Goel, 1986)

Fifty ml of sample was taken in conical flask and 2ml of K_2CrO_4 solution was added in it. The contents were titrated against 0.002N $AgNO_3$ till permanent red tinge appears.

Chlorides was calculated with the following formula.

$$\text{Chlorides (mg/L)} = \frac{\text{(ml. X N } AgNO_3 \text{ solution) X 1000 X 35.5}}{\text{ml. of sample}}$$

14) Inorganic Phosphorus: -(Trivedi and Goel, 1986)

Inorganic phosphorus was estimated according to **Stannous Chlorides method.**

Fifty ml filtered clear sample was taken in a conical flask (If the sample contains colour and colloidal impurities, these were removed by adding a spoonful of activated charcoal and then filtered the sample). Two ml. of ammonium molybdate was added, followed by 5 drops of $SnCl_2$ solution. As soon as blue colour appear then readings were noted at 690 nm on spectrophotometer, using distilled water as blank with same amount of ammonium molybdate and $SnCl_2$ solutions. Readings were taken after 5 minutes but before 12min. of addition of the last reagent. Concentrations were found with the help of standard curve.

15) Nitrates:- (Trivedi and Goel, 1986)

For the determination of Nitrates **colorimetric method** was used.

Fifty ml of filtered sample was taken in conical flask. An equivalent amount of Silver Sulphate solution was added to remove chlorides. (1mg/lit Cl = 1ml AgSo4 solution.) Then heat slightly and filtered the precipitate of AgCl. Evaporate the filtrate in porcelain dishes to dryness. The residue was cooled and dissolved in 2 ml phenol disulfonic acid and diluted the content to 50ml. Six ml of liquid ammonia was added to develop a yellow colour. Readings

were taken at 410 nm. Concentration of nitrate nitrogen was calculated from the standard curve. Standard curve between concentration and absorbance was prepared from 0.0 mg N/lit to 1.0 mg N/lit at the interval of o.1 mg N/lit. Absorbance of the standard solutions were taken by using the same procedure.

16) Oil and grease: -

The water sample was collected buçket with wide mouth separately. Two hundred ml of sample was taken in a separating funnel. Ten ml of sulphuric acid and 25 – 50 ml of petroleum ether was added to the sample. Shaken well, and if suspension prevails, small amount of ethanol added. Set was kept for some time to separate the two distinct layers; the upper layer of petroleum ether and lower of the water sample. Lower layer was discarded of the sample through separating funnel. A pre-weighed dish was taken the petroleum ether from the separating funnel through a filter paper filtered. The paper was already been moistened with fresh petroleum ether. Little more petroleum ether was added on the filter paper to remove any residual oil and grease on the filter paper. Evaporated the petroleum ether on a water bath and the final weight of the dish was taken after cooling in a dessicator.

The oil and grease were calculated by using following formula.

$$\text{Oil and grease, (mg/L)} = \frac{A - B \times 1000}{V}$$

A – Final weight of dish in g.

B - Initial weight of dish in g.

V – Volume of sample taken in ml.

17) Minerals: - (Trivedi and Goel, 1986)

Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Manganese (Mn),

for the heavy metals and trace elements following method was used. Fifty ml. of water sample was filtered by using Whatman filter paper number 42. These samples were analysed with the help of **atomic absorption spectrometer (AAS)** Perkin Elmer A. analyst 300 model from USIC Shivaji University, Kolhapur.

18) Bacteriological Study: - (Rozar, 2002).

The standard plate count (SPC) value provide the density of aerobic and facultative bacteria in water sample, which can grow at 37^0C. The SPC values are useful to give information about bacterial growth in water also to know potability of water. The procedure for measurement of total bacterial number is as follows.

Water sample was collected in sterilized stopper bottle and immediately brought to laboratory. The dilutions were made for the inoculation (1:10 and 1:100). Nutrient agar medium was prepared and poured into petriplates. One ml of (1:100 dilution) sample was transferred on plate containing nutrient agar. The plates were incubated for 48 hrs at 35^0C. Visual counting was done.

The results were recorded the SPC/ ml was calculated by using following formula.

$$\text{SPC/ ml} = \frac{\text{Colonies counted}}{\text{Dilution Factor}}$$

For the confirmation of coliform bacteria seperation of colonies was carried out on Eosine Methylene Blue (EMB) agar. Eosine Methylene Blue contains the dye methylene blue, which inhibits growth of gram +ve organisms. In the presence of an acidic environment EMB forms a complex and pecipitates out onto the coliform colonies, producing dark centers and a metallic appearance. This reaction is characteristic of fecal pollution

One ml of sample with appropriate dilution was inoculated on an EMB agar plate from each sample and all petriplates were incubated for 48 hrs at 30^0 +- 2 ^{0}C .

19) Phytoplankton:- (Trivedi and Goel, 1986)

Fifty litres of water was filtered through plankton net and phytoplankton samples were collected, preserved in 4% formaline and observed under microscope for three seasons. Qualitative analysis of phytoplankton was performed under Carl Zeiss, Axiscope 80 Fluorescence microscope and microphotographs were taken. The specialized literature was used to identify the phytoplankton such as APHA (1992), Prescott (1982), Edmondson (1963).

Numerical sequence was determined by using Smith's classification. The similarity index (S) was calculated by using following formula.

Similarity Index (S) = 2C/ A + B

Where, A:- Number of Species recorded at one site

B:- Number of Species recorded at second site

C:- Number of common species recorded between two sites.