Chapter 2

MATERIALS AND METHODS

2.1 DESCRIPTION OF THE STUDY AREA

The areas of investigation were the mangrove-fringed canals in the Cochin backwaters, a major estuary on the south west coast of India. Cochin backwaters situated at the tip of the northern Vembanad lake is a tropical positive estuarine system extending between 9° 40' and 10° 12N' and 76° 10' and 76° 30E' with its northern boundary at Azhikode and southern boundary at Thanneermukkam bund. The lake has a length of 80 km and the width varies from 500m and 4000 m. Water from two major rivers viz., Periyar and Muvattupuzha drain into this estuary, whereas Thanneermukkam bund regulates the flow from four rivers namely Meenachil, Manimala, Achenkovil and Pamba. During south-west monsoon, the estuary is virtually converted into a freshwater basin even in areas around barmouth where saltwater penetration occurs below 5 m depth only. The two mangrove locations on Vypeen island which were sampled experience only a weak tidal flow. The narrow creeks had with obstructions that restricted the free flow of water. Semidiurnal tidal range of Cochin estuary has been reported to be 1m. No perceptible tidal range was observed in the two selected mangrove locations in Vypeen island.

Around Cochin, good mangrove formation are seen in areas like Vypeen, Kannamali, Maradu, Elamkulam and Vallarpadam. Small patches and isolated strands are seen at Kumbalam, Nettoor, Panangad, and Kundannur. Most
extensive and highly developed mangroves are found on Vypeen island. Among the flora Rhizophora mucornata is the most dominant species, followed by Avicennia officinalis and Avicennia ilicifolius. Rhizophora mucornata is the largest species which grows up to nine metre height. The exact nature of early mangrove vegetation on the banks of Vembanad lake is not fully known. This is because the vegetation has undergone considerable disturbances during the last few years due to human interferences. They have been destroyed and used for fuel, and the land has been used for paddy cultivation, prawn culture, coconut plantation and other purposes. The destruction of mangrove plants leads to soil erosion and silting in Cochin backwaters. When accretion along the coast takes place, colonization by mangroves is rapid. In places devoid of human interferences were not affected, colonization of mangrove takes place along some stretches of Cochin backwaters.

Three important mangrove locations around Greater Cochin were sampled (Fig.2.1). Station 1 is situated between latitudes 9°59' North and longitude 76°14' East and is located at Murikkumpadam in Vypeen island. In Vypeen, the areas bordering the canals are densely populated. Station 1 located near the terminus of a 5-7 feet wide canal. It is about 1km distant from the adjoining estuarine water body. It is characterized by a community of dwellings and therefore prone to sewage inputs. Tidal amplitudes do not vary more than a feet. Along the water course a few bushy clumps of Acanthus ilicifolius and few relic stands of Avicennia could also be seen. Rhizophora existed as isolated individuals. No submerged floras were noticed at any part of the year.

Station 2 is situated between latitudes 9°58' and North longitude 76°11' East and is located at Puthuvypu on the southern tip of the Vypeen Island located on the North Western Bank of Cochin bar mouth. This Station represents a carefully preserved mangrove habitat inside the premises of Kerala Agricultural University campus. The mangrove vegetation consisted primarily of Avicennia species which is growing gregariously on the Western side with Rhizophora spp. and Bruguiera spp. constituting occasional growth. Effective area under mangrove vegetation is about 10 ha.
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The sampling site was located at the terminus of the canal, which extend for about 1.5km from open waters into the Kerala Agricultural University campus. An extent of 10 ha of land supporting mangroves in patches exists at Puthuvypu. This forms part of Vypeen Island having 300 ha in extent. The entire area of 101 ha is marshy containing natural basins; sand pits, crevices and canals support good mangrove vegetation. This area is being used by the university for research in the field of brackish water fish farming. The land is regularly inundated by the tidal rhythm of Cochin bar mouth and the tidal waters bring in lot of fish seed of commercially important species like *Mugil cephalus*, *Chanos*, *Lates calcarifer*, *Eleutheronema tetradactylum*, and prawns such as *Penaeus indicus* and *Penaeus monodon* (Purushan, 1989). Since there is not much destruction from outside the succession of mangrove vegetation is progressing unhindered. There is even some trial of artificial regeneration of *Rhizophora* spp. and *Bruguiera* spp. in order to

![Map of Cochin estuary showing location of sampling sites](image_url)
speed up the growth of mangroves. This is the biggest mangrove area available in
the Kerala coast (Basha, 1991).

Station 3 is situated between latitudes 9°54' North and longitude 76°18' East
and is located at Aroor which is in the southern part of Cochin estuary. This site
has only moderate amount of plants. An estuarine site (Station R) on the Vypeen
Island was also sampled during the study to facilitate comparison of the dissolved
nutrient profiles.

2.2 SAMPLING AND STORAGE

Monthly samplings were done at three mangrove stations from December
1999 to December 2000 except in June 2000. The water samples for different
nutrient analysis were collected in different polythene bottles directly from the water
sampler. Surface sediment samples were collected at low tides with clean polythene
scoop. Sediment cores were sampled to a depth of 20cm, since the mangrove plants
are shallow rooted. A PVC corer was used to collect core sediment samples from the
same Stations to a depth of 10-20cm. The core samples were taken with little
compaction as possible. The core sediment was cut into five fragments: 0- 2cm,
2-4cm, 4-6cm, 6-10cm, and 10-20cm. All of them including surface samples were
kept in plastic bags and carried in iceboxes to the laboratory. These sediment
samples were homogenized and kept deep frozen until analysis.

The dissolved ammonium analyses were done without delay. 50ml of the
water samples were preserved by adding 2ml of phenol reagent in the same vessel
in which the analyses were carried out. Nitrite analyses were also done within a day.
The water samples for analysis of dissolved phosphate were filtered using 0.45μm-
poresize glassfibre filter paper (Whatman GF/F) and stored frozen till analyses. The
filter papers were stored for the analysis of total particulate phosphorus. The water
samples of total phosphorus, nitrate, nitrite etc were kept without filtration.

2.3 ANALYTICAL PROCEDURE

All glass wares used in the analysis were washed, soaked in dilute
hydrochloric acid and rinsed with distilled water. All reagents used were of
analytical grade, reagents and standard solutions were prepared with Milli-Q water.
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Dissolved nutrients

Dissolved phosphate, nitrate, nitrite, ammonium and total phosphorus were analysed colorimetrically according to the methods described in Grasshoff et al. (1983a). Nitrite was analysed as azodye after reaction with ethylene diamine and sulphanilamide. Nitrate was first reduced in cadmium column and analysed as nitrite with 5cm flow cell at 540nm. Ammonium analyses were carried out following the indophenol procedure. The absorption of indophenol blue was measured in a 5cm cell at 630nm. The dissolved inorganic phosphate (DIP) was determined according to Murphy and Riley as phosphomolybdate complex (5cm/880nm). Total phosphorus (TP) in water sample was obtained after oxidation by persulphate and analysed as phosphate as above. Dissolved organic phosphates (DOP) were calculated by subtracting the DIP from the TP value. All standard colorimetric methods were carried out using Hitachi model 160-20 UV-Visible spectrophotometer.

Particulate total phosphorus (PP)

Particulate phosphorus (PP) was determined by the method of drying a sample with magnesium sulphate and baking the residue at a high temperature to decompose organic phosphorus compounds (Solorzano and Sharp, 1980). The residue is then treated with hydrochloric acid to hydrolyze poly phosphates and the resulting ortho phosphate is measured by the molybdate method.

General hydrography

Water samples were analysed for general hydrographic parameters like salinity and dissolved oxygen, following the standard methods (Grasshoff et al., 1983b, 1983c). pH was measured using a portable pH meter and temperature by a sensitive thermometer.

Total suspended solids (TSS)

A known volume of water was filtered through a Whatman GF/F 25mm filter paper held in a filter holder. All filter papers were pre ashed (450°C for 4 hours), pre weighed and held in separate numbered petri dishes. The filter papers containing suspended matter were dried at 59°C in an oven for 24 hours.

Particulate organic Carbon (POC)

POC was determined by the wet digestion of the filter paper containing particulate matter, using acid dichromate followed by titration to determine the concentration of carbon (Parson et al., 1984).
Moisture percentage in sediments

Moisture content was estimated by drying approximately 10g of homogenized wet sediment sample in an oven at 90°C for 48 hours. The difference in weights gave the percentage of moisture in the sediment samples.

Total Organic Carbon in sediment

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 Nitrogen in sediment

Total nitrogen was measured by Kjeldahl method (De Lange et al., 1992). About 1gm finely ground air-dried sediment samples were digested with 6ml of concentrated sulphuric acid and catalyst (Mix katalyser, Merck). When the colour of the solution changes to white greenish it was centrifuged and the clear centrifugates and washings were transferred to a sample holder connected to the steam distillation unit. The ammonia was distilled into 1% boric acid after adding 25ml of 10 N KOH. The distilled ammonium was determined by back titration with 0.1N hydrochloric acid. The total nitrogen (TN) concentrations were determined from the equivalents of ammonia obtained in boric acid.

Exchangeable ammonium and nitrate in sediments

Exchangeable ammonium and nitrate are defined as the amount of ammonium and nitrate extracted by a 2N KCl solution. Analyses of exchangeable inorganic nitrogen (ammonium and nitrate) were carried out on wet sediment sample. The extraction of the exchangeable fraction of inorganic nitrogen including ammonium, and nitrate were done using a solution of 2N KCl (Agemian, 1997). This method involves the shaking of a wet sediment sample in a centrifuge tube with 2N solution of KCI at room temperature for an hour. A portion of the centrifugate was analyzed for ammonium, colorimetrically, using the indophenol blue method, in which it reacts with phenate in the presence of hypochlorite and nitroprusside as catalyst. Another portion was analyzed for nitrate by reduction to nitrite by the method of spongy cadmium (Jones, 1984). The nitrite was determined colorimetrically by reacting with sulphanilamide under acidic conditions to form a diazo compound that couples with N-1 (Naphthyl)–ethylene diamine dihydrochloride to form reddish purple azodye.
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Grain size analysis

Texture analysis was carried out by sieving and pipette analysis. A known weight of wet sediment was dispersed overnight in .025N sodium hexameta phosphate (Calgon) solution. The sand fraction was separated from the dispersed sediments by wet sieving using a 230 mesh (63μm) ASTM sieve (Carvar, 1971). The filtrate containing silt and clay fraction was subjected to pipette analysis (Krumbein and Pettijohn 1938; Lewis 1984).

Total iron in sediments

The oven dried sediment samples were ground 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. Metal concentrations in the solution were determined by atomic absorption spectrophotometry (Perkin-Elmer 3110 AAS), calibrated using secondary standard solutions prepared by appropriate dilution of 1000mgL⁻¹ standard solutions (Merck). Analytical blanks were prepared using the same procedures and reagents.

Chemical fractionation of phosphorus

Phosphorus fractionation was performed by sequential extraction with chelating compounds (Golterman, 1996). This procedure was slightly modified in accordance with validation of different fractionation procedures by Pardo et al. (1998). The different fractions extracted were water exchangeable inorganic phosphate (W-IP), water exchangeable organic phosphate (W-OP), iron bound inorganic phosphate (Fe-IP), iron bound organic phosphate (Fe-OP), calcium bound inorganic phosphate (Ca-IP), calcium bound organic phosphate (Ca-OP), acid soluble organic phosphate (Ac-OP), alkali exchangeable organic phosphate (Alk-OP) and residual organic phosphate (ROP). The extractions were carried out according to the scheme depicted in Fig. 2.2. All organic phosphates were obtained as the difference of total phosphate (TP) and dissolved inorganic phosphate (DIP) in the extract.
Phosphorus Extraction Scheme

Fig. 2.2 Phosphorus fractionation scheme illustrating the sequential extractions
Data analysis

Monthly variations of dissolved nutrients and general hydrographic parameters are presented with a view to find the variations of these parameters within the Stations. Spatial variations are discussed mainly using seasonal distributions which are presented in tables and graphs. The three seasons categorized in this study are pre-monsoon (Feb-May), monsoon (June-Sept) and post-monsoon (Oct-Jan) unless otherwise specified. Correlation analysis was carried out to find the influence of various hydrographic parameters on the distribution of dissolved nutrient concentrations (Snedecor and Kocharan, 1962). Comparison of dissolved nutrients among stations and seasons was carried out using two-way analysis of variance (ANOVA). The nutrient profile of surface and core sediments are illustrated as annual mean variations as well as seasonal variations. The annual mean variations of each parameter was used to compare the spatial variation, while seasonal variations are depicted in a view to identify within the system variations. Comparison of nutrient concentrations between stations, seasons and sediment depth were carried out using three-way anlysis of variance. ANOVAs were followed by a least significant difference test (LSD) if a significant temporal or spatial effect was found. All statistical analyses were conducted as described by Freud and Wilson (1992).

REFERENCES


* Not referred in original