CHAPTER 1
METHODOLOGY

1) SAMPLING:

Surface water samples were collected using a non-metallic sampler from two selected stations in the south-west coast of India, stn. 1 Kannamaly (9° 52' N; 76° 16.5' E) and stn. 2 Fort Cochin (about 10 nautical miles north of stn. 1), and transported immediately to the laboratory using 50 litres capacity black plastic containers. Monthly collections were made during the period from February 2000 to June 2001.

2) SEPARATION OF PLANKTONIC ALGAE:

Five litres of water samples were filtered through the separation tower which is comprised of seven sieves of 500, 250, 150, 105, 53, 25 and 10 μm from the top to the bottom respectively, and built on six litres capacity plastic container (Figure 2).

Each sieve was washed thoroughly in a wide-mouthed plastic tray with sea water collected from the same locality and filtered through GF/F filter paper and the volume is made to 500 ml. From the 500 ml, sub samples were taken for the estimation of primary productivity, chlorophyll a and for identification and enumeration of planktonic algae.

By filtering large volume of sea water (5 litres) and washing the sieve with 500 ml filtered sea water, the planktonic algae will be concentrated (10 times), so even low productivity can be estimated. This would also render the estimation of chlorophyll, identification and enumeration of planktonic algae easier.
3) ANALYSIS OF SAMPLES:

A- PRIMARY PRODUCTIVITY

Measurements of primary productivity were made using Gaarder and Gran's light and dark bottles method (Strickland and Parsons, 1972). A series of three BOD bottles (60 ml) were used for the estimation of primary productivity of each size group. One bottle was used for the determination of the initial oxygen concentration, the second (the dark bottle) for measuring the respiration by planktonic algae and the third one (the light bottle) for the estimation of primary productivity. The three bottles were filled with water sample and the dissolved oxygen concentration was fixed in the initial bottle. The light and the dark bottles were incubated under the natural light for a period of three hours centred around the local noon. After the incubation, the concentration of the dissolved oxygen in the dark and the light bottles was fixed. The concentration of the dissolved oxygen in the three bottles was determined using Winkler's method.

The concentration of the dissolved oxygen was calculated using the following formula:

\[
\text{Oxygen concentration (mg / l)} = y \times x \times 8 \times 1000 \times \frac{s \times (b - c)}{b}
\]

Where:

- \(y\) = normality of sodium thiosulphate solution.
- \(x\) = volume of sodium thiosulphate solution.
- \(s\) = volume of sample titrated.
- \(b\) = volume of BOD bottle.
- \(c\) = volume of reagents added (Winkler's A & B).
The difference in the concentration of oxygen in the three bottles was used for the estimation of respiration, net and gross primary production in terms of oxygen as shown below.

Respiration = \( L_b - D_b \)

Net primary production = \( L_b - L_b \)

Gross primary production = \( L_b - D_b \)

Where \( L_b \), \( D_b \) and \( L_b \) are the concentrations of oxygen (mg / l) in the initial, the dark and the light bottles, respectively.

And in terms of carbon fixed:

\[
\text{Net primary production (mg C/ m}^3/ \text{hr)} = \frac{(L_b - L_b) \times 375}{(t \times 1.2)} \]

\[
\text{Gross primary production (mg C/ m}^3/ \text{hr)} = \frac{(L_b - D_b) \times 375}{(t \times 1.2)}
\]

Where:

375 = is a factor for the conversion from mg \( O_2 \) / l to mg C / m\(^3\)

t = the incubation time (hours).

1.2 = the photosynthetic quotient.

The actual primary productivity of each size group is \( 1/10^{th} \) of that was obtained from the equations 1 and 2, that is, because each size group was concentrated 10 times before measuring primary productivity.

B- CHLOROPHYLL \( a \)

Chlorophyll \( a \) was estimated using Strickland and Parsons (1972) method. The water sample (200 ml) containing the specific size group of planktonic algae was filtered through GF/F filter paper under moderate vacuum, and the filter paper then transferred into a clean stoppered - test tube and 10 ml of 90 % acetone was added. The test tube was placed in a refrigerator for about 24
hours in order to facilitate the complete extraction of chlorophyll. The chlorophyll-acetone solution was centrifuged for about 20 minutes at 5000 rpm, and the absorbance of the clear chlorophyll-acetone solution was measured at 664, 647 and 630 nm wavelengths using U-2001 spectrophotometer. and 90% acetone solution as a blank. The correction has been done by subtracting the absorbance value at 750 nm from the values obtained at 664, 647 and 630 nm wavelengths.

**CALCULATIONS:**

\[
Ca = 11.85 E_{664} - 1.54 E_{647} - 0.08 E_{630} \quad \text{------------------ 1}
\]

Where \(E\) is the absorbance at the respective wavelength.

\[
\text{Chlorophyll } a \ (\text{mg} / \text{m}^3) = (Ca \times v) / (V \times l)
\]

Where:

\(Ca\) = the calculated value of equation 1.

\(v\) = volume of acetone (ml).

\(V\) = volume of water sample filtered (litre).

\(l\) = path length of the cuvette (Cm).

**C- IDENTIFICATION AND ENUMERATION OF PLANKTONIC ALGAE**

After preliminary observation of the live material, samples were transferred to polythene bottles and preserved with Lugol's solution (Lugol's solution was added, so that the concentration in the sample will be 1%), and the organisms were identified microscopically.

Enumeration of microalgae was done using a Sedgwick–Rafter counting cell. The concentrated sample was mixed very well, and 1 ml was taken and transferred into the counting cell, and made to cover the all squares (1000
squares). Planktonic algae in 200 squares were counted using a microscope. Each size group was counted three times and the average was recorded.

4) MEASUREMENTS OF THE HYDROGRAPHIC PARAMETERS:

Temperature, transparency, salinity and nutrients were measured using standard methods.

A- TEMPERATURE:

Surface water samples were collected using an ordinary plastic container and temperature was measured in the shadow in a place protected from air currents using precision mercury thermometer.

B- TRANSPARENCY:

Light intensity at the sea surface was measured using a calibrated lux meter (LUX-101). Transparency was measured using Secchi disc, which was lowered vertically from the sunny side of the ship, and the depth at which the disc was disappeared was recorded. The depth of the euphotic zone then calculated using the following formula:

\[
\text{The depth of the euphotic zone (meters)} = \frac{(\ln 100 - \ln 1)}{K}
\]

Where: \( K = \frac{1.7}{D} \)

\( D \) = Secchi disc depth (meters)

C- SALINITY:

Salinity was estimated immediately after collection using a calibrated salinometer.
D- NUTRIENTS:

1- NITRATE (NO₃ – N)

Nitrate was reduced to nitrite (Grasshoff, 1970), and estimated spectrophotometrically.

2- NITRITE (NO₂ – N)

The determination of nitrite concentration in sea water was done using Bendschneider and Robinson (1952) method.

3- PHOSPHATE (PO₄ – P)

The concentration of inorganic phosphate in sea water was determined spectrophotometrically using the method of Murphy and Riley (1962) as modified by Koroleff (1963) and Koroleff (1968).

4- SILICATE (SiO₄ – Si)

Silicate concentration in water samples was estimated spectrophotometrically using Koroleff (1971) method.
Figure 2 - The separation tower