CHAPTER III
MATERIALS AND METHODS

3.1. Introduction

Aquatic sediments are principally derived from weathering processes, with major transportation from terrestrial sources under high runoff. Land use, geology and topography of the catchment determine the erosion and transport processes. In addition, discharges from urban, industrial and mining activities are potential sources of particulates. Anthropogenic contaminants, including metals, organics and nutrient elements are associated with particulate and dissolved inputs to natural waters. Typically, sediments are characterised as coarse material, clay/silt and sand fractions, higher fractions may consist of shells, rocks, wood and other detritus materials and are usually not a source of bio available contaminants\textsuperscript{135}. Sediment is transported as suspended load, bed load and dissolved load. As earth material, sediment is obviously an important material affecting the physical, chemical and biological conditions of the environment. Sediments act as potential sink for many hazardous chemicals, which once adsorbed or incorporated into the particulate matter become deposited on the bottom, from which the solid-liquid equilibrium generally results in an enrichment of toxic elements and compounds in interstitial waters\textsuperscript{136,137}. Sediment quality assessment is considerably more complex than water quality assessment because of the site-specific parameters, interacting hydro mechanical, biological and geochemical processes, which are not a factor for water quality assessment\textsuperscript{138}. The transport properties and various chemical properties of the sediment are strongly related to the sediment mineralogy and particle size, which are influenced by various factors.
including the source of sediment and the flow rate in the sediment. There is little export of fluvial material in estuaries, but on the other hand most of the materials delivered by rivers to the ocean are trapped in estuaries. Estuaries are the regions where fine sediments accumulate and for this reason, most of these tend to be muddy. The biogeochemistry of estuaries is strongly influenced by temporally variable events occurring at their riverine and marine environments, as well as those impinging on the estuarine water column and sediments. Estuarine responses to temporal variability are complex and often difficult to understand. As mentioned above, estuarine bed sediments act as a net trap for sediments and associated trace metals from land-based sources over the long term. However, over the short term, freshwater and saltwater intrusion during flood tides dramatically influence the master variables of the water column, such as suspended particulate matter, redox potential and sediment dynamics. Sediments conserve important environmental information and are increasingly recognized as both a carrier and a possible source of contaminants in aquatic systems. Estuaries are particularly vulnerable to input changes because of the limited water exchange and sediments are effectively trapped by estuarine circulation processes, but may be eroded, transported and deposited many times before they accumulate below the actively reworked surface layer. Mineralogical studies of the estuarine sediments help to model the sources, transport paths and depositional sites of argillaceous sediments. According to Kemp et al., the data on sediments are subdivided into five major groups as major elements, carbonate elements, nutrient elements, mobile elements and trace elements. Major elements (Si, Al, Ti, K, Na, Mg and Cl) are conservative and make up the bulk (about 70%) of the sediment matrix.
and are unlikely to be affected by diagenesis or eutrophication. In fact, they reflect the major mineralogical species derived from terrigenous sources. Carbonate elements (Ca, Mg and \( \text{CO}_3^2- \)) are the second largest group in sediments, constituting about 15% of the weight. Organic carbon, nitrogen and phosphorus are the nutrient elements essential to the biota, accounting approximately 5% of the sediment composition. Iron, sulphur and manganese are the mobile elements and make up about 10% of the total sediment weight. Mobile elements can easily change redox status and solubility, hence precipitating or dissolving when the physico-chemical conditions at the water-sediment interface change. Trace metals (Cd, Pb, Cu, Cr, Co, Ni, Hg, Zn) are the smallest group constituting less than 0.1% of the sediments. But they are the active agents in the geochemical processes.

This chapter describes the variability of the physico-chemical parameters in the sediments of the study region along with their textural characteristics. Since sediments are highly complex mixture of discrete minerals and organic compounds to which a number of ions are differently attached, their complete analysis is impractical.

A brief outline of the various methods employed in the collection, processing and analysis of sediment samples used for this investigation are given in this chapter. Various procedures adopted for the present study can be broadly be grouped into three a) sampling and storage b) analytical methods and c) computation and compilation of results.

3.2. Sampling and Storage

Location map of Manakudy estuary (Fig.1.2) indicates the area of investigation and location of sampling stations (1-10) in Manakudy estuary. Similarly
location map of Thengapattinam estuary (Fig. 1.3) indicates the area of investigation and location of sampling stations (1-10) in Thengapattinam estuary. The stations were so fixed as to give a fairly good coverage of the prevailing complex environmental condition. The sampling stations were selected based on the criteria that they were near the urban and domestic effluent discharge points, agricultural dewatering areas, places of tourism activities, industrial discharge points and estuarine region. A detailed fieldwork has been carried out in the Manakudy and Thengapattinam estuaries for the collection of primary and secondary data (from the field and various offices) as well as sediment samples for laboratory investigation. This information was updated using necessary field checks / verifications as and when required.

A total of 20 sediment samples were collected systematically from the Manakudy (10 samples) and Thengapattinam (10 samples) estuaries for detailed textural and physico-chemical studies (Fig. 1.2 & 1.3). A hand operated Van Veen grab was used to collect sediments from the two estuaries. All samples were carefully transferred to neatly labelled polythene bags and preserved for further analysis. All the samples were brought to laboratory and analysed for various parameters for achieving the objectives of the study. A portion of the sediment samples was air-dried for chemical analysis. Utmost care was taken to avoid contamination of samples during collection and handling. All the reagents used were analytical grade and deionised water was used for the preparation of standard and reagent solutions.

3.3. Analytical Methods

3.3.1. Sediment Texture

The air dried samples were subjected to textural study by wet sieving and International pipette method\textsuperscript{157,158}. Known weight of sediment sample was dispersed
overnight in distilled water by adding 10 ml of 10% solution of sodium hexametaphosphate. Sediment fraction coarser than 63 μ was separated by wet sieving. The sieve relents were dried and weighed. The suspension passing through the sieve (230 mesh) was transferred into a measuring jar of 1000 ml capacity and distilled water was added to bring the volume exactly to 1000 ml. The suspension was well stirred for about a minute to obtain homogeneity and then left undisturbed. 20 ml aliquot was withdrawn from the suspension using a pipette from a specific depth after the lapse of time duration given for the desired fraction at the room temperature. The aliquot was then transferred into a previously weighed 50 ml beaker. It was dried in an air oven at 60°C and weighed after cooling in a desiccator. The dried aliquot weight was multiplied by a factor of 50 and the weight of sodium hexametaphosphate added was then subtracted. Weight of sand-silt-clay fractions were calculated from the above results and converted into percentages. Triangular textural diagram was used to describe the sediment texture\textsuperscript{159}.

3.3.2. Physico-chemical Characteristics

pH of the sediment (1: 2.5 sediment-water suspension) was measured by a pH meter. Soil pH was determined as per the standard procedure\textsuperscript{160}. Electrical conductivity (EC) was determined by conductivity meter using the method adopted by Clarson\textsuperscript{161}. Redox potential (Eh) of the sediment samples was determined using a platinum electrode attached to pH - Eh meter after proper calibration. Total soluble salts (TSS) was determined using 1: 2.5 sediment: water solution. Moisture content of the sediment was estimated by oven drying the sediment at 105°C for 24 hours. Bulk density and Particle density were determined by the standard procedure followed by
Blake and Hartge\textsuperscript{162}. Cation exchange capacity (CEC) was determined by modified Perkin’s method \textsuperscript{163,164}.

### 3.3.2.1. Nutrient elements (Organic carbon, Total Nitrogen, Phosphate-Phosphorus)

#### Organic carbon:

The sediment organic carbon was determined by wet oxidation method\textsuperscript{165}. Organic matter was oxidized by a known quantity of chromic acid and the amount of chromic acid used was then determined by back titration with standard ferrous ammonium sulphate solution. Diphenylamine was used as indicator.

#### Total Nitrogen:

The total nitrogen was determined by Kjeldahl method\textsuperscript{166}. The nitrogen content of sample was converted to acid ammonium sulphate by digestion with H\textsubscript{2}SO\textsubscript{4} in the presence of a catalyst. On making the reaction mixture alkaline, NH\textsubscript{3} was liberated, which was removed by steam distillation and absorbed in boric acid solution containing methyl-red-methylene -blue indicator and titrated with standard HCl (0.01 N HCl).

#### Phosphate-Phosphorus:

Phosphorus as phosphate (P\textsubscript{04} - P) form was determined colorimetrically based on the reaction of the ions with an acidified molybdate reagent to yield a phosphomolybdate complex\textsuperscript{167}. It was then reduced to a highly blue coloured compound. The intensity of the colour developed is proportional to the concentration of the P\textsubscript{04} - P in the solution. This blue coloured solution exhibits maximum
absorption at 880 nm. The amount of P0.- P was determined by comparison with a set of standard samples.

3.3.2.2. Major elements (Sodium, potassium and Chloride)  

Sodium and potassium:  

Sodium and Potassium were determined using a flame photometer based on the procedure as described in APHA\textsuperscript{168}. The solution was aspirated for the estimation of Sodium and Potassium. Calibration curves for Sodium and Potassium were drawn separately and concentrations of the metals were estimated.

Chloride:  

Chloride content was estimated by the Mohr method of titration using standard silver nitrate solution and potassium chromate indicator\textsuperscript{169}.

3.3.2.3. Carbonate elements (Calcium and Magnesium)  

Calcium and Magnesium in the sediment sample solution were determined titrimetrically with standard EDTA solution by Versanate method\textsuperscript{161}. Calcium forms a stable complex with EDTA at pH 12. Calcium in solution can be titrated with 0.01 M EDTA using murexide indicator in presence of sodium hydroxide solution. The end point is the change of colour from orange red to purple. Magnesium in solution can be titrated with 0.01M EDTA using Eriochrome black T-dye as indicator at pH 10 in the presence of ammonium chloride and ammonium hydroxide buffer. The end point is the change of colour from wine red to blue. From the titre values calcium and magnesium content were calculated.
For assessing sea water intrusion into coastal aquifer, the ratio $\text{Ca}^{2+}/\text{Mg}^{2+}$ can be used\textsuperscript{170}. Since the concentration of Mg present in sea water is much greater than calcium, $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio is recommended as a parameter for determining the salt water contamination. If the ratio is less than 1, then the area is considered to be highly affected by salt water intrusion.

3.3.2.4. Mobile elements (Iron, Manganese and Sulphur)

**Iron and Manganese** were analyzed using an Atomic Absorption Spectrophotometer (Perkin Elmer Model 2380) following the method suggested by Rantala and Loring\textsuperscript{171}.

**Sulphur** was determined as sulphate gravimetrically using Barium chloride in HCl medium\textsuperscript{161}.

3.3.2.5. Trace metals (Cd, Pb, Cu, Cr and Zn)

Trace metals (Cd, Pb, Cu, Cr and Zn) in the sediment samples were analyzed using an Atomic Absorption Spectrophotometer (Perkin Elmer Model 2380) following the method suggested by Rantala and Loring\textsuperscript{171}. The digested samples were directly aspirated into the flame (Air-Acetylene fuel mixture). Using the absorption mode, the concentration corresponding to the absorption in the digest was determined.

**Precision and accuracy:** The precision and accuracy of the AAS analysis were checked against two USGS standard rock samples G 2 and W1 to find their agreement with the published values of Rantala and Loring\textsuperscript{171} and Flanagan\textsuperscript{172}. 
3.4. Computation and Compilation of results

The voluminous data generated in this study has been processed using various statistical procedures / computational techniques. The results obtained were evaluated in the light of available published research works in India and abroad for drawing valuable conclusions.

3.4.1. Inter-relationship between various parameters

Inter-relationship between various parameters, between stations and seasons were studied using statistical methods. Following analyses were carried out on non transformed data:

i) Karl Pearson's coefficient of correlation was adopted to analyse and establish inter-metal relationship and physico-chemical characteristics of the estuarine sediments and tested using 't' statistics.

ii) Observations recorded for all the parameters were statistically scrutinized to test the significance of difference between stations and seasons and their interaction effects by the procedures suggested by Gomez and Gomez\textsuperscript{173} and Sokal and Rohlf\textsuperscript{174} by the two way ANOVA. ANOVA was employed to determine whether groups of variables have the same means on data that are continuous or normally distributed and with homogeneous variance. Additionally, it was employed to assess the relationship between heavy metal concentrations and their elemental interaction between stations of the estuaries and seasons. Wherever the results were found to be significant, critical difference were worked out at 5% level (p=0.05) and 1% level (p=0.01).
3.4.2. Assessment on Sediment contamination

Sediment analyses, which normally do not furnish quantitative data on the absolute degree of pollution, however, indicate relative factors of enrichment and source of pollution in the aquatic environment. Metal pollution in estuarine environment is usually caused by land run-off, mining activities, dredging activities and anthropogenic inputs. Traces of heavy metals such as Pb, Mn, Fe, Cr etc. have been identified as deleterious to aquatic ecosystem and human health. The mechanism of accumulation of pollutants in the sediments is controlled strongly by the nature of the substrate as well as the physico-chemical conditions causing dissolution and precipitation. In the present study, an attempt has been made to get an overall idea about the extent of heavy metal pollution in the Manakudy and Thengapattinam estuaries by comparing the metal concentrations with TEC/PEC guidelines, with the sediment quality guideline proposed by USEPA/WHO and by using statistical tools like Geoaccumulation Index ($I_{geo}$), Contamination Factor ($C_f$), Degree of contamination ($C_d$), Modified degree of contamination ($mC_d$), Enrichment Factor (EF) and Pollution Load Index (PLI).

3.4.2.1. TEC/PEC guidelines

Numerical sediment quality guidelines (SQGs) can be used for assessing the degree to which the pollutants can adversely affect the aquatic organisms. The metal concentrations were thus compared with TEC/PEC guidelines. The concentration of heavy metals in sediments can be a secondary source of water pollution, once environmental condition is changed. Therefore, an assessment of heavy metal contamination in sediments is an indispensable tool to
assess the risk of an aquatic environment. To assess metal concentrations in sediment, Numerical Sediment Quality Guidelines (SQGs) were applied. SQGs include a threshold effect concentration (TEC) and a probable effect concentration (PEC). If the metals in sediments are below the TEC, harmful effects are unlikely to be observed. If the metals are above the PEC, harmful effects are likely to be observed.\textsuperscript{179}

3.4.2.2. United States Environment Protection Agency (USEPA) and World Health Organisation (WHO)

The chemical contamination in the sediments was evaluated by comparison with the sediment quality guideline proposed by USEPA/WHO.

3.4.2.3. Geoaccumulation Index ($I_{geo}$)

Geo-accumulation index introduced by Muller\textsuperscript{180} was used to assess the degree of metal pollution in aquatic sediment studies.\textsuperscript{18, 181, 182} $I_{geo}$ was used to determine metal contamination in sediments, by comparing current concentrations with pre-industrial levels and can be calculated by the following equation.\textsuperscript{183}

$$I_{geo} = \log_2 \left( \frac{C_n}{1.5B_n} \right)$$

Where, $C_n$ is the measured concentration of a heavy metal in sediments, $B_n$ is the geochemical background value in average shale of element n and 1.5 is the background matrix correction due to terrigenous effects. The geo-accumulation index classification consists of seven classes (0-6), ranging from unpolluted to extremely polluted: $\leq 0$ (class 0) unpolluted, 0-1 (class 1) unpolluted to moderately polluted, 1-2 (class 2) moderately polluted, 2-3 (class 3) moderately to strongly polluted, 3-4 (class 4) strongly polluted, 4-5 (class 5) strongly to extremely polluted, 5-6 (class 6) extremely polluted.\textsuperscript{180}
3.4.2.4. Contamination factor (C_f), Degree of contamination (C_d) and modified degree of contamination (mC_d)

Contamination Factor (C_f) analysis is another important tool for the assessment of heavy metal pollution in estuarine study. For C_f computation, the values like Fe, Mn, Cd, Pb, Cu, Cr and Zn are normalized using the corresponding average metal values of shale reported by Turekian and Wedepohl\textsuperscript{184}. This is because, the world shale average is considered as background values. The Contamination factor (C_f) was evaluated using the equation:

\[
C_f = \frac{\text{Metal concentration in polluted sediment}}{\text{Background value (shale) of the metal}}
\]

C_f < 1 refers to low contamination, 1 \( \leq \) C_f < 3 means moderate contamination, 3 \( \leq \) C_f < 6 indicates considerable contamination and C_f \( \geq \) 6 indicates very high contamination\textsuperscript{185}.

Hakanson\textsuperscript{185} analysed seven specific heavy metals (As, Cd, Cu, Cr, Hg, Pb, Zn) and the organic pollutant PCB and thus considers eight possible measures of contamination. Hakanson's study also proposed that the numeric sum of the eight specific contamination factors expressed the overall degree of sediment contamination (C_d) using the following formula:

\[
C_d = \sum_{i=1}^{n} C_{fi}
\]

The calculated C_d is therefore defined as the sum of the C_f for the eight pollutant species specified by Hakanson\textsuperscript{185}. The C_d is aimed at providing a measure of the degree of overall contamination in surface layers in a particular core or sampling site. However the Hakanson\textsuperscript{185} classification terminologies and calculation formula is restricted to the seven metals plus PCB specified in Hakanson's study. Furthermore all
eight species must be analysed in order to calculate the correct $C_d$ for the range of classes defined by Hakanson$^{185}$.

As a result of the above limitations, Abrahim$^{186}$ presented a modified and generalised form of the Hakanson$^{185}$ equation for the calculation of the overall degree of contamination at a given sampling or coring site as follows. The modified formula is generalised by defining the degree of contamination ($mC_d$) as the sum of all the contamination factors ($C_f$) for a given set of estuarine pollutants divided by the number of analysed pollutants. The modified equation for a generalised approach to calculate the degree of contamination is given below:

$$mC_d = \frac{\sum_{i=1}^{n} C_f^i}{n}$$

where $n = \text{number of analysed elements}$ and $i = i^{th}$ element (or pollutant) and $C_f = \text{Contamination factor}$. Using this generalised formula, $mC_d$ allows the incorporation of as many metals as the study may analyse with no upper limit. For the classification and description of the modified degree of contamination ($mC_d$) in estuarine sediments the following gradations are proposed: $mC_d < 1.5$ to Nil $\text{Very low degree of contamination}$, $1.5 \leq mC_d < 2$ $\text{Low degree of contamination}$, $2 \leq mC_d < 4$ $\text{Moderate degree of contamination}$, $4 \leq mC_d < 8$ $\text{High degree of contamination}$, $8 \leq mC_d < 16$ $\text{Very high degree of contamination}$, $16 \leq mC_d < 32$ $\text{Extremely high degree of contamination}$, $mC_d \geq 32$ $\text{Ultra high degree of contamination}$. 
3.4.2.5. Enrichment Factor (EF)

EF as proposed by Simex and Helz\textsuperscript{187} was employed to assess the degree of contamination and to understand the distribution of the elements of anthropogenic origin from sites by individual elements in sediments. Fe was chosen as the normalizing element while determining EF values, since in wetlands it is mainly supplied from sediments and is one of the widely used reference element\textsuperscript{182, 188, 189, 190}. Other widely used reference metal elements include Al and Mn\textsuperscript{191, 192, 193}.

\textbf{Enrichment factor} = \frac{(C_n/Fe) \text{ sample}}{(C_n/Fe) \text{ background}}

where, $C_n$ is the concentration of element “n”. The background value is that of average shale\textsuperscript{180}. An element qualifies as a reference one if it is of low occurrence variability and is present in the environment in trace amounts\textsuperscript{188}. Elements which are naturally derived have an EF value of nearly unity, while elements of anthropogenic origin have EF values of several orders of magnitude\textsuperscript{133}. A value of unity denotes no enrichment or depletion of elements relative to earth's crust. Six categories are recognized: $< 1$ background concentration, 1-2 depletion to minimal enrichment, 2−5 moderate enrichment, 5−20 significant enrichment, 20−40 very high enrichment and $> 40$ extremely high enrichment\textsuperscript{194}.

EF values greater than 1.5 have such heavy metals derived from other sources suggesting environmental contamination by those particular heavy metals\textsuperscript{131}. It is presumed that high EF values indicates an anthropogenic source of trace metals mainly from activities such as industrialization, deposition of industrial wastes etc.\textsuperscript{131}. Since the bioavailability and toxicity of any heavy metal in sediment depend on chemical form and concentration of the metal, it can be inferred that trace metals in
sediment samples with high EF values, along with higher labile fractions in sediments are potential sources for mobility and bioavailability in the aquatic ecosystems. EF values indicate that Fe, Zn, Cd and Ni concentrations need to be monitored to avoid potential pollution risk in the future.

3.4.2.6. Pollution Load Index (PLI)

Pollution load index for each site or sample was evaluated as indicated by Tomlinson et al.\textsuperscript{195}.

\[ \text{Pollution load index} = (C_f_1 \times C_f_2 \times \ldots \times C_f_n)^{1/n} \]

Where, \( n \) is the number of metals and \( C_f \) is the contamination factor. The PLI value > 1 indicates pollution whereas PLI value < 1 indicates no pollution\textsuperscript{182, 190}. This empirical index provides a simple, comparative means for assessing the level of heavy metal pollution. A value of zero indicates perfection, a value of one indicates only baseline levels of pollutants present and values above one would indicate progressive deterioration of the site and estuarine quality\textsuperscript{195}.

3.5. Software Used

Microsoft Word and Excel (MS Office version 2007) were used for writing thesis and data processing [(eg. ANOVA, Pearson's correlation analysis). Adobe Photoshop (version 7.0.1) and Microsoft Paint were used to edit and prepare different figures.