Chapter - III: Materials and Methods

3.1. Study area

The Indian coastline is about 7517 km long, 2000 km wide economic exploitation zone, the Bay Island of Andaman and Nicobar, and the atoll Island group of Lakshadweep, India harbors a vast extent of coast and marine habitat. The coastline comprises of headlands, promontories, rocky shores, sandy spits, barrier beaches, bays, marshy land and offshore Islands. According to the naval hydrographic chart, the Indian mainland consist nearly 43% sandy beaches, 11% rocky coast and 46% mud flats and marshy coast. The Indian coastline supports almost 30% of its human population being dependent on the rich exploitable coastal and marine resources.

Among the eight maritime state of India, Gujarat, situated on the western coast of India, has longest coastline about 1650 km long shore which is comprises about 22% coastal stretch of the total coastline of India. Gujarat coastline consist 28% sandy beach, 21% rocky coast, 29% muddy flats, 22% marshy coast. Gujarat coast could be broadly described in to four regions viz. the Gulf of Kachchh, the Saurashtra Coast, the Gulf of Khambhat and the South Gujarat Coast. Among that Saurashtra is a region located south-western part of Gujarat, which is an arid peninsula and also called as Kathiawar. Saurashtra coast occupies a total stretch of 865 km., which is situated between two Gulfs encircled by the open sea. The continental shelf is vast, varying in width from 58 to 191 miles, of the coast of Saurashtra. With the gentle gradient the littoral zone is 0.5 to 1.5 km wide and covered with loose calcareous sand. Long shore currents, high wave energy with surf action and tides generally low in the range of 2 to 3 m characterized the coastline.

The south coast of Saurashtra from Dwarka to Kodinar segment stretches for about 250 km with smooth and sandy beaches. The beaches are usually calcareous and dominated by bio-clasts, the consolidated ancient equivalent of these biogenic sands are famous milliolite rocks. The milliolite underline the beach sands and occur as cliffs, wave cut platforms and submerged dunes, all along the shoreline indicating quaternary sea level fluctuations. The shoreline is straight and consciously rocky platform backed by sandy beaches.
3.2. Study Localities

Before the selection of study sites, locations of the sampling sites were selected according to a preliminary study of the coastline in view of different anthropogenic pressure on coastal area. Now a day’s especially Saurashtra coast is being hot-spot for various mega industries, fishery related opportunities and further more tourism is also one of the related problems on the coastal zone of Saurashtra peninsula.

For present investigations, four different sites were selected along the South Saurashtra coastline off Arabian Sea, viz. Dwarka, Mangrol, Veraval and Kodinar (Plate 1). Each of these sites was chosen because they are accessible, on the open coast, and all these sites are influenced by the direct pressure of humans and pollutants from both harbors and terrestrial sources. The selected location of Saurashtra coast was surveyed extensively to monitor the coast characteristics, from both physical and biological approach.

3.2.1. Dwarka

Dwarka (22° 13’ N, 68° 58’ E) is situated on the west coast of India and a major pilgrim town owing to that it is also be a tourist place on the coastal area. It is nearest about Okha port which is well known as entry to western India and Gulf of Kachchh (GoK), around 175 km west of Veraval and around the northernmost corner of the Kathiawar peninsula. There are small scales fishing industries also available. The local community mainly depends on the tourism and fishing related opportunities. The total length of the sampling site was about 1.5 km.

3.2.2. Mangrol

On the other hand, Mangrol (21° 07’ N, 70° 07’ E) is a small hamlet and important harbor around 50 km west of Veraval with predominantly fisherman population. There are many small scale fisheries industries located along the coastline and it exports to many other countries. It having a proper landing place included all infrastructure facilities such as storage of catch, ice factories, repairing of boats and engines etc. The total length of this area is about 2 km. The local communities which live nearest about the coastline mainly depend on the fishing related opportunities.
3.2.3. Veraval

Veraval (21° 35’ N, 69° 36’ E) is one of the largest fish landing site of India situated around 35 km east of Mangrol, surrounded by a large chemical factory, a medium scale cement factory, number of small to medium scale industries and fish processing units. It involves port activities like transport, boat manufacture and receive waste from different sources. In addition to that, the area, being one of the most developed spot from industrialization point of view, is a hot spot for both heavy and small scale chemical industries. The area favors the fish processing industries too due to its proximity to the landing centre and easy supply of the raw materials. The total length of the study area is about 3 km.

3.2.4. Kodinar

Kodinar (20° 41’ N, 70° 46’ E) is a small town, situating southeastern part of Junagadh district, on the southern coastal region of Saurashtra peninsula. Tourists find ample places to visit in Kodinar and its nearby region. Here Gujarat Ambuja Cement Group has established its flagship cement factory and the company have also developed the port of Muldwarka. It is also well-known for the sugar factory, which is situated near about the coastline and also minute level of fish catching unit located near the selected site. Total length of the selected coastal stretch of the sampling site is about 1.5 km. The coastal area between Kodinar and Veraval is fast emerging as an industrial hot-spot and few mega industries are already in operation.

3.3. Study Period

The study survey was made from November 2007 to August 2009. During this period, each sampling sites along Saurashtra coastline were survey regularly for the macrofaunal diversity for qualitative assessment. The monthly data were collected for population ecological study during September 2008 to August 2009. Simultaneously, sea water samples were also collected for heavy metal analysis. The monthly surveys were summed up to four seasons viz., winter (December to February), summer (March to May), monsoon (June to August), post monsoon (September to November). During this study, it was also observed and note down the different anthropogenic disturbance on the coastal zone.
3.4. Coast characteristics

The Saurashtra shoreline is straight and conspicuously rocky platform backed by sandy beaches and dune ridges and occasionally cut by river or creek; where marshes are present. Generally the sediments are of carbonate sands. The rocky portion is generally formed of rocks of miliolite and laterite stone. Extensive limestone deposits are seen to occur in the coastal areas of Saurashtra. Rocky shores are rarely smooth slabs of rock. The intertidal belt is interspersed with many tide pools, puddles, crevices and small channels. The upper portion of the intertidal belt is generally covered with an admixture of silt and sand mixed with pieces of broken shells. Depended upon geology they will be crossed with cracks, crevices and pools. Hard rock like granite provides a more secure anchorage for plants and animals than the soft rocks, such as sandstone or chalk. The seabed is comprised of natural rock formation, which is covered with densely grown seaweeds. Thick deposition of sand also noticed in the small channels. On the sea front, the intertidal zone where the sea and the shore are pitted against each other is a unique habitat. The sequence of alternating tides result in the inevitable submergence and emergence of this habitat. The sandy beach gradually slopes into the rocky intertidal belt from landward side. The intertidal area is mostly spongy calcareous or spongy rocky substratum and the substratum of the shore area is of hard rocky.

3.5. Intertidal Zonation

Conditions on the Intertidal zone change, becoming wetter or drier, more or less exposed, more or less steeply sloped etc. as one moves around. These changes influence the composition, abundance and distribution of the population (Lewis, 1972; Prescott, 2006). Intertidal organisms are subject to a vertical gradient of increasing stress with increasing exposure to air higher on the shore (Crowe et al., 2000). This leads to increased abundance within zones where conditions are favorable to species survival; abundance decreases outside of this zone as the physical environment becomes less suitable. In 1949 the ecologists Stephenson and Stephenson devised a classification scheme of zonation of all rocky shores, where they distinguished four major zones. For the zonal study intertidal area was divided in to three major littoral zone viz. upper littoral zone, middle littoral zone and lower littoral zone, as per the sampling area accessibility.
3.6. Sampling Procedure

3.6.1. Sampling Method for Macrofaunal Diversity

The intertidal zone of each sampling sites were surveyed regularly on monthly basis and all the macrofauna and flora encountered were recorded properly. Extensive photography was employed for the identification of the animal species with the identification keys, literature available in the form of books, journals, reports and with extensive use of internet. The complete study was conducted in a non-destructive manner in which the organisms were disturbed to the bare minimum, let alone killing any. Once the organisms were identified, during the successive surveys just the record of the encounter was made. However, few algal samples were collected and stored immediately in 10 % formaldehyde. They were then brought to the laboratory and washed in running tap water, and then it was subjected for temporary herbarium preparation. During the study, all sampling sites were frequently surveyed at regular intervals during the lowest tides. All intertidal macrofauna and algae observed were recorded properly and later classified systematically. Thus animals under various phyla were recorded and checklist was prepared.

3.6.2. Sampling Method for Population Ecology

**Transect and Movement**

Transect sampling is one of the most widespread ecological techniques for sampling both plants and animals. The structural attributes of the intertidal fauna were studied by transect method (Misra, 1968). Gonor and Kemp (1978), Littler and Littler (1985), and Creese and Kingsford (1998) provide good previous accounts of non-destructive sampling methods for quantifying the abundances of intertidal populations which were followed in this study. Foot transect method was primarily used for generating the population database. The main problem in using any other method like belt transect as that all these methods would come out with the result of avoiding a major proportion of the area. The greatest advantage of foot transect method was that it took the maximum available ground into consideration. At all the sites, criss-cross direction was followed to cover the maximum exposed area on the intertidal belt. The visits were made at the lowest tides of the months.
**Quadrate size and number**

Quadrates are extensively used for sampling in all branches of ecology and many approaches are available (Greig-Smith, 1983). Quadrates of 0.25 m$^2$ were laid while following an oblique direction covering maximum area at almost regular occurrence on the preferred intertidal belt. Quadrate frequency was determined on the basis of the total length of the intertidal area along the sampling site. At the each zone of sampling site, ten quadrates were used to be laid during the study period. Sampling used to be started with the start of the low tide and attempts were made to finish two sites within the stipulated duration of about four hours.

**3.7. Study Species**

The Molluscan species were selected on the basis of their occurrence through the study area. As these were found to be the most prominent one and their presence throughout the season, these organisms were selected. More to that, as they were reported to be non-migrant (Inter coast), so long selection of these would ensure a long term study on the same aspect. As the study area was purely a rocky one, so filter feeders and other such organisms those are usually selected were not taken into consideration to work with on these belt. A close look to the animals shows that these organisms were dominant in all the three zones. So, a detailed study on these could reflect the ecological status of the three zones. The selected animals are *Mancinella bufo*, *Conus miliaris*, *Trochus radiatus*, *Turbo coronetus*, *Turbo intercostalis*, *Nerita albicilla* and *Rhinoclavis sinensis*.

**3.8. Seawater Sampling and Metal Analysis**

Sea water samples were collected monthly from each sampling site of the study area. The locations for the collection of samples in a particular coast were fixed using global positioning system (GPS). Water samples were collected from directly from the surface in previously acid-washed glass bottle and stored in HNO$_3$. The sea water samples, analyzed for concentrations of the major trace metals were estimated using an Atomic Absorption Spectrophotometer (AAS). Data was presented in mg/l concentration. The water samples were analyzed for various trace elements based on the procedures described in APHA (1995), Trivedi and Goel (1986).
3.9. Documentation of Anthropogenic Impact

This study was conducted for the various anthropogenic influence on exposed shores and the structural role of few dominant macrofauna on the shores, were demonstrated by field experiments. Extensive field study was regularly carried out along the entire coastal zone of Saurashtra region. The study sites were identified and make a note of the type of various anthropogenic activities such as tourism, fisheries, port activity, industry, sewage and disposal waste and later than classify it’s to the degree of these activities which is actually more affected on the coastal area. For the prediction of likely nature and impact of anthropogenic stress, various environmental indicators were selected subject to the relevance to the study area. Further, direct, indirect, cumulative and unavoidable impacts were examined to assess the predictive impact. The coastal area is mainly polluted by the water pollution; it is directly or indirectly created by human and industrial settlement near the coastal area as well as natural procedure, which is tremendously affected the intertidal community. In the present study heavy metal analysis in sea water which affects the water quality were used for describe the coastal pollution and its effects on the intertidal macrofauna.

3.10. Data Analysis

According to Warwick and Clarke (1991), the available statistical methods for data analysis could be categorized broadly into different methods such as univariate, graphical/distributional and multivariate. This terminology is widely used for the study of benthic ecology.

3.10.1. Similarity Indices

The major groups responsible for the distribution of the species at different localities Sorensen’s index of similarity (Sorensen, 1948) has been used to compare the similarity in species composition of the mollusca between sampling sites. It was calculated using following equation.

\[ QS = \frac{2C}{A + B} \]

Where, A and B are the species numbers in samples A and B, respectively, and C is the number of species shared by the two samples.
3.10.2. Population Ecology

Among the ecological attributes, seasonal variations in the population density and abundance of major prominent molluscan species in each sampling stations were calculated (Misra, 1968). The collected data of ecological attributes were calculated by below formula were treated as raw data from which the total density, total abundance and total frequency values were calculated.

\[
\text{Density} = \frac{\text{Total number of individuals recorded from the sample plot}}{\text{Total number of sample plot studied}}
\]

\[
\text{Abundance} = \frac{\text{Total number of individuals recorded}}{\text{Total number of sample plot where the individuals occurred}}
\]

\[
\text{Frequency (\%)} = \frac{\text{Number of sample plot where the species occurred} \times 100}{\text{Total number of sampled plot where the individual occurred}}
\]

3.10.3. Statistical Analysis

The collected monthly data were presented as seasonally for the seasonal approach like winter, summer, monsoon and post-monsoon then calculated statistically like mean and standard deviation. The obtained data were subjected to different statistical analyses for their cumulative acceptability (Sokal and Rohlf, 1969). All the data was calculated automatically by using Microsoft Office Excel software. Significance of spatial and temporal variations was compared by using single factor ANOVA. Regression coefficients analysis was also performed to find out relationship between various metals in seawater and few prominent molluscan species within a sampling site, to assess the influence of trace metals on the few indicator species in the intertidal zone (Shouthwood, 1978).