CHAPTER-3

MATERIALS AND METHODS
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3.1 FIELD METHODS
Estuary and river are system having various dynamic physiochemical and biological processes taking place in them. Estuaries are the places where fresh-waters from the rivers meet the saline waters form the ocean. The suspended and dissolved materials derived from physical and chemicals weathering of rocks in the catchment with anthropogenic pollutants reaches the system and undergo a variety of physio-chemical and biological changes. Systematic sampling of water, bed sediments and core sediments were done in Zuari, Mandovi and Kalinadi and their estuaries.

The sampling locations were chosen carefully in order to get maximum representation of the diverse environments. The water samples were collected in three seasons namely Post-Monsoon (January-February, 2000), Monsoon (August, 2000) and Post-Monsoon (March-April, 2001). The sampling locations (Table - 3.1) are shown in Figure 3.1. The bed sediment sampling was done in Post Monsoon and in some locations in Pre-Monsoon.

3.1.1 WATER SAMPLES
Water samples were collected from about 10 cm below the surface in cleaned polypropylene bottles. Bottles were completely filled with water sample and capped airtight. For cation analysis, 50 ml of sample was filtered with 0.22μ filter paper and preserved on site with ultra pure HNO₃. These water samples were brought to laboratory for various physico-chemical analysis and stored at 4°C.

3.1.2 BED SEDIMENT SAMPLES
Bed sediment samples were collected from the sampling locations by scooping the top 5-10 cm layer using a small plastic scoop along the riverbed, at sediment water interface. The bed sediment samples were then transferred to a polyethylene bag, which was sealed to air by fastening the mouth immediately. These samples were brought to the laboratory for studying its physico-chemical properties and mineralogy.

3.1.3 CORE SEDIMENT SAMPLES
Core sediment samples were obtained by drilling in 6.25cm diameter PVC pipe into the sediment at certain locations in the river and the estuary region and pulling out the pipe with care as to not to disturb the sediment profile in the core. The pipe was sealed from both the ends and marked
Table 3.1 (a): Sampling Location in Mandovi River.

<table>
<thead>
<tr>
<th>L.No.</th>
<th>Location</th>
<th>Water Samples</th>
<th>Sediment</th>
<th>Core</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre Monsoon Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Sancordem</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Surdai</td>
<td>- ✓ -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Khataki</td>
<td>✓ - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Codli</td>
<td>- ✓ ✓</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Khadapar</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>6</td>
<td>Kothambi</td>
<td>✓ ✓ -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Pombarpar</td>
<td>- - ✓</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Banastari</td>
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<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Old Goa</td>
<td>✓ ✓ -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Rabinder Porto</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Mandovi Bridge</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Municipal Market</td>
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<td>✓</td>
<td>✓</td>
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Table 3.1 (b): Sampling Location on Zuari River.

<table>
<thead>
<tr>
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<th>Sediment</th>
<th>Core</th>
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<tr>
<td></td>
<td></td>
<td>Pre Monsoon Post</td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>Sangum</td>
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<td>-</td>
<td>-</td>
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<td>14</td>
<td>Savardem</td>
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<td>✓</td>
<td>-</td>
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<td>Qupem</td>
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<td>✓</td>
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<td>16</td>
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<td>17</td>
<td>Curtalim</td>
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<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>St. J. Church Island</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>Dona Paula</td>
<td>✓ ✓ -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>Vasco</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
<td>✓</td>
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Table 3.1 (c): Sampling Location on Kalinadi.

<table>
<thead>
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<th>L.No.</th>
<th>Location</th>
<th>Water Samples</th>
<th>Sediment</th>
<th>Core</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>Pre Monsoon Post</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Ganeshgudi</td>
<td>- ✓ ✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>Konkarn Bridge</td>
<td>✓ - ✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>Midstream</td>
<td>✓ - ✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>Kodibag</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>25</td>
<td>Other End</td>
<td>- - ✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>Davbagh</td>
<td>- - ✓</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>Karwar Bay</td>
<td>✓ ✓ ✓</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Pre = Pre-monsoon; Post = Post-monsoon
Figure 3.1a - Sampling locations on Mandovi and Zuari rivers.
Figure 3.1b - Sampling locations in Kalinadi Estuary.
appropriately and brought to the laboratory for further analysis.

3.2 LABORATORY METHODS

3.2.1 SUSPENDED MATTER SEPARATION
Water sample bottles were shaken vigorously to make a homogeneous suspension. Known volume of this suspension was transferred immediately to the filtration unit. Sediments were collected on pre-weighed Millipore, 0.45μ filter paper, which was dried to constant weight at 80°C. Then weight of total suspended matter (TSM) was calculated. The filtered water was used for analyzing anions and silica content.

3.2.2 WATER ANALYSIS

pH and Conductivity
The pH and conductivity were measured in unfiltered water samples. “Rachho” (Model No. 123) pH meter was used to measure the pH. The glass electrode was conditioned and calibrated with buffer solutions of pH 4, 7 and 9. The temperature of the instrument was set according to the temperature of the samples. The samples were stirred continuously to maintain homogeneity before noting the pH. Conductivity was measured in mS/cm using Systronics Conductivity Meter 306. The instrument was calibrated with 0.01 M KCl standard (1413 in mhos/cm at 25°C). The results are reported in mS/m.

Bicarbonate
The bicarbonate content was determined by potentiometric titration method (APHA, 1985). 20 ml of sample and a series of bicarbonate standards ranging from 100 mg/l to 1000 mg/l were titrated against 0.02 N HCl. The end-point was noted at pH 4.5. A graph between bicarbonate standards and volume of HCl consumed was plotted. The readings for the samples were found out from this graph.

Phosphate
Phosphate was determined by the Ascorbic Acid method (APHA, 1985). Phosphate standards ranging from 0.1 to 3 mg/l were prepared from KH₂PO₄. 40 ml of each sample and standard solution was pipetted out in 50 ml volumetric flask and 5 ml of Molybdate Antimony solution (prepared by dissolving 4.8 gm of Ammonium Molybdate and 0.1 gm of Potassium Antimony tartrate in 400 ml 4N H₂SO₄ and making the total volume to 500 ml with same acid) and 2 ml of Ascorbic Acid solution (2.0% w/v) was added and mixed well. The mixture was diluted to 50 ml
and optical density was measured at 650 nm. using Cecil Spectrometer (Model No. 594). A graph was plotted between concentrations of the standards and optical density and sample concentrations were obtained from this graph.

**Dissolved Silica**
The dissolved silica content was determined by the Molybdate Silicate Method (APHA, 1985). Silica standards were prepared, ranging from 0.1 to 10 mg/l from Sodium metasilicate nanohydrate. 20 ml of each sample and standard was pipetted out in 50 ml volumetric flask and 10 ml of Ammonium molybdate solution (prepared by dissolving 2 gm of Ammonium molybdate in 10 ml distilled water, 6 ml concentrated HCl was added and final volume was made upto 100 ml by distilled water) and 15 ml of Reducing Reagent (prepared by mixing 100 ml Metol sulphite solution, 60 ml 10% oxalic acid and 120 ml 25% Sulphuric acid and making the final volume to 300 ml by adding distilled water) was added and mixed well. Metol sulphite was prepared by dissolving 5 gm metol in 210 ml distilled water and 3 gm Sodium sulphate was added and the volume was made upto 280 ml with distilled water. The samples were stirred properly and kept for 3 hrs. to complete the reaction. The optical density was measured at 650 nm using Cecil Spectrometer - 594. Graph between concentration of the standards and optical density was drawn and concentration of samples were recorded from it.

**Chloride, Fluoride, Bromide, Nitrate and Sulphate**
These were analysed using Metrohm Ion Chromatograph with 709 IC Pump, 733.1 IC Separation Centre and 732 IC conductivity detector. The column used for Anion analysis was IC Anion Column Metrosep Anion Dual 2 (6.1006.100). The eluent used was 5mmol/L pthalic acid, 2% acetonitrile, pH= 4.5 (adjusted with NaOH) and a conductivity of approx. 400 μS/cm. The flow rate maintained during the analysis was 0.8mL/min. The anions were eluted out in the order of fluoride, chloride, bromide, nitrate and sulphate. It took 20 min for each run.

**Lithium, Sodium, Ammonium, Potassium, Calcium, Magnesium and Strontium**
These were analysed using Metrohm Ion Chromatograph with 709 IC Pump, 733.1 IC Separation Centre and 732 IC conductivity detector. The column used for cation analysis was IC Anion Column Metrosep Cation 1-2 (6.1010.000). The eluent used was 4mmol/L tartaric acid, 1mmol/L dipicolinic acid and a conductivity of approx. 700 μS/cm. The flow rate maintained during the analysis was 1.0 mL/min. The cations were eluted out in the order of lithium, sodium, ammonium, potassium, calcium, magnesium and strontium. It took 15 min for each run.
Dissolved Organic Carbon (DOC)
Dissolved organic carbon (DOC) was measured by a modified high temperature catalytic oxidation method using a DIMATOC-100 Analyzer System with a combustion temperature of 850°C followed by infra-red analyses of the released CO₂ (Ittekkot and Zhang 1989)

3.2.3 SEDIMENT ANALYSIS
The sediments were analyzed for Total Carbon, Total Nitrogen and Total Phosphorus. Samples were taken out from cold room and were oven dried at 60°C for 24 hrs. (Raaphosst, 1994). Homogenization was done by quartering.

Following analysis were done for the bed, core and suspended sediments:

1. Kjeldahl Nitrogen Analysis
2. Carbon Analysis
3. Analysis for the major and minor elements.

Kjeldahl Nitrogen Analysis
Nitrogen was analysed by Kjeldahl digestion method of Anderson (1993). 0.2 gm sediments was taken in digestion tube and 4.4 ml of digestion mixture was added to each tube. This was digested at 90°C till a solution becomes colourless. The solution was allowed to cool down to room temperature. 50 ml of distilled water was added in each tube and stirred. The final volume was made upto 100 ml by adding distilled water. The clear solution was used for analysis.

Digestion mixture was prepared by dissolving 0.42 gm Selenium powder and 14 mg Lithium Sulphate in 350 ml 30% (v/v) H₂O₂ and mixed. In this mixture 420 ml H₂SO₄ was added carefully while cooling in an ice bath. The digestion mixture was kept at 4°C for further use. Nitrogen was determined by steam distillation. Free ammonia is liberated from the solution by steam distillation in the presence of excess alkali. The distillate is collected in a receiver containing excess Boric Acid indicator and nitrogen is determined by Acid titration.

Distillation process is done by transferring 10 ml of aliquot to reaction chamber and adding 5 ml Alkali mixture and starting distillation immediately. About 25 ml of distillate was collected in 5 ml Boric Acid-indicator solution. The distillate was titrated against N/140 HCl to a grey end point using microburette.
Kjeldahl Nitrogen was calculated using the following equation:

\[
\%N = \frac{(a-b) \times N \text{ of HCl} \times 1.4 \times V}{v \times s}
\]

- \(a\) = ml of HCl used against sample
- \(b\) = ml of HCl used against blank
- \(V\) = ml of total digest titrated
- \(v\) = ml of digest distilled
- \(s\) = Weight of sediment taken

Alkali solution was prepared by dissolving 500 gm NaOH and 25 gm Sodium thiosulphate in water and final volume was made upto 1000 ml with distilled water. Boric acid-indicator solution was prepared by Okalibo (1993) method. 0.3 gm Bromocerol green and 0.165 gm methyl red were dissolved in 500 ml of 95% ethanol to make the Indicator Mixture. 20 gm Boric acid was dissolved in 700 ml distilled water and cooled and 200 ml of 95% ethanol was added. 20 ml of Mixed Indicator was added to it and mixed well by shaking. 0.05N NaOH was added to it till 1 ml of indicator with 1 ml distilled water changes colour from pink to pale green. The solution was then made upto 100 ml.

Carbon Analysis

Carbon was analyzed using ELTRA (CS 1000) Carbon Analyser. Before analysis sediments were made free of halogens by washing with distilled water. Untreated samples were used for determining Total Carbon.

The measuring method is based on the principle of sample combustion and analysis of gases given off through infrared absorption. The sample is put in a ceramic boat, which is made free of any carbon and pushed into the silicon carbide furnace set at a temperature of 1300°C. The combustion occurs in presence of oxygen supplied and the carbon present in the samples is converted to CO\(_2\) and is carried to the infrared absorber by oxygen, which also acts as a carrier gas. The flow of oxygen is maintained to a constant value through an electronic flow regulator. The moisture traps ensure that dry gas mixture reaches the infrared cell. The infrared cell signals are selective and respond to Carbon-di-oxide concentration in the gas mixture. The signals are electronically linearised, integrated and percentage of carbon in the given weight of sample is shown digitally.
Analysis for the major and minor elements

Finely ground homogenized sediments were taken in the beaker and boiled with 30% (v/v) $\text{H}_2\text{O}_2$ (Jackson, 1973) for removal of organic matter. The treatment is repeated until the emission of CO$_2$ stopped.

Analysis of major and minor elements were carried out by the two solution method (Shapiro, 1975). Solution ‘A’ was used for silica and phosphorus analysis and Solution ‘B’ was used for metal analysis.

(a) Solution ‘A’

0.025 gm of finely powder sediment was taken in 50 ml Nickel crucible and 3-4 pellets of NaOH were added to it. The crucible was gently heated on a furnace in order to mix sediment and melt pellets, then the crucible was constantly heated to dull redness for 30 mts. Then, the crucible was allowed to cool down to room temperature. 10 ml of distilled water was added in the crucible and kept overnight. The solution was transferred to 250-ml volumetric flask with help of a policeman. 5 ml of (1:1) HCl and 25 ml water was also added. This solution was boiled till it was clear and the total volume was made upto 250 ml by adding distilled water.

Silica Analysis

0.8 ml of solution ‘A’, standard solution (Canadian soil standards i.e. SO1, SO2, SO3, SO4) and reagent blank were transferred to 50 ml Teflon beaker. 20 ml of distilled water was added to each beaker. 0.2 ml of ammonium molybdate solution was added and kept for 10 mts.). 4 ml of 10% (w/v) tartaric acid was added by swirling the beaker. 0.1 ml of reducing reagent was added and samples properly stirred and kept for 30 mts. Optical density was measured by Cecil Spectrophotometer- 594 at 650 nm.

Ammonium molybdate solution was prepared by dissolving 1.875 gm. ammonium molybdate in 19 ml distilled water and adding 6 ml 20% (v/v) $\text{H}_2\text{SO}_4$. The reducing reagent was prepared by dissolving 0.07 gm. sodium sulphite in 1.0 ml distilled water and then 0.015 gm. 1-amino-2-naphthol-4-sulphonic acid was added and stirred well until dissolved. 9 ml of 10% (w/v) sodium bisulphite solution was added to it and mixed well.

Alumina Analysis

15 ml of Solution ‘A’ (sample, standard and blank) were taken in 100 ml volumetric flasks. 2 ml of Calcium chloride solution (prepared by dissolving 7 gm. $\text{CaCO}_3$, and adding 100 ml of water and 15 ml of concentrated HCl and diluted to 500 ml) was added. 1 ml of hydroxylamine hydrochlo-
ride (prepared by dissolving 5 gm. of hydroxylamine hydrochloride in 50 ml) was added by swilling the flask. 1 ml of potassium ferricyanide (0.75%) was added to each flask and mixed. 2 ml of thioglycolic acid solution (4%) was added and kept for five minutes. 10 ml of buffer solution (prepared by dissolving 100 gm. of sodium acetate in water and adding 30 ml of glacial acetic acid and diluted to 500 ml) was added and kept for 10 minutes. 10 ml of Alizarin Red-S solution (0.05%) was added to each flask and the volume was diluted to 100 ml by adding distilled water. The samples were properly stirred and kept for 50 minutes. The absorbance was measured at 470 nm using Cecil Spectrophotometer- 594.

Phosphorus Analysis
Phosphorus in bed-sediments and suspended sediments were analyzed from solution ‘A’. Phosphate was determined by ascorbic acid method as described in water analysis section. The standards used in this case were standard soil samples, which were digested to make solution ‘A’ 40 ml of each solution ‘A’, and sediment standard was used for analysis.

(b) Solution ‘B’
0.10 gm. of finely ground sample were transferred to the Teflon crucibles and 2 ml of aqua regia (HNO₃ and HCl ratio 1:3 )and 5 ml HF were added to each crucible, these were then sealed in metallic cases. The crucibles were heated for 1 1/2 hrs. at 100°C and allowed to cool down to room temperatures. 5.6 gm. of Boric acid crystal (H₃BO₃) was dissolved in 20 ml distilled water and then added to the bomb contents which was made upto 100 ml. The solution was transferred to polypropylene bottles for storage. The sample was left undisturbed overnight to allow the formation and settling of borosilicate from the solution. This gelatinous precipitate is separated by centrifugation. The solution thus obtained is used for analysis of major and minor elements by GBC-902 double beam Atomic Absorption Spectrophotometer.

3.2.3.1 CHEMICAL FRACTIONATION OF METALS
5 gm of finely grounded sediment samples were extracted with 25% (v/v) acetic acid to remove metals held in amorphous iron oxides, some manganese hydrous oxides and some organic fractions. Samples were shaken with 25% acetic acid for 12 hours, centrifuged and the supernatant filtered through a 0.45-micron millipore filter. The residual sediment was used to determine detrital metal in the sediments. These include metals held in the most resistant fractions, including organic components and silicate lattices. The samples were extracted by 4-hour digestion in hot aqua regia (3:1 HC1: HNO₃). The metals were analysed in the acetic acid solution and in aqua regia extract by using AAS (Loring and Rantala, 1998).
3.2.3.2 GRAIN SIZE ANALYSIS

Mechanical analysis for determining the grain size of surface sediments was done upto 63μm using the standard method of sieving (using ASTM Standard Sieve), less than 63μm fraction were analysed with “FRITCH Laser Particle Sizer” (Analysette 22 model). The size distribution of suspended sediments was also determined using with “FRITCH Laser Particle Sizer” (Analysette 22 model).

3.2.3.3 SURFACE SEDIMENT MINEROLOGY

The surface sediments were oven dried at 80°C. To the homogenised fraction of the sediment was added 30% (v/v) H₂O₂ (Jackson, 1973) and heated on the hot plate. This treatment was done repeatedly until all the organic matter from the sediments was not removed.

Clay was separated from these organic free sediments using Attenborough cylinder. The sediment was crushed to a fine powder with help of pestle and mortar made of agate. The slides were prepared by drop on slide technique (Gibbs, 1965). The samples were run on Philips X-ray Diffractometer (XRD) (Model: PW 1140) using Cu (Kα radiation) source, proportional detector and Ni filter. The chart drive was 1 cm/min, goniometer 1°/min, range 400cps and voltage 16mV/35K V were maintained.

The primary peaks of various minerals were identified by comparing 2θ value for highest intensity peak, as described by Lindholm (1987) (Appendix-I). The major peaks viz. mica, quartz and feldspar were normalised to 100 percent after calculating the respective peak areas. Then, relative abundance of the minerals from these major groups was calculated in terms of percentages.

3.2.3.4 HUMIC SUBSTANCES

Humic substances was determined according to method of Lenhard et al., (1962). Exactly 0.1 gm of sediment was refluxed with 100ml of 10% (v/v) hydrochloric acid for two hrs to destroy carbonates and hydrolyse non-humic organic matter. It was then heated with 0.5%(w/v) sodium hydroxide for 2hrs on a boiling water bath. After centrifuging at 15,000 rev/min, the optical density of supernatant solution was measured at 400 and 460nm.

\[
\text{Weight of Humic Acid} = \frac{\text{optical density (at 400-460nm)}}{1.84}
\]