Chapter 3

MATERIALS AND METHODS

3.1. Study area

India has a coastline of 7517 km (total 4700 mile), which is bounded by the Indian Ocean on the south, the Arabian Sea on the west and the Bay of Bengal on the east. Among that, Gujarat is situated on the north-western part of peninsular India. It has about 1,650 km longest coastline; that accounts for 22 % of the total coastline available to the country, with a continental shelf of 164,200 km² (35.3% of the country) and an Exclusive Economic Zone (EEZ) of 214,000 km² (9.9% of the country). Gujarat coastline consist 28 % sandy beach, 21 % rocky coast, 29 % muddy flats, 22 % marshy coast. Further, the coastal zone of Gujarat state can broadly be divided into three major geographical parts, two major gulfs namely Gulf of Khambhat and Gulf of Kachchh and the Saurashtra coastline, each one with its own distinctive characteristic and diverse geo-environmental features, which embrace diverse coastal habitats as well as biota of ecological significance.

Saurashtra is a region located south-western part of Gujarat which occupies a total coastal stretch of 865 km. Saurashtra is a part of an arid peninsula called as Kathiawar. On an average, it receives 500 mm rainfall annually. The south coast of Saurashtra from Dwarka-Kodinar segment stretches for about 250 km. with smooth and straight sandy of rocky-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 (Stanley, 2004).

People living in the coastal zone of India are considered to generate $1.11 \times 1010 \text{ m}^3$ of sewage annually; a considerable fraction enters the coastal water (Zingde, 1999). There is a high concentration of large to medium industries within the narrow coastal belt of 25 km both the east and west coast of India. The west coast of India is more industrialized than east coast, with the state of Gujarat having the largest concentration of industries (Rejomon, et al., 2008).
The development of the Saurashtra region was driven solely by the trading possibilities offered by its long coastline and ports. It would appear that today the problems consequent to the high degree of industrialization along the Saurashtra coastline are being addressed with even more industrialization. There are two developed fishing harbours with allied at Veraval and Mangrol, which face 62% fish production out of the total fish production. The industrial groups that have greater dominance are cement; food industry and the existing port with facilitate import or export of fish and fishery products, fertilizers, salt, cement, soda ash and lime stone etc.

The whole of south Saurashtra coast was surveyed extensively from physical and biological point of view for their coast characteristics. The main reason behind the selection of the Saurashtra coastline was the difference exists between the four sampling sites which different level of interferences with the human community. It would give a clear indication of the impact of different anthropogenic pressure on marine ecosystem keeping all the physico-chemical parameters constant. The other point of view for selection of this region was differences between locations, in terms of slope, substratum type and the length of the intertidal zone. The Saurashtra shoreline is straight and conspicuously rocky platform backed by sandy beaches.

### 3.2. Sampling sites

Before the selection of study sites, locations of the sampling sites were selected according to a preliminary survey 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 spatial analysis Saurashtra coastline divided in to four sampling site, viz. Dwarka, Mangrol, Veraval and Kodinar (Plate-1). These shores were selected on the basis of their strategic locations, existing industries, infrastructural facilities for the likelihood of being developed as industrial zones, different anthropogenic activities along the entire coastal area of Saurashtra peninsula. The Saurashtra coastline is basically rocky-sandy, being rockier in the east and west, sandier in the central part and more rocky-muddy in the far eastern part. It is remarkable by having milliolite limestone formation along the coastline.
Sampling site: 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 point of the Gulf of Kachchh (GoK) and to western India, and 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.

Sampling site: Mangrol

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

Sampling site: 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 center and easy supply of the raw materials. The total length of the shore area is about 3 km.
**Sampling site: 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. Very near to the coastline, the 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. Coastal characteristics

The selected locations are situated at South Saurashtra coastline off Arabian Sea, which are significantly rocky with irregular patches of sand or mud. 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 Gujarat. The intertidal rocks of Saurashtra shoreline are calcareous sandstone. The selected shoreline of Dwarka and Mangrol experiences wave actions to a heavy extent. The intertidal zone of Saurashtra coasts is not very wide, generally dynamic wave action due to this reason. The selected locations of Saurashtra coastline is rocky with hard substratum having many big cuphols and crevices, whereas, the upper intertidal zone is highly elevated with broad and deep caves because of heavy wave action. The substratum type is varies at the Saurashtra coastline. 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. The intertidal belt is intersected by many tide pools. Since the pools are natural ones, the shape and size are not precisely same. The upper intertidal pools have light accumulation of sand settled over the rocky base.

### 3.4. Zonation

Intertidal zones are transitional coastal regions. The cycling of the tides influences these regions. These littoral areas are located between the high and low tide marks.
Zonal classification is applied to the intertidal area except that it is customary to use a different set of terms for habitats. The intertidal zone has a vertical zonation pattern. These zones are based on height and tidal influence. The rocky intertidal region can be divided into four vertical zones. These four zones include from the highest to the lowest; the splash zone, the high intertidal zone, the mid-intertidal zone and the low intertidal zone; according to their nature and termed as site respectively. Ecologically, the intertidal zone is a diverse community where organisms are divided by the vertical zonation of the tidal zones. Intertidal organisms convey zonation in relation to moving further up the intertidal, and therefore, into more exposed environments. Due to the different zonation patterns, it can harbour different types of organisms. It was the nature of the intertidal zone, which was mainly responsible for exclusion of the idea of studying zone wise distribution patterns of the animals.

3.5. Study Period

The study of the seawater quality was conducted during November 2007 to October 2008, after that, till October 2009 the study was made to the all sites at monthly interval with a purpose of obtained data that matched with the previous year. The quantitative assessment of intertidal macrofauna and flora were done from November 2007 to October 2009, while for the qualitative study of intertidal macrofauna were done from November 2008 to October 2009 at regular monthly intervals. The collected data were presented seasonally as the weather condition of this part of the peninsular India typically represents the periods of winter (December to February), summer (March to May), monsoon (June to August) and post-monsoon (September to November). During this study various anthropogenic disturbance on the coastal area were also note down simultaneously.

3.6. Methodological Approach and Study Groups

Study was intended to conduct the spatial as well as temporal variations of rocky intertidal macrofauna, seawater quality and anthropogenic impact along the Saurashtra coastline. In this regards, four study sites; from Saurashtra coastline were considered as various anthropogenic impacts with different magnitude of human disturbances as seawater quality. Community stress, if any, discriminated by population ecology, community structure and various statistical methods to the recognized anthropogenic
disturbances on intertidal macro-invertebrate assemblages and water quality from Saurashtra coastline, Arabian Sea.

The study focused on intertidal macrofaunal groups existing on rocky intertidal shores of selected locations. The study examined the differences in density, abundance, diversity, richness and evenness of all macro-invertebrate groups between four study localities. For the study of community structure and distribution of various macrofaunal groups on intertidal belt, six groups such as porifera, coelenterata, annelida, arthropoda, mollusca and echinodermata were recognized. These groups are significant on the coastal belt for the detailed study of coastal biodiversity. This investigation was undertaken due to the different anthropogenic pressures affects the intertidal community, which experienced by the coastal ecosystem.

3.7. Sampling Method for Macrofaunal Diversity

*For Quantitative analysis*

The intertidal zone of each sampling sites were surveyed regularly on monthly basis and all the macrofauna and flora encountered were recorded. 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 not at all disturbed and in some cases if disturbed, it was limited 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.
For Qualitative analysis

The structural attributes of the intertidal fauna were studied by transect method (Misra, 1968). Belt and Foot transect methods in were used for generating the data on the selected belt and criss-cross direction was followed to cover the maximum exposed area on the intertidal belt. The surveys were made at the lowest tides of the months. 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 4 hours. Quadrates of 0.25 m$^2$ were laid while following an oblique direction covering maximum area at almost regular occurrence. At least 10 quadrates were laid vertically across the complete intertidal area from upper littoral to lower littoral zone for recording the attributes.

3.8. Sampling Methods for Seawater

Seawater samples were collected at monthly intervals from all the selected sampling sites for the estimation of various physical, chemical and biological parameters, at least six samples of surface seawater during time of high tide were collected from different locations of the same coast. However, the locations for the collection of samples in a particular coast were fixed, which were fixed using global positioning system (GPS) model “Germin GPS-12”. Seawater samples were directly collected from surface into the clean acid-washed polyethylene bottles. Samples for dissolved oxygen and biochemical oxygen demand were collected in glass BOD bottles (300 ml capacity). The sample bottles of DO and BOD were kept in dark. Seawater samples for other selected physical and chemical parameters were stored in sterile polyethylene bottles. Seawater samples for biological parameters such as chlorophyll and phaeophytin were collected separately in dark bottles. The samples were transported to the laboratory in an ice box. In the laboratory, the samples were stored at 4$^\circ$C until analysis. To avoid possible contamination, all glassware and equipment used were acid-washed.

3.9. Methods for Seawater Analysis

The selected seawater quality parameters were analyzed according to standard methods of Trivedi and Goel (1986) APHA (1995), and Grasshoff et al. (1999). The seawater quality parameters taken, their unit of expression and methods are given below.
Temperature, pH and conductivity were measured immediately after collection of water sample with the help of the electronic thermometer, portable digital pH meter and conductivity meter respectively from surface and noted down the final reading. pH is the scale of acidity and alkalinity which defines the medium of samples. Portable pH meter was calibrated using standard pH buffer. On the other hand water is consists of various types of ions, these ions help in passing the electric current through water. Conductivity is defined as the measure of ability of aqueous medium to carry on electric current. Conductivity is totally dependent upon concentration of ions. The unit of measurement for temperature and conductivity are Celsius degrees (°C) and milisiemens/centimeter (ms/cm) respectively. Total solids and total dissolved solids were determined by the gravimetric method. Total solids represents a portion of water sample that are not lost even after evaporation of the unfiltered known volume of sample water, while total dissolved solids are residue left after evaporating the filtered sample through standard filter. Expression unit used for the solids is gram/liter. Nephelometer was used for the determination of turbidity into the seawater. Turbidity is a measure of water clarity. Turbidity measures the amount of light that is scattered or absorbed. The unit of expression is Nephelometric turbidity unit (NTU).

Salinity is formally define as the total amount of the dissolved inorganic solids in seawater, expressed as parts per thousand (ppt, ‰) by weight. Salinity in seawater was determined by titrimetric method, where in the chlorosity was first observed by titration of sample with silver nitrate solution.

Dissolved Oxygen (DO) content of the water samples were analyzed by Winkler’s method. The precipitate of manganese (II) hydroxide is dissolved by acidification, liberating the manganese (III) ions, which reacts with iodine ions, previously added to water sample together with potassium hydroxide. The iodine ions were titrated against sodium thiosulphate. The end point of the titration (blue → colorless) is indicated by using starch as an indicator.

Biochemical Oxygen Demand (BOD) was determined by the same procedure (Winkler’s method) as that for DO, after 5 days of incubation at 25°C in a BOD incubator. The difference in the amount of oxygen on the 1st and 5th day gave the measure of Biochemical oxygen demand.
Chemical Oxygen Demand (COD) was determined by the open reflux method. The sample was refluxed with K$_2$Cr$_2$O$_7$ and H$_2$SO$_4$ in presence of mercuric sulphate to neutralize the effect of chlorides and silver sulphate (catalyst). The excess of potassium dichromate was titrated against ferrous ammonium sulphate using ferroin as an indicator. The amount of K$_2$Cr$_2$O$_7$ used was proportional to the oxidizable organic matter present in sample.

Iodometric method was used for determination of sulphide. Sulphides are stripped from the acidified sample with an inert gas and collected in zinc acetate solution. Excess iodine to solution added to the zinc sulphide suspension reacts with the sulphide under acidic condition. Thiosulphate is used to measure unreacted iodine to indicate the quantity of iodine consumed by sulphide. The unit of expression for dissolved oxygen, biochemical oxygen demand, chemical oxygen demand and sulphide is milligram per liter (mg/l).

Sulphate was analyzed by turbidimetric method. Sulphate ion was precipitated in the form of barium sulphate by adding barium chloride in hydrochloric acid medium. The concentration of the sulphate can be determined from the absorbance of light at 420 nm by barium sulphate and then comparing it with a standard curve. It is represented in gram per liter.

Phosphate content in seawater was estimated by spectrophotometric method. It is represented in milligram per liter. The phosphates in water react with ammonium molybdate and form complex heteropoly acid, which gets reduced to a complex of blue color in the presence of SnCl$_2$. The absorption of light by this blue color can be measured at 690nm to calculate the concentration of phosphates compare with standard curve.

Titration method was used for the estimation of the ammonia, expressed by milligram per liter. Ammonia ion after distillation is dissolved in boric acid + mixed indictor and can be titrated with HCl. Boric acid is so weak an acid that it does not interfere with acidimetric titration.
For the analysis of calcium and magnesium in seawater titration method was followed. Indicator such as ammonium purpurate forms a complex with only calcium at high pH. As EDTA is having a higher affinity towards calcium; the former complex is broken down and a new complex of purple color is formed. Magnesium forms a complex of wine red colour with Eriochrome Black T at pH 10.0. The EDTA has got a stronger affinity for Ca$^{++}$ and Mg$^{++}$; the former complex is broken down and a new complex of blue colour is formed. The value of Mg$^{++}$ can be obtained by subtracting the value of calcium from the total of Ca$^{++}$ + Mg$^{++}$. Calcium and magnesium concentration in sample water is expressed through gram per liter.

Chlorophyll and phaeophytin were estimated by spectrophotometric method, expressed by milligram per cubic meter (mg/m$^3$). For estimation, 500 ml of water sample was filtered through glassfibre whatmann filter paper and extracted using 10 ml of 90% acetone overnight. The spectrophotometric determination of chlorophyll involves filtration, disruption of the cells, and extraction of the chlorophyll, followed by absorbance measurements. The optical density values were converted to chlorophyll $a$ and phaeophytin using the formulae and appropriate calibration factor.

3.10. Method for Anthropogenic Impact Analysis

This study was highlighted the major environmental factors and the importance of biological interactions in structuring shore communities. However, quantitative estimation of anthropogenic impact is remained a non-conclusive controversial one. Thus, in the present study the various anthropogenic influences on exposed shores and the structural role of macro-invertebrate on the shores were qualitatively 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. Including these specific activities furthermore all the stations are also focus point of human settlement very near about 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. Anthropogenic stress is the response of biological entity (individual, population, community etc.) to an anthropogenic disturbance. Stress at one level of organization (e.g. individual, population) may also have an impact on other level, for example, causing alterations in community structure. 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 seawater quality parameters were used for describe the coastal pollution and its effects on the intertidal community.

3.11. Data Analysis

*Biodiversity Indices*

As diversity indices are increasingly used to assess the health of the habitats, presently index of similarity (S), Shannon index of general diversity (H’), species richness indices (d), evenness indices (J’), measures were used to estimate the ecological status of the intertidal area of selected stations.

Sorensen’s index of similarity (Sorensen, 1948) has been used to compare the similarity in species composition of the benthic communities 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.

The most widely used measure of species diversity is the information theory indices. Shannon and Wiener (1963) independently derived functions, known as Shannon index of diversity. The Shannon-Wiener diversity index H’ combines both equitability and species richness by using the proportion of the total count of individuals (p) for each (or the i-th) species.
H' = - \sum \left( \frac{N_i}{N} \right) \log_e \left( \frac{N_i}{N} \right),

Where, the H' = diversity index, N_i = total number of individual species I, N = total number of individuals of all species in stand.

Species richness (d) refers simply to the number of species in a community. Species richness index d is based on the number of species present in a community (S) and incorporates the total number of individuals in the community (N). It is a measure of the number of species present for a given number of individuals. The species richness was calculated using Menhinick (1964).

\[ d = \frac{S}{\sqrt{N}} \]

Where, S = number of species, N = number of individual.

The evenness was calculated using Pielou’s measure. Pielou (1966) describes equitability or the evenness of the distribution of the numbers of individuals among species.

\[ J' = \frac{H'}{\ln S} \]

Where, H’= Shannon index, S = number of species.

**Population Ecology**

Among the ecological attributes, seasonal variations in the population density and abundance of major phylum 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 and total abundance values were calculated.

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

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

The collected monthly data were presented as seasonally for the seasonal approach like winter, summer, monsoon and post-monsoon. The obtained data were initially subjected to various descriptive statistical analyses like mean and standard deviation. The obtained data were further subjected to different statistical analyses for their cumulative acceptability (Sokal and Rohlf, 1969). Significance of spatial and temporal variations was compared by using single factor ANOVA. More advanced analyses like Regression and Correlation Coefficients analyses were also performed to find out relationship between various water quality parameters within a sampling site and to assess the influence of seawater quality parameters on the macrofaunal community structure (Southwood, 1978).