CHAPTER 3. MATERIALS AND METHODS

Regular monthly collections of seaweeds along with bottom water were made from a five study stations located in the Arabian Sea at different geographical position with the help of fishing trawler and motorized outrigger canoe during a period between September, 2001 and September, 2002.

Uttara Kannada is maritime district located on the northern sector of Karnataka State (Fig.1) having a coastal stretch of 144km., is rich in marine algal (seaweeds) resource. This maritime district of Uttara Kannada is blessed with four major riverine systems (Fig. 2) namely, Kali, Gangavali, Aghanashini and Sharavathi, plays important role in escalating the productivity of this coast throughout the biological calendar. River Kali originates in the Kusavali village in the Supa taluka, after meandering about 181 km in the Western Ghat, which joins the Arabian Sea at Karwar and is very much held responsible for the rich resources of fin and shellfishes besides the rich growth of marine algal population in this Karwar bay region.
Description of the Study Stations:

Karwar bay situated on the West Coast of India is geographically demarcated from the rest of the marine systems by extraordinary features. There is great amount of temporal and spatial variation in the magnitude of marine micro and macro algal production in the inshore waters of Karwar and is primarily governed by hydrographical feature. Since our knowledge on marine algae (seaweeds) in this region is fragmentary, the present investigation was carried out to project general picture of spatial distribution of marine algae with respect to their seasonal variation in abundance at different study stations located in the Karwar bay and in Tilmaathi coast. In the present study, totally five study stations were selected, of which four located in the Karwar bay and one at Tilmaathi coast (Fig. 3). Station 1st was located at Karwar Head, Station 2nd at Devgad Island, Station 3rd at Sunghiri Island, 4th at Kurmagad Island and 5th at Tilmaathi coast (Fig. 4-8 and Plate No. 1-5).

Station 1st was selected opposite to the Karwar Head, the bottom sediment was sandy silt. The location of the study station was shown in Fig.4 and its geographical position is given in the Table 1 and Plate No. 1.
Station 2\textsuperscript{nd} situated northern sector of Devgad Island, where bottom sediment was sandy silt. The location of the study station was shown in Fig.5 and the co-ordinates of this station is given in the Table 1 and Plate No. 2.

Station 3\textsuperscript{rd} being located to the western sector of Sunghiri Island, was protected from the physical effects of riverine discharge. Nature of the bottom sediment was sandy silt. In Fig.6, the location of the station is shown while its geographical position is given in the Table 1 and Plate No. 3.

Station 4\textsuperscript{th} was located to the south of Kurmagad Island and was more or less on the axis of riverine discharges. The lowest salinities were recorded at this station as the estuarine mouth of river Kali was just 500 meters away to the east of this place. The bottom sediment was predominantly sandy. Location of the station is given in the Figure 7 and its Latitude and Longitude is given in the Table 1 and Plate No. 4.

Station 5\textsuperscript{th} was situated in the Tilmaathi coast where the bottom sediment was sandy silt with small boulders. The location of the station is shown in the Figure 8 and the geographical position of this station is given in Table 1 and Plate No. 5.
A. HYDROGRAPHY:

Water samples were collected from the respective five study stations by using the mechanized boat (Plate No.6) with the help of Casella (Aqua Sampler) bottle to a depth usually not higher than 20cm from the bottom. Later the water sample was transferred into a separate sampling bottles for estimation of different physico-chemical parameters. Water temperature was recorded by using the ordinary thermometer which was sent to the bottom along with the casella bottle.

Salinity:

Salinity of water sample was determined by Mohr-Knudsen method as described by Strickland and Parsons (1975).

Dissolved Oxygen:

Dissolved oxygen content in the water sample was estimated by Winkler’s method as per the procedure described by Strickland and Parsons (1975).

Suspended matter:

Amount of suspended matter in the sea water was estimated by the filtering one liter of the seawater sample through pre weighed
Whatman GF/A filter paper which was dried to constant weight at 90-105°C and reweighed. The results were expressed as gm per liter.

**Hydrogen ion concentration:**

The hydrogen ion concentration (pH) of the sample was estimated in the site itself by using the portable pH meter (Model LI-120) on the board and recorded the readings.

**Nutrients:**

Collected water sample were used for an estimation of different nutrients such as Phosphate-phosphorus, Nitrate-nitrogen, Nitrite-nitrogen and Silicate-silicon as per the standard method described by Strickland and Parsons (1975).
B. SEAWEEDS:

Seaweeds were collected from the respective study stations using an outrigger boat (Plate No.7) during the low tide period (by referring to the Tidal chart of Karwar coast). Plate No.8-21 explains the exposed study area and the collection of seaweeds from the respective study stations. There are different methods to collect seaweeds, of which, in the present study a random sampling method was opted as it is more common and easy to study the seaweeds in the field (Gowda and Renukumar, 1999). In this random sampling method, a quadrate method is opted (Plate No.13), the different species of seaweeds found within this quadrate were collected along with their holdfast or rhizoid. Some species of the seaweeds were removed with the help of hand but whereas other species which were closely adhering to the substratum were removed with the help of scalpel or with sharp pointed curved picker (Plate No.9-11). Totally collected seaweeds were counted species wise and total number of individual present in the selected quadrate by counting all the collected species and calculated the relative density, abundance, frequency and dominant species in the selected quadrate. Total biomass of the standing crop of given quadrate was estimated by using the monopan balance as per the standard methods given by Gowda and Renukumar (1999). The collected seaweed samples were then transferred to the polyethene bags with detailed labels for further identification in the laboratory.
Collected samples of macrophyte algae (seaweeds) were preserved in wet condition and dry condition. In the former preservation procedure, the collected specimens were washed thoroughly with fresh water to remove the unwanted debris and other flora and fauna which were associated with seaweeds later the sample was preserved in the bottle or in the plastic bags which contained solution of 10% formaldehyde (prepared in seawater). The preserved bottles were closed tightly to avoid leakage of fumes of formaldehyde. The containers were made air tight, labeled with date of collection, locality and time later transported to the laboratory for further identification.

Dry preservation was made in the form of Herbarium. In this technique, the materials like trays (plastic or iron with white coating), forceps, herbarium sheets (mounting paper), muslin cloth, blotting paper, wooden press, camel brush, knife and pencils etc. were made use. The collected seaweeds were dried properly in such a way that the morphological characters are displayed fully and completely as much as possible because the dried herbarium specimen are useful in future for identification of seaweeds. By following the prescribed techniques, a good quality dried herbarium specimens were prepared. Before drying the specimens, following procedures were followed.
The specimens were preserved in 3-5% buffered formalin, (which can retain the specimen's natural colour i.e., original colour). In case of fleshy specimens, utmost care was taken to remove sand particles, unwanted debris, shells of gastropods and larvae of other microorganisms. The specimens which bear thick hold fast, a small portion were retained to facilitate pressing. In some cases, it was very difficult to remove a part of holdfast, in such condition, the entire hold fast was retained, which made a good herbarium specimen. Calcareous specimens were preserved by treating the materials with 3 to 5% formalin and then soaked in 40% glycerene later in 3% buffered formalin prepared in seawater were dried for 10-15 days. In this way, the calcareous specimens were preserved in dry condition without pressing.

i. Herbarium technique:

The collected fresh specimens were cleaned by washing and removing the adhering particles, epiphytic algae, sand particles, mud and other debris etc. A plastic tray with half filled fresh water was taken and mounting board was placed in the water. The herbarium sheet was placed in the tray on which the specimen was displayed properly with the help of brush to avoid overlapping and hiding of the
parts of the specimens. After mounting, the specimens on the herbarium sheets was slowly tilted the herbarium sheets without disturbing the mounted specimens. As the tilting makes the water to run gradually without affecting the herbarium specimen and then removed the sheets from the tray and spread the specimen properly with the help of forceps, then herbarium sheets were transferred on the news paper or blotting paper to remove the remaining water content from the herbarium specimen. After removing the water from the herbarium, cheese cloth was fully placed on the specimen later another sheet of the blotting paper was placed over the herbarium sheets to remove remaining water from the herbarium. After this process, herbarium was piled one above other and finally placed in between the two sheets of wooden press and tied tightly with a rope. The pressed materials were kept at room temperature for the duration of 24 hours. After one full day (24 hrs.), the blotting papers were replaced and this process was continued till the specimen free from the moisture content. While drying of the specimen, some time it gets attached to the paper because of presence of sticky and gummy like substance in the seaweeds. In such a condition, the cheese cloth is carefully removed from the specimen and then herbarium sheet is labeled. The label gives information about the collection number,
Before going for collection of seaweeds, the reference of previous herbarium specimens were made which are available in the herbarium center. The key and reference is very much essential for identification of the seaweed specimens. In addition to the key for morphological characters, it is essential to refer some seaweed books also to know the anatomical peculiarities of the seaweeds specimens, and later the name of the particular seaweed was confirmed.

ii. Method of fixing dried specimens:

The dried specimens were placed on the herbarium sheets, before placing the specimens on the herbarium sheets, applied good quality of gum or resin on the abaxial side (under surface) of the specimen. Rarely the gummed Lenin herbarium tapes may also be used to strap the specimen to the herbarium sheets.

The final step in the herbarium technique is tagging the label of the specimen. The label was pasted on the right hand corner of the herbarium sheet. The completed sheet contain all information regarding the specimens, including the serial number, date, class,
order, family, genus and species, common name, colour, substratum, zone, depth, latitude & longitude, ecological notes, locality, district, state, remarks, brief notes, identified person's name and collector's name. The model data information sheet is shown in Model sheet.

iii. **Wet preservation of seaweed specimen:**

The collected seaweeds were preserved in the glass bottle containing the formalin or ethyl alcohol (C₂H₅OH) but corallines are kept in the 3-5 % formalin (3-5% of formalin is a good preservative for algae). To preserve a specimen for a longer period, it was kept in the dark in a sealed container or polythene bags to prevent bleaching.

iv. **Permanent storage:**

The collected specimen was treated with 3-5% formalin for a period of not less than 24 hours. Before preservation, rinsed with tap water and then transferred the specimen to 70% ethyl alcohol for permanent storage. But, the usage of formalin- acetic acid - alcohol (FAA) to preserve calcified algae is not advisable because the acid causes damage to the calcified specimen. The FAA is a good preservative for flagellate algae. Utmost care was taken to prevent evaporation of preservatives from the containers to avoid desiccation.
and to this a few drops of glycerine was used. The glycerene helps in maintaining the moisture in the specimen. But, few coenocytic algal filaments will shrink when glycerine added to the preservative. Hence, it is advisable to avoid addition of glycerine to coenocytic filamentous algae. In addition to this precaution, care was taken to seal the containers by using the sealing wax. The silicon rubber gasket was also found good materials for sealing the jars.

A field note book was maintained which gives detailed information regarding the habitat, ecological parameters, collection, identification, preservation, labeling and maintenance of herbarium (dry specimen) and wet preservation techniques.
A Model Sheet of Data Information.

<table>
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<tbody>
<tr>
<td>Date:</td>
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<tr>
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<tr>
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<td>Depth:</td>
</tr>
<tr>
<td>Latitude:</td>
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<tr>
<td>Longitude:</td>
</tr>
<tr>
<td>Ecological notes:</td>
</tr>
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<td>District:</td>
</tr>
<tr>
<td>State:</td>
</tr>
<tr>
<td>Identified Person:</td>
</tr>
<tr>
<td>Collected by:</td>
</tr>
</tbody>
</table>


Further, the statistical analysis of Seaweeds like (i) Species Diversity, (ii) Species Eveness (iii) Species Richness (Pielou, 1966 & 1975 and Gleason, 1922) and (iv) Similarity Index (Jaccard, 1908) were carried out by using the following formulas:

(i) **Species Diversity:**

The algal species diversity in each of the study areas was calculated using the Shannon-Weiner Diversity Index ($H'$). (Krebs, 1972).

$$H' = -\sum_{i=1}^{S} p_i \log_2 p_i$$  \hspace{2cm} \text{(Krebs, 1972)}.$$

Where \( S \) = Number of Species

\( p_i \) = proportion of a particular species in a sample which is multiplied by the natural logarithm of itself

(ii) **Species Eveness:**

Shannon-Weiner Index takes into account how evenly the total number of individuals in a sample is apportioned between each species (Equitability).

$$J' = \frac{H'}{\log_2 S}$$ \hspace{2cm} \text{(Pielou, 1966)}.$$

Where \( H' \) = Diversity factor

\( S \) = Number of species
(iii) **Species Richness (SR):**

The number of species present is the species richness, which is a simple index of diversity.

\[ \text{SR} = (S-1) \ln N \] ............................(Pielou, 1975)

Where  \( S = \text{Number of Species} \)
\[ N = \text{Total number of individual in a sample} \]

(iv) **Similarity Index :**

To discuss the compositional similarity between the habitats, Jaccard's (1908) index of similarity (\( J \)) was applied. The similarity index refers to the ratio of number of species shared to total species numbers among the various entities compared.

\[ J = \frac{N_c}{N_1 + N_2 - N_c} \]

Where  \( N_c = \text{Number of species in common} \)
\[ N_1 = \text{Number of species in first entity} \]
\[ N_2 = \text{Number of species in second entity} \]
<table>
<thead>
<tr>
<th>Station No.</th>
<th>Name of the Study Station</th>
<th>Geographical Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Karwar Head</td>
<td>14° 48' 30&quot; N &amp; 74° 05' 36&quot; E</td>
</tr>
<tr>
<td>2</td>
<td>Devgad Island</td>
<td>14° 49' 06&quot; N &amp; 74° 03' 30&quot; E</td>
</tr>
<tr>
<td>3</td>
<td>Sunghiri Island</td>
<td>14° 49' 54&quot; N &amp; 74° 05' 24&quot; E</td>
</tr>
<tr>
<td>4</td>
<td>Kurmagad Island</td>
<td>14° 50' 12&quot; N &amp; 74° 05' 54&quot; E</td>
</tr>
<tr>
<td>5</td>
<td>Tilamathi coast</td>
<td>14° 52' 09&quot; N &amp; 74° 07' 00&quot; E</td>
</tr>
</tbody>
</table>
Fig. 1. Map of Karnataka showing the location of Uttar Kannada District
Fig. 2. Map of Uttar Kannada District showing the locations of four major riverine system.
Fig. 3. Map showing the study stations #1 to #5 located in the Arabian Sea.
Fig. 4. Map showing the study station #1: Karwar Head located in the Arabian Sea.

ARABIAN SEA
KARWAR BAY
Stn. 1 •
KARWAR HEAD

14° 45' 00" N
Fig. 5. Map showing the study station #2: Devgad Island located in the Arabian Sea.
Fig. 6. Map showing the study station #3: Sunghir Island located in the Arabian Sea.
Fig. 7. Map showing the study station # 4: Kurmagad Island located in the Arabian Sea.
Plate No. 6

Mechanised boat used for field work

Plate No. 7

Outrigger boat used for collection of seaweeds
Plate No. 8

Exposed study area of seaweed at Karwar Head

Plate No. 9

Collection of seaweed at northern sector of Karwar Head study site
Plate No. 10

Collection of seaweed at Devgad study site

Plate No. 11

Collection of seaweed in western sector of Devgad island
Plate No. 12

Recording the water temperature at study site

Plate No. 13

Quadrat method for estimating Relative density of seaweed at study site
CHAPTER 4. STUDY SITES AND ENVIRONMENTAL PARAMETERS