Chapter 2

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Cochin estuary is the largest estuarine system in the southwest coast of India. It is a part of Vembanad-Koll wetlands (09°50'N, 76°45'E), which has been identified as the Ramsar Site-1214 at the Convention on Wetlands organised by the UNESCO in the Iranian city of Ramsar in 1981. It is topographically divisible into two arms and lies parallel to the coastline with several islands. Total length of the estuary is about 80 kms and the width varies from a few hundred meters to about 4 km. The depth of the estuary ranges from 2 to 7 m, but the ship channels at the Cochin harbour region are dredged and maintained at 10 to 13 m (Qasim, 2003). Tides are of a mixed semi-diurnal type, with a maximum spring tide range of about 1 m at the mouth (Srinivas, 1999). This low tidal amplitude, perhaps the smallest among the Indian coasts, results in incomplete flushing. This estuary is also under the profound influence of southwest monsoon, which contributes about 71% of annual rainfall (Jayaprakash, 2002). Accordingly, three seasons prevail viz. monsoon (June–September), post-monsoon (October–January) and pre-monsoon (February–May).

2.1 Description of the study region

Mangrove forests are the silent victims of the development boom in Kerala, especially Cochin. Of the total of 1650 hectares of mangrove forests in the State, Ernakulam district ranks third with mangrove vegetation spread over 260 hectares, according to the Forestry Information Bureau of the Forest and Wild Life Department of the State Government. The unprecedented rate of urbanization and the construction boom resulted in the destruction of large patches of mangroves in Cochin. Hence Cochin has been included in the list of metros like Kolkata and Mumbai, where
massive destruction of mangroves has been reported. Mangroves are now mainly found in Mangalavanam, Panangad, Thripunithura, Kumbalam, Nettoor, Panambukad, Puthuvype, Vypin, Mulavukad, Kumbalangi, Kannamaly and Chellanam. Mangalavanam has a core area of 3.44 hectares of mangroves. At Kannamaly and Kumbalangi, mangroves are found in a stretch of around eight hectares each. Panambukad and Puthuvype have a mangrove cover of around 10 hectares. The lack of a permanent monitoring cell is hampering the protection of mangroves in the district.

Three mangrove systems in the northern arm of Cochin estuary were chosen for the present study (Fig. 2.1).

**Station 1: Puthuvype**

It is a mangrove nursery maintained by the fisheries research unit of Kerala Agricultural University and is located about 100 meters away from the estuarine front. It is free from sewage inputs and other pollutants. The dominant mangrove flora found here are *Avicennia officinalis* and *Bruggeria gymnorrhiza* (Sebastian and Chacko, 2006) (Fig. 2.2).

**Station 2: Murikkumpadam**

It is a densely populated fisher-folk settlement. The dominant species in this system are *Acanthus ilicifolius*, *Rhizophora apiculata*, *Rhizophora mucronata*, *Excoecaria agallocha*, and two mangrove associates *Clerodendronica* and *Acrostica* (Sebastian and Chacko, 2006). Discharge of sewage and disposal of garbage and solid waste are the major sources of pollution here. This station is very close to the Arabian Sea.
These two stations form part of the island called Vypin, which is one of the most densely populated coastal zones. Vypin is the largest single stretch of mangroves found in Kerala. It covers an area of 101 hectares. Vypin island is well known for its Pokkali fields. Mixed silvi–agri-aquacultural farming is practiced here. Large areas of mangroves have been destroyed for prawn and fish culture. The pressure of the growing population is also a threat to these mangroves.

Station 3: Mangalavanam

It is a patchy mangrove area (2.74 hectares) in the heart of Cochin City. This habitat consists primarily of *Avicennia officinalis* with occasional patches of *Acanthus ilicifolius* and *Rhizophora mucronata* sp. (Subramaniyan, 2000). This mangrove forest (Fig. 2.2) is home to many exotic and rare varieties of migratory birds. Forty one species of birds were recorded from Mangalavanam representing 12 orders and 24 families and the most common bird species found here are little cormorant (*Phalacrocorax niger*) and night heron (*Nycticorax nycticorax*) (Jayson, 2001). But the urban developmental pressure has spelled doom for the sanctuary. The heavy vehicular traffic, siltation and waste deposition in the area and piling up of non-biodegradable waste in the water body are some of the visible signs of distress regarding this sanctuary. This is an almost closed system with a single narrow canal link to the estuary and this canal is the only source for tidal propagation. During low tide, the water in the system is completely drained. There are very few studies on the biogeochemistry of these systems.
Study conducted by the department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology identified 14 species of mangroves along Kerala Coast. The reported species in Vypin are *Acanthus ilicifolius*, *Avecennia officinalis*, *Acrostichum auretum*, *Avicennia marina*, *B. parviflora*, *Bruguiera gymnorrhiza*, *Derris trifoliata*, *Excoecaria agallocha*, *Kandelia candel*, *Lumnitzera racemosa*, *Rhizophora mucronata*, *Rhizophora apiculata* and *Sonneratia caseolaris*. In Mangalvanam, the identified species are *Acanthus ilicifolius*, *Avecennia officinalis*, *Acrostichum auretum*, *Avicennia marina*, *B. parviflora*, *Bruguiera gymnorrhiza*, *Derris trifoliata*, *Kandelia candel*, *Lumnitzera racemosa*, *Rhizophora mucronata*, *Rhizophora apiculata* and *Sonneratia caseolaris*.
2.2 Sampling and analytical Methodology

Samples of water and sediments were taken from these three locations during December 2005, April 2006 and July 2006. Surface water samples were collected during high tide using a clean plastic bucket. Surface sediment samples were taken from the study areas using a clean plastic spoon. To get a true representation of the system, sediment samples were collected from three different parts of each system and pooled together for analysis. The water samples were stored in previously washed plastic bottles, which were rinsed with the sample at the collection site. The sediment samples were collected in plastic bags. Samples were transported to the lab on ice and stored in a deep freezer till analysis. All the analyses were carried out in triplicates and the average reported.
Figure 2.3 Dominant mangrove fauna of the study region
2.2.1 General Hydrography

General hydrographical parameters and nutrients of the surface waters were analysed using standard methods. pH in the water column was measured in situ and temperature was measured using a sensitive thermometer. Salinity of the water samples was estimated by Mohr-Knudsen method (Muller, 1999). Modified Winkler method was used for the estimation of dissolved Oxygen (Hansen, 1999). Alkalinity of the water samples was estimated by the method of Koroleff (Anderson et al., 1999). Nutrients (nitrite, nitrate phosphate and silicate) were estimated spectrophotometrically using UV-VIS Genesis Thermospectronic. Nitrite was converted to an azo dye with sulphanilamide and N- (1-naphthyl) ethylene diamine dihydrochloride (Grasshoff et al., 1999). Nitrate was reduced to nitrite using copper-coated Cadmium column and estimated as nitrite (Grasshoff et al., 1999). Formation of phospho- molybdate complex using ascorbic acid as reductant was used for phosphate determination (Grasshoff et al., 1999). Silicate was analyzed by converting it into silicomolybdate complex, which is reduced, using ascorbic acid and oxalic acid, to produce a blue solution (Grasshoff et al., 1999).

2.2.2 Geochemistry

Redox potential of the fresh wet sediment was measured using Zobell’s solution for the calibration of the electrodes (Brassard, 1997). The sediment textural characteristics (sand, silt, and clay) were determined by pipette analysis (Krumbein and Pettijohn, 1938) after removing the inorganic carbonates using 10% HCl and organic matter using 15% H₂O₂. This analysis is based on Stoke’s law. Sediment was dispersed in sodium
hexametaphosphate overnight and then wet sieved through a 63 μm sieve to collect the sand fraction. The mud fraction was divided into silt and clay fractions by the timed gravimetric extraction of dispersed sediments (Folk, 1974). Sediment samples were air dried and finely powdered using agate mortar for further analyses. Powder X-Ray Diffraction analysis was carried out to find the mineralogy of the sediments (Moore and Reynolds, 1997). Total Carbon, Nitrogen and Sulphur were determined using Vario EL III CHNS Analyser. Sediment organic carbon was estimated by the procedure of El Wakeel and Riley modified by Gaudette and Flight (1974). The amount of total organic matter (TOM) was obtained by multiplying the organic carbon values with 1.724 (Nelson and Sommers, 1996).

Representative samples were analysed using X-Ray Fluorescence (XRF) for finding the major elements. Major elemental composition of the sediment in station 3 was also analysed using SEM-EDS. Thermo Gravimetric Analysis (TGA) was carried out to find out the loss of ignition. Heavy metals in the sediment were estimated using Flame AAS (Perkin Elmer-3110) after digestion using di-acid mixture (1:5 HClO₄:HNO₃). Accuracy of the analytical procedure was checked using standard reference material BCSS-1 (standard reference material for marine and estuarine sediments). Triplicate analysis of BCSS-1 showed a good accuracy and the recovery rate ranged between 82.7 % for Mn and 103.9 % for Zn (table 2.1).
Table 2.1 Analysis of standard reference material for heavy metals (BCSS-1)

<table>
<thead>
<tr>
<th>Metal (µg/g)</th>
<th>Certified Value</th>
<th>Obtained Concentration (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>11.4 ± 2.1</td>
<td>10.67 ± 2.68</td>
</tr>
<tr>
<td>Cr</td>
<td>123 ± 1.4</td>
<td>112 ± 0.65</td>
</tr>
<tr>
<td>Cu</td>
<td>18.5 ± 2.7</td>
<td>18.2 ± 0.25</td>
</tr>
<tr>
<td>Fe (%)</td>
<td>4.7 ± 0.14</td>
<td>4.64 ± 0.41</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>2.44 ± 0.23</td>
<td>2.32 ± 0.36</td>
</tr>
<tr>
<td>Mn</td>
<td>229 ± 15</td>
<td>189.47 ± 10.75</td>
</tr>
<tr>
<td>Ni</td>
<td>55.3 ± 3.6</td>
<td>49.16 ± 2.01</td>
</tr>
<tr>
<td>Pb</td>
<td>22.7 ± 3.4</td>
<td>24.9 ± 0.08</td>
</tr>
<tr>
<td>Zn</td>
<td>119 ± 12</td>
<td>123.64 ± 2.51</td>
</tr>
</tbody>
</table>

The sequential extraction scheme by Golterman (1996) using chelating agents was employed for estimating different phosphorus fractions (Fig. 2.4). Compared with the other methods, chelating agents allow a specific extraction of inorganic phosphorus with less destruction of organic phosphorus (Golterman, 1996). Iron bound phosphorus (Fe-IP) was extracted with buffered Ca-EDTA/dithionite and calcium bound fraction (Ca-IP) subsequently with Na-EDTA. In the next step, acid soluble organic phosphorus (ASOP) was extracted with H₂SO₄ and then alkali soluble organic phosphorus (Alk-OP) with 2M NaOH at 90°C for 2 hours. Residual organic phosphorous (ROP) was measured after 1 hour K₂S₂O₈ digestion in acid medium. All the extractions were carried out under mild continuous shaking and the results are expressed on the dry weight basis. Generally, iron and calcium bound inorganic fractions and acid soluble organic fractions of phosphorous are considered to be bioavailable (Diaz-Espejo et
al., 1999). But Fe-IP is more important than Ca-IP in terms of potential availability of phosphorus under the redox (Eh) variations observed in the mangroves sediments (Caraco et al., 1989; Silva and Mozeto, 1997).

Figure 2.4 Sequential extraction scheme for phosphorus fractionation

2.2.3 Biochemical Composition

Colorimetric methods were employed for the determination of biochemical compounds. Proteins (PRT) analyses were carried out following the procedure of Lowry et al., (1951), as modified by Rice (1982) to account for the reactivity of phenolic compounds, with albumin as the standard. The amount of protein nitrogen was obtained by multiplying protein with 0.16 (Mayer et al., 1986). Total carbohydrates (CHO) were
analysed according to Dubois et al., (1956), using glucose as the standard.
Total lipids (LPD) were extracted according to Bligh and Dyer (1959), and
estimated according to Barnes and Blackstock (1973) using Cholesterol as
the standard. All the analyses were carried out on triplicates and the
average reported. The sum of all PRT, CHO and LPD was defined as the
labile or easily assimilable organic fraction (Danovaro et al., 1993;
Cividanes et al., 2002). PRT, CHO and LPD concentrations were converted
to carbon equivalents by using the following conversion factors: 0.49, 0.40
and 0.75 g of C/g, respectively (Fabiano and Danovaro, 1994). The sum of
PRT, CHO and LPD carbon is referred to as biopolymeric carbon (BPC)
(Fichez, 1991; Fabiano et al., 1995).

Stable carbon isotope analysis of Total Organic Matter ($\delta^{13}$C_{TOM})
was carried out using Flash EA interfaced with IRMS (FINNIGAN DELTA
PLUS XP, Thermo Electron Corporation). Stable carbon isotope abundances
are presented as $\delta^{13}$C values and are expressed relative to the PDB (Pee Dee
Belemnite) standard:

$$\delta^{13}$C = \frac{13C/12C_{Sample}}{13C/12C_{PDB Standard}} - 1 \times 100$$

### 2.2.4 Fatty acid Biomarkers

Fatty acids extraction using a method described by Harvey (1994)
was selected for the present study. Dry sediment samples were soxhlet
extracted for 72 hrs with a mixture of dichloromethane (DCM): methanol
(MeOH) (1:1). The extracts were combined and evaporated to dryness
using rotary evaporation. The extracted residue was subjected to mild
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alkaline hydrolysis using 0.5 M KOH/MeOH and gentle heating (70 °C for 30 min). After cooling the sample, the neutral lipids were partitioned from the alkaline solution into hexane, which was separated and stored for further analysis. The remaining aqueous layer containing the fatty acid salts was acidified to pH 2, where the fatty acids in this polar-lipid fraction were partitioned into hexane. The polar lipid fraction containing the fatty acids was evaporated to dryness using rotary evaporation and treated with 10 ml of 12% BF3/MeOH (Sigma Aldrich) while heating at 70 °C for 30 minutes to form the fatty acid methyl esters (FAMEs). The FAMEs were subsequently partitioned from the reaction solution into hexane. The hexane layer was evaporated to dryness, and the extract was then re-dissolved into hexane. The analysis of FAMEs was performed using a Trace GC Ultra (Thermo Electronic Corporation) gas chromatograph (GC) equipped with flame ionization detector (FID). FAMEs were separated with a Perkin-Elmer Elite 225 capillary column (30 m length, 0.25 mm internal diameter, 0.25 mm film thickness). After injection at 260°C (split ratio 1:20), the oven temperature was held at 110°C for 4 minutes and then was programmed to increase to 240 °C at a rate of 2.7 °C /min. Then it was held at 240°C for 5 min. The flame ionization detector was maintained at 275 °C during the analysis. FAMEs were identified by comparing the retention times with a standard (Supelco 37 Component FAME Mix, 18919-1AMP).

2.2.5 Statistical Analyses

All data were subjected to statistical analysis wherever necessary. Pearson correlations were determined to find out the inter relations between different parameters. Statistical significance of the observed spatial and
temporal variations in sediments is checked using Two way ANNOVA (stations X seasons). Principal component analysis (SPSS 15.0) was done to find out the factors contributing to different biogeochemical processes occurring in mangrove sediments.

2.3 Results of the General Hydrography

The southwest monsoon has had a profound influence on the study region, creating seasonal variations in the hydrographical parameters (Table 2.2). Salinity varied widely and the near fresh-water condition seen during the monsoon season was gradually transformed to a marine condition during the post-monsoon in the first two stations. However, at station 3, true marine situation could be seen only during the pre-monsoon. pH varied from 6.6 to 7.6 and alkalinity varied from 68 to 216 mg CaCO3/l. Dissolved oxygen varied from hypoxic to saturated conditions (1.4 to 10.2 mg O2/l). Inorganic phosphate were higher during the pre-monsoon season and varied between 5.3 and 49.7 μmol/l, while nitrite and nitrate varied from 0.43 to 2.1 μmol/l and from 1.4 to 8.1 μmol/l respectively. Silicate ranged from 3.6 to 63.0 μmol/l. The variations in the hydrographical parameters at these stations could be attributed to the environmental setting. Station 3 was less alkaline and showed lower silicate and higher nitrate concentration. This could be due to the limited water exchange with the estuary because of its almost closed nature. The first two stations are much closer to the estuarine front when compared to the third, especially the second station which is very close to the bar mouth.
Table 2.2 Seasonal variations of hydrographical parameters in the study region

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Station 1</th>
<th></th>
<th>Station 2</th>
<th></th>
<th>Station 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Mon</td>
<td>Post</td>
<td>Pre</td>
<td>Mon</td>
<td>Post</td>
</tr>
<tr>
<td>pH</td>
<td>6.6</td>
<td>7.1</td>
<td>7.4</td>
<td>7.1</td>
<td>7.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>34.0</td>
<td>1.6</td>
<td>28.5</td>
<td>34.0</td>
<td>1.3</td>
<td>29.2</td>
</tr>
<tr>
<td>Alkalinity (mgCaCO$_3$/l)</td>
<td>164</td>
<td>132</td>
<td>144</td>
<td>216</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>DO (mgO$_2$/l)</td>
<td>6.4</td>
<td>3.0</td>
<td>1.4</td>
<td>10.2</td>
<td>4.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Nitrite (μmol/l)</td>
<td>1.2</td>
<td>1.4</td>
<td>0.43</td>
<td>1.2</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Nitrate (μmol/l)</td>
<td>2.2</td>
<td>2.4</td>
<td>2.7</td>
<td>1.4</td>
<td>1.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Phosphate (μmol/l)</td>
<td>49.7</td>
<td>14.8</td>
<td>16.4</td>
<td>28.5</td>
<td>16.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Silicate (μmol/l)</td>
<td>50.0</td>
<td>63.0</td>
<td>61.2</td>
<td>20.2</td>
<td>43.4</td>
<td>23.0</td>
</tr>
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</table>

References


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