CHAPTER III

MATERIAL AND METHODS
(WITH 2 FIGURES)

This section of the thesis deals with the various requisites and procedures used in the study of different physico-chemical properties of the lake water and qualitative and quantitative measurements of periphyton communities on the natural and artificial substrates. The research was carried out for a period of three years from April, 2010 to March, 2013. The first one year period was used in standardization of methodology, identification of the flora and fauna, selection of sites, etc. The quantitative data were collected for a period of one and half years from April, 2011 to September, 2012, and the remaining 6 months period was used in data synthesis and computation, filling the gaps, etc.

Sampling stations

To collect necessary data on species composition, periphyton abundance, etc. on natural substrates two sites namely ‘mallital’ and ‘tallital’ were selected in Nainital Lake. For the study of periphyton on artificial substrate, only one site was selected in the lake in ‘tallital’ region (Fig 3.1). All sampling stations were situated in the inshore areas (littoral zone) around the lake. The sampling stations were marked by their popular names, viz. ‘Naina Devi’ Temple and Pump House. In Lake Bhimtal, the sampling stations for natural substrates were selected in ‘tallital’ region and near Boat Stand, in ‘mallital’ area. For artificial substrate (glass slide), one sampling station near ‘Durga Mata’ temple was selected (Fig 3.2). For the analysis of physico-chemical properties of the water, water samples were collected from the same stations (from which the periphyton were collected) (See Figs. 3.1 and 3.2).
Fig. 3.1. Sketch map of Lake Nainital showing sampling stations.
Fig. 3.2. Sketch map of Lake Bhimtal showing sampling stations.
Sampling, presentation and transportation of water Samples

The water samples for the analysis of various physico-chemical properties were collected at monthly intervals between 9:00 am and 11:00 am. Various techniques of sample collection were adopted as described in literature (APHA, 1989; Welch, 1948). Water samples for various analyses were collected in wide mouth plastic bottles and carefully brought to the laboratory under ideal conditions. The biological samples were preserved in 4% formaldehyde solution, or in Lugol’s solution, when required. Attempts were made to analyze the live samples on the same day of collection. The samples were transported to the laboratory within shortest possible time, usually within 1-2 hrs under ideal conditions (Wetzel and Likens, 1979).

Analysis of water samples

The water pH (hydrogen ion concentration), surface water temperature, dissolved oxygen (DO) and free carbon-di-oxide (CO₂) concentration were analyzed on the spot. The analyses of other physico-chemical parameters were done in the laboratory on the same day of collection. Surface water temperature was measured by a good grade thermometer (±0.1°C). The hydrogen-ion concentration (pH) was measured by a photometer (YSI make, model- 9100).

Free carbon-dioxide (CO₂)

It was measured on the spot by titration method. Samples of 100 ml water were taken in a conical flask. Few drops of phenolphthalein indicator were added in the sample and then it was titrated against N/44 NaOH solution. Phenolphthalein solution was used as indicator.

Dissolved oxygen (DO)

Winkler’s unmodified method (Welch 1948, Wetzel and Likens 1979) was used to determine DO contents. The samples were immediately processed after collection. A 250 ml water sample was taken in a conical flask and fixed on the
spot by adding 1 ml of manganous sulphate and 1 ml of alkaline potassium iodide solution. The precipitate was dissolved in concentrated sulphuric acid. Up to this stage the work was done on the spot. Then, 200 ml of the treated sample was titrated against N/44 sodium thio-sulphate using starch as an indicator in the laboratory. The amount of sodium thio-sulphate used in the titration was equal to the amount of oxygen present in the water.

**Biochemical oxygen demand (BOD)**

Biochemical oxygen demand (BOD) of lake waters was determined following APHA (1989). Three hundred ml of water samples were incubated at 20±1 °C for 5 days in a BOD incubator. The initial and final concentrations of DO were measured by Winkler’s method. The following formula was used to calculate the BOD concentrations:

$$\text{BOD mg/l} = D_1 - D_2 - (B_1 - B_2)/p \times f$$

Where:

- \(D_1\) = initial concentration of DO in lake water sample,
- \(D_2\) = DO concentration after 5 days of incubation of sample,
- \(B_1\) = initial concentration of DO in blank sample,
- \(B_2\) = DO concentration in blank sample after 5 days of incubation,
- \(p\) = decimal fraction of sample used, and
- \(f\) = ratio of blank to sample.

**Nitrate-nitrogen (NO\(_3\)-N) and phosphate-phosphorus (PO\(_4\)-P)**

The concentrations of nitrate-nitrogen and phosphate-phosphorus were measured by YSI make photometer model- 9100 as well as by HACH-Spectrophotometer (model: DR- 5000). These equipments are preprogrammed and
the reagents for analysis are supplied by the manufacturers themselves and are calibrated with equipment to display direct readings. These equipment follow the methods of APHA (1989).

**Periphyton analysis**

Periphyton were collected from two types of natural substrates, i.e. stone and wooden sticks. Qualitative study of periphyton in natural substrates was made by scrapping the superficial populations with fine metallic bristles and a sharp edged razor. The scrapped materials were collected in petri-dishes and then transferred to a wide-mouth bottle which was transported to the laboratory. The periphytic species were identified in the laboratory with the help of literature (Edmondson, 1959; Fitter and Manuel, 1986; Biggs and Kirloy, 2000). For the quantitative measurements of periphyton abundance, a well specified known area of about 3 cm X 3 cm was scrapped out from the stones (Zebek, 2009). In case of wooden sticks, the dimensions of sticks were recorded carefully to further calculate the periphyton density (Laal et al, 1982). The scrapped samples were diluted as per the requirements.

The glass slides were used for the study of periphyton on artificial substrate. Two slides were installed in the lake on a thermocol holder at a depth of 0.5 to 1.5 m at regular intervals (Acs et al, 2000). These slides were removed at regular intervals from the lakes, generally after a period of 25 to 30 days. The slides showed less to moderate colonization of periphyton in comparison to the natural substrates. The slides were washed or rinsed carefully to collect the superficially grown populations. To calculate the density, area of the slide was measured (7.5 cm X 2.5 cm). Similar to the natural substrates, sample dilutions were adapted for artificial substrate also as per requirements.

The collected samples of periphytic populations were carefully examined under a high magnification microscope. The density of periphyton was calculated
using a Sedgwick-Rafter cell which is composed of 1000 chambers. All the chambers were scanned carefully and counting of three slides of 1 ml aliquots each, were enumerated for each substrate. The density was finally calculated and expressed in terms of number of individuals per square centimeter area (ind/cm²).

Community analysis

To get the information on community structure in terms of diversity and dominance following indices were calculated:

Diversity indices

Species diversity of periphyton was calculated by the Shannon-Wiener index (Shannon and Weaver, 1963) as follows:

$$H = \sum_{i=1}^{S} P_i \log P_i,$$

Concentration of dominance

It was calculated by Simpson’s index (Simpson, 1949):

$$C = \sum_{i=1}^{S} (P_i)^2$$

where, $P_i = \text{the proportion of the abundance of species}$
i and $s = \text{number of species}$.

Frequency of occurrence

Percent frequency of occurrence was calculated by the formula:

$$\frac{\text{No. of samples in which the species occurred}}{\text{Total no. of samples taken}} \times 100$$
Importance Value Index (IVI)

Identification of dominant/important species was done by calculating the IVI of each species, as follows:

\[ \text{IVI} = \% \text{ frequency of occurrence} + \text{relative abundance} /2 \]

Presentation of data and statistical methods

The arithmetic means and standard deviations were calculated from 3 replicates. The correlation and regression analyses were made to determine inter-relationships among different parameters. For the analysis of arithmetic means, standard deviation, analysis of variance, etc., ‘Statistical Methods’ by (Snedecor and Cochran 1967) was followed. Also, for their calculation and data representation, statistical programme Microsoft Excel was used in a computer.

REFERENCES


