CHAPTER 3
MATERIALS AND METHODS
Materials and Methods:

The study was carried out from Pune urban area on three riverine wetlands using key parameters. Studies on the physico-chemical analysis of water are undertaken with the objective of collecting data regarding water quality, influence of human activity on water quality. The study, in general, was undertaken in three steps; periodic collection of surface samples at fixed stations, analyses of collected samples by the standard methods and finally evaluation of data obtained. Each step, being significant in its own way, was undertaken with utmost care.

3.1 Selection of Sampling Stations:

Selection of the sampling stations is very important since it gives an idea of the source and types of pollutants entering in to the rivers. The water quality status of rivers Mula, Mutha and Pavana flowing through Pune city was determined by collecting the water samples at 10 different sampling stations along these rivers and subjecting the samples to physico-chemical and biological analysis.

On each river, one upstream sample was collected to know status of river water just before the river enters urban limits. Two stations such as Wakad and Aundhgaon were located on river Mula, two stations, Thergaon and Sangvi were located on river Pavana and three stations, Vitthalwadi, Garware Causeway and Sangam Bridge were located on river Mutha. While, one station, namely Dapodi, was located at the Pavana-Mula confluence, one, at the Holkar Bridge after the Pavana-
Mula confluence, and the other called as Bund Garden, was located after the confluence of Pavana- Mula and Mutha rivers (Map 3.1).

3.2 Description of Sampling Stations on River Mula, Mutha and Pavana:

1. Wakad (Station No. 1).

This sampling station is just before the Mula river enters in the urban area and is more or less in the natural status. Fishing is done at this site. The water body is almost clear and shows the very poor growth of macrophytes.

2. Aundhgaon (Station No. 2).

This station is located near the Aundh Bridge and slow flowing and receives waste up streams.
The surroundings area shows lot of defecation all over. The activities which are observed at this station include washing of cloths and utensils and fishing. At the upstream small tributary Ram stream joins Mula and carries waste from Pashan and Baner.

3. Thergaon (Station No. 3).

At this station water samples were collected near the Thergaon Bridge. The river is completely stagnant at this station. The water body is covered with algae. Growth of Water hyacinth is seen on the water body at certain points. Lot of domestic waste including the non-biodegradable such as plastic is seen in the water body. The water is used for washing of cloths and utensils. Leaching from nearby industrial units could also be one of the sources of pollution at this point.

4. Sangvi (Station No. 4).

This station is located behind the Dapodi area. At this sampling point, the water is black, turbid and has slow flow. The major activities observed include, washing and fishing. The water body receives domestic and industrial waste from the Pimpri-Chinchwad area, Kaarwadi and
Fugewadi. The water body is covered with water hyacinth at certain points in summer months.

5. Vitthalwadi (Station No. 5).

This is the first downstream station after the Khadakwasala dam. Vitthalwadi is like a sub-urban area situated along the banks of the river Mutha, and now growing at a very fast rate. There are numerous activities, into its catchments area such as new constructions. Local people wash their cloths, animals and also undertake fishing. Water is
flowing at certain locations and stagnant at certain points. The growth of aquatic plants on the banks of the river was visible.

6. Garware Causeway (Station No. 6).

This station is located behind Garware College in the centre of the city. It receives waste from Parvati and Kothrud area. The flow is channelized by constructing walls and water is almost turbid due to inflow of sewage.

7. Sangam Bridge (Station No. 7).

This station marks the confluence of rivers Mula and Mutha. At this station the additional domestic waste joins from Kasba area.
At this point the river water is more stagnant due to weir at Bund Garden and because of the confluence of Mutha with the river Mula. River Mula carries domestic waste and industrial waste from the Pimpri-Chinchwad, Aundh and Khadki area. The water is turbid at this station. Profuse growth of water hyacinth has totally covered the river bed at certain points while at certain points there is a growth of *Lemna*.

8. **Dapodi (Station No. 8).**

   At this station the water is stagnant, dark coloured, turbid, with smell like that of hydrogen disulfide (H$_2$S). Extensive growth of water hyacinth is seen on the waterbed.

9. **Holkar Bridge (Station No. 9).**

   This station is situated after the Pavana-Mula confluence. The water is stagnant at certain locations, dark and turbid. There being a cremation ground, the rituals concerned with the river are performed at this station. This station receives waste from Pimpri-Chinchwad area,
Khadki and its surrounding area. The growth of water hyacinth is observed.

10. Bund Garden (Station No. 10).

This station is situated after the Pavana and Mula rivers meet Mutha. The water is stagnant due to weir. While on the other side of the weir, it is forcefully flowing leading to lot of stable, white colored froth formation. Lot of activities were visible at this station, which included, washing use, swimming and recreation. This station carries all the discharges from Pune urban area.
3.3 Collection of Water Samples:

Water samples for analysis of physico-chemical parameters, were taken from all 10 stations of Pune rivers from October 2004 to September, 2005. For DO analysis the samples were collected in a 300 ml glass stoppered bottles. While for remaining parameters a bulk sample for three liters was collected in plastic container. The water temperature of each site was recorded periodically by using mercury centigrade thermometer at depth of 4 to 6 cm from the surface layer of water. pH of water was examined in the field at the time of collections by using universal pH paper and also checked by using digital systronic pH meter. Water samples were not collected due to heavy flooding of river in the month of July 2005.

3.4 Collection of Algal Samples:

Algal samples were collected from same stations at monthly intervals during October, 2004 to September, 2005. The attached epiphytic and floating forms of algae were collected in acid washed collection bottles and preserved in 4% formalin for further taxonomic investigations.

3.5 Algal Analysis:

The algal samples of 10 stations of river were collected at monthly intervals. The population of 4 groups of algae was estimated by drop count method (Lackey 1938) for quantitative study of algae. The density of population of 4 groups of algae viz. Chlorophyceae (Green-algae),
Cyanophyceae (Blue-green), Bacillariophyceae (Diatoms) and Euglenophyceae (Euglenoids) was estimated at every month from October 2004 to September 2005.

Similarly, the percentage of different algal taxa was calculated every month by choosing monthly means of cells ml$^{-1}$ for quantitative study of algae. Samples were preserved in 4% formation for further study of identification of algae. The identification of phytoplankton was done with the help of standard books and monographs (Allan, 1984; Turner, 1982; Smith, 1950; Prescott, 1954; Ward and Whipple, 1959; Desikachary, 1959; Philipose, 1967; Prescott, 1951). Phytoplanktons were counted by drop count method and the results were converted to organisms per ml of water.

**Pollution Index**

The pollution tolerant genera and most pollution tolerant species of algae are recorded for each station of river. Algal pollution indices of palmer, based on genus and species were used in rating water samples for high organic pollution (Table 3.1 and 3.2). A list of all significantly occurring algae in the samples was made for all stations 20 most frequent genera were taken into account. A pollution index factor was assigned to each genus by determining the relative number of total points scored by each algae for rating of water samples as high or low organically polluted observations were made according to Palmer (1969).
The following numerical values for the individual zones have been followed:

0 - 10 suggests lack of organic pollution.

10 - 15 indicates moderate pollution.

15 - 20 indicates probable high organic pollution; 20 or more confirmed high organic pollution.

The lower figures below 15 indicate that:

1) Organic pollution is not high.

2) Sample is not representative.

3) Some substances of factor interfering with algal persistence are present and active.

**Table 3.1**: Algal genus pollution index (Palmer 1969)

<table>
<thead>
<tr>
<th>Algal Genus</th>
<th>Pollution Index</th>
<th>Algal Genus</th>
<th>Pollution Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anacystis</td>
<td>1</td>
<td>Micractinium</td>
<td>1</td>
</tr>
<tr>
<td>Ankistrodesmus</td>
<td>2</td>
<td>Navicula</td>
<td>3</td>
</tr>
<tr>
<td>Chlomydomonas</td>
<td>4</td>
<td>Nitzschia</td>
<td>3</td>
</tr>
<tr>
<td>Chlorella</td>
<td>3</td>
<td>Oscillatoria</td>
<td>5</td>
</tr>
<tr>
<td>Closterium</td>
<td>1</td>
<td>Pandorina</td>
<td>1</td>
</tr>
<tr>
<td>Cyclotella</td>
<td>1</td>
<td>Phacus</td>
<td>2</td>
</tr>
<tr>
<td>Euglena</td>
<td>5</td>
<td>Phormidium</td>
<td>1</td>
</tr>
<tr>
<td>Gomphonema</td>
<td>1</td>
<td>Scenedesmus</td>
<td>4</td>
</tr>
<tr>
<td>Lepocinclis</td>
<td>1</td>
<td>Stigeoclonium</td>
<td>2</td>
</tr>
<tr>
<td>Melosira</td>
<td>1</td>
<td>Synedra</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 3.2: Algal species pollution index (Palmer 1969)

<table>
<thead>
<tr>
<th>Algal Species</th>
<th>Pollution Index</th>
<th>Algal Species</th>
<th>Pollution Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankistrodesmus falcatus</td>
<td>3</td>
<td>Nitzschia palea</td>
<td>5</td>
</tr>
<tr>
<td>Arthrospira jenneri</td>
<td>2</td>
<td>Oscillatoria chlorina</td>
<td>2</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>2</td>
<td>Oscillatoria limosa</td>
<td>4</td>
</tr>
<tr>
<td>Cyclotella meneghiniana</td>
<td>2</td>
<td>Oscillatoria princeps</td>
<td>1</td>
</tr>
<tr>
<td>Euglena gracilis</td>
<td>1</td>
<td>Oscillatoria putrida</td>
<td>1</td>
</tr>
<tr>
<td>Euglena viridis</td>
<td>6</td>
<td>Oscillatoria tenuis</td>
<td>4</td>
</tr>
<tr>
<td>Gomphonema parvulum</td>
<td>1</td>
<td>Pandorina morum</td>
<td>3</td>
</tr>
<tr>
<td>Melosira varians</td>
<td>2</td>
<td>Scenedesmus quadricauda</td>
<td>4</td>
</tr>
<tr>
<td>Navicula cryptocephala</td>
<td>1</td>
<td>Stigeoclonium tenue</td>
<td>3</td>
</tr>
<tr>
<td>Nitzschia acicularis</td>
<td>1</td>
<td>Synedra ulna</td>
<td>3</td>
</tr>
</tbody>
</table>

**Trophic State Indices**

Nygaard's (1949) trophic state indices (Table 3.3) are used to determine trophic state of 10 stations of rivers. These indices are helpful for determining the nature of water. The values of index for different categories of algae for oligotrophic and eutrophic conditions were calculated.

For determining the indices the number of genera in each group of algae was determined upto species level for each sampling stations of rivers and used for calculating biological index of water quality. From this value the degree of eutrophication is determined for each station.
Table 3.3: Nygaard’s trophic state indices

<table>
<thead>
<tr>
<th>Index</th>
<th>Calculation</th>
<th>Oligotrophic</th>
<th>Eutrophic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myxophycean</td>
<td>Myxophycean Desmideae</td>
<td>0.0 – 0.4</td>
<td>0.1 – 3.0</td>
</tr>
<tr>
<td>Chlorophycean</td>
<td>Chlorococcales Desmideae</td>
<td>0.0 – 0.7</td>
<td>0.2 – 9.0</td>
</tr>
<tr>
<td>Diatom</td>
<td>Centric diatoms Pennate diatoms</td>
<td>0.0 – 0.3</td>
<td>0.0 – 1.75</td>
</tr>
<tr>
<td>Euglenophyte</td>
<td>Euglenophyta Myxophyceae + Chlorococcales</td>
<td>0.0 – 0.2</td>
<td>0.0 – 1.0</td>
</tr>
<tr>
<td>Compound</td>
<td>Myxophyceae + Chlorococcales + Euglenophyta Desmideae</td>
<td>0.0 – 1.0</td>
<td>1.2 – 2.5</td>
</tr>
</tbody>
</table>

Saprobity Index

The saprobity indices for 10 different stations were calculated only, according to Pantle and Buck (1955).

\[
S = \frac{\sum s \cdot h}{\sum h}
\]

Where:  
- \( S \) is the mean saprobity index  
- \( s \) is the degree of saprobity  
- \( h \) is the frequency with which single species occurs.

For the calculation, the following numerical degrees are used:

- Oligo-saprobic indicator organism \( S = 1 \)
- \( \beta \)-mesosaprobic indicator organism \( S = 2 \)
- \( \alpha \)-mesosaprobic indicator organism \( S = 3 \)
Poly mesosaprobic indicator organism $S = 4$
Species found by chance $h = 1$
Species found by frequently $h = 3$
Species occurring in abundance $h = 5$

The following numerical values for the individual zones have been followed:

1.0 – 1.5 denotes oligo (O) saprobic zone.
1.5 – 2.5 denotes $\beta$ mesosaprobic zone.
2.5 – 3.5 denotes $\alpha$ mesosaprobic zone.
3.5 – 4.0 denotes polysaprobic zone

**Diversity Index**

It was calculated by Shannon and Weaver (1949). It is adopted to analyse the structure and algal community. This index can be expressed by the formula as follows:

$$H = -\sum \frac{n_i}{N} \log_e \left( \frac{n_i}{N} \right)$$

Where:

- $H$ = Index of species diversity.
- $n_i$ = Individual number in the species.
- $N$ = Total number of individuals.

It was calculated monthly for 10 sampling stations.

**3.6 Aquatic Macrophytes Analysis:**

In the present investigation for the herbaceous species quadrat method was employed by following the methods of Raunkaier (1934) and Stromberg (1993). The identification of aquatic plants was done with the

**Procedure:**

1. Lay down a quadrat $1m^2$ was laid on the riparian areas along the bank of rivers.
2. Number of species occurring in each quadrat and recorded.
3. The number of individuals of each species was also recorded.
4. In all 5 quadrats were taken from each site.

The data collected was used to analyse. From those Frequency, Relative frequency, Density, Relative density, Abundance, Diversity index and Important value index as per the following method.

**Frequency**

Is the number of sampling units in which a species occurs. Which indicate how frequent a particular species occur in the study area is measured and calculated as follows.

\[
\text{% Frequency} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100
\]

Relative frequency can be calculated by the formula:

\[
\text{Relative frequency} = \frac{\text{Number of quadrats in which species occurred}}{\text{Total number of quadrats occupied by all species}} \times 100
\]
The presence or absence of plant species was recorded. This data was also used to determine the homogeneity/heterogeneity of the vegetational stand of the study area. The species types are divided into five frequency classes depending on Raunkier’s (1934) frequency classification as below.

Class-A= 1 to 20 %  
Class-B= 21 to 40 %  
Class-C= 41 to 60 %  
Class-D= 61 to 80 %  
Class-E= 81 to 100 %

Each species is classified in above-mentioned classes. A histogram was drawn with percentage of the total number of species on Y-axis and frequency classes A to E on X axis. It is compared with the law of frequency as follows. $A > B \geq C \geq D < E$.

**Density**

It is defined as the number of individuals of a particular species per unit area. Counts are usually made in a number of replicate quadrats. Density is the average number of species per quadrat and is calculated as follows

$$\text{Density} = \frac{\text{Total number of individuals of species}}{\text{Total number of quadrats used in sampling}}$$

The density value, thus obtained for each species, was expressed as individuals per unit area. Density may be defined as the number of species per specified collection area.

$$\text{Relative Density} = \frac{\text{Total number of individuals of species}}{\text{Sum of all individuals of all species}}$$
**Abundance**

Abundance of a species is defined as the number of individuals per quadrat and is calculated as follows

\[
\text{Abundance} = \frac{\text{Total number of individuals of the species}}{\text{Number of quadrats in which they occurred}}
\]

**Diversity Indices**

Diversity is one of the central themes of ecology, there has been considerable disagreement about how it should be measured (Magurran 1988). Measures of diversity are frequently seen as indicator of the well being of ecological systems. In general, it is agreed that high species diversities indicate more complex and stable habitats (Brower and Zar 1984).

**Shannon Diversity Index**

Shannon-Weaver index assumes that individuals are randomly sampled from the sample. It is calculated from the formula

\[
H' = -\sum P_i \ln P_i
\]

Where \(P_i\) is the proportional abundance of \(i^{th}\) species = \((n_i/N)\).

**Simpson’s Diversity Index**

Simpson (1949) gave the probability of any two individuals drawn at random from an indefinitely large community belonging to different species as;

\[
D = \sum \left( \frac{n_i (n_i - 1)}{N(N - 1)} \right)
\]
Where:

\[ n_i = \text{the number of individuals in the } i^{\text{th}} \text{ species.} \]

\[ N = \text{the total number of individuals.} \]

This index is referred to as dominance index since it weighted towards the abundance of the commonest species rather than providing a measure of species richness. As \( D \) increases, diversity decreases and Simpson index is therefore usually expressed as \( 1 - D \) or \( 1/D \).

**Important Value Index (IVI)**

The important value is an analytical phytosociological analysis which represents the relative richness in terms of density and frequency of each species.

**Important value** = Relative frequency (RF) + Relative Density (RD)

### 3.7 Water Analysis:

The methods described in the ‘Standard Methods for the Examination of Water and Wastewater (1985)’ as prescribed by American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF) was used for water analysis.

The parameters were selected for water analysis, viz. water temperature, pH, DO (Dissolved Oxygen), BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), Free CO\(_2\), Chloride, Total alkalinity, Total hardness, Sulphate, Phosphate and Nitrate. Method used for each parameter is summarised in Table 3.4.
<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Parameters</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temperature</td>
<td>0.1 °C to 0.2-°C mercuric thermometer.</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>Systronics, reference glass-electrode and digital pH meter.</td>
</tr>
<tr>
<td>3</td>
<td>Dissolved Oxygen (DO)</td>
<td>Azide modification of Winkler's method.</td>
</tr>
<tr>
<td>4</td>
<td>Biological Oxygen Demand (BOD)</td>
<td>Winkler's method.</td>
</tr>
<tr>
<td>5</td>
<td>Chemical Oxygen Demand (COD)</td>
<td>Volumetric titration method.</td>
</tr>
<tr>
<td>6</td>
<td>Chloride (Cl)</td>
<td>Silver nitrate titration method.</td>
</tr>
<tr>
<td>7</td>
<td>Phosphate (PO₄)</td>
<td>Stannous chloride method, using spectrophotometer at 690 nm.</td>
</tr>
<tr>
<td>8</td>
<td>Sulphate (SO₄)</td>
<td>Turbidimetric method using, spectrophotometer at 420 nm.</td>
</tr>
<tr>
<td>9</td>
<td>Nitrate (NO₃)</td>
<td>Brucin method, using spectrophotometer at 410 nm.</td>
</tr>
<tr>
<td>10</td>
<td>Free Carbon Dioxide (CO₂)</td>
<td>Titration method.</td>
</tr>
<tr>
<td>11</td>
<td>Total Alkalinity (TA)</td>
<td>Strong acid titrate with samples.</td>
</tr>
<tr>
<td>12</td>
<td>Total Hardness (TH)</td>
<td>EDTA method.</td>
</tr>
</tbody>
</table>