Chapter III
METHODOLOGY
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Methodology

Different physico-chemical parameters were analyzed monthly from March, 2007 to April, 2008 in three selected ponds. Each chemical parameter was repeated at least three times and average of the three readings was taken for the sake of accuracy.

*Air and water temperatures* were recorded with the help of mercury thermometer graduated upto 100°C at 10 am, every month.

*Transparency*, the limit upto which light can penetrate in water body, was measured by using standard Secchi disk having a diameter of 30 cm and divided into black and white quadrants. The average of two depth readings at which secchi disc disappeared and reappeared was noted as transparency.

*Electrical conductivity* was measured by conductivity meter in \( \mu \text{Scm}^{-1} \).

*Dissolved Oxygen (D.O)* was determined at the sites by Winkler's modified technique (APHA, 1998).

*Free Carbon dioxide (CO}_2\) was determined by titrating a 100 ml water sample with 0.025 N NaOH using phenolphthalein as an indicator.

*pH* of water was determined at the sites by using a portable electronic digital pH meter.
Alkalinity was estimated by titrating 100 ml water sample with 0.02 N Sulphuric acid using phenolphthalein and Methyl orange as indicators (Theroux et al., 1943).

Hardness of water was estimated by titrating the water sample with 0.01N EDTA solution using Murexide as indicator (Trivedy and Goel, 1984).

Calcium and Magnesium was estimated by titrimetric method (Trivedy and Goel, 1984).

Nitrate-Nitrogen ($NO_3-N$) was determined following the phenol-disulfonic acid method (Theroux et al., 1943 and Trivedy and Goel, 1984).

Inorganic phosphorus ($PO_4-P$) was estimated by the ammonium molybdate blue method using stannous chloride ($SnCl_2$) as an indicator (Barnes, 1959).

In order to analyze the periphytic flora and fauna of the pond, samples were collected from the natural substrata by scrapping submerged stones, sticks and parts of macrophytes.

Artificial substrata of different objects were suspended in the pond in surface water at a depth of about 50 cm with the help of nylon thread and two iron rods. These artificial substrata include glass slides (7.5 x 2.5 cm), wooden blocks (7.5 x 2.5 cm) and stones (7.5 x 2.5 cm).

Each substratum was suspended in triplicates so that the average values of periphytic communities attached to particular substratum
may give almost a correct number of the concerned basis and the average of this has been expressed in terms of No./cm$^2$ per month. After the completion of incubation period each substratum was taken out and with the help of scalpel and brushes the organisms were detached from the substratum and transferred into plastic vials. All these were fixed in formaldehyde solution and then analyzed under the inverted microscope.

**Calculation** for the densities of periphytic organisms per unit area of the surface water was made after following the work of Adoni (1985) and using the following formula:

$$\text{Periphyton/cm}^2 = A \times \frac{V}{v} \times \frac{1}{S};$$

where

- $A =$ average no. of organisms per ml.
- $V =$ volume of scrapings (ml)
- $v =$ volume of one drop (ml) and
- $S =$ area of scrapping (in cm$^2$)

**Periphyton species diversity** was determined following Shannon-Wiener's Index (Ludwig & Reynolds, 1988) using the formula:

$$H = - (\sum \pi \ln \pi);$$

where

- $\pi =$ $n/N$
- $n =$ no. of individual species,
- $N =$ Total density of all organisms.
Species diversity was calculated by Menhinick’s Index (Menhinick, 1964) method using the formula:

\[ D_{mn} = \frac{S}{\sqrt{N}}; \text{ where} \]

\[ S = \text{Total number of species} \]

\[ N = \text{total density of all the species} \]

Percentage similarities between various periphytic communities were calculated using Sorenson’s index (Sorenson, 1948) formula:

\[ C_s = \frac{2C}{a+b} \times 100; \text{ where} \]

\[ C = \text{species common in two samples} \]

\[ a = \text{number of species of one collection} \]

\[ b = \text{number of species of next collection.} \]

Species dominance was calculated using Berger-Parker’s Index (Berger and Parker, 1970) formula:

\[ d = \frac{N_{\text{max}}}{N}; \text{ where} \]

\[ N_{\text{max}} = \text{density of most dominant species,} \]

\[ N = \text{density of all the species} \]
Evenness was calculated using formula:

\[ E_1 = \frac{H^i}{\ln S} \] (Pielou, 1975); where

\( H^i \) = species diversity

\( S \) = species richness.

ANOVA was applied to ascertain significance of variations of the recorded abiotic and biotic parameters during the studied period.