CHAPTER 3

METHODOLOGY

The spatio-temporal distribution of foraminifera assemblages of coastal plain, offshore, and estuary/lagoon sediments of southern Kerala, India has been addressed in this research work. In order to focus on the objectives of the research studies, the methodology of the work is defined with: record of field geological setting, sediment surface and core sample collection for analysis of physical features and associated foraminifera in five diversified environmental zones. The field sites selected for the study are; Ashtamudi estuary (A-15), Kayamkulam lagoon (K-1), Kayamkulam offshore (KO-5), Kollam coastal plain (BH-8) and Kollam offshore (QO-1). The standard procedure is followed for field observation and sampling process in the study area. The main objective in this work is foraminifera assemblage studies and its diversity, population to define the biozones, biotopes for discussion of the depositional environment and chronological status of the sediments and its validation with carbon isotope dates and to develop a linkage to understand the Ashtamudi estuary evolution, geologic history and possible pollution by anthropogenic system.

3.1 FIELD INVESTIGATION AND SEDIMENT SAMPLE COLLECTION

30 grab from the Ashtamudi estuary proper and 16 sediment core samples from the Ashtamudi estuary and its Kayal (08°56′32″N: 76°32′44″E) were collected using a Van-Veen grab sampler and shallow sediment corer.
The sediment core of 0.44m from Ashtamudi estuary (A-15) site at a water depth of 1.75 m was sub-sampled to 5cm interval and analyzed. The A-15 core sample is comprised of clay and siltyclay material.

Kayamkulam lagoon is located between the coordinates: 9°10'25"N and 76°23'17"E. The total length of the core is 0.65m and the depth of water column is 3m. This core sample is composed of clayey sand (0-5cm), sandy clay (5-10cm), siltyclay (10-30cm) and clay (30-65 cm).

Kayamkulam offshore site is located between 9° 09' 32" N and 76°25'27"E. The length of the offshore sample is 0.55m and the water depth is 10m. This core sample is comprised of clay (0-35cm) and siltyclay (35-55cm).

Coastal plain borehole (BH-8) located at the place of Kollam (08°52'52"N: 76°36'50"E). It is located on the bordering of Arabian Sea. Kollam is also called as Quilon. This district is covered by an Ashtamudi lake, mountains, lagoons, back waters and rivers. The top soils are removed and drilled up to 38 m every 1m sample was collected by a container. These sediment samples are composed of 3m sand, 32 m lateritic clay.

The sediment core of 0.85m of Kollam offshore samples (15-5m depth) collected for the present work (8° 57' 28" N and 76° 29' 12" E). The core samples are sub-sampled at every 5cm interval, these sub samples composed of siltyclay from top 5cm and 6 to 85cm composed of clay.

3.2 LABORATORY WORK

The collected sediment core was stored at core ice box. The sediment core was cut by using the core cutter and recorded the sediment color, grain size variation through the core length, macrofossils like Mollusca,
gastropod, and wood and peat fragments. The core sediment sub-sampled at every 5cm interval. Subsamples are labeled with study area codes and used for textural, sand-silt-clay contents, separation of foraminifera and heavy metal and carbon date studies.

3.3 FORAMINIFERAL SEPARATION METHOD

3.3.1 Sediment Processing Method

The collected samples were processed for the separation of foraminifera using standard microfossil separation method. The sediment is soaked in water for 6-10 hours. The consolidated sediment disintegrated through boiling process and washed through ASTM 230 sieve. Rinsed the sediment into filter paper placed within a funnel, allowed the sample to drain, and then air dried in safe place from contamination. Added 3% H₂O₂, washed sample over ASTM 230 mesh and dried in the oven at 45°C. The sample is then sprinkled sparsely across a picking tray and examined under a Leica binocular microscope.

3.3.2 Picking and Sorting of Foraminifera

The processed sample is examined in three fractions of 60, 100 and 200 mesh size sample. A24 chambered slides coated with tragacanth gum, needle is used to sort and separate foraminifera from the sediment matrix. The foraminifera are picked by wetting a 000 size paint brush in water and allowing the foraminifera to get attached to the brush. The foraminifera shells are transferred to one of the chambers in the slide and arranged genus and species wise for identification and quantitative studies (Figure 3.1).
3.3.3 Repository System and Identification Procedures

Illustration of the morphological features of separated foraminifera from the sediment samples were done by using Leica binocular microscope and SEM. The test, chamber shape, size and umbilical plug in planktic and benthic foraminifera, coiling pattern, number of whorls, aperture opening, suture, ornamentation and pores are observed to identify up to genus/species (Table 3.1) by referring to Loeblich & Tappan (1988). The chronostratigraphic biodatums were recognized using recent literatures. The taxonomic identifications of foraminifera are after Bolli et al (1985) and Berggren et al (1995). Benthic foraminiferal identifications are after Barker
(1960) and Loeblich & Tappan (1988). The 24 chambered slides containing the foraminifera tests are systematically catalogued and stored in a repository in the Department of Geology, Anna University, Chennai, India.

Table 3.1 Characteristic features for the recognition of species and genera

<table>
<thead>
<tr>
<th></th>
<th>Test composition</th>
<th>Pseudochitinous, agglutinated, siliceous, calcareous Hyaline, Calcareous Porcelaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>General shape of the test</td>
<td>Tabular, spherical, fusiform, globular, radiant etc.</td>
</tr>
<tr>
<td>3</td>
<td>Shape of the chamber</td>
<td>Spherical, ovate, cleavate, Flash shaped etc.</td>
</tr>
<tr>
<td>4</td>
<td>Arrangement of chambers</td>
<td>Uniserial, biserial, triserial etc.</td>
</tr>
<tr>
<td>5</td>
<td>Coiling</td>
<td>Planispiral, trochospiral steptospittra, sinistral/dextral</td>
</tr>
<tr>
<td>6</td>
<td>Number of whorls</td>
<td>1, 2, 3, 4 … evolute, involute</td>
</tr>
<tr>
<td>7</td>
<td>Number of chambers</td>
<td>In the entire test, in the last whorl</td>
</tr>
<tr>
<td>8</td>
<td>Suture</td>
<td>On the dorsal side, on the ventral side, limbate, elvate, depressed etc</td>
</tr>
<tr>
<td>9</td>
<td>Umbilicus</td>
<td>Open/Depressed in filled, limbate</td>
</tr>
<tr>
<td>10</td>
<td>Ornamentation</td>
<td>Open/depressed in filled, bosses, pillars, bullae etc</td>
</tr>
<tr>
<td>11</td>
<td>Wall Texture</td>
<td>Granular, radial, Porcelaneous</td>
</tr>
<tr>
<td>12</td>
<td>Pores</td>
<td>Coarsely Perforate, Finely perforate</td>
</tr>
<tr>
<td>13</td>
<td>Aperture</td>
<td>Primary/Single / Multiple / supplementary</td>
</tr>
<tr>
<td>14</td>
<td>Aperture face</td>
<td>Round, Ovate</td>
</tr>
</tbody>
</table>
3.3.5 **Taxonomy**

Taxonomic descriptions are appended in the order of taxonomical format by presentation of the Kingdom, Subkingdom, Phylum, Subphylum, Super class, Class, Order, Super family, Family, Subfamily, Genus and Species.

3.3.6 **Sand/Silt/Clay Ratio Analysis**

3.3.6.1 **Pipette method**

The size analysis of naturally occurring fine sediment obtained from wet sieving of sediment containing fines is satisfactorily achieved by the use of the pipette method. This technique relies on the fact that in a dilute suspension, particle settles through a column of water at velocities which are dependent upon their size. As particles decrease in sizes they become increasingly cohesive as surface ionic charges grow on relative significance (McManus 1988). To determine the size of the flocculated sediments, the Sodium hexa-meta-phosphate (NaPO$_3$)$_6$ is used as dispersing agent for sediment analysis.

The sediment samples are separated as sand (>63 m) and mud (<63 m) fractions through wet sieving with the help of 230 ASTM mesh. The sand fraction is dried and calculated separately as sand fraction. The mud fraction of each sample is collected in a 1 liter graduated measuring jar and filled with distilled water. The suspension in the measuring jar is then agitated using a stirring device in order to obtain a uniform distribution of particles. As soon as the agitation is stopped, the time is noted and after exactly 2 hours and 3 minutes, a 20 cc pipette is inserted to a depth of 10 cm in solution and from that level the sample is withdrawn with uniform suction. The pipette out sample is transferred to a 50 cc beaker and dried in an oven. Later, the sediment retained on the sieve is dried and weighed as the sand fraction. After
complete drying the weights of sand, silt and clay are converted into weight percentage and interpreted by plotting on a trilinear diagram after Trefethen (1950) (Figure 3.2).

3.3.6.2 Organic matter estimation

The selected core samples were oven dried at 50°C and finely powdered in agate mortar for the determination of organic carbon content. The organic carbon content of sediment samples were determined by using a modified Elwakeel and Riley method (Gaudette et al 1974) which is based on the exothermic heating and oxidation of organic matter. The principle behind the method is that the organic matter in the sample is oxidised by a known quantity of chromic acid and the amount of chromic acid used is determined by titration against ferrous ammonium sulphate solution.

Approx. 0.5 g of sample is weighed and treated with 10 ml of potassium dichromate solution and 20 ml of Conc. H$_2$SO$_4$ with Ag$_2$SO$_4$ which is mixed gently by rotating the flask for a minute. The mixture is kept for an hour and then diluted by adding 200 ml of distilled water. After acquiring normal conditions, 7 or 8 drops of ferroin indicator is added. The solution is titrated against the 0.5N Ferrous Ammonium Sulphate in a burette to a one drop end point (brilliant red wine). A standarisation blank without sediment is run with each new batch of samples. Organic matter is calculated by multiplying with a scale factor of 1.74.

3.3.6.3 Determination of calcium carbonate

The percentage of CaCO$_3$ in sediment samples was determined following the method of Hutchinson and Mcheman (1947). The principle is that, treating the sample with known amount of HCl acid and estimating the excess of HCl by back titration with standard NaOH using pethnolphthalein indicator.
A known weight (approx. 1g) is taken in an Ermelyer flask and 50 ml of 0.2 N HCl is poured in to it. It is mixed gently and kept for 1 hour. Exactly 25 ml dissolved carbonate solution is taken out using pipette and poured into another Ermelyer flask. Approximately 3 to 4 drops of phenolphthalein indicator is added and then finally titrated against 0.2 N NaOH solutions. The end point indicates light pink in colour.

Figure 3.2 Sand-silt-clay separation method
3.4 QUANTITATIVE ANALYSIS OF FORAMINIFERA POPULATION

3.4.1 Cluster Analysis

Clustering is the classification of objects into different groups, or more precisely the partitioning of a data set into subsets (clusters), so that the data in each subset (ideally) share some common trait—often proximity according to some defined distance measure. Data clustering is a common technique for statistical data analysis, which is used in many fields. The diagrammatic representation of this approach is dendrogram, which shows the relative size of the proximity coefficients at which cases were combined. Trees are usually depicted horizontally, not vertically, with each row representing a case on the Y axis, X axis is a rescaled version of the proximity coefficients. Case with low distance/high similarity is close together. Cases showing low distance are close, with a line linking them a short distance from the left of the dendrogram, indicating that they are agglomerated into a cluster at a low distance coefficient, indicating similarity. When, on the other hand, the linking line is to the right of the dendrogram. The linkage occurs at a high distance coefficient indicating the cases/clusters were agglomerated, even though much less alike (Zumlot 2006).

These are based on mathematical manipulation of the data and provide a means of confirming the trends noted qualitatively by foraminifers, as well as allowing their numerical importance to be judged. The quantification of the data and numerical basis of the results allow to be directly correlated with other data to determine to understand the physical, chemical and ecological parameters role in environment studies with time and space. Variable points will show the correlation between the variables similarly, clusters of sample points will be interpreted as the result of the faunal species of similar adaptability belonging to a specific group.
The present study attempts to quantitatively analyze the relationship of recent benthic foraminifera in the estuarine/lagoon, coastal plain, offshore sediments with different parameters of the Kollam, Kayamkulam and Ashtamudi estuary. The multivariate study has been inspired from the results of the faunal abundance data from the area. The faunal abundance data has been iterated statistically using Q-mode Cluster analysis. The purpose of cluster analysis is to show the inter-relationship within the similarity coefficient matrix. This may be accomplished in its simplest form by arranging the variables in a hierarchical dendrogram in which the different foraminifera species–are clustered to understand their interrelationships, as contained in the matrix of coefficients, are shown with greatest simplicity. Ideally the relationship is such that they can be completely represented by a dendritic network that is completely contained in two dimensions.

### 3.4.2 Principal Component Analysis

Principal Component Analysis (PCA) is a technique used to reduce multidimensional data sets to lower dimensions for analysis. The applications include exploratory data analysis for generating predictive models. PCA involves the computation of the eigen value decomposition. The results of a PCA are usually discussed in terms of scores and loadings. Each principal component is calculated by taking a linear combination of an eigen vector of the correlation matrix with a standardized original variable. The eigen values show the variance of each component. The set of principal components has the same total variation and structure as the original variables. Rotated components are a set of the larger principal components that have been transformed (rotated) so that the components tend to point in directions in common with sets of variables. This is done in a quest for interpreting “factors” in the data that is represented by several variables. Directions for
variables that are opposite are considered close as well as those for directions that are the same (Zumlot 2006).

3.4.3 Factor Analysis

A factor analysis is a data reduction technique to summarize a number of original variables into a smaller set of composite dimensions, or factors. It is an important step in scale development and can be used to demonstrate construct validity of scale items. We will then move onto cluster analysis techniques. Cluster analysis groups individuals or objects into clusters so that objects in the same cluster are homogeneous and there is heterogeneity across clusters. This technique is often used to segment the data into similar, natural, groupings.

3.4.4 Correlation Matrix

In probability theory, statistics and correlation, (also called correlation coefficient) indicates the strength and direction of a linear relationship between two random variables. In addition, correlation refers to the departure of two variables from independence, measuring the degree of correlation, adapted to the nature of data from different stations. Performed by using the computer software package SPSS 13 on each data set to determine if statistically significant differences existed between sampling sites (Zumlot 2006).

3.5 SCANNING ELECTRON MICROSCOPE ANALYSIS

SEM photographs of the foraminifer that have identified from the studied samples have been taken by Scanning Electron Microscope (S-3400N). The selected foraminifera specimens were placed in a stud (35 species in each stud). The stub is then placed in the SEM instrument, which has been vacuumed. Electron is transmitted to the stub with foraminifer
samples and the image recorded on the screen. The magnification and resolution for the selected image is documented for foraminifera morphological studies. The SEM instrument used in this study is S–3400N with resolution of 4.0 nm (30KV), magnification 5X to 300,000X, accelerating voltage (0.3-30kV, maximum specimen size200mm in diameter, specimen stage type II -X : 0– 100mm, Y: 0 – 50mm, Z : 5– 65mm, rotation : 360°, tilt : -20° - +90°, maximum Specimen Height: 80mm at WD = 10mm. Scanning electron microscope images are used to identify the foraminifera species based on its morphological characteristics; such as shape of the test, chamber, coiling pattern, whorls, aperture, suture pattern, umbilicus, ornamentation, and pores.

3.6 HEAVY METAL ANALYSIS

Total metals (Cd++, Cr³⁺, Cu⁺⁺, Mn⁺⁺, Ni⁺⁺, Pb⁺⁺, Fe³⁺ and Zn⁺⁺ were determined by Atomic Absorption Spectrophotometer technique after acid digestion. Samples were allowed to dry at 40°C in an electrical oven for a minimum of 48 hours in coated metal pans to simulate air-drying. Once dry, the sediment was ground into a fine powder using agate mortar and pestle and using 2mm plastic sieve. The sieved material was then placed in sterilized plastic bags and labeled for storage at room temperature.

For digestion, 1 g of dried sample was put into a PTFE vessel with 2 ml of Perchloric acid (HClO₄) and l0 ml of hydrofluoric acid (HF) to near dryness, subsequently a second addition of 1 ml HClO₄ and 10 ml HF was made and again the mixture was evaporated to near dryness. Finally, 1 ml of HClO₄ alone was added and the sample was evaporated until the appearance of fumes. For each digestion, a blank was prepared with the same amount of acids. After digestion and cooling below extractor hood, samples were filtered through a Whitman No.42 filter paper and diluted to 100 ml with distilled water and analyzed (Tessier et al 1979).
3.7 CARBON DATING TECHNIQUE

The process involved several steps. The combustion of the cleaned and pre-treated (using 10% HCl) sediment sample was carried out in the quartz electrical furnace in the presence of clean and dry oxygen flow at 750 to 900°C. To convert carbon monoxide, if any was left over, to the carbon dioxide, this was followed by allowing the reaction with copper oxide. The sample carbon dioxide is may be containing several impurities like sulfur and halides in addition to moisture. Therefore multi-stage cleaning using acidified potassium permanganate, potassium dichromate, silver chloride in definite concentrations and moisture absorbers like cooled silica gel and molecular sieve was carried out. The cleaned and dried carbon dioxide was collected using liquid nitrogen. It was freed from oxygen followed by formation of lithium carbide in the presence of catalyst (molten lithium) and slow hydrolysis to obtain acetylene. In the presence of a catalyst, the acetylene was trimerised to obtain benzene. The benzene was obtained by heating the catalyst at 110 degree C and collecting in a liquid nitrogen trap. It was placed in a vial and stored in deep freeze and allowed to cool to get rid of low half-life radioactivity (such as radon) which might disturb the counts. After careful weighing of sample benzene, a calculated amount of scintillator (PBD Butylepurum) was added to benzene vial and the vial was placed in the counter Quantulus 1220 for 25 cycles of counting. Appropriate SQP correction was applied to estimate the radiocarbon amount and ages calculated using the standard formula based on radioactive decay of radiocarbon. In calculating age, the half-life used was 5530 years as per tradition though the accepted present value is 5730 years. In order to correct for variation in the radiocarbon production rate in the atmosphere during the sample history, calibration was carried out. The obtained radiocarbon dates were calibrated using IntCal09 software of the Washington University.