III. MATERIALS AND METHODS

3.1. Environment

The coastal water of Mangalore which is a part of southwest coast of India is regarded as highly productive region of the Northern Indian Ocean. Biological production in these parts experience seasonal variation in tune with the intensity of monsoonal wind force. The river Nethravathi and Gurpur river discharge freshwater at 8170 m$^3$ sec$^{-1}$ and 1140 m$^3$ sec$^{-1}$ respectively into coastal waters of Mangalore with a maximum flow during May to September and minimum during the rest of the year. The Nethravathi joins Arabian sea almost at right angle, whereas the Gurpur river runs parallel to the coast for a short distance before joining the Nethravathi river and form a simple estuary. Finally both the rivers enter the sea together at a place south of New Mangalore Port (ENVIS, 2002).

For the investigation Chitrapur was selected, to the north of the New Mangalore port (Fig. 1). The coastal waters of Chitrapur receive treated effluents from Baden Aniline and Soda Factory (BASF) and Mangalore Refinery and Petrochemicals Limited (MRPL). The daily discharge of effluents from industries in and around the study area include Kudremukh Iron Ore Corporation Limited (KIOCL) 40,000m$^3$, MCF 7200m$^3$, MRPL 13000m$^3$ in addition to municipal waste water as well as contaminants arising out of harbour operation.

Hydrographic parameters and phytoplankton taxonomic data and respective abundances were assembled from a monitoring program during the period 1990-2009. The monitoring was conducted at fixed stations along a depth transect of Chitrapur at 4, 8, 12 and 15 (12°56'47.50" N, 74°44'45.85" E) meters depth during 1990-2011. The data
was collected from surface water at all stations once a month, excluding the period of Southwest monsoon months, i.e. June to September.

Sampling stations were selected at four different depth contours namely 4m, 8m, 12m and 15m depth (Plate 1). Water samples and phytoplankton samples were collected from each fixed station once a month and collection of samples for various parameters was made onboard the R.T.V. Nethravathi, the research vessel of our institution. The present study consisted of both field work and laboratory work. Field work involved collection of water and phytoplankton samples and in the laboratory, analysis of various physico-chemical parameters and qualitative and quantitative analysis of phytoplankton was carried out.

3.2. Field work

The surface water samples for the determination of various physical and chemical parameters were collected in a clean plastic bucket.

3.2.1. Rainfall

Rainfall data (1990-2011) from two stations in coastal Karnataka, India, was downloaded from the data archive of Indian Institute of Tropical Meteorology (http://www.tropmet.res.in/static_page.php?page_id=53).

3.2.2. Air temperature

Air temperature was recorded at the beginning of every sampling using a precision centigrade thermometer and expressed in degree Celsius.
3.2.3. Water temperature

Immediately after the collection of surface water sample, the water temperature was recorded by using a precision centigrade thermometer and expressed in degree Celsius.

3.2.4. pH

pH of surface water samples was estimated using a pH meter (WTW pH 320).

3.2.5. Extinction co-efficient

The Sacchi disc values from each station were used to calculate extinction coefficient by using the empirical relation, $k=1.7/D$, where, $d$ is the depth of disappearance of the disc in meters and $K$ is the extinction coefficient (Poole and Atkins, 1929).

3.2.6. Dissolved oxygen

The water samples were collected in clean glass bottles following all the precautions prescribed for determination of dissolved oxygen in the water sample. Two fixatives such as manganous sulphate and alkaline iodide (Strickland and Parsons, 1972) were added to get the precipitate.

3.2.7. Nutrients

For plant nutrients such as nitrite and nitrate water samples were collected in clean polythene bottles. The collection of water samples for determination of ammonical nitrogen was made in Amber color bottle. In the field, the collected water samples were fixed by adding Phenol, Sodium nitropruside and an oxidizing reagent (Sodium
Hypochlorite and alkaline reagent). Acid washed glass bottles were used for collection of water samples for determination of phosphate.

### 3.2.8. Phytoplankton collection

Phytoplankton from the columnar waters at each station were collected using a Heron-Tranter net with a mouth area of $0.25\text{m}^2$ and having a filtering cone of 1.2 m length with 60µ pore size. The collected phytoplankton samples were filtered through 200µ pore filtering cloth to separate zooplankton. The filtrate along with the phytoplankton was preserved in lugol’s solution.

### 3.3. Laboratory analysis

#### 3.3.1. Hydrographical parameters

#### 3.3.1.1. Dissolved oxygen

Winkler’s method (Strickland and Parsons, 1972) was followed for estimation of dissolved oxygen. The titration for determination of dissolved oxygen was done within 6 hrs of collection after all the precipitate had settled.

#### 3.3.1.2. Salinity

Mohr’s method (Strickland and Parsons, 1972) was followed for the determination of salinity of water sample, which was collected in polythene bottles.
3.3.1.3. Nutrients

Plant nutrients such as ammonia, nitrite, nitrate, phosphate and silicate were estimated following standard methods described by Strickland and Parson’s (1972). Absorbance of the developed color was read by using spectronic21 with 1cm path line cuvette and the values were expressed in μg-at/l.

3.3.2. Phytoplankton analysis

For the qualitative analysis the samples were resuspended and from three aliquots of phytoplankton sample, 1 ml was drawn for qualitative analysis. The phytoplankton cells present were identified, counted and recorded employing Sedwickrafter type of cell and a compound microscope fitted with closed circuit television camera (CCTV camera). Phytoplankton cells were identified up to generic level and expressed in terms of number of cells/m$^3$ of water. As the numbers were too high to depict graphically so the log$_{10}$ transformation was done.

3.3.3. Diversity indices of phytoplankton

The data generated on phytoplankton abundance were treated with different diversity indices to study its community structure. The various diversity indices employed were Shannon’s diversity index, Pielou’s evenness index and Margalef species richness.
3.4. Statistical analysis

3.4.1. Correlation Coefficient

Correlation coefficient is used to measure the strength of association between two continuous variables. This tells if the relation between the variables is positive or negative that is one increase with the increase of the other or one decreases with increase of the other. Thus, the correlation measures the observed co-variation. The simple correlation was determined between various hydrographical parameters and dominant groups of phytoplankton.

3.4.2. Analysis of Variance (ANOVA)

The basic principle of ANOVA is to test for differences among the means of the populations by examining the amount of variation within each of these samples, relative to the amount of variation between the samples (Kothari C.R., (1985). The technique of Analysis of Variance (2-way ANOVA) was applied to phytoplankton and water quality data to establish the relation between years and depth by using SPSS Software package.

3.4.3. Multidimensional Scaling (MDS) Analysis

The Multidimensional Scaling technique (MDS) is a set of data analysis techniques, which allow one to infer the dimensions of the perceptual space of subjects. The primary outcome of MDS analysis is a spatial configuration, in which the objects are represented as points. The points in this spatial representation are arranged in such a way, that their distances correspond to the similarities of the objects: similar objects are represented by points that are close to each other, dissimilar objects by points that are far
apart (Wunderlin et al., 2001, Bulut et al., 2008). In this study, MDS analysis was used to determine which physico-chemical variables play roles in similarities or dissimilarities between depth and years.

3.4.4. Hierarchy theory

Hierarchy theory provides an intellectual vehicle for applying ecosystem management philosophy to real world problems by explaining how ecosystem linkages are to be understood. Under this theory, individual ecosystem components, each with a specific set of spatial and temporal scales, are nested within larger layers, forming a hierarchy. So this technique was used for the present study as the hierarchy theory provides an important intellectual framework for the research design.