CHAPTER III

MATERIAL AND METHODS
SAMPLING STATIONS:

The present study on Sagar lake, Bila reservoir and Chittora reservoir was carried out from July 1982 to June 1983. After preliminary observations, in all four stations were selected for further intensive and extensive studies. In Sagar lake two stations, one each at littoral and limnetic zones, and only one station each in the limnetic zone of Bila and Chittora reservoirs was selected. In Sagar lake the littoral station was located near the Bus Stand, where thick macrophytic growth was present; while the limnetic station was situated towards the Fort in the deepest area, which was devoid of macrophytic vegetation. In Bila reservoir the sampling station was situated some distance from the dam, whereas in Chittora reservoir it was not very far off from the embankment.

SAMPLING PROGRAMME:

During the present investigation all the sites were visited regularly in the last week of every month with the time difference of two days. Diurnal studies were started from 0900 hrs and continued for next 24 hrs with an interval
of three hours period. Samples from different depths were analysed for physico-chemical characteristics, and phyto- and zooplankton enumerations. Simultaneously, experiments for phytoplanktonic productivity were also conducted. The sampling stations were visited with the help of a boat in a sequence which was followed throughout the investigation period. The sampling was started after a lapse of few minutes on arrival at the sampling station, to minimise the effect of disturbance caused in water due to rowing.

ANALYSIS OF PHYSICO-CHEMICAL PROPERTIES:

The water samples from different depths (surface, 0.5m, 1.0m, 2.0m and bottom) were collected in 250 ml and 500 ml plastic bottles with the help of Ruttner water sampler. Some of the physico-chemical properties such as air and water temperature, pH, free carbon dioxide, alkalinity, chloride, calcium and total hardness and dissolved oxygen contents were recorded on the spot in the temporary laboratory established in the boat. The samples for nitrate and phosphate estimations were fixed with a few drops of chloroform (to check subsequent biological activities), and were brought to University Laboratory and analysed within 24 hrs of the collection.

The following methods were used to analyse different
physico-chemical parameters.

Transparency:

It was measured by a Secchi disc of 20 cm diameter, having alternate quadrants of white and black colours. The disc was lowered down with the help of a graduated rope till it disappeared from the view on shaded side of the boat and then was lifted till it reappeared. The depth of disappearance and reappearance of Secchi disc was noted and the average of these two recordings was taken as Secchi transparency.

Temperature:

The air temperature was recorded with the help of a good grade, mercury filled celsius thermometer having the accuracy of 0.1°C and the water temperature was recorded from the thermometer fixed in the Ruttner’s sampler which had the specifications as mentioned above.

pH:

It was measured colorimetrically with the help of a Lovibond pH comparator box using BDH Universal pH indication.
Free carbon dioxide:

It was analysed titrimetrically with the help of N/44 NaOH solution and using phenolphthalein as indicator (APHA, 1976).

Alkalinity:

It was analysed titrimetrically with the help of N/50 HCl, using the phenolphthalein and methyl orange as indicators. Carbonate alkalinity and bicarbonate alkalinity were calculated following the method as given in APHA (1976).

Total carbon dioxide:

It was calculated from the free carbon dioxide, carbonate alkalinity and bicarbonate alkalinity (APHA, 1976).

Chloride:

The chloride content was estimated by Argentrometric method (APHA, 1976), titrating 50 ml of water sample with 0.0141N silver nitrate solution and using the solution of potassium chromate as indicator.
Hardness:

It was analysed using EDTA titrimetric method as detailed in APHA (1955, 1976). Sodium salt of EDTA was used as titrant with sodium purpurate plus sodium chloride mixture and Eriochrom Black T as indicators. The values for calcium hardness and total hardness were calculated according to APHA (1976).

Dissolved oxygen:

It was analysed using Winkler's method. The samples were collected in 250 ml Winkler's bottles and immediately winklerized by manganous sulphate and alkaline potassium iodide solutions. The resultant brown coloured precipitate was dissolved by adding one ml of conc. $\text{H}_2\text{SO}_4$. Then 50 ml of this treated sample was titrated against N/40 sodium thiosulphate solution using starch as an indicator (APHA, 1976).

Percent oxygen saturation:

It was calculated with the help of the method given by Golterman (1969). The solubility of oxygen at different temperatures was calculated with the help of tables given by Montgomery et al. (1964). The solubility of oxygen was modified for altitudinal correction of atmospheric pressure.
(Dussart and Francis-Boeuf, 1949) by multiplying with the constant factor to get corrected values for the study sites. From these values, per cent oxygen saturation was calculated by the following formula:

$$\text{Per cent oxygen saturation} = \frac{\text{Observed } O_2 \text{ ppm } \times 100}{\text{SX}}$$

where,

$$\text{SX} = \text{St} \times \text{Cf}$$

$$\text{St} = \text{solubility of } O_2 \text{ at the temperature 't' of water sample}$$

$$\text{Cf} = \text{Altitudinal correction factor.}$$

**Phosphate:**

It was determined in the laboratory by stannous chloride method (APHA, 1976). Two ml of ammonium molybdate reagent was added to 50 ml of water sample. Then three or four drops of freshly prepared stannous chloride solution were added, which produced blue colour after some time. Soon after, reading was recorded on a Elico Spectrocolorimeter at 690 nm wavelength against a distilled water blank; which provided exact values on comparison with standard curve.
Nitrate:

It was analysed in the laboratory by evaporating 50 ml of water sample in a conical flask and dissolving the residue in 2 ml of phenol-disulphonic acid. Further, strong alkaline solution (12N NaOH) was added into the flask to neutralize the resulted acidity. The content of flask was diluted to 10 ml, and on the per cent transmittance was recorded on a Elico Spectrocolorimeter at 410 nm wavelength (APHA, 1955) which on comparison with standard curve provided the exact values of nitrate.

Plankton collection and analysis:

Samples for the plankton analysis were collected from the deep water zone at all the study sites from where the samples for physico-chemical parameters were collected.

Phytoplankton collection and sedimentation:

The surface and sub-surface (0.5m and 2.0m) water samples were collected in the same way as described for the physico-chemical analysis. An amount of sub-sample, depending on its phytoplankton concentration, was fixed by adding Lugol's solution and was then kept for sedimentation. After twenty-four hours or so, the supernatent liquid was decanted down to
a few milliliters and the rest was subsequently centrifuged to obtain the concentrated sample of the desired volume. Two to three drops of glycerine were added to each sample.

**Zooplankton collection and centrifugation:**

Samples for zooplankton analysis were collected by filtering the water sample through conical bolting silk net (No. 25-mesh size 60 um) having a diameter of 30 cm and a length of 60 cm, for surface, 0.5m, 2.0m and bottom waters. In Sagar lake fifty litres of water and in Bila and Chittora reservoir hundred litres of water samples were carefully passed through the net. The collected sample was further concentrated by means of centrifugation at 2500 rpm or by sedimentation. The concentrate was preserved in 5% formalin containing two or three drops of glycerine. And the final volume of the concentrated sample was noted.

**Qualitative analysis:**

Identification of phyto- and zooplankton was done with the help of standard books and published taxonomic articles (Smith, 1933, 1950; Fritsch, 1935, 1945; Hymen, 1940, 1951; Pratt, 1951; Mellanby, 1951; Desikachary, 1959; Ward and Whipple, 1966; Philipose, 1967; Ruttner-Kolisko, 1974; Pennak, 1978; Pontin, 1978; Koste, 1978; Tonapi, 1980; Seghal, 1983).
Quantitative analysis:

During the present investigation counting for plankton done only up to the generic level. Unicellular as well as colonial and filamentous forms at the time of counting were treated as a single unit. For the counting of plankton the differential count sedimentation method (APHA, 1976) was applied. One drop from thoroughly shaken plankton concentrate was kept on a clean microslide with the help of a vertically held dropper and was covered by 22 mm coverslip in such a way that it covers the drop completely and no sample runs out of it. After enumeration of the larger forms in the low power of the compound microscope, the smaller forms contributing particularly to the phytoplankton were counted in about twenty visual 'field' (focus) under the high power. The same process was repeated at least for five times.

Number of individual species were calculated per litre with the help of the following formulae.

\[
\text{Total number of individuals/drop} = \frac{\text{Area of the cover glass}}{\text{Area of the one focus}} \times \frac{\text{Average of the individuals per field}}{n}
\]

\[
\text{Individuals/litre} = A \frac{1}{L} \times \frac{n}{V}
\]

where,

A = number of individuals/drop
\[ V = \text{volume of one drop (ml)} \]
\[ n = \text{total volume of the concentrated sample (ml)} \]
\[ L = \text{volume of the original sample (l)} \]

**PHYTOPLANKTON PRODUCTIVITY**

At present it has widely been recognised that \( 14_C \) method is the most valuable tool in the determination of organic production, but due to the lack of facilities for employing \( 14_C \) techniques, it could not be used. Another method that has been widely employed for the estimation of primary productivity of phytoplankton is the light and dark bottle technique of Gaarder and Gran (1927). Though, the limitations of this method are well known (Ryther and Vaccaro, 1954; Strickland, 1960; Fogg, 1963) yet, still it has been widely used due to its simplicity; and moreover, it is best suited for productive waters. Hence in the present investigations, the 'light and dark bottle method' was used to assess the primary productivity of the phytoplankton.

To assess the production potential of the water bodies under investigation, clear and darkened bottles were suspended at surface, 0.5m, 1.0m and 2.0 meter depths (containing the water sample of the respective depths) and the oxygen changes resulting from the metabolism of the plankton were measured.
Dissolved oxygen was determined by Winkler’s method. For this, samples were siphoned in 250 ml capacity ground glass stoppered bottles. One bottle was immediately analysed for initial oxygen content, whereas one set of clean and darkened bottle was suspended with the help of graduated rope at the depth from which the sample was collected. After incubating for a period of three hours duration they were taken out and dissolved oxygen content was estimated. Simultaneously, production estimation from long exposure of 24 hrs duration was also made.

The change in the dissolved oxygen content from the initial in the suspended clear bottle indicates the net photosynthesis or net oxygen production. The decline of dissolve content in dark bottle (from the initial) indicates the amount of respiration and it is referred to as community respiration. The difference between the final oxygen content of clear and darkened bottles gives an estimate of total photosynthesis and it is referred as gross primary production. It is becoming popular to express primary production in terms of carbon fixed rather than oxygen evolved. Therefore, oxygen values are converted in carbon assuming that one mole of oxygen is released for each mole of carbon dioxide fixed, as implied in the simple photosynthetic formula:

$$6\text{CO}_2 + 12\text{H}_2\text{O} \xrightarrow{\text{light, chlorophyll}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + 6\text{O}_2$$
Carbon with an atomic weight of 12 compared with oxygen's molecular weight of 32, allows the factor $12/32 = 0.375$ which has been used for the conversion. The oxygen values in mg/l (or g m$^{-3}$) multiplied by 0.375 gives the carbon in mg/l/Δt (or g/m$^3$/Δt) where Δt is the incubation period.