Material and Methods

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Water quality of estuary is changing day by day due to improper methods of disposing and discharging solid and liquid waste through the thick growing population belt on the bank of estuary. Continuous increasing solid and liquid waste leads to problems of its disposal. Tourism business and increasing number of hotels are choosing estuaries for disposal of their waste. Insufficient provision for disposal of solid and liquid waste is changing nature of estuarine water quality bearing direct and indirect impact on aquatic life and human beings. Water quality and aquatic environment will be at risk due to anthropogenic sources. It is necessary to predict the concentration and distribution of the pollutant loads in estuary at different location.

2.1 Study area:

Malvan is well-known town for the historical and cultural heritage with picturesque setting, is situated in the southern part of the Konkan coast in Sindhudurg district of Maharashtra. It is located approximately 35 km from NH-17 Mumbai - Goa National Highway. The coast line of the town is about 6.5 sq km and of Sindhudrg district is 121 km. Sindhudrg district is on 15^0 7' and $16^0$40' North latitude and 73^0 19' and 74^0 18' East longitude. Maximum temperature of Sindhudrg district is 34^0C and minimum temperature is 16^0C. Average rainfall is 3280 mm and rainfall of the year 2007 is 3750 mm. Malvan is a compact town situated on coast of Arabian sea and has beautiful beaches around such as Tarkarli beach, Sindhudurg fort, Kunakeshwar temple which enhances the Tourism quotient. Climate of Malvan can be generally classified as warm and moderately humid. Average temperature range between 16^0C - 33^0C, while relative humidity ranges from 69% to 98%.

Malvan has Kolam and Achara creek on the north and Tarkarli creek on the south. There are coral reefs near the rockshore adjoining the Sindhudurg fort. These estuaries are main attraction of tourist and tourism business due to its picturesque value, good vegetation and climatic conditions. The coastal aquatic habitats around Malvan are known to harbor

excellent diversity of aquatic fauna and estuaries of Malvan are very rich in biodiversity.

Coastal zone has different biotopes as estuaries, mangroves, coral reefs and Lagoons endowed with splendid beauty and high productivity. Variety of fishes, crustaceans and molluscan animals are found in large number. Major and minor fishery is carried out in the estuary. Therefore, it can be said that estuaries of Sindhudurg district are more productive in nature.

Achara is a long river having few tributaries throughout its length (about thirty miles). It originates near Phonda Ghat. A prominent headland separates the estuary of this river from the Deogad river. The river is navigable only up to a few miles. Karli river is known as Sarambal in upper reaches and as Karli only near its mouth. It originates near Manohargad in the Sahyadri, and after a winding course of about fifty miles, joins the sea about eight miles south of Malvan. Kolam is a small creek originates 7 km away from Malvan at North. Sandpits and shallow mouth have considerably reduced the importance of this river though it is navigable for about fourteen miles. These rivers are almost flooded in rainy season and having more fresh water inflow. Quality of estuary water of Sindhudrg district is changing and therefore, it is necessary to analyze physico-chemical parameters to understand these changing patterns regularly.

Physico-chemical parameters were analyzed in the laboratory of Center for Marine Environment (CME), Malvan established by the Department of Environmental Science, Shivaji University, Kolhapur. The Field research facility and infrastructure for water and soil analysis is available in the well equipped laboratory of CME for physical, chemical and biological investigation.

2.2 Methodology:

2.2.1 Water sampling:

Water samples were collected in clean polythene cans of 2 liters capacity. Physico-chemical analysis was carried out within 24 hrs after collection of the samples. Proper care was taken during sampling with due consideration to the material of the container, gaseous exchange, sample analysis time, preservation required etc. The sample containers were washed properly in the laboratory. During the sample collection containers were rinsed 2 – 3 times with the sample to be analyzed before filling. The containers were directly dipped into the water and filled. The container was tightly capped and carried to the laboratory for analysis. For transportation of sample bottles were placed in sturdy, insulated plastic box. The temperature was maintained at 4^0C during transportation. In the laboratory it was kept in refrigerator at 4^0C for further analysis. For field parameters glasswares and reagents were carefully taken to the field without contamination and tests were carried out.

2.2.2 Sampling Frequency:

The samples were collected monthly to detect noticeable changes in the quality of the estuary water and no change remains unnoticed. Water samples were collected monthly for a year from Nov (2006) to Oct (2007) for three upstream and three downstream locations of Tarkarli, Kolam and Achara estuaries during low tide conditions.

2.2.3 Physico-chemical Parameters:

An element or a compound present in a natural water system will generally be distributed between a variety of Physico-chemical states. The distribution among these states, as well as the total concentration, varies between different environments e.g. between the river and sea waters which form the end-members of estuarine mixing series, between coastal seas and the open ocean, surface and deep oceanic waters, sediments with their associated pore waters and the overlying waters. Furthermore, there will usually be major differences in physicochemical forms between pollutant inputs and receiving waters and sediments. It is important in water

management to know the concentration of the various constituents of water. Environmental physicochemical condition play a major role governing the the ecological distribution, productivity and health of the organisms.

Following Physico-chemical parameters were selected for the study

a) Physical Parameters:

1) Temperature
2) Color

b) Chemical Parameters:

1) pH
2) Salinity
3) Dissolved Oxygen (DO)
4) Biochemical Oxygen Demand (BOD)
5) Chemical Oxygen Demand (COD)
6) Total Dissolved Solid (TDS)
7) Total Suspended Solid (TSS)
8) Chlorides
9) Nitrogen Nitrate (NO_3-N)
10) Oil and Grease.

C) Heavy Metals:

1) Copper	2) Nickel
3) Lead	4) Iron
5) Manganese	6) Magnesium
7) Zinc	8) Sodium
9) Potassium	10) Calcium

All these parameters were measured by standard methods described in the hand book of APHA 20th edition, (2001), Grassoff 1985 for sea water analysis, Handbook of methods in Environmental studies by S.K Maiti, Vol 1 (2001).

2.2.4 Physical Parameters:

1. Temperature:

For determination of surface temperature mercury glass thermometer was used. Temperature was measured on site. 3 to 4 reading were taken from different locations.

2. Colour:

Colour of water may be result of the presence of natural metallic ions (iron and manganese), humus, peat material, plankton, weeds, suspended and dissolved particulate matter. For the determination of Colour samples were collected in clean glass bottle and kept for some time to check its change visually.

2.2.5 Chemical Parameters:

1. pH :

pH is the measurement of the intensity of acidity or alkalinity and measure the concentration of hydrogen ion in water. For pH determination electronic pH meter was used in the laboratory. On the field sampling site the pH strip was used to measure the pH.

2. Salinity:

Salinity was measured by online Elico digital Salinometer 100 ml sample was taken for analysis. After collection of sample test was carried out within 2 to 4 hrs. Mean of three samples were taken. Values are expressed in percentage.

3. Dissolved Oxygen (DO):

Dissolved oxygen was determined by standard Winkler's method.

Reagents :

1. Monohydrate manganous sulfate
2. Pottasium iodide
3. Conc Sulphuric acid (sp.gr.1.84)
4. Sodium thiosulphate (0.025 N)
5. Pottasium dichromate

6. Sodium hydroxide
7. Sodium azide and Starch.

Protocol :

1. Sample was collected in well rinsed BOD bottle (300 ml capacity). Care was taken to avoid bubbling.
2. Dissolved oxygen was fixed at site by adding 1 ml manganous sulphate and alkaline potassium iodide solution and 3 to 4 samples were taken from different location to check the variation.
3. Carefully removed stopper and immediately added 1 ml conc. H_2SO_4 mixed with gentle inversion until the precipitate completely dissolve.
4. Titrated 100 ml sample contents of the bottle with fresh Sodium thiosulphate (0.025 N) solution using starch as an indicator.
5. End point was dark blue to colorless.

DO was calculated by using formula

$$\text{Dissolved oxygen} = \frac{\text{ml of titrant x Normality x 8 x 1000}}{V2}$$

where,

V2 = Volume of the contents titrated in ml.

Results were expressed as mg/l of DO

4) Biochemical Oxygen Demand (BOD):

Amount of oxygen required by the microorganisms in stabilizing the biologically degradable organic matter in a sample under aerobic condition at 20^0C over period of 5 days. The test for biochemical oxygen demand (BOD) is a bioassay procedure that measures the oxygen consumed by bacteria for the decomposition of organic matter

BOD was measured by 5 days BOD test.

Reagents:

1. Phosphate buffer
2 .Magnesium sulphite
3 .Calcium chloride
4. Ferric chloride
5. Sodium sulphite solution (0.025 N)

Protocol :

1. Sample was collected in well rinsed BOD bottles (300 ml capacity) care was taken to avoid bubbling.
2. Sample was aerated by providing sufficient air by air bubbler.
3. Dissolved oxygen of one set was carried out for initial DO measurement.
4. The second set of the bottles were kept in BOD incubator at 20^0C for 5 days.
5. DO was determined in the sample bottle immediately after the completion of 5 days incubation.
6. Biochemical oxygen demand was calculated by difference in initial and final dissolved oxygen.

BOD was calculated by using formula

BOD = (D1 - D5) x Dilution factor

D1= Initial DO in the sample

D5= DO after 5 days.

Results were expressed in mg/l of BOD

5) Chemical Oxygen Demand (COD):

COD is the amount of oxygen required by the organic and inorganic substances in water to oxidize them by strong oxidant.

COD was analyzed by Reflux method.

Reagents:

1. Standard Potassium dichromate
2. Conc. sulphuric acid
3. Sulphamic acid
4. Silver sulphate
5. Ferrous ammonium sulphite
6. Mercuric sulphate.

Protocol:

1. 20 ml well diluted sample was taken in a 250 ml COD flask (Round bottom flask).
2. Add pinch of $HgSO_4$ and shake thoroughly to maintain the ratio 10:1 for high chloride sample.

3. Add few glass beads followed by 10 ml 0.25 N Potassium dichromate solution.
4. Add slowly 30 ml of conc. H_2SO_4 and pinch of Ag_2SO_4 reagent and mixed thoroughly.
5. After connection of flask to reflux condenser, sample was refluxed for 2 hrs at 150^0C.
6. The sample was titrated against 0.1 N Ferrous ammonium sulphite using starch as indicator. Sharp colour changed blue green to wine red was indicated as end point.
7. Similar procedure was carried out for blank sample.

$$\text{Chemical Oxygen Demand} = \frac{(\text{Blank - Sample}) \times M \times 8 \times 1000}{\text{ml of Sample}}$$

Where,

8 = Miliequivalent weight of oxygen.

Results were expressed as mg/l of COD.

6) Total Dissolved Solid (TDS):

Dissolved solid denote mainly the various kinds of minerals present in the water.

Total dissolved solid was analyzed by online Elico Digital TDS meter.

1. 100 ml sample was taken for analysis.
2. Sample was filtered with Whatman filter paper No 42.
3. TDS meter was calibrated according to manual.
4. Mean of three samples were taken.

Total Dissolved solid = ------ mg/l

Values are expressed in mg/l.

7) Total Suspended Solid (TSS):

Total suspended solid found in suspended form. TSS was analyzed by gravimetric method.

1. Initial and final weight of evaporating dish was taken carefully on electronic balance.

2. 100 ml sample was taken for filtration. Filtration was carried out by Whatman filter paper.
3. Wash the sample with distilled water for complete drainage.
4. Sample was dried in hot air oven for 2 hrs at 103^0C to 105^0C. Cooled in dessicator and final weight was taken.
5. TSS calculated by using difference between initial and final weight.

$$\text{Total suspended solid} = \frac{\text{Initial wt. - Final wt.}}{\text{Vol of sample}} \text{ mg/l}$$

TSS = Total solid - Total dissolved solid.

8) Chloride:

Chloride was determined by Silver nitrate method.

Reagents:

1. Silver nitrate 0.02 N
2. Potassium chromate 5%

Protocol :

1. 50 ml sample was taken in a conical flask and add 2 ml 5% potassium dichromate solution.
2. Sample diluted 10 to 50 times due to presence of high chloride contents.
3. Sample titrated against 0.02 N silver nitrate.
4. Reddish brown color appears as end point.

Chloride was calculated by using following formula.

$$\text{Chloride} = \frac{(\text{ml X N}) \text{ of } AgNO_3 \text{ X } 1000 \text{ X } 35.5}{\text{ml of sample}} \text{ mg/l}$$

Where,

ml = Titration reading

N = Normality of $AgNO_3$

Results are expressed as mg/l

9) Nitrogen (NO_3 - N):

Brucine Sulphanilic acid method was used to determine the nitrate.

Reagents :

1. Brucine Sulhpanalic acid
2. Sulphuric acid
3. Sodium chloride
4. Sodium arsenite solution
5. Standard nitrate solution.

Protocol :

1. 50 ml cleared sample was taken in large tube and put on wire rack.
2. Rack was placed in a cool water bath with the addition of 10 ml H_2SO_4 in the sample, the contents were mixed thoroughly swirling by hand.
3. 0.5 ml Brucine reagent was added and placed on a hot water bath for 20 minutes.
4. Standard curve of nitrate (1 to 10 mg/l) was prepared in different concentrations.
5. Similar procedure was carried out for the blank.
6. Absorbance of the sample and blank was recorded at 220 nm. Nitrate was calculated by standard curve with absorbance.
   Results are expressed as mg/l

10) Oil and Grease:

Oil and grease was analyzed by separating funnel method.

Reagents:

1. Hydrochloric acid
2. Petroleum ether (Boiling temp 65^0C)
3. pH meter
4. Water bath or drying oven

Protocol:

1. 200 ml sample was collected in a separatory funnel and added 10 ml of sulphuric acid and 20 - 25 ml petroleum ether solution.

2. Shack the sample gently to form distinct two layers in which upper layer of petroleum ether and lower one is sample and added petroleum ether for proper filtration
3. Discarded lower layer was taken for final weight. Initial and final weight was taken on electronic weight balance.
4. Evaporate petroleum ether on water bath and final weight was taken after cooling of beaker.

Oil and Grease was calculated by using formula

$$\text{Oil and grease} = \frac{\text{Initial wt} \times 1000}{\text{Volume of sample}} \text{ mg/l}$$

Results are expressed as mg/l of oil and grease

2.3.6 Heavy metal:

Heavy metals were detected by Perkin Elmer Atomic Absorption Spectrophotometer (AAS) The Facility is available in the Common Facility Center (CFC), Shivaji University, Kolhapur.

Protocol:

1. Sample was collected from selected site and kept in refrigerator for heavy metal analysis. 100 ml sample was taken for each element analysis.
2. Sample was carefully filtered by using Whatman filter paper No 42.
3. Sodium, Calcium, Potassium and Magnesium elements were estimated by proper dilution at 100 to 500 times.

Standard solution was prepared according to element detection method.

1. Copper (Cu) (1ml =100 µg Cu): Dissolve 0.1g copper metal in 2 ml conc. HNO_3 add 10 ml conc. HNO_3 and dilute it to one liter.
2. Iron (Fe) (1ml = 100 mg Fe): Dissolve 0.1 g iron wire in a mixture of 10 ml (1+1) HCl and 3 ml conc. HNO3 and then add 5 ml HNO_3. Dilute it to one liter.
3. Lead (Pb) (1 ml=100 µg Pb): Dissolve 0.1598 g lead nitrate in HNO_3 solution and add 10 ml conc. HNO_3 soln .Dilute it to 1 Liter.

4. Manganese (1 ml =100 µg Mn): Dissolve 0.1 g Mn metal in 10 ml conc. HCl mixed with 1 ml conc. HNO_3 and dilutes it to one liter.
5. Zinc (Zn) (1 ml =100 µg Zn) : Dissolve 0.1 g zinc metal in 20 ml 1+1 HCl and dilute it to one liter.

2.3.7 Statistical analysis:

Physico-chemical analysis results were analysed statistically. Standard deviation (± S.D.) was calculated with the mean of 3 samples. The student t - test was used for determining the significance of the mean. The level of significance was set at $p < 0.05$ considering 5% error.

2.3.8 Social Survey:

Social survey was conducted to know the disposal facility of solid and liquid waste at three locations. People in and around Malvan are unaware about water quality and its changing nature. Insufficient and improper disposal provision from local government produces undesirable change in water quality and its associated problems. This study was carried out to evaluate awareness among the people about waste and its proper disposal.

Data collection:

A sample size of 25 individual was taken from each location. Initially sample schedule was implemented as pilot study. In the questionnaire (Appendix I) total area of village, source of water, sanitation, health, information from people was gathered. Secondary information was collected from public works department, Grampanchayat, Municipal council. On the basis of pilot survey and field data sheet, results were prepared.

Plate - I

Map of Sindhudurg District

Plate - II

Satellite Image of Malvan

Map of Malvan