Chapter 2

MATERIALS AND METHODS

2.1 Description of the study area

2.2 Sampling and storage

2.3 Laboratory procedure

2.4 Statistical analysis

2.1 DESCRIPTION OF THE STUDY AREA

The area of study, the Chitrapuzha tidal river system forms part of the Cochin backwaters as well as the National Inland waterway No.III (9°52' - 10°00'N; 76°15' - 76°25'E), is shown in the Fig. 2.1. The Cochin backwaters are the largest of the backwaters on the Kerala coast. The hydrography of these backwaters is controlled mainly by discharges from Periyar, Muvattupuzha and Chitrapuzha rivers and also by tidal action through the Cochin barmouth. Saline water intrusion to the southern parts of the estuary is regulated by the Thanneermukkam bund, a saltwater barrier commissioned in 1975. A large number of heavy industrial units are situated on either banks of River Periyar and hence northern parts of the backwaters receive large quantities of treated and untreated industrial effluents. Chitrapuzha River hosts diverse aquatic organisms and many areas have been transformed into breeding pools so as to increase fish production for commercial exploitation. The river originates as a small stream from the upper reaches of high
ranges in the eastern boundary of Kerala and passes through the valley and finally joins the southern arm of Cochin backwaters. The depth of the study area varies considerably. While the shipping channels of Cochin port are maintained at a depth of 10 – 13m, the major portion of the study area has a depth range of 3-5m. Numerous industrial units including a diesel power project, fertilizer manufacturing unit and a petrochemical unit, are located along the banks of the Chitrapuzha River. Effluents from these industrial units along with agricultural and other anthropogenic effluents find their way into Chitrapuzha River ultimately into Cochin backwaters. There are long standing local complaints about water pollution causing fish mortality and serious damage to agricultural crops resulting in extensive unemployment in the area. The lower reaches of this river became part of National Waterways in 1993 and is now mainly used for transporting chemicals from Cochin Port to the industrial units located on the banks of the river. The river Chitrapuzha is thus of considerable social and economic importance. Though numerous studies were carried out to elucidate the metal distribution in the aquatic system of Cochin estuary, very little is known about the same in the Chitrapuzha aquatic system.

Based on specific geographical features, water flow regimes and anthropogenic activities, 9 sampling locations were selected (Fig. 2.1). Samples were collected at monthly intervals between January and December 1999. Stations 1 - 3, (Zone 1) are saline, Stations 4 - 6 (Zone 2) are of intermediate salinities and Stations, 7 - 9, (Zone 3) are fresh water Zones. In addition to these nine Stations samples were collected from a far upstream station (Station R), which is free from industrial pollution and is therefore regarded as a reference site.

Station 1 is at the Cochin bar-mouth. Station 2 is near the oil tanker berth. Station 3 is near Thoppumpady fishing harbour. All the three Stations in the Zone 1 are saline through out the year. Station 4 is Thevara Ferry point, Station 5 is near Thykoodam N H Bridge and Station 6 is near Kaniyampuzha Railway Bridge. These Stations in the Zone 2 are of intermediate salinities; salinity at Station 6 was practically nil during the monsoon season. Station 7 is near Eroor Bridge Station 8 is near the discharge outlet of FACT - Cochin Division and Station 9 is near FACT Barge jetty. These three Stations constitute Zone 3, which is the recipient of industrial effluents. The reference Station R is near Mamala.
Fig. 2.1 Map of Chitrapuzha River showing location of sampling stations (R - Reference Station)
2.2 SAMPLING AND STORAGE

The sampling surveys were conducted using a fibreglass research vessel "King Fisher" thus eliminating all possible metal contamination from the collecting vessel. A stainless steel plastic-lined Van Veen grab was used to collect sediment samples. At each Station five grab-full of sediments were sampled and the top 5 cm layer was carefully skimmed from all the grabs using a polyethylene scoop, homogenized and stored at -5°C, in double polyethylene containers. Surface water samples were collected in a 2-litre conventional polyethylene container. A pre-cleaned Teflon High-tech water sampler was used to collect bottom water samples. Water samples meant for metal analysis were filtered immediately after collection, through thoroughly acid washed; pre weighed 0.45 μm Whatman membrane filters. The filtrate was used for the analysis of dissolved metals while the residue retained on the membrane filters was dried to constant weight.

2.3 LABORATORY PROCEDURE

All glassware and plasticware used in the experiments were previously washed, soaked in dilute nitric acid and then rinsed with Milli-Q water. All reagents used were of analytical grade and were checked and excluded for possible trace metal contamination. Reagents and standard solutions were prepared with Milli-Q water and the whole laboratory procedure for metal analysis was carried out under laminar flow hood with caution to avoid contamination.

Dissolved metals

Dissolved chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and lead were pre-concentrated using solvent extraction of the chelates formed with a mixture of complexones (ammonium pyrrolidine dithiocarbamate and diethylammonium diethyl dithiocarbamate (APDC-DDDC) into chloroform, followed by back-extraction into nitric acid (Danielsson et al., 1978,1982). Determinations were carried out on the concentrates by graphite-furnace atomic absorption spectrometry (Perkin Elmer model 3110, with HGA 600), calibrated using standard solutions prepared by dilution of 1000 mg/l standard solutions (Merck). Analytical blanks were prepared using the same procedures and reagents.
Particulate metals

The particulate matter separated as above was digested according to the APHA (1995) procedure. The dry residue in the membrane filter was leached with 10 ml of concentrated acid solution (HClO₄, HNO₃ and HCl in the ratio 1:1:3) at 90°C for 6 hours. The resultant solution was centrifuged and then made up to 25 ml with 0.1 M HCl for analysis on the (Perkin Elmer- Model 3110) AAS.

Total metals in the sediment

The oven dry sediment samples were ground to a fine powder in an agate mortar and 0.5g aliquots were weighed into beakers for estimation of total metal. Each sample was carefully digested with 10 ml of an acid solution (HClO₄, HNO₃ and HCl in the ratio 1:1:3) at 90°C until complete digestion and evaporated to incipient dryness. After cooling, the sides of the beaker were rinsed with Milli-Q water, centrifuged and the centrifugate made up to 50 ml. The metal concentrations in the solution were determined by atomic absorption spectrophotometry (Perkin-Elmer 3110 AAS), calibrated using standard solutions prepared by dilution of 1000mg/l standard solutions (Merck). Analytical blanks were prepared using the same procedures and reagents.

Moisture percentage

Known amounts of wet sediment samples were dried at 105°C in an electric oven to constant weight (Hakanson and Jansson, 1983). The moisture content was expressed as the percentage weight of the sample. From the percentage moisture values, the sediment metal values were recalculated and the same was expressed in terms of oven dry weight basis.

Sediment organic carbon

The organic carbon content in the sediment was estimated by the dichromate method (Walkley and Black, 1934) as modified by El Wakeel and Riley 1957).
Total phosphorus

The total phosphorus was determined in the nitric acid - perchloric acid extract using phosphomolybdic acid method (Strickland and Parsons, 1977).

Grain size analysis

Textural analysis was carried out to study the variations in grain sizes of sediments. A known amount of wet sediment was dispersed overnight in sodium hexametaphosphate. The sand was separated from the dispersed sediments by wet sieving using a 230 mesh (63 μm) sieve (Carver, 1971). The filtrate containing the silt and clay fractions was subjected to pipette analysis (Krumbein and Pettijohn, 1938).

Complimentary analysis

Concurrent recording of water quality parameters such as salinity, dissolved oxygen pH and suspended solids were performed by standard procedures as detailed below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Portable pH Meter (Merck)</td>
</tr>
<tr>
<td>Salinity</td>
<td>Argentometry (Strickland and Parsons, 1977)</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>Iodometry (Strickland and Parsons 1977)</td>
</tr>
<tr>
<td>Suspended solids</td>
<td>Gravimetry (Butler and McManus, 1979)</td>
</tr>
</tbody>
</table>

Chemical partitioning

Trace metals in the sediments were extracted according to the scheme depicted in the Fig. 2.2. The extractants, the sequence and the procedure followed were adapted from the method employed by Tessier et al. (1979). The different metal species studied were:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Exchangeable</td>
</tr>
<tr>
<td>2</td>
<td>Bound to Carbonates</td>
</tr>
<tr>
<td>3</td>
<td>Easily reducible fraction (metals bound to Fe-Mn oxides)</td>
</tr>
<tr>
<td>4</td>
<td>Bound to organic matter (oxidizable)</td>
</tr>
<tr>
<td>5</td>
<td>Residual</td>
</tr>
</tbody>
</table>
Fig. 2.2 Sequential extraction scheme
Four to five gram aliquots of the wet sediment samples were used for the sequential chemical extraction. Wet sediments were analyzed, as the drying process is known to significantly alter metal speciation (Kerster and Forstner, 1986; Jones and Turki, 1997). The extractions were done in 250 ml Erlenmeyer flasks with continuous agitation. The phases were separated by centrifugation at 6000 rpm for 10 minutes. The centrifugate was analysed for metal concentration by atomic absorption spectrophotometry (Perkin-Elmer 3110 AAS), calibrated using standard solutions prepared by dilution of 1000 mg/l standard solution (Merck), whereas the residue was carefully transferred to the container with the next extractant to be used. Washings in between the extractions were dispensed with to avoid excessive solubilisation of solid phases. The moisture content in the sediment was determined separately and simultaneously and the concentrations were expressed in mg/kg dry weight. The samples were analysed in duplicate and the mean of the two determinations has been reported.

2.4 STATISTICAL ANALYSIS

The annual mean, standard deviation and percentage coefficient of variation for all the parameters recorded were computed along with minimum and maximum values to get an idea of the spread of the data. Both spatial and temporal variations were significant. The spatial variations are discussed mainly on the basis of annual mean concentrations recorded at each Station as well as in each Zone. Correlation study was carried out to find out the influence of various hydrographic parameters on the distribution of dissolved and particulate metals. Three seasons viz. Pre-monsoon (February - May), Monsoon (June - September) and Post-monsoon (October - January) have been recognized. The seasonal and annual mean values are represented graphically, Station-wise and Zone-wise to bring out seasonal and spatial variations. Multiple regression analysis was performed on the total metal concentrations to assess the role of different parameters in determining distribution pattern of heavy metals. An attempt was also made to quantify the natural and anthropogenic contribution of heavy metals to the sediment.
REFERENCES


