CHAPTER – IV

MATERIALS AND METHODS
4. Materials Methods

In the present study work has been concentrated on the two freshwater tanks namely Sambhaji tank and Hotagi tank were selected for the investigation of the physico-chemical parameters and zooplankton diversity. These tanks are situated in and around Solapur city, Maharashtra.

4.1 Selection of sampling sites

In the present study total six sampling sites were selected. Three sampling sites from each tank to study the physico-chemical parameters and zooplankton.

4.1.1 Sambhaji tank

There was three sites selected for the study from this tank such as,

1) **Site A (STS-A):** It is situated at the side of national highway No. 13 where boating and recreational activities go on.

2) **Site B (STS-B):** It is at the middle of the tank were the railway track is bisecting the tank where certain domestic activities like washing of clothes and buffalos, cattle, etc will carried.

3) **Site C (STS-C):** It is at the source of rain water inlet where, washing of clothes (Dhobi ghat) and source of entry of domestic sewage takes palce.

4.1.2 Hotagi tank

The sites selected to collect the samples are

1) **Site A (HTS-A):** It is located towards the outlet of the tank (Sourth direction) where washing of the clothes and human interference is maximum.
2) **Site B (HTS-B):** It is selected from north side of the tank, where the water overflows.

3) **Site C (HTS-C):** It is selected from the catchment area of the tank (east direction) through the water is coming inside to the tank.

### 4.2 Collection of Samples

Surface water sample were collected directly from each selected site from the both tanks. The samples were carefully transferred to the separate two litre bottle all sample were packed in a cane basket protecting bottles from the exposure to sunlight, contamination and transported to the laboratory without disturbance.

The samples were collected once in month for two consecutive days from all 6 stations of two tanks for a period of two year from July 2008 to June 2010. The samples were collected during morning hours to study physico-chemical and biological parameters. The sampling schedules are as follows.

#### Sampling Schedules at two tanks

<table>
<thead>
<tr>
<th>Site</th>
<th>Physico-chemical sampling</th>
<th>Biological sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTS-A</td>
<td>6.30 a.m. to 7.00 a.m.</td>
<td>7.00 a.m. to 7.30 a.m.</td>
</tr>
<tr>
<td>HTS-B</td>
<td>7.30 a.m. to 8.00 a.m.</td>
<td>8.00 a.m. to 8.30 a.m.</td>
</tr>
<tr>
<td>HTS-C</td>
<td>8.30 a.m. to 9.00 a.m.</td>
<td>9.00 a.m. to 9.30 a.m.</td>
</tr>
<tr>
<td>STS-A</td>
<td>6.30 a.m. to 7.00 a.m.</td>
<td>7.00 a.m. to 7.30 a.m.</td>
</tr>
<tr>
<td>STS-B</td>
<td>7.30 a.m. to 8.00 a.m.</td>
<td>8.00 a.m. to 8.30 a.m.</td>
</tr>
<tr>
<td>STS-C</td>
<td>8.30 a.m. to 9.00 a.m.</td>
<td>9.00 a.m. to 9.30 a.m.</td>
</tr>
</tbody>
</table>
4.3 Physico-chemical analysis

The physico-chemical parameters were studied with the help of different analysis methods.

4.3.1 Temperature

Temperature (°C) of air and water was read from a mercury thermometer at the time of sampling.

4.3.2 pH

pH (Hydrogen ion concentration) was measured with Elico-pH meter CL01/03 using glass electrodes. (APHA, 1998).

4.3.3 Turbidity

Turbidity was estimated by Turbidometer method. (APHA 1998).

4.3.4 Total dissolved Solids (TDS)

TDS were estimated by following the procedure: some amount of the water sample was filtered through Whatman No. 1 filter paper and 50 ml of filtered water was evaporated at 105 °C and desiccated to constant weight finally calculated as mg/L (APHA, 1998).

4.3.5 Total hardness

Total hardness was calculated by titrating sample one ml of ammonia buffer, pinch of NaCN and pinch of Erichrome black- T indicator with EDTA. (APHA, 1998).

4.3.6 Alkalinity

Alkalinity was measured by titrating the sample with 0.1 N H₂SO₄ using phenolphthalein and methyl orange as an indicator.
4.3.7 **Dissolved oxygen (DO)**

Dissolved oxygen (DO) of water sample was fixed in the field by Wrinklerization and analysed by titrometric method and finally expressed as mg/L at surrounding temperature. APHA (1998).

4.3.8 **Free CO₂**

Free CO₂ was calculated by NaOH titration method using phenolphthelian indicator and calculated as mg/L. (APHA 1998).

4.3.9 **Nitrates**

Nitrates was determined with diazotization method colorimetric with spekol at 520 mm and calculated as mg/L (APHA, 1998).

4.3.10 **Chlorides**

Chlorides was calculated in 50 ml sample using yellow light and expressed ad mg/L (Wilcox and Hatcher, 1950).

4.3.11 **Sulphates**

Sulphates was measured by turbidometric method (APHA 1998). After developing the turbidity by addition of barium chloride crystals to the acidified water samples, the optical density was measured at 420 µm on spectrophotometer. With the help of standard graph the value of sulphates was calculated.

4.3.12 **Iron**

Iron was determined by colorimetric method with an operating range of 400-700 µm. And calculated in mg/L. (APHA, 1998).
4.3.13 Fluorides

Fluorides was determined by using colorimetric method in spectrophotometer at 570 µm with at least one centimetre light path and calculated in mg/L. (APHA, 1998).

4.4 Biological Parameters

The collections of zooplankton sampling were done on monthly basis for period of two years from July 2008 to June 2010 at six sampling sites of both tanks (Sambhaji tank and Hotagi tank). Zooplankton were collected by filtering known quantity (100 litre) of water was filtered from sampling site through zooplankton net which is made up of fine mesh and collected zooplankton in to 100 ml bottle which is attached at the bottom of net. The samples were preserved 4% formaldehyde solution and studied for diversity by using standard key, other literature (APHA, 1998, Harding and Smith, 1974, Tonpi, 1980, Pennak, 1989, Dhanpati, 2000) and by sending to ZSI. For population density studies 10 Bucket (100 litter) of water filtered through the plankton net (mesh size 25) by using the Sedgwick-Rafter cell counted the organism by using inverted microscope and expressed populations as Org./L.
Plate 4: - Different sites of collection at Sambhaji tank and Hotagi tank

- **Sambhaji tank site- A (STS-A)**
  - Boating alignment at STS-A

- **Sambhaji tank site- B (STS-B)**
  - Railway track bisecting at STS-B
  - Washing of cloths at STS-B
  - *Eichhornia crassipes* covering the surface water of tank

- **Sambhaji tank site C (STS-C)**
  - Washing of cloth at STS-C
Plate 5: Different sites of collection at Sambhaji tank and Hotagi tank

Domestic sewage released in Sambhaji tank

Hotagi tank site A (HTS-A)

Washing of cattle at Sambhaji tank

Washing of cloths at HTS-A

Overflow outlet at HTS-B

Hotagi tank site B (HTS-B)

Hotagi tank site C (HTS-C)

Water inlet at HTS-C