FATTY ACIDS AS BIOMARKERS IN THE MANGROVE SEDIMENTS OF COCHIN

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To my Parents....

For their love and support

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Certificate

This is to certify that the thesis entitled "Fatty acids as Biomarkers in the Mangrove sediments of Cochin" is an authentic record of the research work carried out by Mrs. Manju Mary Joseph, under my supervision and guidance at the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for Ph.D degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any university.

Kochi - 16,
October, 2009.

Dr. N. Chandramohanakumar
(Supervising Guide)

Declaration

I hereby declare that this thesis entitled "Fatty acids as Biomarkers in the Mangrove sediments of Cochin" *is an authentic record of the research work carried out by me under the guidance and supervision of Dr. N. Chandramohanakumar, Professor and Head, Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, and no part of this has previously formed the basis of the award of any degree, diploma, associateship, fellowship or any other similar title or recognition.*

Manju Mary Joseph

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List of Abbreviations

TOM	-	Total Organic Matter
Fe-IP	-	Iron bound Inorganic Phosphorus
Ca-IP	-	Calcium bound Inorganic Phosphorus
ASOP	-	Acid Soluble Organic Phosphorus
Alk-OP	-	Alkali Soluble Organic Phosphorus
R-OP	-	Residual Organic Phosphorus
EF	-	Enrichment Factor
Igeo	-	Geoaccumulation Index
PRT	-	Proteins
CHO	-	Total carbohydrates
LPD	-	Total lipids
LOM	-	Labile Organic Matter
BPC	-	Biopolymeric carbon
TFAs	-	Total Fatty acids
SFAs	-	Saturated Fatty acids
MUFAs	-	Monounsaturated Fatty acids
PUFAs	-	Polyunsaturated Fatty acids
LCFAs	-	Long chain Fatty acids
FAMEs	-	Fatty acid Methyl Esters

Preface

Mangroves ecosystems are recognized as biogeochemically active regions, where organic carbon inputs from a variety of sources undergo intense biogeochemical processing. In addition to their carbon sequestration, these ecosystems are also hotspots in terms of mineralization. Due to their dynamic ecotonal location, these environments display strong spatial and temporal variability of major biogeochemical characteristics. Many aspects of carbon dynamics in mangrove ecosystems still remain unravelled.

Knowledge of the sources and reactivities of organic matter, as well as the factors controlling its distribution, are important to understand the role of mangrove ecosystems in global biogeochemical cycles. In general, bulk parameters are relatively reliable proxies of organic matter origin. Elemental and isotopic compositions of sedimentary organic matter are commonly used to distinguish its sources. Measurement of the biochemical composition of organic matter - proteins, carbohydrates, and lipids- can also help to determine both sources and the quality of organic matter. However, these tools are effective only to differentiate the relative contributions of autochthonous and allochthonous organic matter sources.

Due to the complex nature of organic matter in mangrove sediments, these bulk parameters are not completely successful in revealing the sources. An effective tool for the source characterization of organic matter in mangrove systems is the biomarker approach. Molecular organic biomarkers can serve as proxies to provide insight into how aquatic systems process, metabolise and sequester carbon in both the water column and the sediments that is spread over decadal to geological time scales. They are extremely useful in resolving the complexity of systems with multiple organic carbon sources. Fatty acids are abundant in living organisms and their source specificity with respect to individual compounds and their relative stability when compared to aminoacids and carbohydrates make them ideal as biomarkers.

The studies pertaining to the biogeochemistry of organic matter with special emphasis on source characterisation of Cochin estuary and mangrove areas still remains poorly documented. The main objective of the study was to investigate the sources of organic matter in three mangrove systems of Cochin estuary and to identify the major biogeochemical pathways. Fatty acid biomarkers, $\delta^{13}C$ of total organic matter and the elemental composition were used to characterize the sources of organic matter in these mangrove systems. Assessment of biochemical composition (total lipids, proteins and total carbohydrates) were employed to study the quality and quantity of organic matter. The geochemical characteristics of the systems were also assessed using mineralogy, trace metal analysis and phosphorus fractionation.

The thesis is divided into five chapters. Chapter I is ***Introduction*** and it deals with the aim and scope of the present study. Chapter II, ***Materials and Methods*** describes the general geographical features of the study area and the details of the sampling and analytical methodology. Results of the general hydrographical condition of the study region are also included here.

Chapter III is ***Geochemistry*** and it deals with the seasonal and spatial variations of the geochemical parameters of the surface sediments. It includes the general sedimentary characteristics, mineralogy, elemental composition, heavy metal and phosphorus geochemistry. Chapter IV is ***Biochemical composition***. It discusses the biochemical composition of organic matter in the surface sediments to examine the quality and quantity of organic matter. Attempt has also been made to characterise the sources of organic matter with the help of bulk parameters. Chapter V, ***Fatty Acid Biomarkers***, deals with the source characterisation of organic matter in these complex ecosystems with the help of fatty acid biomarkers. All the biogeochemical characteristics of the three mangrove systems under study are summarised at the end of the thesis.

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Chapter 1
Introduction

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Vegetated coastal ecosystems are critical components of global ocean carbon and nutrient budgets. Despite their relatively small areal extent, their carbon sequestration represents a large inventory of organic matter; comparable to global riverine carbon discharges (Ludwig et al., 1996; Gattuso et al., 1998). Mangrove forests are one of the most productive and biodiverse wetlands on earth. Growing in the inter-tidal areas and estuary mouths between land and sea, mangroves provide critical habitat for a diverse marine and terrestrial flora and fauna. Healthy mangrove forests are key to a healthy marine ecology. Yet, these unique coastal tropical forests are among the most threatened habitats in the world. Due to increased population and urbanization around coasts, the surface area of mangrove ecosystems worldwide declines by approximately 2% per year, implying major changes in the coastal carbon cycle (Duarte et al., 2005).

1.1 Mangrove Ecosystem

Mangroves are specialized coastal ecosystems existing in the intertidal zones of sheltered shores, estuaries, tidal creeks, river mouths, lagoons, and mud-flats of the tropical and sub tropical regions of the world. They form an important ecological asset and economic resource of the coastal environment. Mangroves form a very special association of plants dominated by the mangrove forest as the primary producers interacting with associated fauna and the physical environment. They support unique ecosystems, especially on their intricate root systems. Mangrove areas are ecologically sensitive and provide physical protection for the communities, more importantly they are believed to play a major role in supporting tropical estuarine and coastal food webs (Alongi and Christoffersen, 1992). In areas where roots are permanently submerged, mangroves may be host to a wide variety of organisms, including algae, barnacles, oysters, sponges, and bryozoans. The ecosystem and its biological components are under the influence of both marine and freshwater conditions and have developed a set of physiological adaptations to overcome the problems of anoxia, salinity and frequent tidal inundations. This has led to the assemblage of a wide variety of plant and animal species of special adaptations suited to the ecosystem. Mangrove ecosystems serve as nursery ground for juvenile fish, shellfish crabs, shrimps and molluscs. In addition to the marine organisms, both terrestrial organisms and birds utilize the forest floor, root complex and the canopy.

High primary productivity, efficient biological nutrient recycling and a permanent exchange with terrestrial and marine ecosystems are the common features of mangrove ecosystem (Jennerjahn and Ittekkot, 2002). This ecosystem is considered as the most productive and biodiverse, providing significant functions in the coastal zones (Clough, 1992). The organic matter from mangrove systems is highly important in the coastal food webs and the litter from mangroves and the subsequent formation of detritus and its tidal export to the adjacent coastal waters have also profound effect on promoting biodiversity richness in the coastal environment (Odum and Heald, 1972, 1975; Twilley, 1998; Alongi et al., 1989; Alongi, 1990; Wattayakorn et al., 1990; Robertson et al., 1992).

Mangrove systems act as a buffering zone against natural episodic events such erosion, storm surge and tsunamis (Danielsen et al., 2005).

Mangroves are important stabilizers of sediments, in some areas creating new areas of land over long period of time. Mangroves may intercept terrestrial-derived nutrients, pollutants and sediments before they reach the coastal waters, alleviating problems due to high loading of these anthropogenic constituents (Valiela and Cole, 2002).

Anthropological pressures and natural calamities are the enemies of this ecosystem. The substantial reduction in the global mangrove cover may significantly alter the biogeochemical cycling of elements in tropical coastal waters (Ong, 1982; Vannucci, 1988; Diop, 1993; Lacerda, 1993; Whitten et al., 1996).

1.1.1 Global Distribution

The occurrence of mangroves is largely limited to the regions between 30° north and south of the Equator. A few mangroves out of this area worth mentioning are in the North up to the Bermudas (32°20'N), Japan (31°22'N) as well as in the South, in Australia (38°45'S), New Zealand (38°03'S) and the East Coast of South Africa (32°59'S). There are two center of mangrove diversity- the Eastern group (Australia, Southeast Asia, India, East Africa and the Western Pacific) where the total number of species is approximately 40 and the Western group (West Africa, Caribbean, Florida, Atlantic South America, pacific North and South America) where the number of species is only eight. The most diverse biogeographical regions are in the Indo-West Pacific (Alongi, 2002). According to estimate made by F.A.O. / UNDP a total area of 7.1 million hectare is covered under the mangrove formation in the world. The largest mangrove area occurs in Indonesia (30%) followed by Nigeria (10%), Australia (8%) and Mexico (7%).

Mangroves are one of the most threatened ecosystems of the world. Growing human populations are increasingly converting, polluting, or otherwise disturbing mangrove ecosystems, often with greater or longer-term impacts than natural disturbances (Farnsworth and Ellison, 1996, 1997; Twilley, 1998). Globally, about one third of mangrove forests have been lost within the past 50 years (Alongi, 2002). The establishment of shrimp farms has been the main cause of mangrove loss in many countries over the past 30 years (Rodríguez, 2001). Mangrove deforestation contributing to fisheries declines, degradation of clean water supplies and salinisation of coastal soils, erosion, and land subsidence, as well as the release of carbon dioxide into the atmosphere. This unique ecosystem needs immediate protection and conservation and urgent steps are to be taken to save this fragile ecosystem.

1.1.2 Status of Indian Mangroves

The coastal zone of the mainland of India and Andaman and Nicobar islands is endowed with the presence of extensive and diverse mangrove wetlands. On the macro scale, geomorphic settings of the mangrove wetlands of the east coast of India are different from those of the west coast (Ahmad, 1972). The costal zone of the west coast is narrow and steep in slope due to the presence of the Western Ghats. Secondly, there is no major west-flowing river. As a result, mangrove wetlands of the west coast of India are small in size, less in diversity and less complicated in terms of tidal creek network.

According to Forest Survey of India (FSI, 1999), out of 4, 87,100 ha of mangrove wetlands in India, nearly 56.7% (2,75,800 ha) is present along the east coast, and 23.5% (1,14,700 ha) along the west coast, and the remaining 19.8% (96,600 ha) is found in the Andaman and Nicobar islands (Selvam, 2003). The Sunderban's of India and Bangladesh put together forms the single largest block of mangroves of the world. It covers an area of about 1 million ha, of which 60% is located in Bangladesh and the remaining western portion, lies in India (Choudhuri and Choudhury, 1994). Mangroves are under the serious threat of degradation; India has lost about 40% of its mangrove cover within this century (Krishnamurthy et al., 1987). The recognition of the environmental and economic importance has led the Supreme Court of India to categorize mangrove habitats under the coastal zone regulation – 1 (CRZ – 1) area to ensure that they are sustainably utilized and conserved.

Forest Survey of India (FSI, 2003) reported 800 ha area of mangrove cover in Kerala state, with 300 ha moderately dense and 500 ha open mangrove vegetation. FSI showed that mangrove vegetation in Kerala is now confined largely to river mouths and tidal creeks and that there has been no significant mangrove cover south of Cochin in Kerala coast. This is probably due to the more accelerated destruction of the ecosystem in the southern part as compared to the northern part of the State.

A recent study by Radhakrishnan et al., (2006) showed that mangrove vegetation in four northern districts of Kerala - Kasargod, Kannur, Kozhikode and Malappuram – is approximately 3,500 ha, which represents about 83 per cent of mangrove cover in the State. At Kannamaly and Kumbalangi, mangroves are found in a stretch of around 8 hectares each. Panambukad and Puthuvype have a mangrove cover of around 10 hectares (Suma and Joy, 2003). It is reported that 17 true mangrove species occur in the State (Unni and Kumar, 1997). The dominant mangrove species are *Avicennia marina, Rhizophora mucronata, Acanthus ilicifolius, Excoecaria agallocha, Acrostichum aureum and Cerebra manghas.*

1.2 Mangrove Biogeochemistry

Mangrove ecosystems are complex, with highly interactive plant, animal and microbial life. Mangrove systems are recognized as highly productive, biogeochemically active regions, where organic matter inputs from a variety of sources undergo intense biogeochemical processing and play an important role in the carbon balance of coastal environment (Jennerjahn and Ittekkot, 2002). Mangrove forests have the capacity to efficiently trap the suspended materials from the water column. Litter from trees and subsurface root growth provide significant inputs of organic carbon to mangrove sediments (Alongi, 1998). Besides this, other important organic carbon inputs; including allochthonous riverine or marine material, autochthonous production by benthic or epiphytic micro- or macroalgae, and local water column production by phytoplankton (Bouillon et al., 2004; Bouillon and Boschker, 2006). As a consequence, mangrove environments are sites of intense carbon processing with a potentially high impact to the global carbon budget (Borges et al., 2003; Dittmar et al., 2006; Alongi, 2007).

Mangrove-derived detritus is an important food source for decomposer food webs including many macroinvertebrates (Fratini et al., 2000; Cannicci et al., 2008). Irrespective of the pathways of organic matter consumption and food web structure, organic matter that is not exported by tidal action enters the sediment where it is consumed, degraded and chemically modified. The degradation of organic matter in mangrove sediments is mediated by both aerobic and anaerobic microbial processes using a variety of electron acceptors. A fraction of mangrove detritus escapes degradation and is permanently buried within the mangrove sediments or adjacent ecosystems. While some mangrove forests largely retain detritus within their sediments, others lose a major fraction of their net primary production to the adjacent coastal waters mainly through tidal forcing. Despite their relatively small areal extent, the mangroves are known to be potentially significant sources of organic matter to the adjacent estuaries and coastal waters on a global scale (Jennerjahn and Ittekkot, 2002; Dittmar et al., 2006).

In addition to their carbon sequestration, these ecosystems are also "hotspots" (McClain et al., 2003) in terms of mineralization (Middelburg et al., 2005). Mangroves may also act as sinks for other elements including nitrogen and phosphorus (Nedwell, 1975; Odum et al., 1982; Robertson and Duke, 1987).

The mangrove sediments are generally anaerobic and highly reduced. Anaerobic processes are of major importance in the mangrove sediments and sulfate reduction along with aerobic respiration account for almost all the diagenetic carbon degradation in mangroves. Generally, sulfate reduction is the major diagenetic pathway in mangroves (Alongi et al., 1998; Alongi et al., 2000), but in some cases aerobic degradation predominates (Alongi et al., 2000; Alongi et al., 2001). The role of anaerobic processes in mangrove element cycling however is largely unresolved. Organic matter produced or deposited on the sediment surface in mangrove forests supports aerobic and anaerobic detritus food chains (Kristensen et al., 2000). Organic matter belongs to the most dynamic component of sediment and they participate in a variety of biogeochemical processes that significantly alter molecular structures and distributions. Climate, tidal flooding, vegetation evolution and bioturbation are parameters that also contribute to the complexity of the geochemistry of mangrove inhabited deposits (Marchand et al., 2004).

The amount of organic matter found in the sediment is a function of the amount of various sources reaching the sediment surface and the rates at which different types of organic matter are degraded by microbial processes during burial. Degradability can further be modified in time as less available fractions remain (Middelburg, 1989) or decreased by adsorption to clay minerals (Keil et al., 1994). The majority of organic matter produced in surface waters by autotrophic organisms is not incorporated in to the surface sediments, but is recycled in the water column or at the sediment water-interface (Harvey, 2006).

The sources of organic carbon stocks in mangrove sediments have rarely been studied in detail, although this should be an important factor when constructing any carbon budget of the mangrove ecosystems. The carbon sequestration estimates are not well constrained and our understanding of the ecological fate of these large quantities of organic matter is far from complete (Bouillon et al., 2004). So far, budgeting and productivity studies in the mangrove areas have primarily been based on litterfall estimates (Jennerjahn and Ittekkot, 2002), which obviously leads to underestimation of carbon dynamics.

1.3 Biomarkers

The biogeochemical properties of mangroves are the least understood because of their sediment complexity due to the tidal influx of allochthonous organic matter and also the autochthonous inputs. Bulk parameters are relatively reliable proxies of organic matter origin, in general. Elemental and isotopic compositions of sedimentary organic matter have been commonly used to distinguish organic matter from different sources (Meyers, 1994; Schelske and Hodell, 1995).This could only differentiate the relative importance of two end member organic matter sources-autochthonous and allochthonous (Middelburg et al., 1997). When more detailed information is required, molecular source indicators, i.e. "biomarkers" can be applied.

Biomarker is defined as "a molecule whose carbon skeleton can unambiguously be linked to that of a known biological precursor compound" (Killops and Killops, 2005). They are complex organic compounds, which originated from formerly living organisms (Simoneit, 2002). Biomarkers are characteristic of an organism/plant which can be used to indicate the presence of the organism/plant in the environment and to estimate its biomass (Parrish et al., 2000; Mfilinge et al., 2005).

Molecular biomarkers are easily determined compounds that tell us about the history of a sample. They can be signatures of the condition of a sample, they can tell us about the past events and even about the future when certain compounds are used as early warning signals (Parrish et al., 2000). Three principal characteristics permit biomarkers to be distinguished from many other organic compounds (Peters et al., 2006). These are 1) biomarkers have structures composed of repeating subunits, indicating that their precursors were components in living organisms; 2) each parent biomarker is common in certain organisms; and 3) these biomarkers can be abundant and widespread.

Molecular biomarker analyses have been extensively used in geochemical studies (Villanueva et al., 1997; Guzman-Vega and Mello, 1999) but there is now increasing interest in their use in ecological studies. Biomarker studies of modern environments will greatly aid source identification in sediments (Volkman et al., 1998; Parrish et al., 2000). Lipids are one such group that receives increasing amounts of attention in ecological (Sargent et al., 1987) and biogeochemical (Saliot et al., 1991; Conte et al., 1995) studies.

1.3.1 Lipid compounds as Biomarkers

Lipids are a broad group of naturally-occurring molecules which includes fatty acids, terpenes, terpenoids, sterols, steroids, fat-soluble vitamins, phospholipids and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules. The molecular distribution of lipid biomarkers provides particularly useful information about the source, diagenetic alteration, preservation and historical changes in organic matter, as well as changes in trophic status (Hayashi and Takii, 1977; Wakeham and Canuel, 1990; Laureillard and Saliot, 1993; Canuel et al., 1995; Budge and Parrish, 1998; Sun et al., 2000; Koch et al., 2003; Meyers, 2003; Muri et al., 2004; Alfaro et al., 2006). Although lipids make up only a small percentage of bulk organic matter (Meyers, 2003), they have been widely used in many geochemical studies (Cranwell, 1981; Meyers et al., 1984; Findlay et al., 1995; Canuel and Martens, 1996; Koch et al., 2003; Burns et al., 2004; Muri et al., 2004).

An astonishing variety of different lipids have been found in marine sediments and the water column attesting to the diversity of biosynthetic pathways employed by the aquatic organisms. Many of the compounds have distinctive structures allowing them to be used as biomarkers for particular sources of organic matter in the marine ecosystems. Microalgae synthesize many unusual compounds, such as long chain alkenones, alkenoates and alkenes, long chain alkyl diols, highly branched isoprenoid alkenes as well as distinctive sterols and unsaturated fatty acids, thus enabling inputs of microalgal organic matter to be easily recognised (Han et al., 1968; Volkman, 1986; Pond et al., 1998; Rontani et al., 2001). The input of terrestrial organic matter to marine environments can be recognized from lipids of higher plant origin, such as long chain alcohols, alkanes and fatty acids, and C_{29} sterols (Volkman, 1986; Boon and Duineveld, 1996; Volkman, 2006). Bacteria synthesise a diverse range of compounds such as branched fatty acids, hopanoids and isoprenoids, many of which are particularly stable, for instance those that contain an ether bond (Waples et al., 1974; Brassell et al., 1981; Claustre et al., 1989; Kannenberg and Poralla, 1999). Qualitative assignments of organic matter sources are thus reasonably straightforward. The combination of lipid biomarker data with information from stable isotopes can provide good estimate of organic matter sources (Volkman, 2006). Some of the lipids and their derivatives which can be used as biomarkers in the biogeochemical studies of aquatic environments are described here.

1.3.1.1 Steroidal Compounds

Sterols and their diagenetic derivatives are ubiquitous lipid compounds in sediments. Their structures contain a number of unique features such as positions of double bonds, alkylation in the ring system and the side chain, and stereochemistry which makes them ideal for assigning sources of organic matter and for studying its short-term fate (Volkman et al., 1998; Volkman, 2003). Microalgae are the primary source of sterols in the marine environment. Some species show a predominance of a single sterol, such as cholesterol in marine eustigmatophytes, 24-methylcholesta-5,22E-dien-3β-ol in some diatoms and a mixtures of 4-desmethyl and 4-methyl sterols in some species of dinoflagellates (Volkman, 1986). Dinoflgellates are the major source of 4-methyl sterols in marine systems and the C_{30} sterol, 4ά, 23,24-trimethyl-5ά-cholest-22E-en-3β-ol(dinosterol) is often used as a biomarker for dinoflagellates (Volkman et al., 1993). Sterols with a fully saturated ring system (5ά(H)- stanols) occur in all marine sediments where they are thought to be formed by bacterial reduction of stenols (Volkman, 2006). The presence of 5β(H)-stanols in sediments is often taken as evidence for the presence of fecal-derived organic matter (Nishimura, 1982). 5β(H)-stanols are formed in sediments under highly reducing conditions and its presence can be considered as an indicative of reducing condition (Venkatasan and

Santiago, 1989). Phyto-sterols like β-sitosterol have recently been identified as biomarker for mangroves (Koch et al., 2003).

1.3.1.2 Terpenoids

The terpenoids sometimes called isoprenoids, are a large and diverse class of naturally-occurring lipid compounds similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups, but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are particularly useful to assign sources of organic matter (Volkman, 2006). The most common terpenoids in sediments include triterpenoids and hopanoids. Many pentacyclic triterpenoids such as taraxerol, germanicol, ά- and β- amyrin have been considered as biomarkers for mangroves (Killops and Frewin, 1994; Koch et al., 2003). Hopanoids are synthesized in bacteria and their presence in sediments are considered as bacterial origin (Kannenberg and Poralla, 1999).

1.3.1.3 Hydrocarbons

Hydrocarbons conceal a great variety of chemical structures that can be found in marine organisms and the multitude of structures formed by degradation of functionalized lipids (Moldowan et al., 1992). Alkanes isolated from marine environments typically fall in to two categories. Those with odd chains such as n-C_{15}, n-C_{17} and n-C_{19} are indicative of algal inputs (Han et al., 1968). Long chain (n-C_{20} to n-C_{35+}) alkanes that display strong predominance of odd chain lengths indicates a contribution from terrestrial plants (Volkman et al., 1997). Hydrocarbons from eroded sediments often display distinctive sterane and hopane distributions (Rowland and Maxwell, 1984).

The C_{19} isoprenoid alkane, pristane is common in marine samples, reflecting its abundance in some zooplankton species (Blumer et al., 1963). C_{20} isoprenoid phytane can also be found, either reflecting petroleum inputs or a contribution from Archaebacetria (Volkman and Maxwell, 1986). Another isoprenoid that is ubiquitous in sediments is lycopane (Sinninghe Damste et al., 2003). It seems to be particularly abundant in sediments that were deposited under anoxic conditions suggesting that the lycopane/ C_{31} n-alkane ratio could be used as a proxy for oxic conditions (Sinninghe Damste et al., 2003).

Simple branched alkenes such as 7-and 8-methyl heptadecane are found in many species of cyanobacteria (Han et al., 1968), and in algal mats and lagoonal sediments. The most common unsaturated hydrocarbon found in marine sediments is the hexa-unsaturated alkene n- $C_{21:6}$, which are produced by many species of microalgae by decarboxylation of the $C_{22:6\ (n-3)}$ fatty acid (Lee and Loeblich, 1971). n-$C_{21:5}$ and n-$C_{21:4}$ alkenes are also present in microalgae such as diatoms and dinoflagellates' (Volkman et al., 1994). The presence of such highly labile alkenes in sediments usually indicates intact (perhaps living) algal cells.

An unusual class of highly branched isoprenoid alkenes (HBI alkenes) has been recognised in many studies of marine sediments (Gearing et al., 1976). HBIs have an unusual coupling of C_5 isoprene units producing a "T" shaped molecule and typically have 2-4 double bonds (for C_{25}

alkenes) or 4-6 double bonds (for C_{30} alkenes). A diatom origin was suggested by the high abundance of C_{25} HBIs in sediments and sea water from the Peru upwelling (Volkman et al., 1983).

1.3.1.4 Aliphatic Ketones

A surprising diversity of aliphatic compounds containing carbonyl groups has been found in sediments and sea water. Some of these are natural products, while others are formed by diagenetic reactions (Volkman, 2006). Distributions of long-chain (C_{19}-C_{35}) ketones having a carbonyl group at the 2-positions (metyl ketones) have been found in some coastal marine sediments (Volkman et al., 1983). These compounds can be derived from oxidation of n-alkanes via the intermediate alkan-2-ols (Cranwell et al., 1987).

Very long straight chain (C_{35}-C_{40}) unsaturated methyl and ethyl ketones with trans double bonds are termed as alkenones (Volkman et al., 1995). Microalgae contain several new alkenones including monounsaturated homologs, as well as the corresponding long chain alkenols (Rontani et al., 2001). Alkenones are ubiquitous in marine sediments and the ratio has been found to vary systematically with the seawater temperature in which the microalgae grow (Marlowe et al., 1984; Prahl and Wakeham, 1987). This has prompted many paleoceanographic studies that have used the ratio of concentrations of tri- to di-unsaturated C_{37} ketones in sediments (some over 100 million years old) to estimate the paleotemperature when the sediments were deposited (Brassell et al., 1986; Prahl and Wakeham, 1987; Sikes et al., 1997).

1.3.1.5 Fatty acids

Fatty acids are long chain alkanoic acids and refer principally to straight chain, saturated or unsaturated monocarboxylic acids with carbon numbers, usually ranged from 12 to 28. Fatty acids are the major constituents of lipids in living organisms. The myriad structures of fatty acids biosynthesized by organisms (van Vleet and Quinn, 1979; Bobbie and White, 1980), and their source specificity make them useful as biomarkers (Lee and Wakeham, 1988; Wakeham and Lee, 1993). Numerous studies have used the source information provided by fatty acids to estimate the relative contributions of terrestrial, algal or planktonic and bacterial fatty acids to the total pool in marine sediments (Volkman et al., 1980; Smith et al., 1983; Prahl et al., 1989; Gong and Hollander, 1997; Wakeham et al., 1997; Budge and Parrish, 1998; Canuel, 2001; Zimmerman and Canuel, 2001; Camacho- Ibar et al., 2003).

Fatty acids with carbon atoms $\geq$ 22 are synthesized mostly by vascular plants and are considered indicative of higher plant markers in sediments (Kolattukudy, 1970; Scribe et al., 1991; Colombo et al., 1996; Carrie et al., 1998). The odd carbon-numbered and branched-chain (iso- and anteiso-) fatty acids are generally considered to be synthesized by bacterial communities (Jeffries, 1972; Volkman et al., 1980), and are therefore used as biomarkers of bacteria (Parkes, 1987). Unsaturated fatty acids are generally associated with algae (Colombo et al., 1996; Meziane and Tsuchiya, 2000).

However, the majority of such studies have been focused on marine and lacustrine sediments, while only a few have been carried out on mangrove sediments (Killops and Frewin, 1994; Meziane and Tsuchiya, 2000; Koch et al., 2003; Bouillon et al., 2004; Versteegh et al., 2004; Mfilinge et al., 2005).

1.4 Aim and scope of the study

Mangrove forests, one of the most productive and biodiverse wet lands on earth, are among the most threatened habitats in the world. They provide an excellent supply of organic matter in the early food chain of coastal and insular habitats. The biodiversity and the nursery character shown by them authenticate the evaluation of the biogeochemistry of these ecosystems. Since mangroves are considered to be major supporter of the coastal aquatic life, the present study has a special significance in predicting the management requirements of the aquatic system. Cochin estuary requires a special reference; as it is a part of the large Vembanad-Kol wet land ecosystem (a Ramsar site), which is one of the largest polluted water bodies.

The biogeochemical properties of mangroves are least understood because of their sediment complexity due to the tidal influx of allochthonous organic matter and the autochthonous inputs (Bouillon et al., 2004). Due to their dynamic ecotonal location, these environments display strong spatial and temporal variability of major biogeochemical characteristics. In order to understand the relative importance of biogeochemical processes, it is necessary not only to characterise and quantify the organic matter but also to identify its major sources.

Many biogeochemical studies in the mangrove ecosystem focus on mangrove trees, as mangrove litter fall and root biomass have been implicated as the ultimate source of organic matter (Kristensen et al., 1995; Alongi, 1996; Bouillon et al., 2000).This obviously leads to underestimation of carbon dynamics as the organic matter in these sediments mostly consists of different sources, including locally produced macrophyte material, microphytobenthos, and suspended organic matter imported to the ecosystem during tidal inundation (Bouillon and Boschker, 2006). Due to the complex nature of organic matter in these sediments, the bulk parameters are not completely successful in revealing the sources of organic matter in mangrove sediments. Biomarkers have proved themselves as an effective tool for the source characterisation of organic matter in mangrove systems. Fatty acids, because of their abundance in living organisms, their source specificity with respect to individual compounds and their relative stability when compared to aminoacids and carbohydrates are ideal biomarkers. The relative abundances of individual fatty acids are useful in evaluating the respective importance of inputs from microalgae, marine fauna, bacteria and higher plants (Sargent et al., 1987).

The studies pertaining to the biogeochemistry of organic matter with special emphasis on source characterisation of Cochin estuary and mangrove areas still remains poorly documented. The present study investigates the sources of organic matter in three mangrove systems of Cochin estuary to identify the major biogeochemical pathways. The objectives of the study are

- To find the geochemical characteristics of the systems using mineralogy, heavy metal analysis and phosphorus fractionation.
- To assess the quality and quantity of organic matter in the mangrove systems using biochemical composition (total lipids, proteins and total carbohydrates).
- To characterise different fatty acids in the surface sediments of mangrove systems.
- To identify the sources of organic matter in the mangrove systems using fatty acid biomarkers, $\delta^{13}C$ of total organic matter and the biochemical composition.
- To evaluate the efficacy of fatty acids as biomarkers of mangrove ecosystem.

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Chapter 2

Materials & Methods

Contents

Cochin estuary is the largest estuarine system in the southwest coast of India. It is a part of Vembanad-Kol 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 1m 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 Kolkatta 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: Puthuvyppu

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 gymnorhiza* (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 ilicifolis*, *Rhizophora apiculata*, *Rhizophora mucronata*, *Excoeacaria 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 (*Phalacrocroax niger*) and night heron (*Nychcorax nychcorax*) (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.

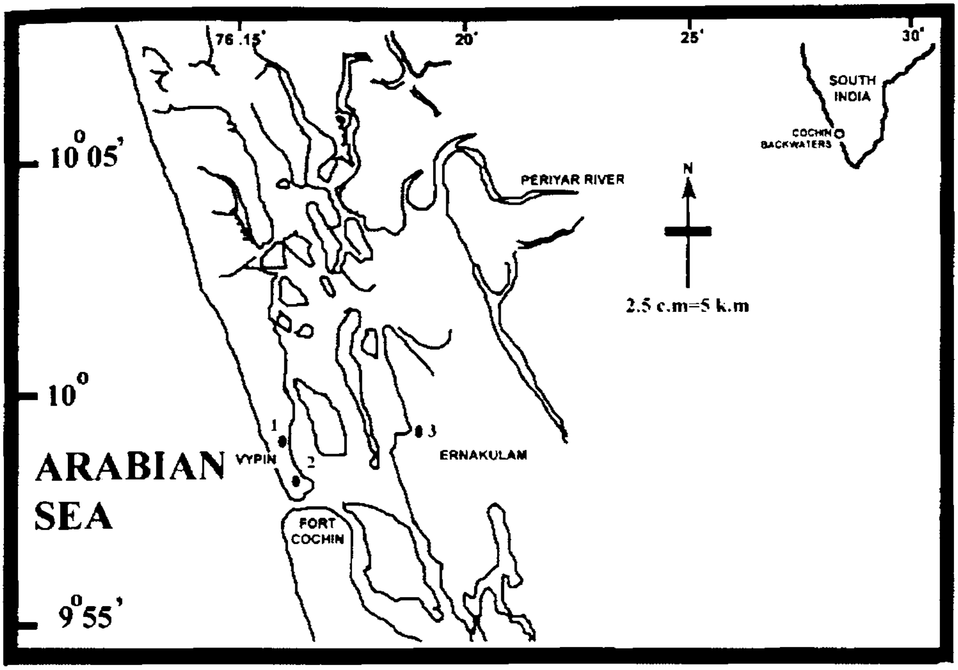

Figure 2.1 Location of the mangrove stations

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 trifoliate, Excoecaria agallocha, Kandelia candel, Lumnitzera recemosa, 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 trifoliate, Kandelia candel, Lumnitzera recemosa, Rhizophora mucronata, Rhizophora apiculata* and *Sonneratia caseolaris*.

Figure 2.2 Mangrove vegetation in the study region

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 a 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.

Rhizophora mucronata *Avicennia officinalis*

Acanthus ilicifolius Linn *Bruguiera gymnorrhiza*

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 spectophotometrically 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% H_2O_2. This analysis is based on Stoke's law. Sediment was dispersed in sodium hexametaphoshate 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 $HClO_4$:HNO_3). 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)

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

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 H_2SO_4 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 $K_2S_2O_8$ 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).

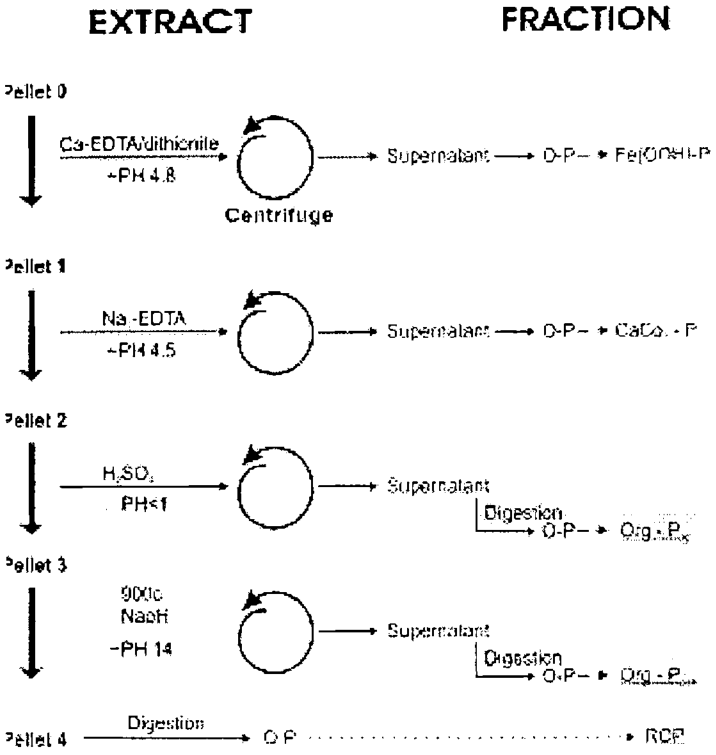

Figure 2.4 Sequential extraction scheme for phosphorus fractionation

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 ($\delta^{13}C_{\text{TOM}}$) was carried out using Flash EA interfaced with IRMS (*FINNIGAN* DELTA $^{\text{PLUS}}$ 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:

$$\delta 13\mathrm{C} = \frac{13C/12Csample}{13C/12CPDBS\tan dard} - 1\mathrm{X}100$$

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 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% BF_3/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 $CaCO_3$/l. Dissolved oxygen varied from hypoxic to saturated conditions (1.4 to 10.2 mg O_2/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

Parameters	Station 1			Station 2			Station 3		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
pH	6.6	7.1	7.4	7.1	7.0	7.6	7.5	-	7.2
Salinity (psu)	34.0	1.6	28.5	34.0	1.3	29.2	33.8	-	13.9
Alkalinity (mg$CaCO_3$/l)	164	132	144	216	132	132	92	-	68
DO (mgO_2/l)	6.4	3.0	1.4	10.2	4.2	2.9	4.2	-	4.2
Nitrite (μmol/l)	1.2	1.4	0.43	1.2	1.4	1.2	1.5	-	2.1
Nitrate(μmol/l)	2.2	2.4	2.7	1.4	1.6	4.4	4.4	-	8.1
Phosphate (μmol/l)	49.7	14.8	16.4	28.5	16.2	5.3	18.4	-	7.5
Silicate (μmol/l)	50.0	63.0	61.2	20.2	43.4	23.0	3.6	-	4.0

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Chapter 3

Geochemistry

Contents

3.1 Introduction

Mangrove ecosystems play an important role in the biogeochemistry of nutrients, organic matter and heavy metals in the coastal environment. The potential role of the mangrove ecosystems as sinks for anthropogenic contaminants in the tropical and subtropical areas has been widely recognised (Silva et al., 1990; Badarudeen et al., 1996; Machado et al., 2002; Alongi et al., 2004). Mangrove systems have a high adaptation capacity to the extreme environmental conditions and are characterised by the high rates of organic carbon accumulations (Marchand et al., 2004).Mangrove ecosystems play an important role in nutrient cycling and energy flow in most of the tropical coasts (Odum and Heald, 1974; Lacerda et al., 1993; Silva and Mozeto, 1997). However, the knowledge of biogeochemical processes in the sediments of the tropical mangrove forests are still limited (Kristensen et al., 1995; Robertson and Phillips, 1995).

3.1.1 Heavy Metals in Mangrove Sediments

Tidal activities, land runoff and rainfall are the major sources of heavy metals into the mangrove systems. Besides this, the mangroves are at risk from heavy metals that are associated with a diverse range of anthropogenic activities including shipping, dredging, and urban wastewater discharges. Many mangrove ecosystems are close to the urban development areas and are impacted by urban and industrial run-off, which contains trace and heavy metals in the dissolved or particulate form (Tam and Wong, 2000; MacFarlane, 2002; Preda and Cox, 2002). Therefore concentrations of metals in the mangrove sediments could reflect the degree and sources of anthropogenic pollution (Tam and Wong, 1995). Mangrove sediments, being anaerobic, reduced and rich in organic matter and sulphide, favour the retention and accumulation of heavy metals (Lacerda et al., 1991; Tam and Wong, 1995; Badarudeen et al., 1996). Several studies indicate that these anoxic, fine-grained, and organic-rich mangrove sediments can reduce the potentially deleterious effects of metal contamination because of the low availability of metals accumulated within the sediments for remobilization and biotic uptake (Lacerda et al., 1991; Sadiq and Zaidi, 1994; Tam and Wong, 1995). However, the degradation of mangrove forests tends to increase worldwide (Farnsworth and Ellison, 1997), which can promote the remobilization of sediment-trapped metals and facilitate its transport to adjacent areas (Lacerda, 1998).

The adsorption and desorption of heavy metals in the mangrove sediments depend on one or a combination of sediment properties including pH, redox potential, cationic exchange capacity, organic matter, clay content, salinity, iron and manganese oxides and the presence of other metals (Lacerda et al., 1993; Tam and Wong, 1995). The cycling of organic matter through litter production, decomposition and tidal transport, may eventually export a fraction of the accumulated heavy metals, and therefore convey it to detritus food chains in the adjacent coastal waters (Murray, 1985; Silva, 1988).

3.1.2 Phosphorus Geochemistry

Phosphorus is an essential element that limits marine primary productivity, by which it is intimately involved in the marine biogeochemical cycles (Howarth et al., 1995; Tyrell, 1999). Phosphorus cycle in tropical mangroves is multifarious because of the periodic flooding of sediment with both fresh and saline waters (Salcedo and Medeiros, 1995). Phosphorus retention and release in mangrove sediments depends on several factors including pH, redox potential, tidal inundation, the nature of phosphorus compounds supplied to the sediment-water interface, sedimentation rate, bioturbation, diagenetic processes etc. (Ingall and Van Cappellen, 1990; Ruttenberg and Berner, 1993; Silva and Sampaio, 1998; Schenau and de Lange, 2001). Sediment phosphorus is particularly important in shallow systems as various forms of bioavailable phosphorus in the upper sediments can be a major source of phosphorus to the water column biota via numerous physically, chemically and biologically mediated processes (Reddy and D'Angelo, 1994; Kufel et al., 1997). Analysis of total phosphorus loading alone does not provide information regarding the phosphorus availability to the biota or the potential for recycling through sediment-water interactions as it is transported to downstream locations (James et al., 2001). An exact stoichiometric and structural identification and quantification of inorganic phosphorus species are very complicated (Luderitz and Gerlach, 2002). A common route to estimate the stock of potentially accessible forms is to fractionate phosphorus based on the extractability by leaching reagents of increasing aggressiveness (Golterman, 1996; Ting and Appan, 1996; Perkins and Underwood, 2001; Tiyapongpattana et al., 2004).

Sequential leaching extractions were proposed to understand the association of phosphorus with various sedimentary components and to estimate its mobility, solubility, or biological availability (Reynolds and Davies, 2001). Phosphorus fractionation in the sediments can provide valuable information on the origin of phosphorus in sediments, diagenetic transformations of organic phosphorus in sediments, the degree of pollution from anthropogenic activities, the bioavailability of phosphorus in sediments and the biogeochemical processes (Andrieux and Aminot, 1997; Jensen et al., 1998; Schenau and De Lange, 2001; Filippelli et al., 2003). Although many different extraction schemes may exist, the underlying extraction mechanisms are the same (Table 3.1).

It must be kept in mind that sequential fractionation schemes are rather crude methods; with many extractants causing the dissolution of more than one type of phosphorus solid phase as various extractants are unlikely to be either exhaustive or unique with respect to the target compounds (Turner et al., 2005). Sequential extraction techniques are rather laborious, tedious, time consuming and it is subjected to several potential errors including the risks of contamination due to sample manipulation and underestimation of given fractions due to re-adsorption phenomena (Buanuam et al., 2006).

In spite of these shortcomings these fractionation schemes are used widely since these schemes use only small quantity of soil samples ($\leq$0.5 g), relatively simple to perform, and require only basic laboratory equipments. To avoid disadvantages like the uncertainty in the bioavailable and the non-specific nature of the extraction solutions, an extraction scheme using chelating agents was developed by Golterman, (1996). This "EDTA method" is useful for the extraction of inorganic phosphorus pools with lesser modifications of the organic pool (Golterman, 1996).

Table 3.1 Some of the commonly used sequential extraction schemes

Scheme	Extractants	Phosphorus Fraction
1. Chang and Jackson (1957) modified by **Hartikainen (1979)**	a) 1 M Ammonium Chloride b) 0.05 M Ammonium Fluoride c) 0.1 N Sodium Hydroxide d) KCl-$C_6H_8O_6$-EDTA	Loosely bound P Aluminium bound P Iron bound P Occluded P
2. Hieltjes and Lijklema (1980) and **Nurnberg (1998)**	a) 1 M Ammonium Chloride b) 0.11 M Sodium bicarbonate and Dithionate c) 0.1 N Sodium Hydroxide d) 0.5 N Hydrochloric acid e) Persulphate digestion of NaOH extraction f) Persulphate digestion of remaining sediment	Loosely bound P Iron bound P Aluminium bound P Calcium bound P Labile organic/Polyphosphate P Refractory Organic P
3. Psenner et al., (1984) Modified by **Hupfer et al., (1995)**	a) NH4Cl- at neutral pH b) Buffered dithionite c) NaOH d) HC e) Digestion	Labile, loosely bound or adsorbed P Reductant soluble P, bound to Fe oxides and hydroxides P adsorbed to metal oxides and bound to humic substances P bound to carbonates, apatite-P and released by the dissolution of oxides Organic and refractory P
4. SEDEX procedure Ruttenberg, (1992) Modified by **Anderson and Delaney (2000)**	a) CDB (citrate-dithionite-bicarbonate) b) 1 M sodium acetate buffered to pH 4 c) 1 N hydrochloric acid d) 50% magnesium nitrate to sediments, drying in oven at 80°C, and ashing at 550°C, 1 N HCl	Labile and oxide-associated P Authigenic CFA Detrital Apatite P Organic P
5. De Groot and Golterman (1993)	a) Buffered CaEDTA/dithionite b) Na_2EDTA c) H_2SO_4 d) 2M NaOH pH 1 with H_2SO_4 e) Digestion	Iron bound P Calcium bound P Acid solubile P Alkali solubile P Humic& Fulvic P Residual Organic P
6.Jensen and Thamdrup (1993)	a) $MgCl_2$ b) Buffered dithionite extraction c) NaOH d) HCl Digestion	Loosely sorbed P Iron bound P Al/clay mineral and humic bound Calcium bound P Refractory organic P

Sediments conserve important environmental information (VonGunten et al., 1997), and are increasingly recognised as both a carrier and a possible source of contaminants in aquatic systems (Forstner and Salomons, 1991; Tessier et al., 1994).The distribution of heavy metals in the mangrove sediments were assessed to find the geochemical characteristics and also the degree of anthropogenic influence on these systems. The phosphorus fractions in the mangrove systems were analysed to quantify the different forms of phosphorus in these systems and to find the processes leading to the fractional distribution of phosphorus.

3.2 Results

The spatial and seasonal variations of different geochemical parameters in the surficial sediments of the study region, including texture, mineralogy, major and trace elemental composition are explained in this chapter. Different fractions of phosphorus and their seasonal variations are also detailed.

3.2.1 General Sedimentary Characteristics

General sedimentary characteristics of the surficial sediments of the mangrove systems are given in Table 3.2. Texture analysis revealed that silt was the major fraction during all seasons in these mangrove sediments. By applying Folk (1974) classification (Fig.3.1), station 1 is silty during pre-monsoon and changes to sandy mud during monsoon. It was muddy during post-monsoon season. Station 2 did not show any seasonal variations in texture and was muddy in nature. Station 3 was sandy mud in nature during pre-monsoon and changed to silty in post-monsoon. The sediment pH ranged from 5.85 to 7.1 in the mangrove stations. Eh values showed that the sediments were generally anoxic and station 3 was found to be highly reducing.

Table 3.2 General sedimentary characteristics of the study region (± SD)

Parameters	Station 1			Station 2			Station 3		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
Sand (%)	6.73 ± 1.9	36.60 ± 4.8	8.87 ± 2.86	4.34 ± 1.1	2.32 + 0.91	0.94 ± 0. 09	23.59 ± 2.6	-	10.46 + 1.92
Silt (%)	71.79 ± 6.8	28.51 ± 3. 0	58.92 ± 4.17	59.97 ± 2.1	63.78 ± 6.7	59.38 + 6.9	55.56 ± 3.1	-	70.47 ± 5.7
Clay (%)	21.45 ± 2.21	34.89 ± 2.69	32.20 ± 2.32	35.69 ± 2.71	33.90 ± 2.78	39.68 ± 2.79	20.97 ± 3.1	-	19.07 ± 2.22
pH	6.2	5.85	7	6.46	6.48	7.1	6.65	-	6.6
Eh	-98	-16	-10	12	-41	-53	-337	-	-398

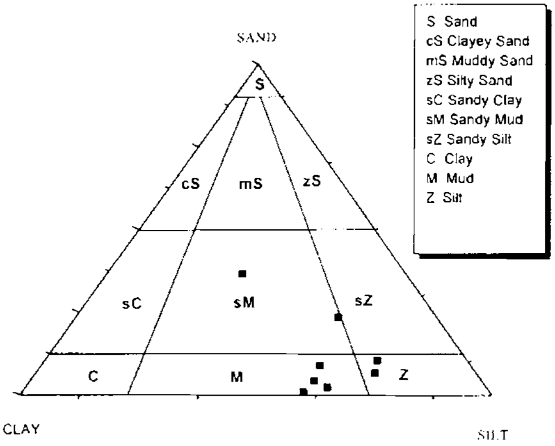


Figure 3.1 Ternary diagram showing the grain size distribution. Sediment nomenclature after (Folk, 1974)

3.2.2 Mineralogy

X-ray diffraction (XRD) is an important tool in the mineralogy for identifying, quantifying and characterising minerals in complex mineral assemblages (Moore and Reynolds, 1997). Powder X-ray diffraction analyses of the representative sediments of the study region showed that perovskite and sodalite were the dominant minerals at station 1 and 2 respectively. Presence of monetite, a rare calcium phosphate mineral, was observed at station 3 along with silica. (Fig.3.2).

3.2.3 Major Elements

Since the major elements are conservative in nature and unlikely to be affected by diagenesis, only the post-monsoon samples were analysed to access their background levels (Table 3.3). XRF analysis showed that Silicon was the major element in all the three stations, followed by Aluminium and Iron. XRF analysis revealed very high calcium and phosphorus at station 3, when compared to the other two stations. The SEM-EDS analysis of station 3 sediment (Fig. 3.3) also showed high mass percentages of calcium (9.79) and phosphorous (5.33). Sulphur content was also significantly higher at station 3. But Silicon, Aluminium and Iron were comparatively lower at station3, compared to the other stations. TGA analysis showed a moisture content of 7 % and the weight loss after 900^0 C was about 25%.

CHN-S analysis of the mangrove sediments (Table 3.4 and Fig. 3.4) showed that they were rich in total carbon. It ranged from 2.91 to 7.64 % and was higher at station 3. Generally, post-monsoon season was found to have higher carbon content, except for station 1. Organic carbon constituted 75 to 93% of total carbon and was also higher at station 3. It also followed the seasonal trend of total carbon. Total nitrogen content ranged from 0.27 to 0.66 % and was higher during the post-monsoon season, except at station

1. It was also higher at station 3 during all the seasons. Total sulphur varied from 0.22 to 1.96 % and was very high at station 3. It was found to be higher during the post-monsoon season at station 1 and 2, but station 3 showed high concentration during pre-monsoon.

Analysis of variance (ANOVA, Two-Factor without replication) showed that sediment texture did not have any significant seasonal variations in these ecosystems. Carbon and nitrogen showed no significant variations in mangroves, while sulphur was significantly higher at station 3.

Table 3. 3 Major elemental composition and TGA results (weight %) of the mangrove sediments

Compound	Station 1	Station 2	Station 3
SiO_2	40.030	39.570	35.060
TiO_2	0.748	0.820	0.552
Al_2O_3	16.63	17.94	13.14
MnO	0.033	0.04	0.034
Fe_2O_3	7.99	8.96	4.86
CaO	**1.434**	**0.807**	**7.278**
MgO	2.901	2.799	1.947
Na_2O	3.250	2.325	2.302
K_2O	1.547	1.316	0.885
P_2O_5	**0.630**	**0.622**	**6.760**
SO_3	0.096	0.158	1.25
Cr_2O_3	0.034	0.033	0.02
CuO	0.009	0.007	0.01
NiO	0.010	0.010	0.000
Rb_2O	0.006	0.006	0.003
SrO	0.029	0.016	0.054
ZnO	0.000	0.012	0.044
ZrO_2	0.013	0.010	0.012
Loss of Ignition at 110°C	7.40	7.67	7.34
Loss of Ignition at 900°C	24.61	24.63	26.66

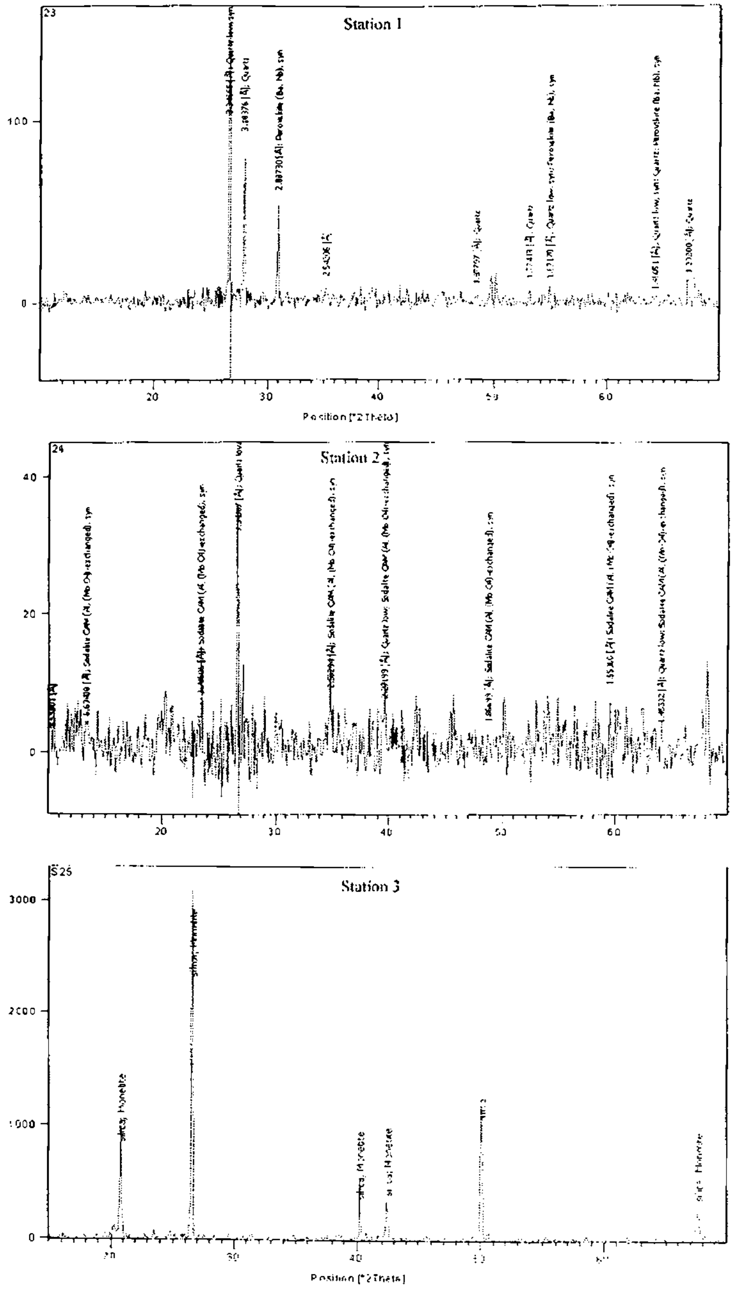


Figure 3. 2 XRD spectra of the sediments in the study region

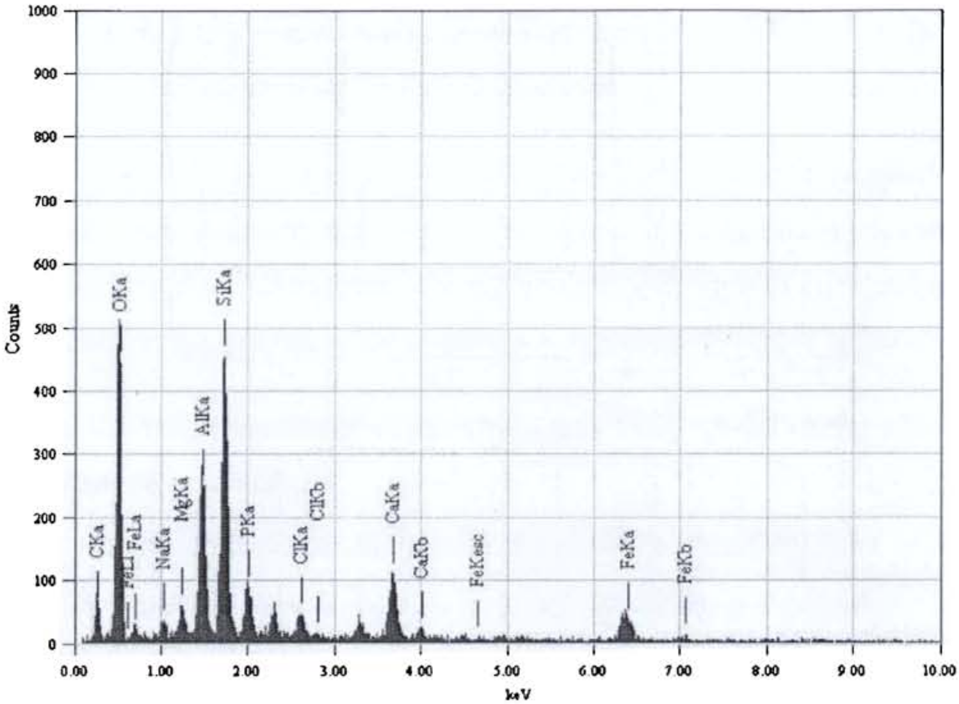


Figure 3.3 SEM-EDS spectrum of station 3 sediment

Table 3. 4 Seasonal variations of Carbon, Nitrogen and Sulphur in the study region.

Parameters	Station 1			Station 2			Station 3		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
Total Carbon (%)	3.73 ± 0.11	6.75 ± 0.07	4.72 ± 0.04	2.91 ± 0.06	3.31 ± 0.05	6.25 ± 0.11	5.52 ± 0.09	-	7.64 ± 0.09
Organic Carbon (%)	2.8 ± 0.01	6.3 ± 0.13	3.9 ± 0.03	2.2 ± 0.02	2.5 ± 0.03	4.9 ± 0.05	5.0 ± 0.06	-	6.7 ± 0.13
Total Nitrogen (%)	0.34 ± 0. 07	0.50 ± 0.01	0.32 ± 0.01	0.27 ± 0.05	0.29 ± 0.006	0.46 ± 0.009	0.47 ± 0.01	-	0.66 ± 0.01
Total Sulphur (%)	0.32 ± 0.006	0.25 ± 0.005	0.62 ± 0.1	0.35 ± 0.012	0.22 ± 0.004	0.63 ± 0.013	1.96 ± 0.02	-	1.14 ± 0.023

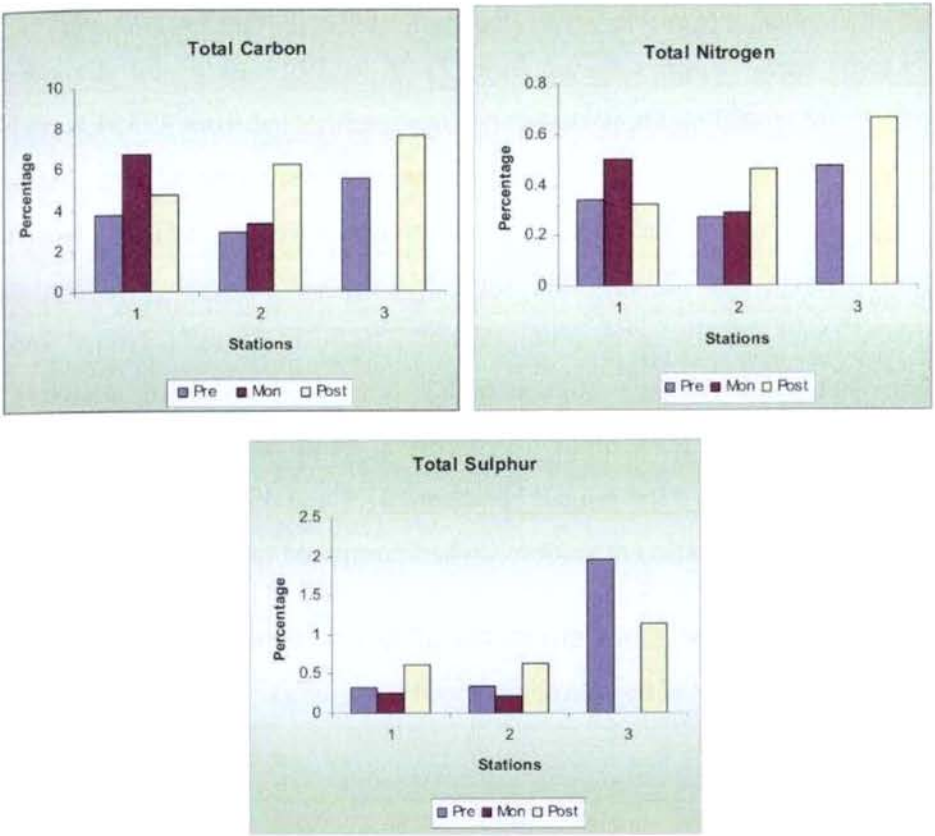


Figure 3.4 Seasonal variations of Carbon, Nitrogen and Sulphur

3.2.4 Heavy Metals

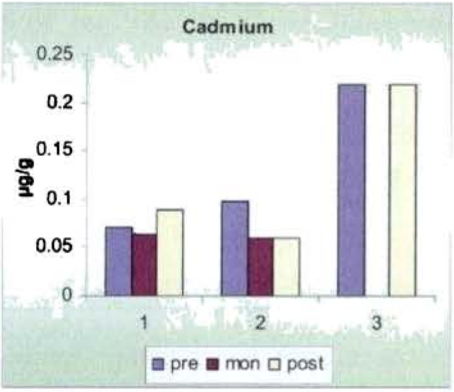
The heavy metals analysed during the study were Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn. The total concentrations of the various heavy metals estimated in the surface sediments of the three mangrove ecosystems are furnished in Table 3.5 and Fig.3.5. Cadmium ranged between 0.06 and 0.22 μg/g and was higher at station 3. Seasonal variations were not prominent in the case of Cd. Cobalt ranged between 12.83 and 23.08 μg/g and did not show any seasonal variations. Co was found to be lower at station 3, compared to the other stations. Chromium was low at station 3 and the range was from 53.30 to 90.23 µg/g. Cr also did not show any seasonal trend. Copper varied from 23.98 to 39.13 µg/g and showed no significant spatial and temporal variations. Iron varied from 42554 to 58304 µg/g and did not show any variations between seasons and stations. Magnesium was found to be comparatively lower at station 3 and the overall range was 12386-17817 µg/g. Mn did not show any seasonal and spatial trend and the range obtained was 210.5-315.35 µg/g. Nickel varied from 30.60 to 69.35 µg/g and was higher at station 2 and lower at station 3. Lead also showed the similar distributional trend and the range was from 19.5 to 39.50 µg/g. Zinc ranged between 101.3 and 455.68µg/g and showed very high concentration at station 3 when compared to other stations.

Table 3.5 Average Concentrations of heavy metals estimated in the surface sediments of the mangrove systems (µg/g)

Metal	**station 1**			**station 2**			**station 3**		
	pre	**mon**	**post**	**pre**	**mon**	**post**	**pre**	**mon**	**post**
Cd	0.07	0.064	0.09	0.10	0.06	0.06	0.22	-	0.22
Co	22.3	17.73	21.43	22.2	22.8	23.08	12.83	-	15.7
Cr	90.23	73.18	76.08	85.28	89.78	89.05	53.3	-	63.28
Cu	30.75	24.98	23.98	27.75	31.38	31.58	29.18	-	39.13
Fe	58304	42554	45617	57554	57429	57117	56179.3	-	57429
Mg	17817	12386	15299	17242	16886	16836	12386.1	-	12686
Mn	315.33	227.83	257.83	227.2	210.5	299.08	260.95	-	225.95
Ni	55.5	55.43	54.75	68.75	69.35	65.58	30.6	-	40.25
Pb	35.25	21.25	37.5	39.5	33.25	32.5	25.25	-	19.5
Zn	128.8	101.3	111.3	116.3	112.55	132.55	315.05	-	455.68

3.2.5 Phosphorus Fractionation

Fractionation of phosphorus in mangrove sediments (Table 3.6, Fig. 3.6) revealed significant spatial variations for different phosphorous fractions among the three systems under study. Fe-IP varied from 825 to 2080 µg/g among the mangrove systems. Fe-IP was the major fraction in the first two stations accounting for 38.2 % and 37.57 % of the total phosphorus. Ca-IP ranged from 505 to 24764 µg/g. Station3 was exclusively dominated by Ca-IP contributing to about 87% of total phosphorous. ASOP varied from 201 to 1555 µg/g and was almost uniformly distributed in all the three systems with a percentage range of 5.2-13. Alk-OP ranged from 428 to 738 µg/g. This fraction was very low at station 3 (2.2 %), while it showed similar distribution pattern in other two stations. R-OP ranged from 48 to 92 µg/g. R-OP was the smallest fraction (0.2-3.8% of total phosphorous).


Cadmium
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3
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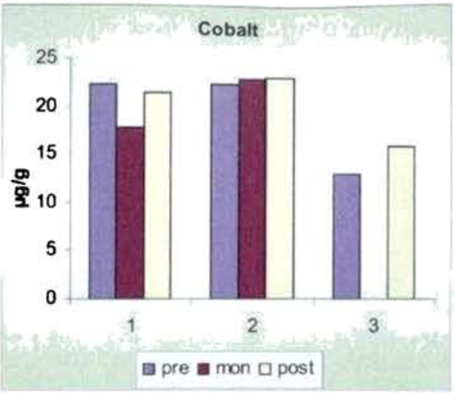
Cobalt
µg/g
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20
15
10
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1
2
3
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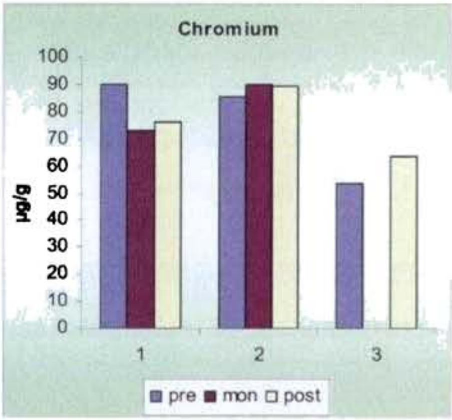
Chromium
µg/g
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1
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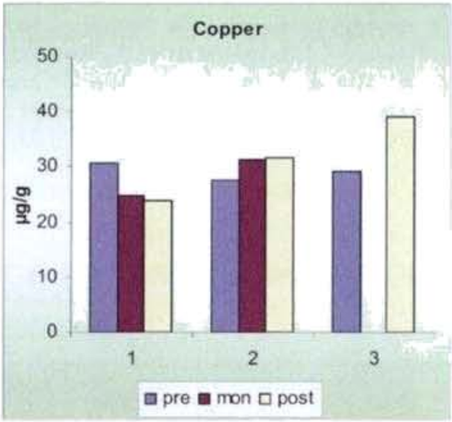
Copper
µg/g
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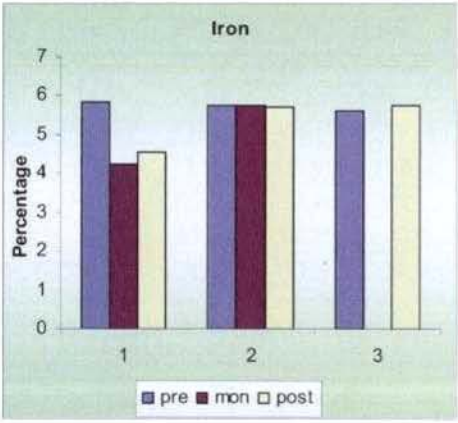
Iron
Percentage
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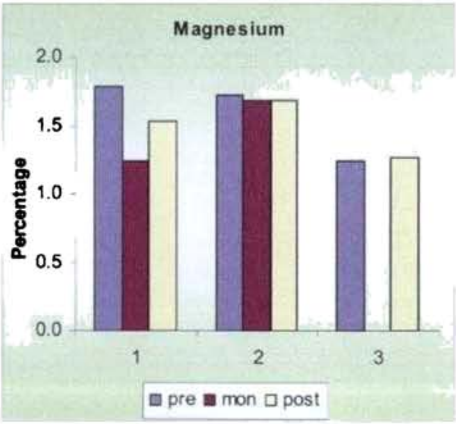
Magnesium
Percentage
2.0
1.5
1.0
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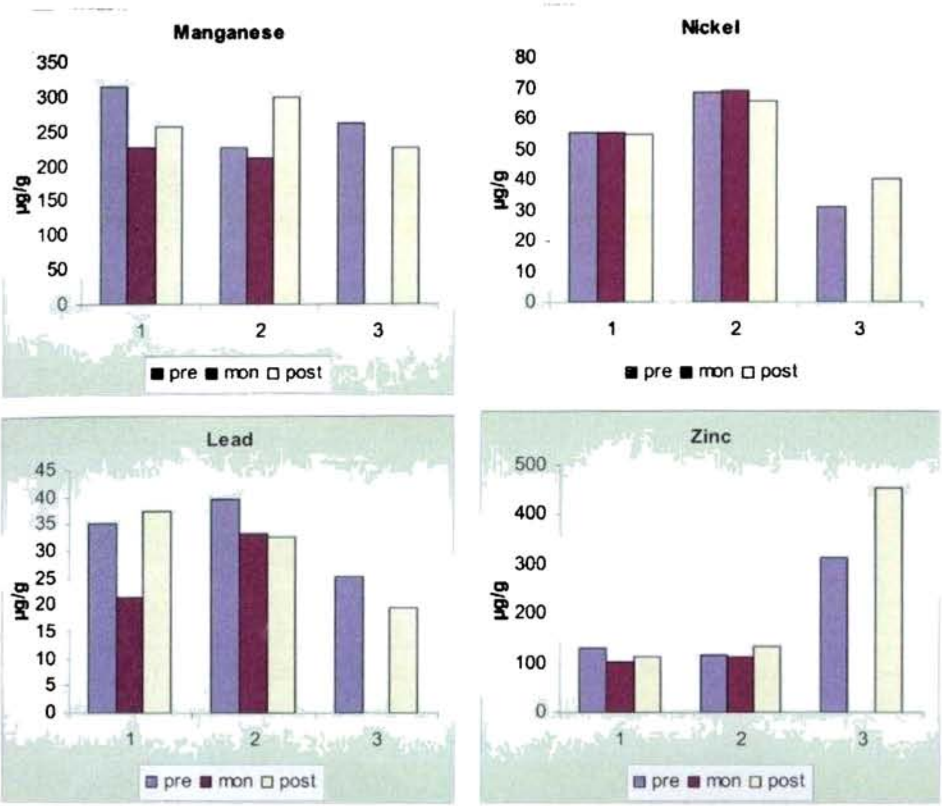

Figure 3.5 Seasonal variations of Heavy metals in the study region

Total phosphorous, calculated as the sum of all fractions, varied between 2226 to 28665 µg/g. Comparing to other two stations, station 3 showed about 10 fold increases in total phosphorous content. First two stations did not show any noticeable difference in phosphorous fractions and inorganic fractions constituted to about 65% of total phosphorus. But at station 3, inorganic fractions were about 92%. Bioavailable fractions of phosphorus at stations 1 and 2 were about 75% whereas at station 3, these were about 98%. Analysis of variance (ANOVA) showed that there are no significant seasonal variations for any of the phosphorus fractions in the study region. But considerable spatial variations were observed for Ca-IP and ASOP ($p<<0.001$), which were significantly higher at station 3. Fe-IP did not show any significant variation between stations, while ROP was significantly ($p= 0.01$) lower at station 3.

Table 3.6 Different fractions of phosphorus in the study region (µg/g ± SD)

Parameters	Station 1			Station 2			Station 3		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
Fe-IP	856 ± 22	825 ± 19	909 ± 24	1142 ± 34	979 ± 26	982 ± 32	1129 ± 36	-	2080 ± 31
Ca-IP	727 ± 21	541 ± 13	532 ± 17	1019 ± 26	505 ± 28	803 ± 17	24764 ± 171	-	24287 ± 189
ASOP	201 ± 3	289 ± 7	236 ± 5	322 ± 8	274 ± 10	318 ± 6	1555 ± 16	-	1499 ± 27
Alkali-OP	452 ± 13	501 ± 21	506 ± 12	428 ± 11	539 ± 17	738 ± 21	504 ± 10	-	735 ± 18
R-OP	72 ± 1.5	70 ± 2.1	66 ± 1.9	76 ± 1.2	90 ± 2.1	92 ± 2.7	48 ±1.4	-	64 ± 1.6
Total-P	2308 ± 58	2226 ± 54	2249 ± 56	2987 ± 73	2387 ± 59	2933 ± 71	28000 ± 192	-	28665 ± 209

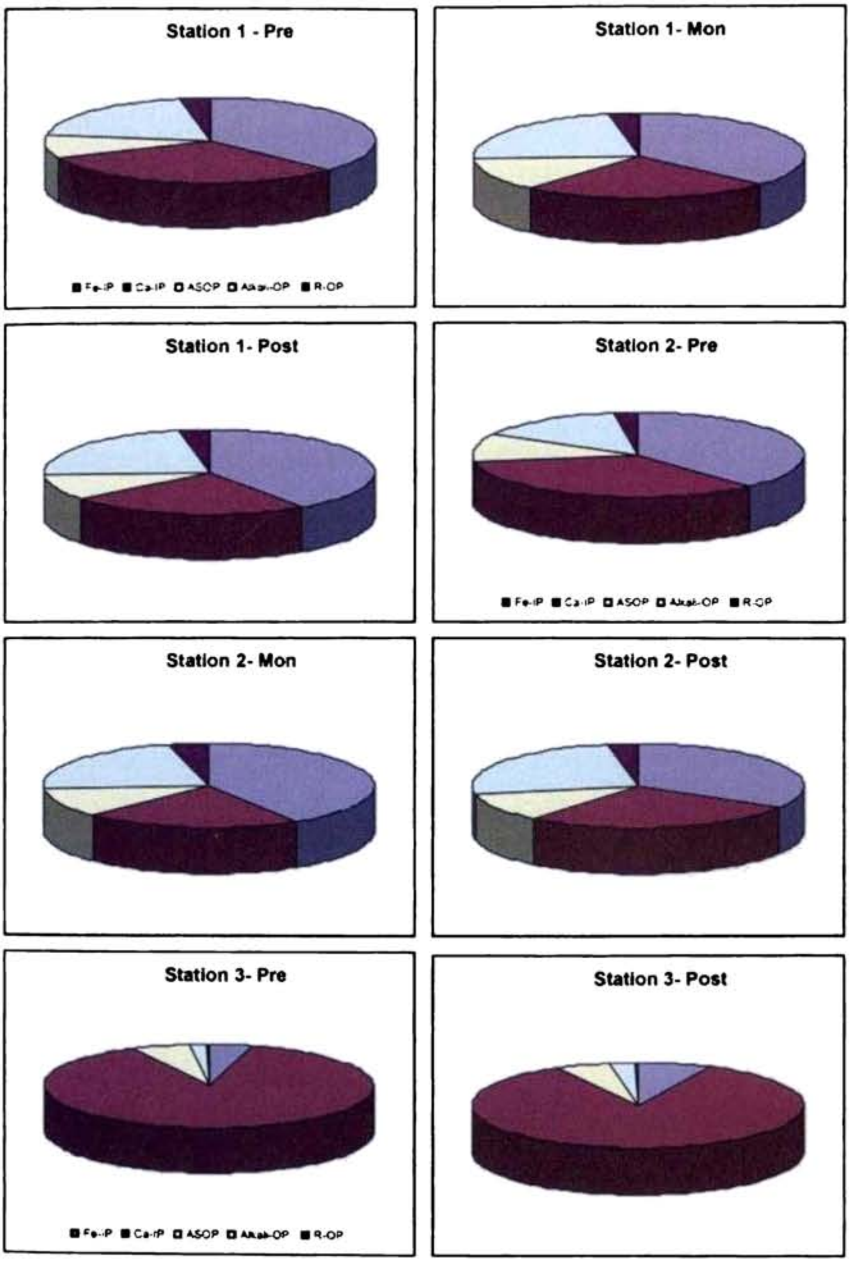

Figure 3.6 Seasonal variations of different phosphorus fractions. Pre, Mon and Post denote pre-monsoon, monsoon and post-monsoon seasons respectively

3.3 Discussion

General sedimentary analysis showed the predominance of fine substratum and silt was the major fraction in all the seasons in these mangrove sediments. The texture of the sediments has a significant role on the physico-chemical processes as well as on the species diversity of the depositional environment (Badarudeen et al., 1996). It is well documented that fine particles often concentrate metals due to their greater surface area and amounts of organic carbon, clay, iron, or aluminium (Santschi et al., 2001) and also proportionally higher concentrations of associated trace elements (Myers and Thorbjornsen, 2004). The sediments were slightly acidic also. High redox potential indicates that the sediments were generally anoxic in nature and station 3 was found to be highly reducing.

Mineralogical fingerprints might be employed as tools to explore the dynamics and transport of particle and sediments associated with local environments. XRD analysis showed that perovskite and sodalite were the dominant minerals at station 1 and 2 respectively. Perovskite is a calcium titanium oxide mineral ($CaTiO_3$) and Sodalite is a Sodium aluminium silicate chloride mineral ($Na_4Al_3(SiO_4)_3Cl$). Monetite, an anhydrous calcium phosphate mineral ($CaHPO_4$) was detected at station 3.

Station 3 is a congregation of communally breeding birds. The bird excreta and their remains are not effectively flushed away by tides as this mangrove system is linked to the adjacent micro-tidal estuary by a narrow canal, the only source for tidal propagation. This results in the accumulation of bird guano and death and decay of the birds, rich source of phosphorous. Monetite formation is interpreted as being the result of reaction between guano and clay mineral or carbonate rocks (Onac and Veres, 2003). Eh analysis revealed that this unique system is highly reducing during tidal influx. There are also reported evidences for high anoxic condition of this unique ecosystem. Authigenic filamented pyrites have been reported at Mangalavanam (Rosily, 2002), the presence of which can be taken for the highly anoxic conditions (Jeng and Huh, 2001). 5α-cholestan-3β-ol, which is reported in anaerobic sediments (Volkman et al., 1998) is also found in Mangalavanam (Narayanan, 2006). During low tide, the sediments get exposed to the atmosphere for considerably longer periods. These periodic fluctuations in redox potential create a metastable condition, which might favour the formation of monetite. Drier and acidic conditions of the sediment favour the formation of monetite (Onac and Veres, 2003). The presence of monetite indicates the complexity of the system as it is a thermodynamically metastable calcium phosphate mineral (Effler, 1987).

Major elemental analysis using XRF and SEM-EDS revealed high mass percentages of calcium and phosphorous at station 3, which was about 10 times higher than the other two stations. This could be taken as a confirmation for the existence of the rare mineral monetite.

Nutrient elements in the study region did not show any seasonal trend. No correlation was observed between CNS composition and texture indicating the absence of any granulometric dependence. The positive correlation of sediment carbon with total nitrogen indicates a common source of organic matter to these elements. These elements also showed significant negative correlations with the redox potential suggesting their preservation in the anoxic condition. It is reported that organic matter is better preserved under anoxic conditions (Lehmann et al., 2002).

Stoichiometric ratios of nutrients are utilised to determine the origin and transformation of organic matter (Yamamuro, 2000). By mass, Redfield (1958) predicts a C:P ratio of 40 and N:P ratio of 7 for algal material, while Hecky et al., (1993) indicated that a wider range of C: P and N:P ratios in aquatic sediments can still be considered to follow Redfield (C:P = 28 to 56 and N:P = 4 to 9). The C: P ratio varied widely in the study region (1.8 to 28.3) and was very low at station 3. N:P ratio also showed considerable variations among stations and ranged between 0.17 and 2.25. It was also very low at station 3, indicating high enrichment of phosphorus at station 3. Both these ratios did not show any seasonal variations and were far below than Redfield ratio. Hence there is phosphorus enrichment in all the systems. The lower N:P ratios also indicate that benthic nitrogen recycling is in excess of phosphorous and denitrification and the benthic release of nitrogen might play a role in sustaining the productivity of the system. The very high silt + clay content in these systems might favour the higher retention of phosphorus. Clay mineral, such as kaolinite which is abundant in tropical sediments is very efficient in phosphate adsorption (Alongi et al., 1992).

The overall order of heavy metal concentration in sediments was Fe > Mg > Mn > Zn > Cr > Ni > Pb >Cu > Co > Cd. The heavy metal composition in the study region was comparable to the other reported works (Table 3.7), but showed higher values for zinc. ANOVA showed that cadmium was significantly higher at station 3 ($p=0.0040$) and no significant

Table 3.7 Previous reported works on the heavy metal distribution of different aquatic sediments

Location	Heavy metals (µg/g)										Reference
	Cd	Co	Cr	Cu	Fe(%)	Mg (%)	Mn	Ni	Pb	Zn	
Cochin Estuary	0.8 - 18			5.0-61	4.4-29.1		15-640			116-1385	Paul and Pillai, 1983
Cochin Estuary	0.3-8.4	1-25	4.0-100	1-50	2.8-20.2		7-170		3-50	14-259	Nair, 1992
Mangroves of Panama	6.6-10		10-23.3	4-56.3	0.17-0.98		143-295	27.3-91.8	32.5-78.2	10.9-105	Guzman and Jimenez, 1992
Cauvery estuary			6-85	2-46	0.58-2.93	1.20-1.70	264-1800		12-47	14-120	Ramanathan et al., 1993
Cochin Estuary	0.06 - 4.20	1.40 - 5.30	0.50 - 3.50	0.17-1.12	0.114-2.694		0.50-217.0			0.20-192.0	Jayasree and Nair, 1995
Veli Mangroves		20-25		23-61	0.83-1.95		60-320		16-87	44-80	Badarudeen, 1997
Kochi Mangroves		44-122		12-69	1.56-6.78		60-171		20-49	15-83	Badarudeen, 1997
Pichavaram mangrove	2-10	19-54	89-186	20-81	2.34-4.69	0.41-0.52	385-1248	38-86	6-17	50-130	Ramanathan et al., 1999
Vellar estuary	4-9	14-36	28-43	4-20	2.03-3.10	0.39-0.57	86-130	15-31	4-8	71-122	Ramanathan et al., 1999
Mangrove sediments , U.A.E.	3.12-6.94	6.14-14	8.28-18.9	5.31-29.4			28.8-169	14.9-109	13.2-49.8	4.59-22.4	Shriadah, 1999
Singapore's mangrove sediment	0.18-0.27		16.61-32.07	7.06-32				7.44-11.65	12.28-30.98	51.24-120.23	Cuong et al., 2005
Brisbane and Logan Estuaries			1-85	BDL-67	0.12-3.93		57-751	6-75	7-107	1-246	Cox and Preda, 2005
Mangroves of Kerala	BDL-2.98	7.0-22	1.91 - 163.72	0.13 - 243.3	0.53-95.44		12.16 - 325.98		0.73 - 144.68	3.88 - 439.70	Sarika, 2005
Cochin Estuary		5.6-26.1	15-121	5.4-53.2	1.41-6.18		141-337	16-66.5	19.3-71.3	92-1266	Balachandran et al., 2006
Cochin Estuary		3.05-37.8	0.35-110.5	0.08-44.14	0.21-8.26	0.015-1.36	7.3-361.6	3.24-110.6	5.96-81.56	2.71-181.84	Renjith and Chandramohanakumar, 2009
Cross River Estuary mangrove swamp			19.2-37.9	23-33.4	0.06-.094	.0003-.0004		23.1-35	18.3-26.6	126.4-153.4	Essien et al., 2009
Cochin Mangroves	0.06-0.22	15.70-23.08	53.30-90.23	23.98-39.13	4.25-5.83	1.24-1.78	210.5-315.35	30.60-69.35	19.5-39.50	101.3-455.68	**Present Study**

seasonal variations were observed. Cobalt also did not show any seasonal trend, but was significantly lower (p= 0.0055) at station 3. Cr also followed the trend of Co and was also significantly lower (p= 0.0156) at station 3. Copper, iron, magnesium and manganese did not show any significant spatial and seasonal variations. But nickel was significantly lower (p= 0.0008) at station 3. Lead showed no significant seasonal and spatial variations. Zinc was significantly higher (p=0.0023) at station 3.

Correlation analysis of the chemical parameters of the mangrove sediments (Table 3.8) revealed that there was no granulometric dependence on heavy metal distribution. Mg and Pb showed highly significant negative correlation with organic carbon and Zn showed significant positive correlation with total nitrogen. No correlation with pH was observed for all the metals. Co, Cr and Ni had significant positive correlations with redox potential and negative correlation with sulphur. Cd and Zinc showed significant inverse correlation with Eh and positive correlation with sulphur. The interrelations between the heavy metals are tabulated in Table 3.9.

Mangrove systems play an important role in the biogeochemistry of heavy metals in tropical coastal areas, and are considered to have the capacity to act as a sink or buffer and to remove or immobilize heavy metals before they reach nearby aquatic ecosystems (Tam and Wong, 1995).The major processes of metal retention in aquatic sediments include cation exchange, complexing with organic molecules, precipitation as oxides, oxyhydroxides and carbonates, and precipitation as sulphides (Dunbabin and Bowmer, 1992).

Table 3.8 Correlations of heavy metals with other sedimentary parameters

	Sand	Silt	Clay	pH	Eh	Total C	Org. C	Total N	Total P	Total S	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Sand	1																			
Silt	-0.86	1																		
Clay	-0.16	-0.32	1																	
pH	-0.59	0.39	0.17	1																
Eh	-0.18	-0.17	0.81	-0.1	1															
Total C	0.48	-0.32	-0.22	0.06	-0.55	1														
Org. C	0.61	-0.43	-0.25	-0.05	-0.56	0.99	1													
Total N	0.43	-0.17	-0.43	-0.03	-0.74	0.95	0.94	1												
Total P	0.25	0.04	-0.73	0.14	-0.97	0.53	0.56	0.71	1											
Total S	0.26	-0.1	-0.57	0.35	-0.83	0.42	0.45	0.51	0.89	1										
Cd	0.23	0.06	-0.73	0.16	-0.93	0.45	0.49	0.62	0.98	0.89	1									
Co	-0.69	0.42	0.62	0.16	0.81	-0.6	-0.68	-0.69	-0.87	-0.82	-0.86	1								
Cr	-0.62	0.39	0.56	-0.01	0.77	-0.58	-0.67	-0.64	-0.86	-0.86	-0.87	0.97	1							
Cu	-0.36	0.6	-0.5	0.14	-0.69	0.35	0.28	0.57	0.58	0.28	0.49	-0.2	-0.12	1						
Fe	-0.66	0.71	-0.3	0.24	-0.36	-0.3	-0.37	-0.06	0.29	0.21	0.26	0.12	0.2	0.69	1					
Mg	-0.8	0.59	0.37	0.19	0.61	-0.76	-0.85	-0.75	-0.69	-0.61	-0.66	0.92	0.91	-0.06	0.45	1				
Mn	-0.23	0.25	-0.14	0.26	0.03	-0.02	-0.1	-0.07	-0.16	0.07	-0.18	0.23	0.25	-0.03	0.21	0.37	1			
Ni	-0.52	0.18	0.79	0	0.86	-0.55	-0.61	-0.65	-0.87	-0.87	-0.87	0.92	0.92	-0.24	0.04	0.78	-0.07	1		
Pb	-0.67	0.41	0.42	0.33	0.67	-0.85	-0.89	-0.91	-0.66	-0.45	-0.56	0.79	0.69	-0.41	0.2	0.87	0.28	0.65	1	
Zn	0.12	0.21	-0.74	0.15	-0.97	0.59	0.59	0.78	0.96	0.76	0.93	-0.76	-0.74	0.74	0.33	-0.61	-0.16	-0.78	-0.67	1

Table 3.9 Significant inter relations between the heavy metals

Metal	Positive	Negative
Cd	Zn	Co, Cr, Ni
Co	Cr, Mg, Ni	Cd, Zn
Cr	Co, Mg, Ni	Cd, Zn
Cu	Zn	-
Fe	-	-
Mg	Co, Cr, Ni, Pb	-
Mn	-	-
Ni	Co, Cr, Mg	Cd, Zn
Pb	Co, Mg	-
Zn	Cd, Cu	Co, Cr, Ni

Transformations during early diagenesis and changing redox state characterize the chemical forms of metals in aquatic sediments.The major changes that occur in redox conditions between oxic waters and anoxic sediments had profound influences on the speciation and bioavailability of many heavy metals. Sulfate is the major electron acceptor driving organic matter oxidation in anaerobic marine sediments, after oxygen utilization in the top few millimeters (Gaillard et al., 1989). Under oxic conditions, Pb, Ni and Co can be easily adsorbed on oxide fractions (Lienemann et al., 1997; Zwolsman and van Eck, 1999; Dong et al., 2000). On the contrary, in anaerobic conditions, active sulphide co-precipitation rapidly removes Co, Cu, Ni, Pb and Zn from the dissolved phase (Balistrieri et al., 1994; Clark et al., 1998; Schlieker et al., 2001).These specific behaviour of the heavy metals under varying redox conditions can be used as tracers of geochemical characteristics. The mangrove sediments can be considered as a massive, suboxic bed reactor (Aller, 1998), the repetitive redox cycling may induce dissolution of some forms of heavy metals.

Pyrite is an important sink for heavy metals since many of them are incorporated during its formation (Boulegue et al., 1982). As soon as sulphate-reduction began, dissolved Fe concentrations will decrease, reflecting the co-precipitation of Fe and S in the form of framboidal pyrite (Marchand et al., 2003). Active sulphide co-precipitation during anaerobic conditions results in preferential rapid removal of metals from the dissolved phase (Clark et al 1998; Schlieker et al 2001). This seems to be the plausible mechanism for higher concentrations of heavy metals in the study region. The presence of rich organic matter and the reducing environment in this substratum creates a selective affinity for heavy metals.

Cd showed highly significant negative correlation with Eh and positive correlation with sulphur. Adsorption and desorption of Cd is highly variable depending on the type of colloid and local pH-Eh conditions. Cd can be adsorbed in larger quantities by organic matter or Fe oxyhydroxides, but the presence of other metals, especially Zn, can inhibit the adsorption of Cd (Alloway, 1990). Zinc is a very mobile element under oxidizing and acid conditions; however in reducing environments, Zn substantially decreases in mobility due to its affinity for sulphur and the tendency to form sulphide phases (Thornton, 1983; Alloway, 1990). Its significant negative correlation with Eh and positive correlation with sulphur support the retention under anoxic conditions.

Under oxic conditions, Pb, Ni and Co can be easily adsorbed on Mn oxides (Lienemann et al., 1997; Zwolsman and van Eck, 1999; Dong et al., 2000).Chromium is of low geochemical mobility in any conditions of pH-Eh, although at low pH the presence of Mn oxides can promote Cr oxidation to more mobile phases (McGrath and Smith, 1990). This particular heavy metal which does not form sulphide minerals is immobilized in refractory organic compounds in mangrove sediments (Lacerda et al., 1991). In the present study, the poor association of Mn with other metals suggests that Mn-oxide may be only a minor host phase for these elements in this reducing mangrove environment. Lead is the only chalcophile element that is immobile under any pH- Eh conditions, although acidic conditions can trigger Pb desorption to a greater degree than alkaline environments.

Enrichment Factor (EF)

Normalisation is a powerful tool for the regional comparison of heavy metals content in sediments and can also be applied to determine enrichment factors for the studied metals with respect to crusted average (Nolting et al., 1999). However, the most readily interpretable information on anthropogenic contamination is often provided by examining the concentrations of single elements, after adjustment for grain size effects. Differentiating the metals originating from human activity and those from natural weathering is an essential part of geochemical studies. One such technique largely applied is 'normalisation' where metal concentrations were normalised to a textural or compositional characteristic of sediments. Iron (Fe) was chosen as geochemical normaliser because of its conservative nature during diagenesis (Berner, 1980).

The enrichment factor was calculated for each metal using iron as normalising element by the following equation,

$$EF - (\text{metal}/\text{Fe})\ \text{sediment}/\ (\text{metal}/\text{Fe})\ \text{crust}$$

EF values were interpreted as suggested by Birth (2003) for metals studied with respect to natural background concentration. EF < 1 indicates no enrichment, EF < 3 is minor enrichment, EF = 3–5 is moderate enrichment, EF = 5–10 is moderately severe enrichment, EF = 10–25 is severe enrichment, EF = 25–50 is very severe enrichment and EF >50 is extremely severe enrichment.

An EF value less than 1.5 suggests that the metals may be entirely from crustal materials or natural weathering processes (Zhang and Liu, 2002; Feng et al., 2004). However, an EF value greater than 1.5 suggests that a significant portion of the heavy metal is delivered from non-crustal materials or non-natural weathering processes and that the heavy metals are provided by other sources (Feng et al., 2004).

The analysis of the estimated enrichment factors of the various heavy metals in the study area (Table 3.10) indicated a minor enrichment for Pb and Zn and no enrichment for other metals.

Table 3.10 Enrichment of heavy metals in the mangrove sediments.

metal	station 1			station 2			station 3		
	pre	mon	post	pre	mon	post	pre	mon	post
Cd	0.18	0.23	0.3	0.26	0.16	0.17	0.6	-	0.59
Co	0.26	0.28	0.32	0.26	0.27	0.27	0.15	-	0.18
Cu	0.54	0.6	0.53	0.49	0.55	0.56	0.53	-	0.69
Cr	0.79	0.87	0.85	0.75	0.79	0.79	0.48	-	0.56
Mg	0.87	0.83	0.96	0.86	0.84	0.84	0.63	-	0.63
Mn	0.29	0.29	0.3	0.21	0.2	0.28	0.25	-	0.21
Ni	0.64	0.88	0.81	0.8	0.81	0.77	0.37	-	0.47
Pb	1.38	1.14	1.88	1.57	1.32	1.3	1.03	-	0.78
Zn	1.06	1.15	1.17	0.97	0.94	1.12	2.7	-	3.82

Geoaccumulation Index (Igeo)

The Geoaccumulation Index (Igeo) introduced by Muller (1979) was used to assess metal pollution in sediments. Igeo is expressed as Igeo = Log2 (Cn/1.5Bn), where Cn = measured concentration of heavy metal in the mangrove sediment, Bn =geochemical background value in average shale (Turekian and Wedepohl, 1961) of element n,1.5 is the background matrix correction in factor due to lithogenic effects. The Average Shale value is a quick and practical means of assessing metal enrichments in fine grained sediments (Forstner and Wittmann, 1981).

According to Igeo classification, pollution degrees can be delimitated as: very strongly polluted (Igeo > 5), strongly to very strongly polluted (Igeo = 4–5), strongly polluted (Igeo = 3–4), moderately to strong polluted (Igeo = 2–3), moderately polluted (Igeo = 1–2), unpolluted to moderately polluted (Igeo = 0–1) and unpolluted (Igeo < 0).

The geo accumulation index estimated for the heavy metals from the sediments of the study area (Table 3.11) exhibited very low values (<0) in the case of almost all metals except Zn (>1.0 in station 3), indicating the sediments of the mangrove ecosystem are unpolluted to moderately polluted.

Table 3.11 Geoaccumlation index for heavy metals in the mangrove sediments.

metal	station 1			station 2			station 3		
	pre	mon	post	pre	mon	post	pre	mon	post
Cd	-6.16	-6.29	-5.81	-5.67	-6.41	-6.33	-4.49	-	-4.49
Co	-2.19	-2.52	-2.25	-2.20	-2.16	-2.14	-2.99	-	-2.70
Cr	-0.58	-0.88	-0.83	-0.66	-0.59	-0.60	-1.34	-	-1.09
Cu	-1.13	-1.43	-1.49	-1.28	-1.11	-1.10	-1.21	-	-0.79
Fe	-0.23	-0.69	-0.59	-0.25	-0.26	-0.26	-0.29	-	-0.26
Mg	-0.43	-0.95	-0.65	-0.48	-0.51	-0.51	-0.95	-	-0.92
Mn	-2.02	-2.48	-2.31	-2.49	-2.60	-2.09	-2.29	-	-2.50
Ni	-0.88	-0.88	-0.90	-0.57	-0.56	-0.64	-1.74	-	-1.34
Pb	0.23	-0.50	0.32	0.40	0.15	0.12	-0.25	-	-0.62
Zn	-0.15	-0.49	-0.36	-0.29	-0.34	-0.10	1.14	-	1.68

Enrichment Factor and geo accumulation index confirm the absence of heavy metal pollution in these systems. Under reducing environments, mobility of Zn substantially decreases due to its affinity for sulphur (Thornton, 1983; Alloway, 1990). The Zn rich contaminant discharging from Zn smelting industries located at the northern part of the study area can enhance the content of Zn in these sulphur rich sediments. The lack of a clear enrichment in mangrove sediments of other metals may be caused by their strong soluble complexes with reduced sulphur (Emerson et al., 1983), which will increase the migration of these elements from sediments to the water column (Huerta-Diaz and Morse, 1992).

The biogeochemical processes operating in the system will lead to chemical transformations of the element and an *in situ* identification and monitoring of individual processes and their contribution to the total dynamicity is an unapproachable task. The speciation study thus can be considered as an indexing of various biogeochemical processes to get a thorough understanding of the processes. If we consider the chemical transformations as redox reactions, a better index can be developed by potential elements, which exhibit a definite redox character and sufficient concentration to monitor the transformations. The advantage with phosphorus is that it is available in various oxidation states (Morton and Edwards, 2005) and the monitoring of phosphorus in various forms can be easily done. Hence phosphorus is taken as the element to identify the geochemistry of the system. The study is organised in such a way to identify all the geochemical forms with an intention to assess the interrelations for the identification of the geochemical processes. The speciation scheme is selected in such a way that the maximum variability and species diversity can be estimated.

Some of the earlier works on the fractionation of phosphorus in the sediments of different aquatic systems and some reported works in India, especially near the Cochin area are tabulated in Table 3.12.

Table 3.12 Important works on the fractionation of phosphorus in the sediments of different aquatic systems

Sl. No	**Aquatic system and reference**	**Extraction scheme**	**Results**
1.	Southwest coast of India (Nair et al., 1993)	8 different schemes	The major forms of P namely exchangeable P, Carbonate bound P, labile and resistant organic P, Fe-, Al- and Ca bound P etc.
2.	Cochin estuary (Balchand and Nair, 1994)	8different schemes	The major forms of P namely exchangeable P, Carbonate bound P, labile and resistant organic P, Fe-, Al- and Ca bound P etc.
3.	Tidal floodplain forest in the Amazon estuary (Silva and Sampaio,1998)	Kurmies (1972), Salomons and Gerritse (1981) and Leeg and	The P combined with iron and aluminum was themain fraction of P in the soil (0.05±0.02 mg/g to 0.20± 0.05 mg/g,).

		Black's (1955)	
4.	.Cauvery Estuary and Pichavaram mangroves (Ramanathan, 1999)	Ramanathan et al, (1996)	In the estuary P was mainly associated with Al-P followed by Ca-P and Fe-P.
5.	Mangrove sediments of French Guiana (Fabre et al., 1999)	Golterman and Booman, (1988) modified by Golterman (1996).	Four stages of mangrove sediments were studied-pioneer, mature, mixed and dead mangrove sediments. The sum of the fractions varied between 638 to 804 µg/g in pioneer and mixed mangroves respectively. In all the stages investigated, the sum of the inorganic fractions (Fe(OOH)-P + $CaCO_3$-P) represented more than 50% of the sum of the fractions
6.	Mediterranean French coastal lagoons (Thau and Méjean) (Paing et al., 1999)	De Groot and Golterman (1993)	Fe-P constituted about 25% of P, Ca-bound (30%)was the most important inorganic fraction. Organic-P represented a substantial proportion of the Total-P (38% of Total-P in Thau and 28% in Méjean).
7.	Kuttanad region, Vembanad Lake (Lizen, 2000)	Hieltjes and Lijkema (1980) and van Eck (1982)	Residual P (40.06-91.65%) dominated in the region with very low contribution of exchangeable P (0.06-0.6%). Fe- and Al- bound P (2.76- 37.25%) and Ca bound P (1.49-33.50%) were the other dominant forms.
8.	Mangrove ecosystems around Cochin (Shaly, 2003)	Golterman (1996) modification by Pardo et al., (1998)	Total P in surficial sediments was in the range from 480.72 to 1967.60 µg/g. Fe-IP was the dominant fraction (21.82-28.31%), followed by Ca-IP (15.55-20.47%) and Alk-OP (14.22-21.35%)
9.	Mattaponi River, Virginia, USA (Morse et al., 2004)	Paludan and Jensen (1995)	Al-Po and Fe-Pi comprising 64% and 53% of total Pat the upstream and downstream sites, respectively. organic P represented a larger percentage of total P – 88% versus 59% for the upstream marsh and 70% versus 50% for the downstream marsh.

10	Different oceanic regimes (Faul et al., 2005)	SEDEX procedure Ruttenberg, (1992) Modified by Anderson and Delaney (2000)	Org. P was the largest contributor to total P, especially in the Ross Sea (average 53% of total P) and detrital P is a more significant contributor to total P on the California Coast.
11	Japan Sea (Cha et al., 2005)	Ruttenberg (1990) modified by Rao (1994)	The total P ranged 13.0–35.5 µmol/g. The concentrations of Det-P were very low (0.7–2.6 µmol/g) accounting less than 10% (by weight) of total P. The Fe-P concentration was 0.9–23 µmol/g, accounting for 7–63% of the total P. The CFA-P concentration was 3.1–6.1 µmol/g (9–44% of the total P), while Org-P was the major form (3.6–10 µmol/g), accounting for more than 40% of the total P.
12.	Eastern Arabian Sea (Babu and Nath, 2005)	SEDEX procedure Ruttenberg, (1992) Modified by Anderson and Delaney (2000)	Total phosphorus ranged between 920 and 2496 ppm, while it was 1070 –1632 ppm in off Cochin region. Porg was low in shelf (6%,) and relatively high in deep-sea sediments (12–17%). In sediments overlain by OMZ, Porg proportion is relatively high in the SE Arabian Sea (10–26%) when compared to the NE Arabian Sea (8–13%), which have less Porg than the deep-sea sediments.

Fractionation analysis of phosphorus in the mangrove sediments showed that station 3 is abnormally enriched with Ca-IP. Presence of monetite at station 3 supports the enrichment of Ca-IP in this station. The diagenesis of sediments containing bird or bat guano can also lead to the crystallization of apatite and other phosphorus minerals (Tiessen et al., 1996).The high mass percentages of calcium and phosphorous by XRF and SEM-EDS analysis confirm the phosphorous enrichment at station 3.

Correlation analysis (Table 3.13) showed that Eh had highly significant negative correlation with Ca-IP and ASOP. pH did not show correlations with any of the parameters. Total sulphur showed highly significant positive correlations with Ca-IP and ASOP. Fe-IP had no correlation with Fe, while it showed highly significant positive correlations with other two bioavailable fractions. Ca-IP showed highly significant positive correlation with ASOP and significant negative correlation with clay.

Calcium bound phosphorus dominate in mangrove sediment (Silva and Mozeto, 1997), due to its stability under the redox (Eh) variations observed in the mangroves (Nriagu, 1976; Silva et al., 1998). Highly significant negative correlation of Ca-IP with Eh and its highly significant positive correlation with sulphur, the redox indicator also suggested that there is a preferential accumulation of Ca-IP when the system is highly reducing. The slight acidic pH of the sediments at the station 3 provides high stability to Ca-IP (Silva and Sampaio, 1998).

The increase in Ca-IP during pre-monsoon at all the stations might be due to the increase in salinity. Similar trend is reported in marine sediments presumably by the accumulation of calcium under high salinity, which favours apatite formation (Ryden et al., 1997). Silva and Mozeto (1997) also suggested that phosphorous combined with calcium under high salinity acts as a principal mechanism for its retention.

Table 3.13 Pearson correlation between the sedimentary parameters in the study region

	Sand	Silt	Clay	pH	Eh	Total Carbon	Organic Carbon	Total Nitrogen	Total Sulphur	Fe	Fe-IP	Ca-IP	Acid -OP	Alkali -OP	R-OP
Sand	1														
Silt	-0.86	1													
Clay	-0.16	-0.32	1												
pH	-0.59	0.39	0.17	1											
Eh	-0.18	-0.17	0.81	-0.10	1										
Total Carbon	0.48	-0.32	-0.22	0.06	-0.55	1									
Organic Carbon	0.61	-0.43	-0.25	-0.05	-0.56	0.99	1								
Total Nitrogen	0.43	-0.17	-0.43	-0.03	-0.74	0.95	0.94	1							
Total Sulphur	0.26	-0.10	-0.57	0.35	-0.83	0.42	0.45	0.51	1						
Fe	-0.66	0.71	-0.30	0.24	-0.36	-0.30	-0.37	-0.06	0.21	1					
Fe-IP	-0.11	0.39	-0.54	0.16	-0.77	0.50	0.47	0.68	0.44	0.37	1				
Ca-IP	0.26	0.03	-0.74	0.13	-0.97	0.52	0.55	0.70	0.90	0.28	0.74	1			
Acid-OP	0.27	0.00	-0.69	0.14	-0.96	0.53	0.56	0.70	0.90	0.28	0.74	1.00	1		
Alkali-OP	-0.23	0.24	-0.01	0.50	-0.44	0.71	0.60	0.70	0.24	0.22	0.58	0.35	0.36	1	
R-OP	-0.59	0.28	0.70	0.13	0.62	-0.29	-0.39	-0.36	-0.74	0.21	-0.28	-0.71	-0.69	0.26	1

Fe-IP was the major fraction at stations 1 and 2. Generally release of this phosphate fraction from the sediment is controlled by sulphate reduction (Caraco et al., 1989) and is considered more bioavailabile under the redox (Eh) variations observed in the mangroves sediments (Caraco et al., 1989; Silva and Mozeto, 1997). Sulphide produced from sulphate respiration may reduce the iron-oxides and thus promote a release of iron-bound phosphorus (Howarth et al., 1995; Jensen et al., 1995). However, sulphate reduction is generally of less importance in intertidal zones because of periodic aeration in the environment (Kristensen et al., 1992; Alongi, 1998; Holmer et al., 1999) causing the formation of $Fe(OH)_3$ (Crosby et al.,1984). Furthermore, the mangrove trees are able to excrete oxygen through their root system, producing an oxygenated microenvironment (Silva et al., 1991) capable of trapping phosphorus as $FePO_4$ through the formation of $Fe(OH)_3$.

The involvement of iron in the dynamic equilibrium between the sediment and water as explained above has led to the suggestion that an iron dependent threshold exists for the sediment's ability to bind phosphorus. Jensen et al., (1992) showed that the retention capacity was high as long as the Fe: P ratio exceeds 15 (by weight) while Caraco et al., (1993) suggested that this ratio should be above10 to regulate phosphorus release. Fe:P ratios of the sediments were higher than 15 in the study region, except at station 3. This justifies higher concentration of Fe-IP at stations 1 and 2. The Fe:P ratio was very low at station 3, resulting in lower percentage of iron bound phosphorous in the system. Periodic fluctuations in the redox potential of this system might also result in lower Fe-IP content at station 3.

ASOP includes apatite bound phosphate and biochemical components such as nucleic acids, lipids and sugars that bound to phosphate (De Groot, 1990). ASOP had highly significant negative correlation with Eh and highly significant positive correlation with total sulphur. This indicates that highly reducing environment is favourable for ASOP similar to Ca-IP. Alk-OP generally constitutes humic phosphate and phytate phosphate (Golterman et al., 1998). Phytate or phytic acid (inositol hexa phosphate) is an organic phosphate that is widely spread in plants (Hess, 1975), soils (Stevenson, 1982) and aquatic sediments (De Groot and Golterman, 1993). Phytate is relatively stable as it can be strongly adsorbed onto iron hydroxide and multivalent cations (De Groot and Golterman, 1993). Irrespective of the higher content of total organic matter, the labile fraction was very low in these mangrove sediments, signalling to the refractory organic matter accumulation (Chapter 4). This might results in the higher concentration of Alk-OP, which is non-bioavailable. As a result of the diagenetic reorganization of phosphorus within sediments, organic phosphorus concentrations usually decrease with time as it is ultimately transformed to authigenic phosphorus during diagenesis (Ruttenberg and Berner, 1993; Anderson et al., 2001).

Organic phosphorous in the study region ranged from 8 (station 3) to 38.6 % (station 1) of total phosphorous. Generally organic bound phosphorus accounted for 6 -19% of total in coastal sediments (Hirata, 1985). The high percentage organic bound phosphorous at stations 1 and 2 indicated that the mineralisation of phosphorus is less, where as at station 3, active mineralization is taking place. C:P ratio at station 3 was very low and it is reported that mineralisation of organic phosphorous and C:P ratio are inversely related (Reddy and Delaune, 2008). Also, organic phosphorous mineralization is high under anaerobic condition than under aerobic conditions (Bridgham et al., 1998).Very high reduction potential at this station results in higher mineralisation and subsequently lower concentration of organic phosphorus.

The variations in the phosphorus content in the three systems could also be favoured by the difference in local vegetation (Alongi, 1989). Organic matter associated with *Avicennia* sediments, because of the presence of more degradable organic matter, can sustain a higher rate of microbial activity than *Rhizophora* and, as a consequence, better recycling of nutrient elements (Lacerda et al., 1995). In *Rhizophora* sediments either microbial conversion is negligible or the organic components are more refractory (Alongi, 1989). Station 3 is dominated by *Avicennia* and the high amount of inorganic phosphorus in this system could be deduced to be a signal of higher levels of diagenetic activity.

3. 4 Conclusions

General sedimentary analysis showed the predominance of fine substratum and silt was the major fraction in all the seasons in these mangrove sediments. XRD analysis of the sediments showed the presence of monetite, an anhydrous calcium phosphate mineral ($CaHPO_4$) at station 3. The high mass percentages of calcium and phosphorous by XRF and SEM-EDS analysis confirm the existence of calcium phosphate mineral at station 3. Stoichiometric ratios of nutrients revealed phosphorus enrichment in all the systems. The overall order of heavy metal concentration in sediments was: Fe > Mg > Mn > Zn > Cr > Pb > Ni > Pb >Cu > Co > Cd. The enrichment factor calculated, using iron as normalizing element, indicated a minor enrichment for Pb and Zn and no enrichment for all other metals. The geo accumulation index estimated for the present study exhibited very low values (<0) in the case of almost all metals, except Zn (>1.0 in station 3), indicating sediments of the mangrove ecosystem are unpolluted to moderately polluted. Phosphorus fractionation analysis showed that these mangrove systems act as a sink of phosphorus, primarily as Ca-IP and Fe-IP fractions. Internal loading in these systems acts as a source of phosphorus to the adjacent coastal waters. The first two stations behave identically and Fe-IP was the major fraction in these stations. Station 3 is unique because of the accumulation of bird guano in this bird sanctuary and it resulted in about 10 fold increase in the total phosphorous content. This station is exclusively dominated by Ca-IP and a rare mineral, monetite was detected in the system.

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Chapter 4

Biochemical Composition

Contents

4.1 Introduction

Mangrove wetlands are one of the most productive aquatic ecosystems and are characterised by high number of primary producers, diversity of microhabitats, complex multispecies interactions and by intensive exchange of organic matter and organisms within and outside the ecosystem (Day et al., 1989; Twilley et al., 1992). Mangroves play an important role in the global carbon cycle and are characterised by high net primary production of organic matter (Odum and Heald, 1975; Clough, 1992), intense turnover (Alongi et al., 1999) and burial. Mangroves can act either as a sink for organic matter accumulation, or as reservoirs able to fertilise adjacent coastal areas, through organic and inorganic nutrient export (Jennerjahn and Ittekkot, 2002; Dittmar et al., 2006). The balance between organic matter export and its accumulation is dependent upon degradation and heterotrophic utilization of the organic matter pools.

Organic matter sources in mangrove sediments are characterised by a large variability in their composition and degradability, ranging from labile sources such as phytoplankton and benthic microalgae to less degradable sources such as mangrove material and terrigenous inputs (Bouillon et al., 2004). Quantity and quality of sedimentary organic matter are largely depend upon several factors including origin, composition and biochemical transformations that occur on organic particles during their descent through the water column (Cowie and Hedges, 1992).

4.1.1 Quality of Organic Matter

Sediments represent a 'recorder' of water column processes and are the final storage for the accumulation of autochthonous and allochthonous organic matter inputs (Fabiano and Danovaro, 1994). Determining the organic matter quality is important both in a biogeochemical perspective as organic matter degradation rates might affect its burial in sediments and from a trophodynamic point of view as organic matter availability influences feeding strategies and the distribution of benthic organisms (Jumars and Penry, 1989; Graf, 1992; Hartnett et al., 1998). Quantity and quality of organic matter in surface sediments is of primary importance in determining the potentially available fraction to consumer organisms, thus affecting benthic fauna dynamics and metabolism (Graf et al., 1983; Grant and Hargrave, 1987; Graf, 1992).

Organic matter in marine sediments is composed of labile and refractory compounds, whose relative importance changes as a function of a complex array of processes, including degradation, heterotrophic utilisation, transformation, accumulation and export (Viollier et al., 2003). Refractory organic compounds (such as humic and fulvic acids, structural carbohydrates and "black" carbon) characterised by lower degradation rates account generally for most of the sedimentary organic matter and are easily accumulated in marine sediments (Middelburg et al., 1999; Zegouagh et al., 1999). Conversely, the labile fraction of organic matter consists of proteins, carbohydrates and lipids that are rapidly mineralized, which are assumed to represent the fraction of organic matter more readily available to benthic consumers (Fabiano et al., 1995; Dell'Anno et al., 2002). The autochthonous production or external inputs of rapidly sinking particles allows the accumulation of organic matter, which have suffered a continuous process of degradation of labile compounds in the water column as well as after sedimentation (Colombo et al., 1996). This results in a preferential loss of the more labile compounds (Downs and Lorenzen, 1985; Neal et al., 1986; Wakeham and Lee, 1989). Thus environmental and biological factors such as the depth of the water column, sedimentation rate, oxygen concentration, primary productivity and bioturbation may be fundamental accounting for the quantity and quality of the organic bulk of sediments (Emerson et al., 1985; Cowie and Hedges, 1992; Danovaro et al., 1999). The labile portion of organic matter could also be suitable to assess the trophic status of coastal marine systems (Dell'Anno et al., 2000; Pusceddu et al., 2003).

Thus determining the labile fraction of organic matter is crucial in assessing food quality and quantity in benthic ecological studies (Incera et al, 2003). Although a universally accepted methodology to assess the labile fraction of sedimentary organic matter does not exist, concentrations of some specific compounds have been used to estimate the nutritional value of the sediment (Buchanan and Longbottom, 1970). This is accomplished with the use of biochemical indices like concentrations of protein, carbohydrates, lipids, protein/carbohydrate ratio and lipid/carbohydrate ratio (Dell'Anno et al., 2000; Gremare et al., 2002). Sedimentary protein concentrations reflect the productivity of a given marine system and it appears to be a good descriptor of the trophic status of the benthic systems at different spatial scales (Danovaro et al., 1999, 2000; Dell'Anno et al., 2002).Carbohydrates, which include polyhydroxylated compounds ranging in size from 5-6 carbon sugars to large biopolymers (starches, cellulose), are much higher in vascular plants than in algae (Cowie and Hedges, 1984). In mangroves, litter is primarily composed of non-nutritive complex carbohydrates that are difficult or impossible for detritivores to digest. Dominance of carbohydrates and lower protein to carbohydrate ratio (<1) is a typical feature of detrital-heterotrophic environments (Danovaro, 1996). Lipids in sediments are derived not only from aquatic biota but also from wax of higher plants. Lipids in surface sediments are abundant in eutrophic systems than in oligotrophic systems. Like proteins, it also indicates the productivity of the system (Gremare et al., 1997).

The C:N ratio has also been used as an organic matter quality indicator (Huston and Deming, 2002), although the elemental quantification does not give any clues to the chemical features of the organic matter and overestimates the nutritionally available nitrogen due to the abundance of indigestible nitrogenous compounds such as humic material (Mayer et al., 1995).

4.1.2 Bulk Geochemical Proxies for Source Characterisation of Organic Matter

Bulk geochemical proxies such as C/N and isotopic compositions of sedimentary organic matter have been commonly used to distinguish sources of organic matter – autochthonous or marine versus allochthonous or terrestrial (Meyers, 1994; Schelske and Hodell, 1995). In addition, biochemical composition of sedimentary organic matter has also been used to gather information on the origin and parameters controlling the diagenetic fate of organic matter (Colombo et al., 1996).

The C/N ratios have been widely used to distinguish the origin of organic matter based on the generalization that algal organic matter has atomic C/N ratios between 4 and 10, where as organic matter from terrestrial vascular plants has C/N ratios of 20 and greater (Prahl et al., 1980; Ishiwatari and Uzaki, 1987; Meyers, 1994; Twichell et al., 2002). This distinction arises from the absence of cellulose in algae and its abundance in vascular plants. However, the selective degradation or the different minerals in sediments can affect the C/N ratios of organic matter (Muller, 1997; Lehmann et al., 2002). Selective degradation of organic matter components during early diagenesis has the potential to modify C/N ratios in sediments. Microbial immobilization of nitrogenous material accompanied by the remineralization of carbon might also result in the lowering of C/N ratios (Sollins et al., 1984).

Stable isotopes have been commonly used to identify origin of organic matter (Peterson et al, 1985; Cifuentes et al., 1988; Dittmar et al., 2001; Bouillon et al., 2002; Bouillon et al., 2003). The basic principle surrounding the application of stable isotopes in natural ecosystems is based on variations in the relative abundance of lighter isotopes from chemical rather than nuclear processes (Hoefs, 1980). Due to the faster reaction kinetics of the lighter isotope of an element, reaction products in nature can be enriched with the lighter isotopes. These fractionation processes have proven to be useful in determining the source of organic matter in ecological studies. The most common stable isotopes used in marine biogeochemical studies are ^{18}O, ^{2}H, ^{13}C, ^{15}N and ^{34}S. The preference for using such isotopes is related to their low atomic mass, significant mass differences in isotopes, covalent character in bonding, multiple oxidation states and sufficient abundances of the rare isotope.

Stable carbon isotope signatures ($\delta^{13}C$) of the various carbon inputs are often different, can be used as powerful tracers of carbon sources in various ecosystems (Fry and Sherr, 1984; Dehairs et al., 2000; Yamamuro, 2000; Dittmar et al., 2001; Bouillon et al., 2003; Bouillon et al., 2004; Goni et al., 2006; Hu et al., 2006). Stable carbon isotopes have been used to distinguish between allochthonous versus autochthonous organic carbon inputs (Peterson and Fry, 1987; Middelburg et al., 1997; Bianchi et al., 2002).

Carbon isotopic ratios are particularly useful to distinguish between marine and continental plant sources of sedimentary organic matter and to identify organic matter from different types of land plants. Enzymatic and diffusional fractionation processes lead to discrimination against ^{13}C during photosynthesis that varies between C3, C4, and CAM plants (Brugnoli and Farquhar, 2000). In C3 plants (woody plants), CO_2 is fixed within the Calvin cycle yielding C3 compounds as first products and an isotopic signature that varies between -21 ‰ and -35 ‰. In C4 plants, CO_2 is first fixed in C4 organic acids that transport the carbon to separate tissues where sugar synthesis within the Calvin cycle takes place. The isotopic composition ranges between -9 ‰ and -20 ‰ (Badeck et al., 2005). CAM plants have an intermediate isotopic signature between C3 and C4 with variation depending on the proportion of carbon fixed in the dark into C4 organic acids or in the light via the Calvin cycle. Marine organic matter consequently typically has $\delta^{13}C$ values between 20 and -22 ‰. The ~ 7 ‰

difference between organic matter produced by C3 land plants and marine algae has successfully been used to trace the sources and distribution of organic matter in coastal ocean sediments (Newman et al., 1973; Gearing et al., 1977).

However, the isotopic source signal can become complicated in coastal areas which receive contributions of organic matter from algae and both C3 and C4 vascular plants (Fry et al., 1977). In these areas addition of C/N ratios to $\delta^{13}C$ determinations allows better discrimination of organic matter sources. These combinations of elemental and carbon isotopic values can be used to distinguish sources of organic matter in sediments and in settling particles.

Although the biochemical composition of sedimentary organic matter has been widely researched in many marine ecosystems such as deep sea (Danovaro et al., 1993), semi-enclosed marine systems (Pusccedu et al., 1999), subtidal sandy sediments (Fabiano et al., 1995), seagrass bed (Danovaro et al., 1994) or estuarine environments (Fabiano and Danovaro, 1994), there is a conspicuous lack of information about concentrations and variability of these compounds in mangrove sediments. Due to their dynamic ecotonal location, these environments display strong spatial and temporal variability of major biogeochemical characteristics.

This chapter investigates the quantity and quality of organic matter in surface sediments in terms of the biochemical composition and thereby to identify the major biogeochemical pathways. Bulk parameters like $\delta^{13}C$ of total organic matter, elemental and biochemical composition were also used to characterise the sources of organic matter in these mangrove systems.

4.2 Results

The spatial and seasonal variations of total organic matter, proteins, total carbohydrates and lipids in the sediments of the mangrove systems under study are described in Table 4.1 and Fig. 4.1. The $\delta^{13}C$ of total organic matter in these mangrove sediments are also described.

4.2.1 Total Organic Matter (TOM)

TOM in the mangrove sediments ranged from 3.8 (station 2, pre-monsoon) to 11.6% (station 3, pot-monsoon). At station 1, it ranged from 4.8 (pre-monsoon) to 10.9% (monsoon). TOM varied between 3.8 (pre-monsoon) and 8.4% (post-monsoon) at station 2. At station 3, TOM was in the range from 8.6 (pre-monsoon) to 11.6% (post-monsoon).

4.2.2 Proteins (PRT)

PRT concentrations ranged from 702 ± 8.4 (station 2, pre-monsoon) to 4608±17.7 μg/g (station 3, post-monsoon) in the study region. At station 1, PRT concentrations varied from 887±13.3 (pre-monsoon) to 1527±16.8 (post-monsoon) μg/g. At station 2, the range was from 702± 8.4(pre-monsoon) to 1325±13.25 (post-monsoon) μg/g. It varied between 887± 14.2(pre-monsoon) and 4608±17.7 (post-monsoon) μg/g at station 3. PRT nitrogen concentrations (protein×0.16) varied from 112.32 to 737.28μg/g in the study region.

Table 4.1 Seasonal variations of sedimentary parameters in the surficial sediments of mangrove ecosystems. Results of ANOVA are also shown

Parameters	Station 1			Station 2			Station 3			ANOVA P-value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Spatial	Seasonal
Total Lipids (μg/g)	2789 ± 155.8	2360 ± 47.2	2244 ± 102.4	1534 ± 23.01	804 ± 34.5	1620 ± 86.2	5931 ± 359.3	-	6816 ± 602.2	0.0003	0.48
Protein (μg/g)	887 ± 13.3	1400 ± 25.2	1527 ± 16.8	702 ± 8.4	870 ± 13.05	1325 ± 13.25	887 ± 14.2	-	4608 ± 17.7	0.228	0.204
Total Carbohydrates (μg/g)	1043 ± 12.5	779 ± 9.3	1790 ± 23.3	1000 ± 34	653 ± 8.5	2458 ± 49.2	505 ± 10.1	-	1716 ± 68.64	0.284	0.007
BPC (μg/g)	2944	2767	3147	1895	1291	2848	5085	-	8057		
C/N Ratio	8.24	12.6	12.19	8.15	8.62	10.65	10.64	-	10.15	0.115	0.16
PRT/CHO	0.85	1.8	0.85	0.7	1.34	0.54	1.76	-	2.68		
LPD/CHO	2.68	3.03	1.25	1.53	1.23	0.66	11.74	-	3.97		

4.2.3 Total Carbohydrates (CHO)

CHO ranged from 505±10.1 (station 3, pre-monsoon) to 2458±49.2 µg/g (station 2, post-monsoon) in the study region. At station 1, CHO showed a range of 779±9.3 (monsoon)-1790±23.3 µg/g (post-monsoon). At station 2, CHO varied from 653±8.5 (monsoon) to 2458±49.2 µg/g (post-monsoon). CHO at station 3, ranged from 505±10.1 (pre-monsoon) to 1716±68.64 µg/g (post-monsoon).

4.2.4 Lipids (LPD)

LPD ranged from 804±34.5µg/g (station 2, monsoon) to 6816±602.2 µg/g (station 3, post-monsoon) in the study region. LPD at station 1, varied from 2244±102.4 (post-mosoon) to 2789±155.8µg/g (pre-monsoon).At station 2, LPD was in the range 804±34.5 (monsoon)-1620±86.2 (post-monsoon) µg/g. At station 3, LPD concentration varied between 5931±359.3 (pre-monsoon) and 6816±602.2 µg/g (post-monsoon).

4.2.5 Stable Carbon Isotope ($\delta^{13}C$)

Stable Carbon isotope ($\delta^{13}C$) analysis of total organic matter was carried out during post-monsoon, the season with higher concentrations of TOM and biochemical composition. $\delta^{13}C$ of TOM was -26.46 ± 0.33 ‰, -25.53 ± 0.07 ‰ and -26.71 ± 0.13 ‰ respectively for station 1, 2 and 3. Station 3 was observed to be more depleted with ^{13}C whereas station 2 showed comparative enrichment.

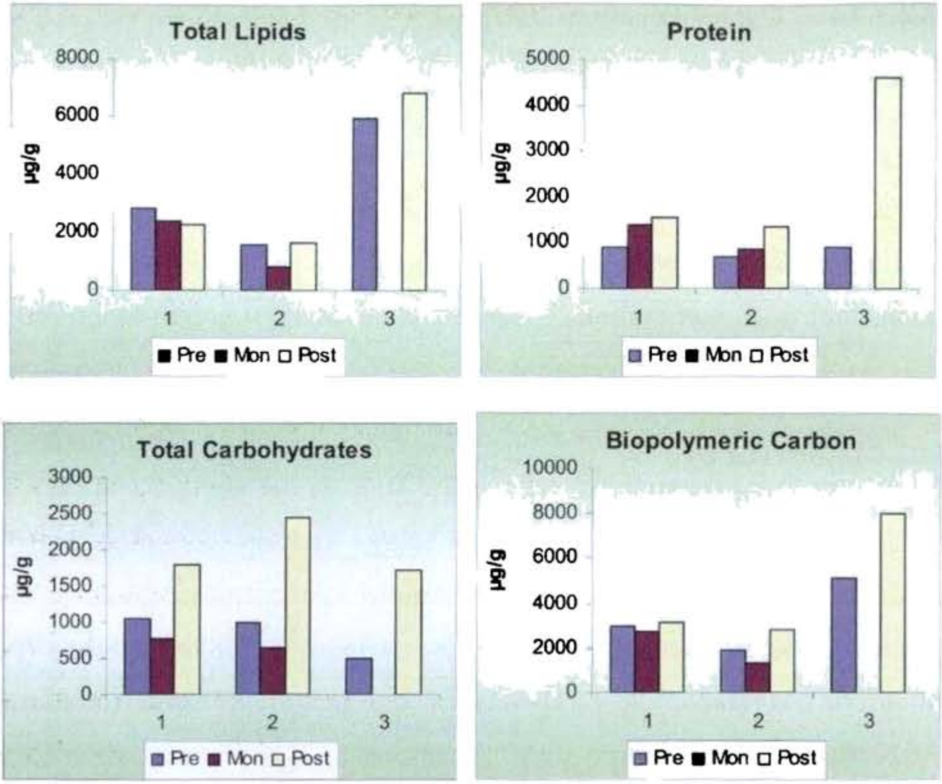

Figure 4.1 Seasonal variation of biochemical compounds in the mangrove sediments

4.3 Discussion

All the biochemical compounds, in general, showed higher concentrations during post-monsoon and lower concentrations during pre-monsoon seasons, except CHO, which was lower during monsoon. Station 3 recorded comparatively higher concentration of biochemical compounds.

Labile organic matter (LOM), the sum of the biochemical compounds, ranged from 2327 to 13140 µg/g in the study region. The BPC fraction of sedimentary organic carbon ranged from 1291 (station 2, monsoon) to 8057μg C/g (station 3, post-monsoon). BPC fraction at station 1 was in the range 2767 (monsoon) – 3147 μg C/g (post-monsoon). At station 2, it varied from 1291(monsoon) to 2848 μg C/g (post-monsoon). BPC fraction at station 3 was from 5085 (pre-monsoon) to 8057μg C/g (post-monsoon). Monsoon season recorded lowest concentration of BPC.

Analyses of variance (Table 4.1) showed that LPD had significant spatial variations in the study region with higher values at station 3, while PRT did not show any variations. CHO showed significantly higher values during the post-monsoon season. Correlation analysis showed that LPD had highly significant negative correlation with Eh and significant positive correlation with total sulphur (Table 4.2). PRT showed significant positive correlation with total carbon and total nitrogen. CHO did not show correlation with any of the sedimentary parameters. BPC showed highly significant positive correlation with LPD, PRT and total nitrogen and significant positive correlation with total carbon and organic carbon. BPC also showed highly significant negative correlation with Eh and significant negative correlation with clay.

The biochemical composition of sedimentary organic matter in the study region showed a dominance of LPD followed by PRT and CHO. LPD contribution to the LOM pool in these sediments at stations 1, 2 and 3 were 50.48%, 37.33% and 66.43% respectively. PRT contribution to the LOM pool at stations 1, 2 and 3 were 25.70%, 27.86% and 23.59 % respectively. CHO contribution at stations 1, 2 and 3 were 23.81%, 34.81 % and 9.98% respectively of LOM.

Table 4.2 Correlation between sedimentary parameters in the mangrove ecosystem (n=8)

	Sand	Silt	Clay	pH	Eh	Total Carbon	Organic Carbon	Total Nitrogen	Total Sulphur	Total Lipids	Proteins	Total Carbohydrates	BPC
Sand	1												
Silt	-0.81	1											
Clay	-0.17	-0.44	1										
pH	-0.59	0.44	0.16	1									
Eh	-0.18	-0.32	0.82	-0.10	1								
Total Carbon	0.48	-0.31	-0.22	0.06	-0.55	1							
Organic Carbon	0.61	-0.41	-0.25	-0.05	-0.56	0.99	1						
Total Nitrogen	0.43	-0.13	-0.43	-0.03	-0.74	0.95	0.94	1					
Total Sulphur	0.26	0.12	-0.60	0.35	-0.83	0.42	0.45	0.51	1				
Total Lipids	0.34	0.19	-0.84	0.03	-0.96	0.61	0.64	0.77	0.82	1			
Proteins	0.03	0.24	-0.45	0.11	-0.65	0.71	0.68	0.80	0.27	0.67	1		
Total Carbohydrates	-0.47	0.27	0.27	0.69	0.05	0.39	0.25	0.26	-0.07	-0.04	0.41	1	
BPC	0.21	0.25	-0.74	0.14	-0.91	0.72	0.72	0.85	0.70	0.95	0.85	0.21	1

Biochemical composition of sedimentary organic matter in the study region seems to be quite different from other coastal systems (Table 4.3), which are usually characterised by the dominance of proteins and carbohydrate over lipids (Meyer-Reil, 1983; Sargent et al., 1983; Fabiano and Danovaro, 1994). At stations 1 and 3, lipids were the dominant class followed by proteins and carbohydrates whereas at station 2 lipids and carbohydrates were dominant when compared to proteins. The high concentrations of sedimentary lipids, proteins and total carbohydrates recorded in the study area could be related to the morphodynamic, hydrological and physico-chemical characteristics of mangrove systems. The shallow water depth and high sedimentation rate of mangrove ecosystems assist the settling of organic matter without significant degradation. Significantly higher values of total lipids in the study region especially at station 3 might be due to its preservation under highly anoxic conditions. Highly significant negative correlation of LPD with Eh and significant positive correlation with total sulphur support this argument. Higher concentrations of CHO during the post-monsoon season point towards the possibility of higher input of vascular plant materials, especially mangrove litter. The absence of any significant correlations of CHO with other sedimentary parameters also suggests different origin and diagentic pathways for carbohydrates.

Table 4.3 Biochemical compositions of different aquatic sediments

Sl.No	Area	Lipids	Proteins	Carbohydrates	Reference
1	Mediterranean Sea	350	350	1600	Albertelli et al., 1999
2	South Pacific	7200	5750	5800	Neira et al., 2001

3	Baltic Sea	-	3800-7700	400-4000	Meyer-Reil, 1983
4	W-Mediterranean	10-660	500-2600	900-4200	Fichez, 1991
5	Tyrrhenian Sea	10-20	300-1700	300-1900	Fabiano and Danovaro, 1994
6	Gulf of Gascogne	220	1850	2440	Khripounoff et al., 1985
7	Intertidal flat of the Galician coast (NW Spain)	50-1483	400-4016	32-675	Cividanes et al., 2002
8	Balsfjorden (Norwey)	-	-	130	Sargent et al., 1983
9	Gulf of Lions	-	100-2300	300-4300	Buscail et al., 1995
10	Ligurian Sea (Zoagli)	20-210	20-70	130-670	Fabiano et al., 1995
11	Ligurian Sea (Prelo)	300-3600	50-1600	300-3600	Danovaro et al., 1994
12	Ligurian Sea	90-630	20-300	300-5300	Danovaro and Fabiano, 1995
13	Northern Adriatic Sea	70-1070	200-4100	70-420	Manini et al., 1999
14	Southern Adriatic Sea	140-620	900-3700	90-640	Manini et al., 1999
15	Tyrrhenian Sea	1-25	200-1700	300-1900	Fabiano and Danovaro, 1994
16	Ionian and Aegean Seas	50-190	70-160	1200-2500	Danovaro et al., 1993
17	Mundaka Estuary	300–5000	0.00–16700	200–5700	Cotano and Villate, 2006
18	Lower St. Lawrence Estuary	820-1470	110-400	7580-10700	Colombo et al., 1996
19	Marsala Lagoon	300-4500	2200-12100	800-70500	Pusceddu et al., 1999
20	Western Continental Shelf of India	-	90-1020	1080-9880	Jacob et al., 2008

21	Eastern Continental Shelf of India	-	170-550	1280-4430	Jacob et al., 2008
22	Mangroves of Cochin	-	200-1240	1550-7860	Geetha. et al., 2008
23	Cochin Estuary	312-2815	205-1924	250-1229	Joseph et al., 2008
24	Mangroves of Cochin	804-6816	702-4608	505-2458	Present Study

The dominance of lipids and proteins over carbohydrates indicated the nutritive or energetic value as well as the freshness of labile organic matter in the mangrove sediments. It has been established that mineralisation of biochemical components are in the order LPD > PRT > TN = THAA > TOC > CHO (Colombo et al., 1996).This suggests that a significant fraction of LOM not only reaches sediments underlying the shallow water column, but also escapes wide scale degradation. LOM was high at station 3 and low at station 2. The high concentration of LOM at station 3 might be due to its almost closed nature resulting in restricted tidal activity that consequently aided the retention of organic matter. Out welling of organic matter from this station is also less. Death and decay of birds and other organisms, excreta, mangrove detritus and other inputs contributed to these elevated levels at station 3. The highly negative correlation of BPC with Eh, suggesting the anoxic preservation of organic matter, might also be contributing to this enrichment at this station.

However, irrespective of the higher content of total organic matter, the labile organic matter was very low in these sediments. The contribution of LOM to TOM pool in the mangrove sediments ranged from 4.16 (station 1, monsoon) to 11.45 % (station 3, post-monsoon). A large fraction of total organic matter in these sediments is represented by refractory material or is uncharacterized. The uncharacterised fraction (88.55-95.84% of TOM) probably contains recalcitrant humic-type geopolymers, which usually account for a major fraction of TOM in sediments (10-70%, Mayer, 1985).

Sedimentary biopolymeric organic carbon (BPC) accounted for 4.4 – 12.09 % of TOC. Tenore and Hanson (1980) suggested that about 5-15% of sedimentary detritus, depending on the environmental characteristics, is generally available at any time for benthic consumers. BPC fraction was found to be higher at station 3. The observed variations in the BPC contribution to TOC is inferred to have resulted mainly from the strong spatial variations in total lipid concentrations, established through ANOVA.

A similar result is obtained by converting protein content to nitrogen equivalents. Labile nitrogen (protein), which is widely considered as the major limiting factor for deposit feeders was in the range 1.42-11.21% of the total nitrogen in these sediments. Hence most of the sedimentary nitrogen was refractory in nature. Nitrogen occurring in sedimentary organic matter mainly derives from living organisms. Proteins and peptides, the most abundant nitrogen containing substances in sediments, have been traditionally considered part of the labile fraction in the environment, although recent studies have shown that proteinaceous material can resist microbial degradation in sedimentary environments and consequently a portion of the nitrogen is incorporated into biologically refractory organic material and removed from the active nitrogen pool (Knicker and Hatcher, 2001; Nguyen and Harvey, 2001; Zang et al., 2001). But it must be noted that TOM, TN and biochemical components are very high in these mangrove systems, even though the labile fraction to total is less in percentage. It is sufficiently high enough to support the benthic fauna.

Protein to carbohydrate ratio (PRT: CHO) is used as an index to determine the origin of materials present in sediments and to distinguish the presence of fresh/aged sedimentary organic matter (Danovaro et al.,1993;Cividanes et al., 2002).Since proteins are more readily used by bacteria than carbohydrates (Williams and Carlucci,1976; Newell and Field,1983), high PRT:CHO ratios indicate living organic matter or " newly –generated " detritus (Danovaro et al.,1993) and the role of proteins as a potentially limiting factor for benthic consumers (Fabiano et al.,1995).This ratio ranges from lower than 0.1 in oligotrophic deep-sea sediments (500-2400 m depth in the Eastern Mediterranean Sea, Danovaro et al., 1993) to higher than 10 in coastal Antarctic sediments (Pusceddu, 1997). Values of the protein to carbohydrate ratio greater than 1 are associated generally with recently produced organic matter; typically they are reported immediately after a microphytobenthic bloom (Fabiano et al., 1995) or after the deposition of freshly produced phytoplankton (Pusceddu et al., 2000). PRT:CHO can also be used for classifying benthic trophic status which in turn reflects patterns of nutrient enrichment (Dell'Anno et al., 2002). PRT:CHO ratio in the study region ranged from 0.54 (station 2, post-monsoon) to 2.68 (station 3, post-monsoon). Higher PRT:CHO at station 3 (>1) indicated the presence of fresh organic matter. Stations 1 and 2 showed >1 PRT: CHO ratios only during monsoon season. During pre-monsoon and post-monsoon seasons, the lower ratios indicate the presence of aged or less degradable organic matter i.e. terrestrial or vascular plant input.

The lipid content and lipid to carbohydrate ratio (LPD:CHO) have been used as good indices to describe the energetic (food) quality of the organic matter in the sediments (Gremare et al, 1997; Fabiano and Pusceddu, 1998; Gremare et al., 2002). Furthermore, lipid concentrations have been associated with the most labile fraction of sedimentary organics and it is considered as the best descriptor for meiofauna abundance and biomass over enzymatically hydrolysable amino acids or protein contents (Gremare et al, 1997; Cartes et al., 2002; Gremare et al., 2002). The sediments showed higher concentrations of LPD and consequently high LPD:CHO ratio. LPD:CHO ratio in mangrove sediments ranged from 0.66 (station 2, post monsoon) to11.74 (station 3, pre-monsoon). This ratio was very high at station 3. High LPD:CHO ratios observed at these sites indicates the high quality of labile organic matter to support the benthic fauna.

Stoichiometric ratios of elements are utilised to determine the origin and transformation of organic matter (Prahl et al., 1994; Jennerjahn and Ittekkot, 1997; Yamamuro, 2000). The range of C/N values are typically lower for aquatic organic matter than that for terrestrial organic matter (Meyers and Lallier- Verges, 1999) and the ranges were 6-9 for planktonic organisms and 20–100 for terrestrial plant tissue and soil (Meyers, 1994; Tyson, 1995). Organic matter should have a C/N ratio lower than 17 in order to be of nutritional use to invertebrates (Russel-Hunter, 1970).

However, the selective degradation of the different minerals in sediments can affect the C/N ratios of organic matter (Muller, 1997).

The C/N ratios ranged from 8.15 (station 1, pre-monsoon) to 12.6(monsoon), which are intermediate values characteristic for autochthonous and terrestrial inputs of organic matter (Muri et al., 2004). Using C/N = 13 and C/N = 6 for the terrestrial and marine end members, respectively (Colombo et al., 1995), it could be deduced that organic matter in these systems is of mixed origin. In shallow coastal ecosystems, most of the organic carbon and nitrogen are produced by microphytobenthos and macroalgae rather than phytoplankton (Barranguet et al., 1996; Lucas et al., 2000). But in case of mangroves, litter fall have also been implicated as a significant source of organic matter (Kristensen et al., 1995). C/N ratios also indicate that the organic matter is of high nutritional value and represents a high- energy source for benthic organisms (Albertelli et al., 1998).

$\delta^{13}C$ values of the mangrove sediments of the study region are comparable to the reported values of mangrove sediments (Table 4.4). Comparatively more enriched value was obtained at station 2 and more depleted values at station 1 and 3. Generally, more enriched values in the low organic carbon sites, and more depleted values, close to those of mangrove derived organic matter, in organic rich sediments were observed. From the observed relationships, it can be assumed that due to the almost closed nature of station 3, much of the mangrove area is rarely inundated. Because of low tidal flushing, this station acts as retention site where mangrove derived organic carbon is a significant contributor to the sediment organic carbon pool. However, the sediments at station 2 reflect a balance between autochthonous carbon inputs and carbon imported from the water column during high tide.

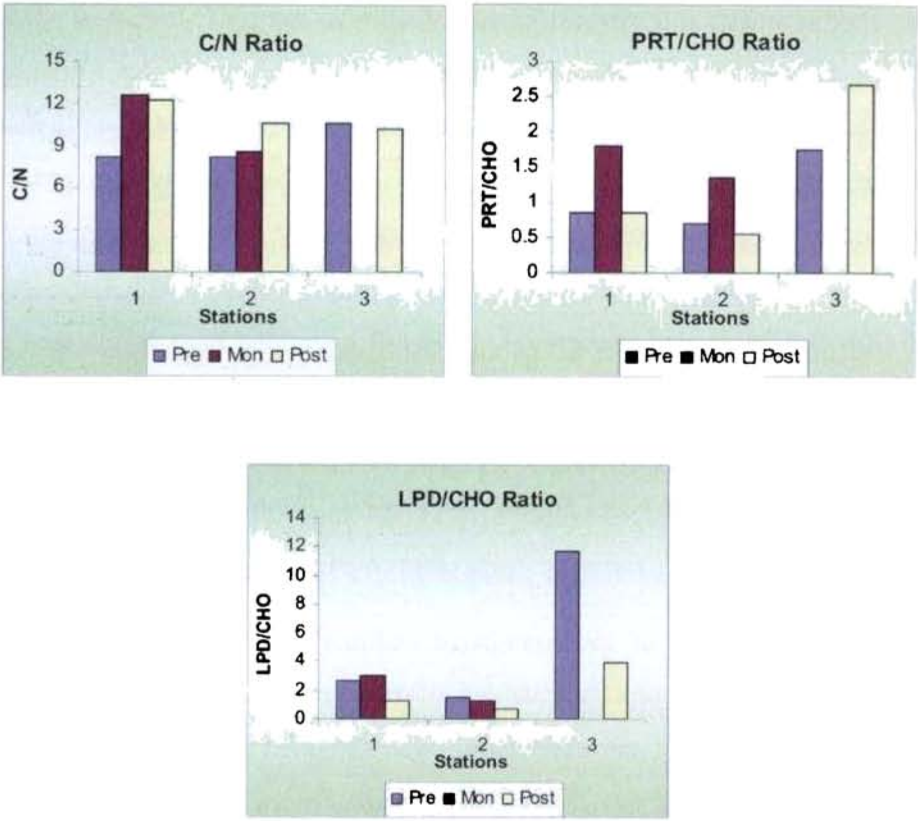

Figure 4.2 Seasonal variations of C/N, PRT/CHO and LPD/CHO in the mangrove sediments of the study region

Table 4.4 $\delta^{13}C$ values of different compartments of aquatic system

Sl.No	Aquatic System	$\delta^{13}C$ value (‰)	Reference
1	Plum Island Sound estuary sediments	-19. 4 to -22 9	Deegan and Garritt, 1997
2	Leaves of *Rhizophora apiculata* Galle, south-west Sri Lanka	−31.5 ± 1.4	Bouillon et al., 2003
3	Leaves of *Avicennia officinalis*, Pambala, south-west Sri Lanka	−30.5	Bouillon et al., 2003
4	Mangrove sediments of Gazi Bay, Kenya	-26.5 to -22.1	Bouillon et al., 2004
5	Seagrass beds of Gazi Bay, Kenya	-25.5 to -16.0	Bouillon et al., 2004
6	Microphytobenthos of Gazi Bay, Kenya	−22.1	Bouillon et al., 2004
7	Suspension feeders of Roebuck Bay, Dampier	-16.15 to -18.61	Compton et al., 2008
8	Coarse POM of Roebuck Bay, Dampier	-26.43 ±0.21	Compton et al., 2008
9	Medium POM of Roebuck Bay, Dampier	-24.91± 0.16	Compton et al., 2008
10	Fine POM of Roebuck Bay, Dampier	-23.72 ± 0.37	Compton et al., 2008
11	Macroalgae green of Roebuck Bay, Dampier	-11.04 ± 0.86	Compton et al., 2008
12	Macroalgae brown of Roebuck Bay, Dampier	-13.81± 0.69	Compton et al., 2008
13	Planktons	-5.51±0.11 to -19.35±0.10	Compton et al., 2008
14	Diatoms	-6.74	Compton et al., 2008

The quantity and quality of sedimentary organic matter in aquatic systems is mainly controlled by the biogeochemical processes taking place in the system. Principal Component Analysis (PCA) was employed here to deduce the geochemical processes in these ecosystems. The main aim of

PCA is to reduce the number of variables that needed to be considered into a smaller number of indices, principal components (PCs), which can be more easily interpreted (Manly, 1997; Panigrahy et al., 1999; Spencer, 2002). The parameters for the PCA were selected in such away that the component of the analysis can give an indication to the significance of processes. The concentrations of individual chemical species will be the net result of these processes and by fixing suitable indicators; it will be possible to identify the relative significance of each process to each species. The possible biogeochemical processes that can operate on the organic matter in aquatic systems are the diagenesis, allochthonous and autochthonous additions and sorption/desorption. Diagenesis is a redox process, largely mediated by sedimentary microorganisms and the suitable indicators to this are the redox element sulphur, organic carbon and nitrogen. The relation of sedimentary parameters with grain size can give indication about the sorption/desorption processes.

PCA analysis of the mangrove sediments shows that three components without significant differences account for a total of 92.84% variance (Fig. 4.3). First component accounts for 35.77% of the total variance and shows very high positive loadings on biochemical compounds (except lipids), total carbon, organic carbon, total nitrogen and BPC. But this component has no significant loadings on the sediment texture and total sulphur, one of the major redox indicators. Hence the first factor seems to be autochthonous input including the mangrove litter and this could be the main source of organic matter in mangrove sediments.

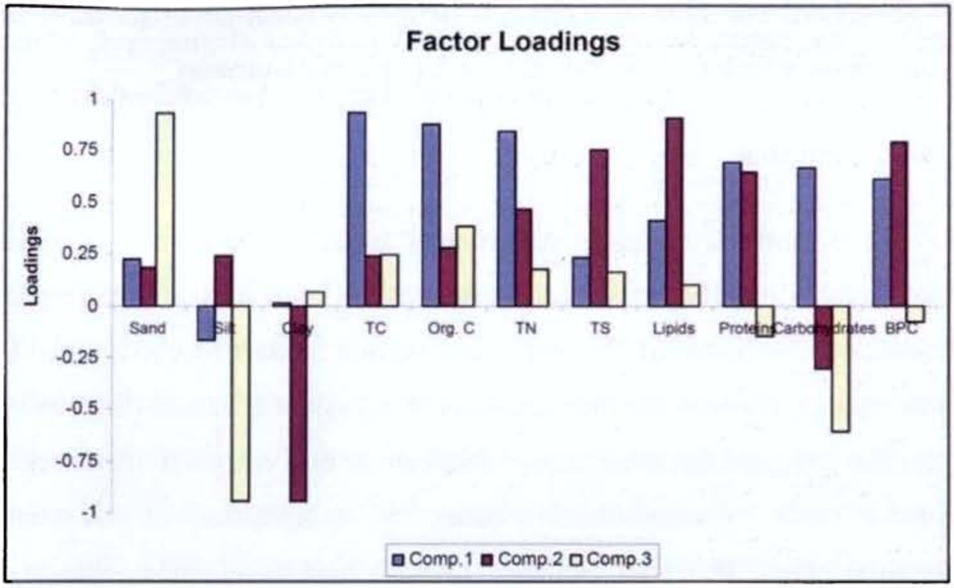

Figure 4.3 Results of factor analysis showing the loading pattern

Component 2 shows high negative loadings on clay and positive loadings on sulphur, lipids and BPC accounts for 34.84% of total variance. It also has a statistically significant negative loading on carbohydrate, which points towards the diagenetic pathway. The low negative loading pattern for carbohydrates might be due to the preferential remineralisation of lipids and proteins. It is established that carbohydrates are diagenetically weaker than proteins under anoxic conditions, but the reverse is true under oxic conditions (Harvey et al., 1995). Thus the anaerobic diagenetic processes account for the second major geochemical processes in these systems.

Component 3 accounts for 22.23% of total variance and shows high positive loadings on sand and negative loadings on silt and carbohydrates. Also from the positive loadings on organic carbon and low loading on clay it seems that the third component is the geochemical processes other than diagenesis, which includes siltation and sorption/desorption.

4.4 Conclusion

Sedimentary organic matter was found to be high in mangrove sediments. However the percentage ratio of the labile to total organic matter indicated that most of the deposited organic matter is refractory. This discrepancy between the high amounts of organic matter and the refractory fraction suggests that these ecosystems behave as a detrital trap and organic matter tends to accumulate. Biochemical composition of sedimentary organic matter provided evidence of high nutritional value, due to the dominance of lipids and protein fractions over carbohydrates in the study region. All the biochemical compounds, in general, showed higher concentrations during post-monsoon and lower concentrations during pre-monsoon seasons, except CHO, which was lower during monsoon. Station 3 recorded comparatively higher concentration of biochemical compounds. Higher PRT:CHO ratio at station 3 (>1) indicated the presence of fresh organic matter. The mangrove sediments showed higher LPD:CHO ratio, indicating the high quality of labile organic matter to support the benthic fauna. $\delta^{13}C$ analysis showed comparatively more enriched values at station 2 and more depleted values at station 1 and 3. From the observed relationships, it can be assumed that due to the almost closed nature of station 3, much of the mangrove area rarely inundated. Because of low tidal flushing, this station acts as retention site where mangrove derived organic carbon is a significant contributor to the sediment organic carbon pool.

Station 2 sediments reflect a balance between local inputs and carbon imported from the water column during high tide.

Biogeochemical analysis revealed that the biogeochemistry of mangrove sediments is very complex and cannot be explained effectively by using biochemical composition, elemental ratios or stable carbon isotope ($\delta^{13}C$) analysis. The bulk parameter analyses revealed that the organic matter in these systems is of mixed origin and it also reflects a balance between the local inputs and the carbon imported from the water column during high tide. Further studies based on the biomarker approach are essential to understand the origin and fate of the large detrital pool in these sediments.

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Chapter 5

Fatty acid Biomarkers

Contents

5.1 Introduction

Knowledge of the sources and reactivities of organic matter, as well as the factors controlling its distribution, are important to understand the role of mangrove ecosystems in global biogeochemical cycles. Organic carbon in mangrove sediments originates from both autochthonous sources (mangroves, microphytobenthos) and tidal inputs (Bouillon et al., 2004). Due to the complex nature of organic matter, the bulk parameter approaches including elemental analysis (e.g. C/N ratios), biochemical composition (Proteins, Lipids and Carbohydrates) and stable carbon isotopic composition of total organic matter ($\delta_{13}C_{TOM}$) are not completely successful in revealing the sources of organic matter in mangrove sediments. An effective tool for the source characterisation of organic matter in mangrove systems is the biomarker approach. Generally, approaches that used multiple techniques-incorporating biomarker approach along with bulk parameters- would be more successful for the source characterisation of organic matter in complex aquatic systems like mangroves.

Biomarkers are compounds or groups of compounds that can be used as signatures of individual organisms or groups of organisms, or of certain environmental processes (Parrish et al., 2000). Molecular organic biomarkers can serve as proxies to provide insight into how aquatic systems process, metabolise and sequester carbon in both the water column and sediments over decadal to geological time scales and are extremely useful in resolving the complexity of systems with multiple organic carbon sources (Hedges et al., 1997; Meyers, 1997; Wakeham et al., 1997b; Belicka et al., 2004).

Lipid compounds often contain the basic skeletal structure and functional group details of the original source (Venkatesan, 1988). Therefore, the investigation of lipid compositions in various recent depositional environments can provide much information about the sources and diagenetic processes of organic matter (Venkatesan, 1988; Rieley et al., 1991; Logan and Eglinton, 1994; Duan and Ma, 2001). Fatty acids constitute an important fraction of the lipid pool in living and dead organic material. Their abundance in living organisms, their source specificity with respect to individual compounds and their relative stability when compared to amino acids and carbohydrates make them ideal as biomarkers. Hence fatty acid biomarkers can be effectively used to trace the origin, transport and diagenetic changes of organic material in water columns and sediments (Lee and Wakeham, 1988; Wakeham and Lee, 1993; Harvey, 1994; Niggemann and Schubert, 2006). The relative abundances of individual fatty acids are useful in evaluating the respective importance of inputs from bacteria, microalgae, marine fauna and higher plants (Sargent et al., 1987).

Cochin estuarine system is one of the intensively studied aquatic systems of India and there are many data sets available with regard to geochemistry, nutrient dynamics and pollution of Cochin estuary and mangrove areas (Qasim et al., 1969; Sankaranarayanan and Qasim 1969; Nair et al., 1993; Seralathan et al., 1993; Seralathan and Padmalal, 1994; Sujatha et al., 1999; Menon et al., 2000; Shajan, 2001; Verma and Subramanian, 2002; Balachandran et al., 2003; Geetha et al., 2006;

Mathews et al., 2006; Renjith and Chandramohanakumar, 2007,2009). However, studies pertaining to the biogeochemistry of organic matter with special emphasis on source characterisation still remain poorly documented. This chapter attempts to identify the sources of organic matter in three mangrove systems of Cochin estuary and thereby to identify the major biogeochemical pathways. Fatty acid biomarkers were used to characterise the sources of organic matter in these mangrove systems.

5.1.1 Fatty acids

Fatty acids are carboxylic acids often with a long aliphatic hydrocarbon tail (chain), which is either saturated or unsaturated. The non-polar aliphatic hydrocarbon chain is an important counter balance to the polar acid functional group. Carboxylic acids as short as butyric acid (4 carbon atoms) are considered to be fatty acids, while fatty acids derived from natural fats and oils may be assumed to have at least 8 carbon atoms, e.g. caprylic acid (octanoic acid). Most of the natural fatty acids have an even number of carbon atoms, because their biosynthesis involves acetyl-CoA, a coenzyme carrying a two-carbon-atom group. Chain-lengths of fatty acids could range from 2 to 80, but most commonly from 12 up to 30. These fatty acids are ubiquitous in nature. They are found only in trace amounts in living cells in their free, unesterified form, and are of greatest importance as components of lipids which, upon alkaline hydrolysis, afford the alkali metal salts of fatty acids.

Fatty acids are simple in structure and can be subdivided into well-defined families. Among straight-chain fatty acids, the simplest are referred to as saturated fatty acids. They have no unsaturated linkages and cannot be altered by hydrogenation or halogenation. When double bonds are present, fatty acids are said to be unsaturated. Fatty acids may be more complex in plants and bacteria, since they can have an odd number of carbon atoms, branched chains or contain a variety of other functional groups, including acetylenic bonds, epoxy-, hydroxy- or keto groups and even ring structures (cyclopropane, cyclopropene, cyclopentene, furan, and cyclohexyl) or a Coenzyme A moiety (acyl CoA).

Saturated Fatty acids

Saturated fatty acids (SFAs) have no double bonds between the carbon atoms of the fatty acid chain and are thus fully saturated with hydrogen atoms. They have the general formula: $CH_3(CH_2)nCOOH$ (Table 5.1). They are named from the saturated hydrocarbon with the same number of carbon atoms, the final -e is changed to -oic. For example, the fatty acid with 18 carbon atoms is termed octadecanoic acid, but it has also a common name stearic acid. This compound may be defined also $C_{18:0}$ or 18:0. Saturated fatty acids are commonly straight chains with even carbon number.

Unsaturated Fatty acids

An unsaturated fatty acid is a fatty acid in which there are one or more double bonds in the fatty acid chain (Table 5.2). A fatty acid is monounsaturated (MUFA) if it contains one double bond, and polyunsaturated(PUFA) if it contains more than one double bond. The conventional nomenclature for fatty acids gives the chain length followed by the number of double bonds separated by a colon. The position of the double bond nearest the methyl terminus is given by a suffix of the form n-x, where x is the distance from the end of the chain. For example, Eicosapentaenoic acid (EPA) is $C_{20:5n-3}$ or 20:5n-3 denoting a 20 carbon fatty acid containing 5 double bonds, with the first double bond at 3 carbons from the methyl end.

Table 5.1 Most common saturated fatty acids

Systematic name	**Common name**	**Designation**	**Chemical Structure**
butanoic	butyric	$C_{4:0}$	$CH_3(CH_2)_2COOH$
pentanoic	valeric	$C_{5:0}$	$CH_3(CH_2)_3COOH$
hexanoic	caproic	$C_{6:0}$	$CH_3(CH_2)_4COOH$
octanoic	caprylic	$C_{8:0}$	$CH_3(CH_2)_6COOH$
nonanoic	pelargonic	$C_{9:0}$	$CH_3(CH_2)_7COOH$
decanoic	capric	$C_{10:0}$	$CH_3(CH_2)_8COOH$
dodecanoic	lauric	$C_{12:0}$	$CH_3(CH_2)_{10}COOH$
tetradecanoic	myristic	$C_{14:0}$	$CH_3(CH_2)_{12}COOH$
hexadecanoic	palmitic	$C_{16:0}$	$CH_3(CH_2)_{14}COOH$
heptadecanoic	margaric (daturic)	$C_{17:0}$	$CH_3(CH_2)_{15}COOH$
octadecanoic	stearic	$C_{18:0}$	$CH_3(CH_2)_{16}COOH$
eicosanoic	arachidic	$C_{20:0}$	$CH_3(CH_2)_{18}COOH$
docosanoic	behenic	$C_{22:0}$	$CH_3(CH_2)_{20}COOH$
tetracosanoic	lignoceric	$C_{24:0}$	$CH_3(CH_2)_{22}COOH$
hexacosanoic	cerotic	$C_{26:0}$	$CH_3(CH_2)_{24}COOH$
heptacosanoic	carboceric	$C_{27:0}$	$CH_3(CH_2)_{25}COOH$
octacosanoic	montanic	$C_{28:0}$	$CH_3(CH_2)_{26}COOH$
triacontanoic	melissic	$C_{30:0}$	$CH_3(CH_2)_{28}COOH$
dotriacontanoic	lacceroic	$C_{32:0}$	$CH_3(CH_2)_{30}COOH$
tritriacontanoic	ceromelissic	$C_{33:0}$	$CH_3(CH_2)_{31}COOH$
tetratriacontanoic	geddic	$C_{34:0}$	$CH_3(CH_2)_{32}COOH$
pentatriacontanoic	ceroplastic	$C_{35:0}$	$CH_3(CH_2)_{33}COOH$

Table 5.2 Most common unsaturated fatty acids

Common name	**Designation**	**Chemical structure**
Myristoleic acid	$C_{14:1\ n\ 5}$	$CH_3(CH_2)_3CH{=}CH(CH_2)_7COOH$
Palmitoleic acid	$C_{16:1\ n\ 7}$	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
Oleic acid	$C_{18:1\ n-9}$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
Linoleic acid	$C_{18:2\ n\ 6}$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
α-Linolenic acid	$C_{18:3\ n\ 3}$	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
Arachidonic acid	$C_{20:4\ n\ 6}$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH^{NIST}$
Eicosapentaenoic acid	$C_{20:5\ n\ 3}$	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$
Erucic acid	$C_{22:1\ n\ 9}$	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$
Docosahexaenoic acid	$C_{22:6\ n\ 3}$	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_2COOH$

Branched Fatty acids

Branched-chain fatty acids are common constituents of the lipids of bacteria and animals, although they are rarely found in the integral lipids of higher plants. Normally, the fatty acyl chain is saturated and the branch is a methyl-group. Branched chain fatty acids (mono- branched) may have also a methoxy or a hydroxy substitution. However, unsaturated branched-chain fatty acids are found in marine animals, and branches other than methyl may be present in microbial lipids. The most common branched chain fatty acids are mono-methyl-branched, but di- and poly-methyl-branched fatty acids are also known. Branched fatty acids have usually either an iso-structure (methyl group at the penultimate carbon atom) or an anteiso-structure (methyl group on the third carbon from the end).

5.1.2 Fatty acids as Biomarkers

Fatty acids are essential components of every living cell and have been used as sediment biomarkers by many researchers (Scribe et al., 1991; Wakeham and Beier, 1991; Harvey, 1994; Colombo et al., 1996; Laureillard et al., 1997). They have great structural diversity coupled with high biological specificity (Parkes, 1987) and have therefore been used as taxonomic indicators (Minnekin and Goodfellow, 1980). Fatty acid biomarkers are usually used to identify sources and fate of organic matter in marine environments (Wakeham and Canuel, 1990; Harvey, 1994; Laureillard et al., 1997; Budge and Parrish, 1998; Carrie et al., 1998; Mudge et al., 1998; Fahl and Stein, 1999). Fatty acid analysis have been used, for example, in the characterisation of microbial populations in polluted marine sediments (Parkes and Taylor, 1985), in the phylogenic classification of microalgae (Zhukova and Aizadaicher, 1995), to provide information on the distribution pattern of aquaculture waste in the environment, (Henderson et al., 1998), in the assessment of reactivity of recently deposited organic matter (Canuel and Martens, 1996), and in the determination of microbial biomass and community structure in sediments (Findlay et al., 1995). The last decade has seen an increase of studies using fatty acids as markers in order to identify the origin of organic matter in coastal environments (Le Blanc et al., 1989; Canual et al., 1995; Mudge and Gwyn Lintern, 1999).

The saturated fatty acids $C_{16:0}$ and $C_{18:0}$ are ubiquitous in the marine environment and can therefore be used as a measure of total community biomass (Parkes, 1987). $C_{14:0}$ fatty acid is present in phytoplankton, especially in diatoms (Reitan et al., 1994) and to a lesser extent in dinoflagellates (Napolitano et al., 1995). The marine phytoplankton shows a characteristic fatty acid composition in the range C_{14-22} (Claustre et al., 1989) and has been reported for settling particles in marine environments (Reemtsma et al., 1990; Colombo et al., 1996).

Unsaturated fatty acids are mainly associated with algae (Volkman et al., 1989; Carrie et al., 1998). For example, $C_{20:5n-3}$ was found rich in diatoms (Pond et al., 1998), and has been used as diatom marker in marine environments (Currie and Johns, 1988; Canual et al., 1995; Colombo et al., 1995). Polyunsaturated fatty acids (PUFAs), $C_{16:2n-4}$, $C_{16:2n-7}$ and $C_{20:4n-6}$ are also found rich in diaoms (Volkman et al., 1989). Some monounsaturated fatty acids (MUFAs) such as, $C_{16:1n-5}$, $C_{16:1n-7}$, $C_{16:1n-9}$ are also used as signals for diatom derived organic matter (Berge et al., 1995; Suzuki and Matsuyama, 1995; Carrie et al., 1998). Similarly, dinoflagellates are usually described as containing elevated amounts of $C_{22:6n-3}$ (Sargent et al., 1987; Harvey et al., 1988; Viso and Marty, 1993) and presence of this fatty acid in sediments usually indicates dinoflagellate origin (Colombo et al., 1996; Budge and Parrish, 1998; Carrie et al., 1998; Parrish et al., 2000). Chlorophyta contain high abundance of C_{18} PUFAs especially with the positional isomers n-3 and n-6 (Volkman et al., 1989; Dunstan et al., 1992; Zhukova and Aizdaicher, 1995) and$C_{18:2n-6}$, $C_{18:3n-3}$ and $C_{18:3n-6}$ have been used as markers of green algae (Dunstan et al., 1992; Kharlamenko et al., 1995; Napolitano et al., 1997; Meziane and Tsuchiya, 2000).However recent fatty acid analyses revealed that $C_{18:2n-6}$ and $C_{18:3n-3}$ are also dominant in mangrove leaves (Hall et al., 2006; Meziane et al., 2007). MUFA, $C_{18:1n-9}$ has been reported as a biomarker for brown algae (Jamieson and Reid, 1972; Johns et al., 1979).

The ratios of $C_{16:1}/C_{16:0}$ and $\sum C_{16}/\sum C_{18}$ as initially proposed by Claustre et al., (1988/1989, 1989), are used to distinguish between diatom and dinoflagellate dominance in the ecosystem. They interpreted an increase in values of both these ratios as representative for increased proportions of diatoms. In conjunction with this, Bodennec et al., (1994) suggested that values of $C_{16:1}/C_{16:0}$ greater than 1.6 can be interpreted as signaling the predominance of diatoms (Budge and Parrish, 1998). Similarly,the ratios of $C_{20:5n-3}/C_{22:6n-3}$ and $C_{16:1n-7}/(C_{18:3n-3} + C_{18:4n-3})$ are also used to predict the dominance of diatoms versus dinoflagellates. MUFA $C_{24:1}$ has been ascribed to a zooplanktonic origin (Wakeham et al., 1997a). $C_{20:1}$ fatty acids are widely found in marine animals such as zooplankton and fish (Ota et al., 1995; Albers et al., 1996).

Long chain saturated fatty acids ($\geq C_{22}$) are generally associated with the waxy leaf coatings of higher plants and are thus considered as an indicative of higher plant inputs (Kolattukudy, 1970; Boon and Duineveld, 1996; Canuel and Martens, 1996; Colombo et al., 1996; Meyers, 1997; Carrie et al., 1998). In addition, long chain fatty acids (LCFAs) also have been found in high concentrations in mangrove leaves (fresh and decomposing) (Wannigama et al., 1981; Meziane and Tsuchiya, 2000, 2002). Mangroves utilize C_3 photosynthetic biochemistry, in which phosphoglyceric acid (PGA), a three carbon compound is produced during the dark reactions. All woody plants are C_3 plants, so use of the C_3 pathway is likely a reflection of the terrestrial ancestry of mangroves. These similarities in carbon fixing abilities result similar fatty acid signatures for mangroves and terrestrial plants (Alongi, 1998). Ratios of longer chain to shorter chain lipids ($C_{24:0}/C_{16:0}$) can be used to assess relative contribution of allochthonous vs. autochthonous components (Meyers et al., 1984).

Odd carbon-numbered and branched-chain fatty acids are commonly produced by bacteria and may be used as bacterial biomarkers (Volkman et al., 1980; Parkes,1987; Wakeham and Beier, 1991; Haddad et al., 1992; Rajendran et al., 1997). Bacteria contain the most distinct fatty acid compositions of all marine taxa, with high proportions of C_{13} to C_{21} odd-numbered fatty acids, often branched and with at most one unsaturation (Claustre et al., 1989). Fatty acid $C_{18:1n-7}$ has also been used as a bacterial biomarker (Volkman et al., 1980; Claustre et al., 1989).

In general, short chain saturated fatty acids (i.e. 14-18) show greater reactivity than saturated fatty acids with more than 20 carbons, and PUFAs are more reactive than saturated fatty acids (Haddad et al., 1992; Canuel and Martens, 1996; Colombo et al., 1997). The long chain fatty acids from terrigenous sources are more refractory than the shorter chained fatty acids from autochthonous planktonic sources , and the overall order of relative reactivity between sources is plankton > bacteria> terrestrial (Camacho-Ibar et al., 2003).

The important fatty acids that can be used as specific biomarkers for source characterisation of organic matter in aquatic environment are encapsulated in Table 5.3

Table 5.3 Specific biomarkers for source characterisation of organic matter in aquatic environment

Fatty acids	**Source**	**Reference**
$C_{14:0}$	Diatoms	Reitan et al.,1994
$C_{14:1}$	Cyanobacteria	Caudales and Wells, 1992; Caudales et al., 1993.
n-$C_{15:0}$, n-$C_{17:0}$, $C_{15:0iso-}$ and $C_{15:0\ anteiso-}$ $C_{15:1}$, $C_{17:1}$	Bacteria	Volkman et al., 1980 ; Parkes, 1987 ;Currie and Johns, 1988 ; Clausture et al., 1989 ; Rajendran et al., 1993
$C_{16:0}$	Ubiquitous biological markers of plankton in the marine environment.	Parkes, 1987
$C_{16:1}$	Marine algal species	Reitan et al.,1994
$C_{16:1n-7}$	Diatoms	Berge et al., 1995; Skerrat et al., 1995; Suzuki and Matsuyama, 1995
$C_{16:1n-5}$	Diatoms	Berge et al., 1995; Suzuki and Matsuyama, 1995; Carrie et al., 1998
$C_{16:1n-9}$	Diatoms	Berge et al., 1995; Suzuki and Matsuyama, 1995; Carrie et al., 1998
$C_{16:4n-1}$	Diatoms	Colombo et al., 1996
$C_{18:0}$	Ubiquitous biological markers of plankton in the marine environment.	Parkes, 1987
$C_{18:1n-7}$	Bacteria	Volkman et al.,1980; Rajendran et al., 1993;.
$C_{18:1n-9}$	Brown algae	Jamieson and Reid, 1972; Johns et al., 1979

$C_{18:2n-6}$	Green algae	Kharlamenko et al., 1995
$C_{18:3n-6}$	Green algae	Volkman et al., 1989; Dunstan et al., 1992; Zhukova and Aizdaicher, 1995; Napolitano et al., 1997
$C_{18:3n-3}$	Green algae	Volkman et al., 1989; Dunstan et al., 1992; Zhukova and Aizdaicher, 1995; Napolitano et al., 1997
$C_{20:1}$	Zooplankton	Albers et al.,1996
$C_{20:5n-3}$	Diatoms	Currie and Johns, 1988; Canuel et al., 1995; Pond et al., 1998; Meziane and Tsuchiya, 2000
$C_{22:6n-3}$	Dinoflagellates	Sargent et al., 1987; Harvey et al., 1988; Viso and Marty, 1993; Colombo et al., 1996
LCFAs($\geq C_{22}$)	Terrestrial plants and mangroves	Kolattukudy, 1970; Wannigama et al., 1981; Boon and Duineveld, 1996; Canuel and Martens, 1996; Colombo et al.,1996; Meyers, 1997
$C_{24:1}$	Zooplankton	Wakeham et al., 1997a
$\sum C_{15}+\sum C_{17}$	Bacteria	Rajendran et al., 1993
$C_{16:1}/C_{16:0} > 1.6$	Diatoms	Claustre et al.,(1988/1989) ;Bodennec et al.,1994 ; Parrish et al., 2000
$\sum C_{16}/\sum C_{18} > 2$	Diatoms	Claustre et al.,(1988-1989), Bodennec et al.,1994 ; Parrish et al., 2000

$C_{16:1n-7}/(C_{18:3n-3}+C_{18:4n-3})$	Increasing biomarker ratio, increasing importance of diatoms vs. dinoflagellates	Budge and Parrish, 1998; Mudge et al., 1998;Tolosa et al., 2004
$C_{20:5n-3}/C_{22:6n-3}$	Increasing biomarker ratio, increasing importance of diatoms vs. dinoflagellates	Claustre et al. 1988/1989;Budge and Parrish, 1998; Mudge et al., 1998 ;Tolosa et al., 2004

5.2 Results

Fatty acids were extracted from the surface sediments of the mangrove stations during the post-monsoon season (the season with higher concentrations of biochemical composition). Fatty acids ranging from C_8 to C_{24} were identified from the study region (Table 5.4, Fig. 5.1). The fatty acids distinguished are broadly classified in to saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). SFAs were the major fraction at station 1 and 3 where as at station 2, MUFAs dominated.

At station1, fatty acids ranging from C_8 to C_{24} were obtained (Table 5.4). A total of 19 fatty acids were characterised and the total fatty acid concentration was 64.91 μg/g dry weight. $C_{16:0}$ was the most abundant fatty acid (9.86 μg/g) contributing 15.1 % of total fatty acids (TFAs). The PUFA, $C_{22:2}$ was the second most abundant (6.01 μg/g), which accounted for 9.3 % of TFAs.

Table 5.4 Fatty acid composition in the surface sediments of the mangrove stations

Fatty acids	Station 1		Station 2		Station 3	
	% of Abundance	Conc. (μg/g)	% of Abundance	Conc. (μg/g)	% of Abundance	Conc. (μg/g)
C8	1.2	0.758	-	-	0.1	0.126
C10	1	0.642	-	-	0.2	0.189
C12	5.3	3.441	3.1	1.16	1.8	1.666
C14	4.7	3.033	2.9	1.075	3	2.673
C14:1	2.2	1.458	1.7	0.623	1.9	1.698
C15	1	0.642	0.8	0.283	1.3	1.132
C15:1	-	-	0.8	0.311	1	0.912
C16	15.1	9.856	15.3	5.716	14	12.64
C16:1	0.5	0.35	0.6	0.226	0.8	0.692
C17	6.6	4.257	-	-	1.3	1.195
C17:1	-	-	-	-	0.3	0.252
C18	6.5	4.199	8.3	3.113	7.5	6.792
C18:1n-9	2	1.283	4.8	1.811	1.7	1.509
C18:3n-3	-	-	-	-	1.4	1.226
C18:3n-6	6.5	4.199	4.2	1.556	2	1.824
C20	7.6	4.957	0.8	0.283	2.4	2.138
C20:1	9	5.832	19.8	7.385	0.9	0.849
C20:3n-3	-	-	-	-	1.2	1.092
C20:5n-3	8.6	5.657	8.7	3.254	22.4	20.155
C21	-	-	-	-	0.7	0.637
C22	3.3	2.158	3.3	1.245	7.4	6.666
C22:2	9.3	6.007	7.7	2.886	5.8	5.22
C24	4	2.566	2.4	0.905	15.5	13.961
C24:1	5.6	3.616	14.8	5.546	5.4	4.842
TOTAL		64.911		37.378		90.086

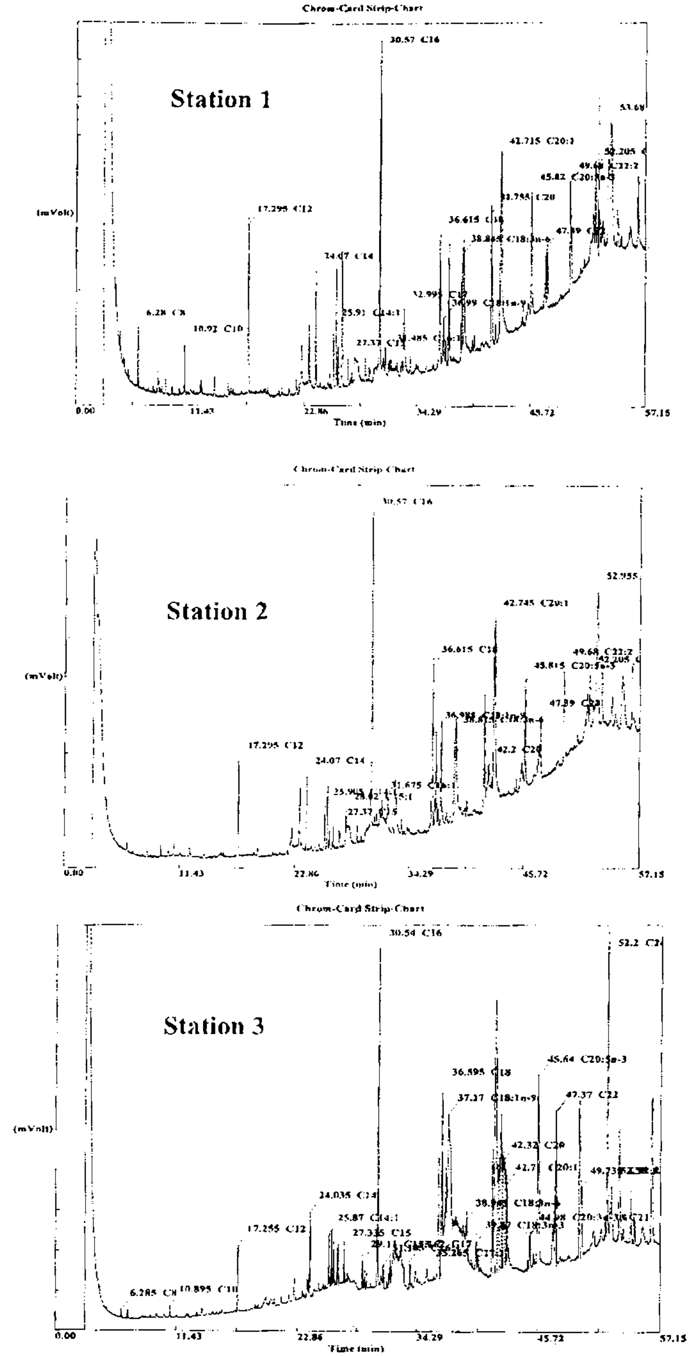

Figure 5.1 Gas Chromatograms of FAMEs of the study region

Fatty acids ranging from C_{12} to C_{24} were identified at station 2. (Table 5.4). A total of 17 fatty acids were recognised and the total fatty acid concentration was 37.38 µg/g dry weight. $C_{20:1}$ fatty acid was the most abundant fatty acid contributing 19.8 % of TFAs, followed by $C_{16:0}$ (15.3 % of TFAs).

At station 3, fatty acids ranging from C_8 to C_{24} were obtained (Table 5.4). A total of 24 fatty acids were characterised and the total fatty acid concentration was 90.09 µg/g dry weight. The PUFA, $C_{20:5n-3}$ was the most abundant fatty acid contributing 22.4 % of TFAs, followed by $C_{24:0}$ (15.5 % of TFAs).

5.2.1 Saturated Fatty acids (SFAs)

Saturated fatty acids were the major fraction at station1 and 3, contributing to 56.3 and 54.5 % of TFAs respectively. At station 2, this fraction was comparatively lower (36.9 % of TFAs).

SFAs ranging from $C_{8:0}$ to $C_{24:0}$ were characterised from station1. $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$,$C_{24:0}$ were the dominant SFAs obtained at this station. $C_{16:0}$ was the dominant SFA (15.1 % of TFAs) at station 1, followed by $C_{20:0}$ (7.6 % of TFAs). At station 2, SFAs ranged from C_{12} to C_{24} were obtained.$C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$, $C_{24:0}$ were the SFAs detected at this station .C_{16} was the dominant SFA (15.3 % of TFAs) at station 2 followed by $C_{18:0}$ (8.3 % of TFAs). SFAs ranged from C_8 to C_{24} were detected at station 3. $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{20:0}$, $C_{21:0}$, $C_{22:0}$, $C_{24:0}$ were the SFAs obtained at this station .$C_{24:0}$ was the dominant SFA (15.5 % of TFAs) at station 3 followed by $C_{16:0}$ (14% of TFAs).

5.2.2 Monounsaturated Fatty acids (MUFAs)

At station 1, MUFAs accounted for 19.3 % of TFAs. $C_{14:1}$, $C_{16:1}$, $C_{18:1n-9}$, $C_{20:1}$, and $C_{24:1}$ were the MUFAs identified at this station. $C_{20:1}$ was the major MUFA here (9.0 % of TFAs) followed by $C_{24:1}$(5.6 % of TFAs). At station 2, MUFAs were the largest class contributing to 42.5 % of TFAs. $C_{14:1}$, $C_{15:1}$, $C_{16:1}$, $C_{18:1n-9}$, $C_{20:1}$, and $C_{24:1}$ were the MUFAs identified at this station. $C_{20:1}$ was the dominant MUFA (19.8 % of TFAs) followed by $C_{24:1}$ (14.8 % of TFAs). At station 3, the MUFAs accounted for 12 % of TFA. $C_{14:1}$, $C_{15:1}$, $C_{16:1}$, $C_{17:1}$, $C_{18:1n-9}$, $C_{20:1}$, and $C_{24:1}$ were the MUFAs identified at station 3. $C_{24:1}$ was the major MUFA (5.4% of TFAs) at this station followed by $C_{14:1}$(1.9 % of TFAs).

5.2.3 Polyunsaturated Fatty acids (PUFAs)

PUFAs accounted for 24.4 % of TFAs at station 1. $C_{18:3n-6}$, $C_{20:5n-3}$, $C_{22:2}$ were the PUFAs identified at this station. $C_{22:2}$ was the major PUFA (9.3 % of TFAs) followed by $C_{20:5n-3}$ (8.6 % of TFAs).The PUFAs at station 2 contributed to 20.6 % of TFAs. $C_{18:3n-6}$, $C_{20:5n-3}$, $C_{22:2}$ were the PUFAs detected here. $C_{20:5n-3}$ was the major PUFA (8.7% of TFAs) followed by $C_{22:2}$ (7.7 % of TFAs).At station 3 PUFAs accounted for 33.5 % of TFAs. $C_{18:3n-3}$, $C_{18:3n-6}$, $C_{20:3n-3}$, $C_{20:5n-3}$, $C_{22:2}$ were the PUFAs detected at station3. $C_{20:5n-3}$ was the dominant PUFA (22.4% of TFAs) followed by $C_{22:2}$ (5.8 % of TFAs).

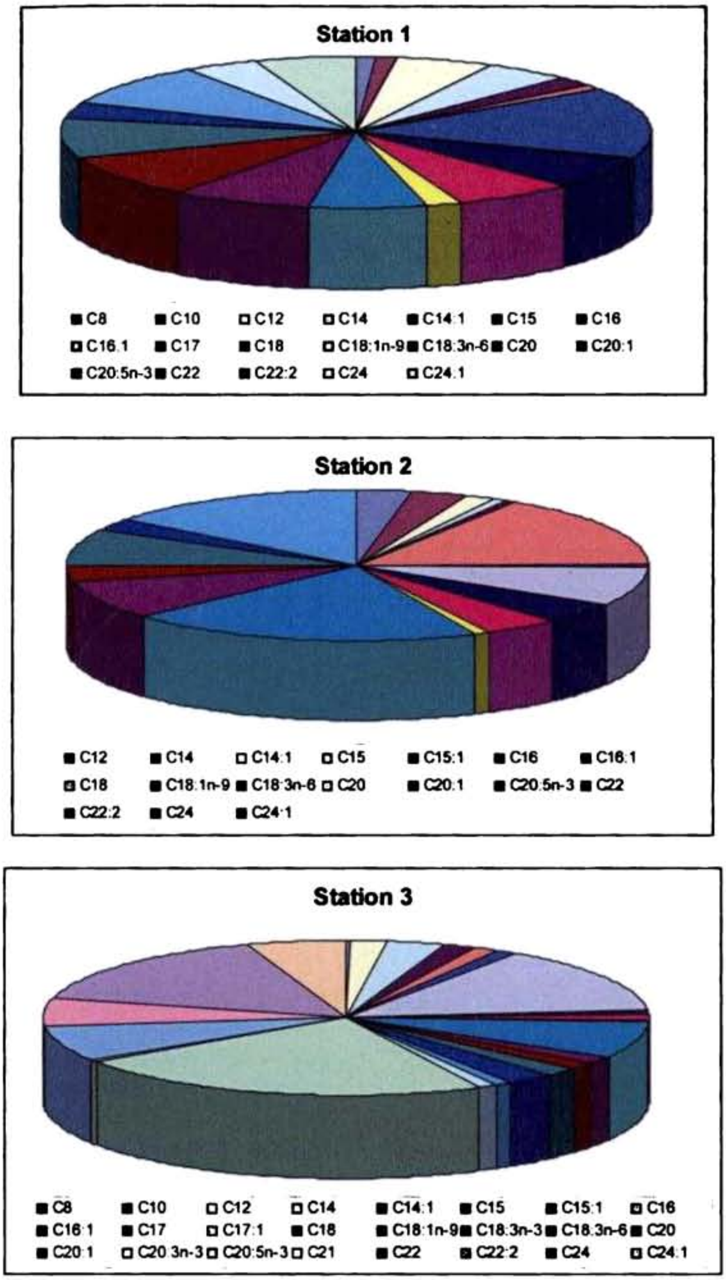

Figure 5.2 Distribution of fatty acids in the mangrove sediments

5.3 Discussion

The results obtained from the three mangrove sediments of Cochin are in agreement with the concentrations and abundance of fatty acids obtained from different aquatic systems of the world (Table 5.5).

Table 5.5 Earlier works using fatty acids as biomarkers

Sl. No.	Study region	Total Fatty acids (µg/g)	Reference	Inference
1	Surficial sediments of Lake Huron	13.2 - 126.8	Meyers and Takeuchi, 1979	Fatty acids were originated from autochthonous sources.
2	Sediments of Hebridean Shelf edge, Scotland	1.9-13.2	Carrie et al., 1998	The fatty acids detected were predominantly marine origin.
3	Surface sediments of Ria Formosa Lagoon, Portugal	0.3-67.5	Mudge et al., 1998	~0.5 % of TOC. Major source was phytoplankton.
4	Surface sediments from Santa Monica Basin	5 - 40	Pearson et al., 2001	The major carbon source of Santa Monica Basin sediments was from marine euphotic zone primary production or subsequent heterotrophic consumption of this biomass.
5	Surface sediments of Arctic Ocean	3-168	Belicka et al., 2002	The organic matter is dominated by substantial autochthonous marine carbon.
6	Surface sediments of Okinawan estuary mangrove estuary, Japan.	89.2-613.9	Meziane and Tsuchiya, 2002.	The export of organic matter from the mangrove litter to the intertidal flat was limited and spatially restricted.
7	Shelf, slope and basin sediments of the Beaufort Sea	3.60-11.1(mg/g OC)	Belicka et al., 2004	Beaufort Shelf system was greatly influenced by the Mackenzie River with significant terrestrial contributions to total organic carbon.

8	Shelf, slope and basin sediments of the Chukchi Sea	1.84-17.0 (mg/g OC)	Belicka et al., 2004	Dominance of marine carbon on the broad, shallow Chukchi Shelf and Slope, and suggest that episodic pulses of marine material may be incorporated with out much degradation into sediments.
9	Sediment traps in the Gulf of Papua	20-2215	Burns et al.,2004	The fatty acids consisted of marine phyto-and zooplankton biomarkers
10	Suspended particles in Alboran Sea (SW Mediterranean Sea)	860-3770	Tolosa et al., 2004	Flagellates and cyanobacteria dominated in the suspended particles of the surface waters. The lower levels of PUFAs suggested that most of these labile fatty acids were effectively recycled in the water column.
11	Sinking particles in Alboran Sea (SW Mediterranean Sea)	2470 -21530	Tolosa et al., 2004	Diatoms dominated at greater depths and in all sinking particles.
12	Hydrothermal vent sediments of western Pacific Ocean.	13.2-184	Yamanaka and Sakata, 2004.	Organic matter in these sediments was rich in bacteria and other vent organisms.
13	Surface sediments of Yalujiang Estuary, North China	4.8–32.9	Wu et al.,2004	The distribution of fatty acids suggested that the dominant sources are phytoplankton, zooplankton, and bacteria.
14	Surface Sediments of subtropical mangrove in Okinawa Island, South Japan (Oura Bay estuary)	222.6	Mfilinge et al., 2005.	Outwelling of mangrove-derived POM varies seasonally and spatially in Oura Bay estuary. High concentrations of LCFAs markers in sediments consistent with the increases in litter fall, suggest a greater input of vascular plant or organic material produced from the mangroves during autumn.

15	Surface sediments of Matapouri estuary, New Zealand.	295.4- 547.4	Alfaro et al., 2006	Bacterial biomarkers showed highest concentration in sediments. The strong representation of diatom was also noted.
16	Particulate organic matter of of Matapouri estuary, New Zealand.	33-64.6 (wet wt.)	Alfaro et al., 2006	Mangrove and brown algae appears to be important contributors of particulate organic matter followed by sea grass.
17	Surface sediments from the subtropical Pearl River estuary and adjacent shelf, Southern China	1.28 - 42.25	Hu et al., 2006	Fatty acids were mainly derived from phytoplankton, bacteria and terrestrial organic matter.
18	Surface sediments from the Chilean coastal upwelling region	20 - 203	Niggemann and Schubert, 2006	Fatty acids were predominantly from marine and bacterial sources.
19	Fatty acids in surface sediments of Florida Bay	5-21	Xu et al., 2006	The organic matter was mainly from algal and bacterial origin.

5.3.1 Distributional characteristics of Fatty acid Biomarkers

The fatty acid molecular distributions in the surficial sediments suggest that there are several sources of organic matter in these mangrove systems. From the individual fatty acid biomarkers, 4 different source signals could be distinguished, viz. algal, zooplankton, bacterial and higher plant signals.

5.3.1.1 Algal Signals

Unsaturated fatty acids are mainly associated with algae (Volkman et al., 1989; Carrie et al., 1998). PUFA, $C_{20:5n-3}$ was present in appreciable levels at all the stations, especially at station 3; it was 22.4% of TFAs.

$C_{20:5n-3}$ has been strongly detected in diatoms (Pond et al., 1998), and has been used as diatom marker in marine environments (Currie and Johns, 1988; Canual et al., 1995;Colombo et al., 1995).Diatoms are a major group of eukaryotic microalgae, and are one of the most common types of phytoplankton. Microalgae are a major source of fatty acids in most sedimentary environments and are important in mangrove ecosystems. They are epiphytic and grow on the aerial roots of the trees and on the sediments. They include green (Chlorophyta), brown (Phaeophyceae), red (Rhodophyta) and blue-green algae (Cyanophyta).The strong representation of diatoms at station 3 was evident from the high concentration of biomarker $C_{20:5n-3}$.

Phytoplanktons generally contain more $C_{16:0}$ (Wakeham, 1995) whereas zooplanktons contain more $C_{18:0}$ (Sargent, 1976). The ratio $\sum C_{16}/\sum C_{18}$ is considered to be an indicator of benthic phytoplankton (Parrish et al., 2000). The ratio was relatively high at station 3 (1.7). This also supports the significant contribution of diatom organic matter at station 3.

The saturated fatty acid $C_{14:0}$ was present at all stations. This fatty acid is also among the major lipid components of phytoplankton, especially diatoms (Reitan et al., 1994). $C_{16:1}$ fatty acid was detected in very low levels at all the stations and this fatty acid is relatively common in marine algal species (Reitan et al., 1994; Berge et al., 1995). The ratio of $C_{16:1}/C_{16:0}$ could be used to distinguish between diatom and dinoflagellate derived organic matter and values >1.6 has been regarded as diatom origin (Budge and Parrish, 1998). The ratios in the study region were in the range 0.03 - 0.06. Due to the higher susceptibility of unsaturated fatty acids to the biological and chemical degradation during the sedimentation, the ratio of $C_{16:1}/C_{16:0}$ in surface sediments is usually well below 1 (Birgel et al., 2004) and hence this ratio could not be considered as a reliable one.

$C_{18:3n-6}$ was also present at all the three stations, where as $C_{18:3n-3}$ was present only at station 3. PUFAs, $C_{18:2n-6}$, $C_{18:3n-3}$ and $C_{18:3n-6}$ have been used as markers of green algae ((Dunstan et al., 1992; Kharlamenko et al., 1995; Napolitano et al., 1997; Meziane and Tsuchiya, 2000). However, recent fatty acid analyses revealed that $C_{18:2n-6}$ and $C_{18:3n-3}$ are also dominant in mangrove leaves (Hall et al., 2006; Meziane et al., 2007).

$C_{18:1n-9}$ was also detected at all the three stations and this MUFA has been reported as a biomarker for brown algae (Jamieson and Reid, 1972; Johns et al., 1979).$C_{20:3n-3}$ fatty acid, which has been found in brown macroalgae (Khotimchenko, 1991), was present only at station 3.

5.3.1.2 Zooplankton Signals

MUFAs, 20:1 and 24:1 has been considered as zooplanktonic in origin (Wakeham et al., 1997a). All the three stations contained these fatty acids but station 2 sediments showed higher concentration. The contribution of this fatty acid at station 3 was very low when compared to other two stations. The mangrove ecosystem is rather a harsh one for plankton owing to a combination of periodic fluctuations and extremes of its physico-chemical parameters, water depth, turbidity, tidal amplitude and organic matter. Hence, the zooplankton standing stock (biomass) values in mangroves were low as compared to contiguous estuarine and neritic biotopes (Goswami, 1992). For this reason, the zooplankton biomarkers in the study region could be considered as estuarine contribution and the lower values at station 3 might be due to the limited tidal exchange. As has been noted, station 1 is located about 100 meters away from the estuarine front and station 2 is on the bank of the estuary. This explains the higher abundance of zooplankton biomarkers at these stations and the dominance at station 2.

5.3.1.3 Bacterial Signals

Odd carbon-numbered and branched-chain fatty acids are commonly produced by bacteria and may be used as bacterial biomarkers (Volkman et al., 1980; Haddad et al., 1992; Harvey, 1994; Rajendran et al., 1997). Bacterial biomarkers in the study region ranged between 1.6 (station 2) and 7.6 % (station1) of total fatty acids. $C_{15:0}$ fatty acids were identified at all the three stations. $C_{15:1}$ fatty acid was present at stations 2 and 3 where as at station 1, it was absent. At stations 1 and 3, $C_{17:0}$ fatty acid was identified. $C_{17:1}$ was identified only at station 3. The n-fatty acids of $<C_{12}$ in sediments are not often reported, but were present at stations 1 and 3. The presence of C_8 to C_{10} fatty acids in the study region indicate that they are derived mainly from bacteria, because surface or shallow sediments contain abundant bacteria (Hayashi and Takii, 1977).

Microbially-mediated degradation processes appear to have a significant effect on the composition of sedimentary organic matter in coastal sediments. These processes diagenetically alter lipid constituents over short geochemical time scales (Canuel and Martens, 1993). At station 2, the percentage of saturated and unsaturated (MUFAs+PUFAs) were 36.9 and 63.1 of TFAs respectively. The low concentrations of both bacterial biomarkers and saturated fatty acids suggested low reworking of organic matter accumulating at station 2. As a result, source signals of labile constituents may be preserved (Canuel and Martens, 1993).

5.3.1.4 Higher plant Signals

Long chain even fatty acids from $C_{22:0}$ to $C_{30:0}$ are generally associated with the waxy leaf coatings of higher plants and are thus considered as indicative of higher plant inputs (Kolattukudy, 1970; Scribe et al., 1991; Colombo et al., 1996, Meyers, 1997). Long chain fatty acids (LCFAs) were found in high concentrations in mangrove leaves (Wannigama et al., 1981; Mezianc and Tsuchiya, 2000; Alfaro et al., 2006). Two higher plant biomarkers, $C_{22:0}$ and $C_{24:0}$ were obtained in this study. Station 3 showed higher percentage of $C_{22:0}$ (7.4% of TFAs) and $C_{24:0}$ (15.5% of TFAs) compared to other two stations. Ratios of longer chain to shorter chain lipids ($C_{24:0}/C_{16:0}$) can be used to assess the relative contribution of allochthonous vs. autochthonous components (Meyers et al., 1984). The ratios ranged from 0.16 (station 2) to 1.11(station 3). The ratio >1 at station 3 also indicated that this system receives a significant amount of organic matter from higher plants including mangroves.

5.3.2 Source Characterisation of the Mangrove Systems

The bulk parameter approach had revealed that biochemical composition of sedimentary organic matter in the study region is quite different from other coastal systems and showed a dominance of LPD followed by PRT and CHO. Significantly higher values of LPD in the study region especially at station 3 might be due to its preservation under highly anoxic conditions, which was evident from its highly significant negative correlation with Eh and significant positive correlation with total sulphur. Higher concentrations of CHO during the post-monsoon season point towards the possibility of higher input of vascular plant materials, especially mangrove litter. The absence of any significant correlations of CHO with other sedimentary parameters also suggests different origin and diagentic pathways for carbohydrates. These analyses also suggest that a significant fraction of LOM not only reaches sediments underlying the shallow water column, but also escapes wide scale degradation. Higher PRT:CHO at station 3 (>1) indicated the presence of fresh organic matter. Stations 1 and 2 showed >1 PRT: CHO ratios only during monsoon season. During pre-monsoon and post-monsoon seasons, the lower ratios indicate the presence of aged or less degradable organic matter. LPD:CHO ratio was also very high at station 3, indicating the high quality of labile organic matter. The C/N ratios were intermediate to that of autochthonous and terrestrial inputs of organic matter, signaling to a mixed origin. $\delta^{13}C$ analysis showed comparatively more enriched values at station 2 and more depleted values at station 1 and 3.

Bulk parameter approach also reveled that station 3 acts as retention site where mangrove derived organic carbon is a significant contributor to the sediment organic carbon pool. This is due to the almost closed nature of the station, where much of the mangrove area is rarely inundated because of the low tidal flushing. However, the other stations reflect a balance between local inputs and carbon imported from the water column during high tide. From the statistical interpretations of bulk parameters, it could also be deduced that the autochthonous inputs including the mangrove litter might be the main source of organic matter in these mangrove sediments. The anaerobic diagenetic processes account for the second major biogeochemical processes in these ecosystems. The geochemical process other than diagenesis, which includes siltation and sorption/desorption was found to be the third major process controlling the biogeochemistry of the mangrove systems under study.

The biomarker approach using fatty acids was found to be highly suitable for the study region since the lipids were the dominant class of labile organic matter. The major organic matter sources in the mangrove systems, as evident from the fatty acid biomarker characterisation are tabulated below (Table 5.5).

Table 5.5 Abundance of specific fatty acid biomarkers (%) in the mangrove systems of Cochin

Source	**Biomarker**	**Station1**	**Station2**	**Station3**
Diatoms	$C_{20:5n-3}$ $\sum C_{16}/\sum C_{18}$	8.6 1.04	8.7 0.91	22.4 1.7
Bacteria	$\sum C_{15}+\sum C_{17}$	7.6	1.6	3.9
Terrestrial plants and mangroves	LCFAs ($C_{22:0}+C_{24:0}$) $C_{24:0}/C_{16:0}$	7.3 0.26	5.7 0.16	22.9 1.11
Zooplankton	$C_{20:1}+C_{24:1}$	14.6	34.6	6.3
Brown algae	$C_{18:1n-9}$	2.0	4.8	1.7

Saturated fatty acids were the major fraction at station1, contributing to 56.3 % of TFAs, followed by PUFAs (24.4 %). Unlike other stations, a clear dominance of specific fatty acid biomarkers was absent at this station. PUFA, $C_{20:5n-3}$ was present in appreciable levels and also the ratio $\sum C_{16}/\sum C_{18}$ was greater than 1, both indicating significant benthic phytoplankton contribution. Bacterial biomarkers were higher at this station, when compared to the other stations, signaling to the significant influence of microbially mediated degradation processes. The zooplankton biomarkers $C_{20:1}$ and $C_{24:1}$ were present in considerable levels, suggesting the tidal influx. This station also showed noticeable levels of higher plant markers.

The major fatty acids at station 2 were MUFAs $C_{20:1}$ and $C_{24:1}$, the zooplankton biomarkers. The percentage unsaturated fatty acids (MUFAs+PUFAs) were 63.1 of TFAs. The low concentrations of both bacterial biomarkers and saturated fatty acids suggested low reworking of organic matter accumulating at station 2. Higher contribution of zooplankton biomarkers in mangroves are considered to be estuarine contribution. The proximity to the estuary explains the higher abundance of zooplankton biomarkers at this station.

SFAs were the major fraction at station 3, contributing to 54.5 % of TFAs, followed by PUFAs (33.5 %).The dominant fatty acids at this station were diatom marker $C_{20:5n-3}$ and the higher plant marker $C_{24:0}$. The ratio $\sum C_{16}/\sum C_{18}$ was also relatively high at station 3. This also supports the significant contribution of diatom organic matter at station 3.The high ratio of $C_{24:0}/C_{16:0}$ (>1) at this station indicated that this system receives a significant amount of organic matter from higher plants including mangroves. $C_{18:2n-6}$ and $C_{18:3n-3}$, dominant fatty acids in mangrove leaves, were also present at station 3. The low contributions of MUFAs $C_{20:1}$ and $C_{24:1}$ suggests the limited tidal exchange.

It can be seen that unsaturated fatty acids constituted a significant portion of the total fatty acids in the study region. It implies that the fatty acid diagenesis during particle settling through the water column is not intense, allowing the deposition of labile fatty acids. Sinking times of organic matter are short in shallow mangrove ecosystems, resulting in a relatively less decomposition of organic matter in the water column. This results in better preservation of organic matter during their faster burial. After burial, degradation of unsaturated fatty acids might also be low due to the anoxic nature of the sediments. Changes in redox condition significantly affect lability of specific organic compounds. Under anoxic conditions bacteria do not efficiently remineralise lipid compounds, results in their preservation (Sun et al., 2002).

5.4 Conclusion

The fatty acid biomarker study of the three mangrove systems of Cochin estuary could substantiate the bulk parameter approaches effectively to characterise the multiple sources of the organic matter and their biogeochemical transformations. The variations in the organic matter sources and the diagenentic pathways due to the environmental settings of the mangrove systems could also be well documented through this approach. From the study, fatty acid biomarkers are proved to be highly effective to find the relative abundance of algae, zooplankton and bacteria. However, vascular plants (mangrove and terrestrial plants) share common fatty acid markers. Therefore, it is difficult to distinguish between mangrove and terrestrial plants organic matter inputs through the long chain fatty acids. Hence other lipid biomarkers like sterols and terpenoids along with the fatty acids could be more effective to differentiate the terrestrial higher plant sources from the mangrove litter.

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Summary

Mangrove systems are recognized as highly productive, biogeochemically active regions, which play an important role in the carbon balance of coastal environment. Due to their dynamic ecotonal location, these environments display strong spatial and temporal variability of major biogeochemical characteristics. There is a conspicuous lack of information about concentrations and variability of these compounds in mangrove sediments. In order to understand the relative importance of biogeochemical processes, it is necessary not only to characterize and quantify the organic matter, but also to identify its major sources.

Many biogeochemical studies in the mangrove ecosystem focus on mangrove trees, as mangrove litter fall and root biomass have been implicated as the ultimate source of organic matter. This obviously leads to underestimation of carbon dynamics as the organic matter in these sediments mostly consists of a mixture of different sources, including locally produced mangrove material, microphytobenthos, and suspended organic matter imported from outside the ecosystem boundaries during tidal inundation. Due to the complex nature of organic matter in mangrove sediments, the bulk parameters are not completely successful in revealing the sources. An effective tool for the source characterisation of organic matter in mangrove systems is the biomarker approach. Fatty acids are abundant in living organisms and their source specificity with respect to individual compounds and their relative stability when compared to amino acids and carbohydrates make them ideal as biomarkers.

The main objective of the study was to investigate the sources of organic matter in three mangrove systems of Cochin estuary and to identify the major biogeochemical pathways. Fatty acid biomarkers, $\delta^{13}C$ of total organic matter and the elemental composition were used to characterise the sources of organic matter in these mangrove systems. Assessment of biochemical composition (total lipids, proteins and total carbohydrates) were employed to study the quality and quantity of organic matter. The geochemical characteristics of the systems were also assessed using mineralogy, trace metal analysis and phosphorus fractionation.

Three mangrove systems in the northern arm of Cochin estuary, southwest coast of India (09°50'N, 76°45'E), which is a Ramsar Site (No.1214), were chosen for the present study. Station 1, Puthuvyppu, is a mangrove nursery maintained by the fisheries research unit of Kerala Agricultural University. Station 2, Murikkumpadam, is a densely populated fisher-folk settlement. These two stations form part of the island called Vypin, which is one of the most densely populated coastal zones. Station 3, Mangalavanam, is a patchy mangrove area in the heart of Cochin City. This mangrove forest is home to many exotic and rare varieties of migratory birds. This is an almost closed system with a single narrow canal link to the estuary and this link is the only source for tidal propagation. During low tide, the water in the system is completely drained.

General sedimentary analysis showed the predominance of fine substratum and silt was the major fraction during all the seasons in the mangrove sediments. XRD analysis of the sediments showed the presence of monetite, an anhydrous calcium phosphate mineral ($CaHPO_4$) at station

3. The high mass percentages of calcium and phosphorous by XRF and SEM-EDS analysis confirm the existence of calcium phosphate mineral at station 3. Stoichiometric ratios of nutrients also support the phosphorus enrichment in all the systems. The overall order in the concentration of heavy metals in the surface sediments of the three mangrove ecosystems was Fe > Mg > Mn > Zn > Cr > Pb > Ni > Pb >Cu > Co > Cd. The enrichment factor calculated, using iron as normalizing element, indicated a minor enrichment for Pb and Zn and no enrichment for all other metals. The geo accumulation index exhibited very low values (<0) in the case of almost all metals except Zn, indicating that sediments are unpolluted to moderately polluted.

Phosphorus fractionation analysis showed that these mangrove systems act as a sink of phosphorus, primarily as Ca-IP and Fe-IP fractions. Internal loading in these systems serves as a source of phosphorus to the water column. The first two stations behave identically and Fe-IP was the major fraction at these stations. Station 3 is unique because of the accumulation of bird guano and it resulted in about 10 fold increase in the total phosphorous content. This station was exclusively dominated by Ca-IP, which is a confirmation for the presence of the calcium phosphate mineral, monetite.

Sedimentary organic matter was higher in these mangrove sediments. However, the percentage ratio of the labile to total organic matter indicated that most of the deposited organic matter is refractory. This discrepancy between the high amounts of organic matter and refractory fraction suggests that these ecosystems behave as a detrital trap and organic matter tends to accumulate. Biochemical composition of sedimentary organic matter showed the dominance of lipids and proteins over carbohydrates. All the biochemical compounds, in general, showed higher concentrations during post-monsoon and lower concentrations during pre-monsoon seasons, except carbohydrates. Carbohydrates were lower during monsoon and significantly higher values were observed during post-monsoon season. Station 3 recorded comparatively higher concentration of biochemical compounds. Higher PRT: CHO at station 3 (>1) indicated the presence of fresh organic matter. Stations 1 and 2 showed higher PRT: CHO ratios only during monsoon season. During pre-monsoon and post-monsoon seasons, the lower ratios indicate the presence of aged or less degradable organic matter i.e. terrestrial or vascular plant inputs. Higher LPD: CHO ratios indicate the high quality of labile organic matter to support the benthic fauna. The C/N ratios were intermediate to that of autochthonous and terrestrial inputs of organic matter, signaling to a mixed origin. $\delta^{13}C$ values also reflects a combination between local inputs and carbon imported from the water column during high tide. Generally, more enriched values in the low organic carbon sites, and more depleted values, close to those of mangrove biomass, in organic rich sediments were observed. From the observed relationships, it can be assumed that due to the almost closed nature of station 3, much of the mangrove area is rarely inundated because of low tidal flushing and subsequently act as retention site, where mangrove carbon is a significant contributor to the sediment organic carbon pool. However, the sediments at station 2 reflect a balance between autochthonous carbon inputs and carbon imported from the water column during high tide.

Fatty acids ranging from C_8 to C_{24} were identified from the study region. SFAs were the major fraction at station 1 and 3 where as at station 2, MUFAs were the major fraction. The fatty acid molecular distributions in the surficial sediments suggested that there are several sources of organic matter in these mangrove systems including algae, zooplankton, higher plants and bacteria.

Unlike other stations, a clear dominance of specific fatty acid biomarkers was absent at station 1. PUFA, $C_{20:5n-3}$ was present in appreciable levels and the ratio $\sum C_{16}/\sum C_{18}$ was greater than 1 indicates significant benthic phytoplankton contribution. Bacterial biomarkers were higher at this station, when compared to the other stations, signaling to the significant influence of microbially mediated degradation processes. The zooplankton biomarkers $C_{20:1}$ and $C_{24:1}$ were present in appreciable levels, suggesting the tidal influx. This station also showed noticeable levels of higher plant markers.

The major fatty acids at station 2 were MUFAs $C_{20:1}$ and $C_{24:1}$, the zooplankton biomarkers. The percentage of unsaturated fatty acids (MUFAs+PUFAs) was 63.1 of TFAs. The low concentrations of both bacterial biomarkers and saturated fatty acids suggested low reworking of organic matter accumulating at station 2. Higher contribution of zooplankton biomarkers in the study region are considered to be estuarine contribution. The proximity to the estuary explains the higher abundance of zooplankton biomarkers at this station.

The dominant fatty acids at station 3 were diatom marker $C_{20:5n-3}$ and the higher plant marker $C_{24:0}$. The ratio $\sum C_{16}/\sum C_{18}$ was also relatively high at station 3. This also supports the significant contribution of diatom organic matter at station 3.The high ratio of $C_{24:0}/C_{16:0}$ (>1) at this station suggested that this system receives a significant amount of organic matter from higher plants including mangroves. $C_{18:2n-6}$ and $C_{18:3n-3}$, dominant fatty acids in mangrove leaves, were also present at station 3. The low contributions of MUFAs $C_{20:1}$ and $C_{24:1}$ suggests the limited tidal exchange.

It can be seen that unsaturated fatty acids constituted a significant portion of the total fatty acids in the study region. It implies that the fatty acid diagenesis during particle settling through the water column is not intense, allowing the deposition of labile fatty acids. Sinking times of organic matter are short in shallow mangrove ecosystems, resulting in a relatively less decomposition of organic matter in the water column. This results in better preservation of organic matter during their faster burial. After burial, degradation of unsaturated fatty acids might also be low due to the anoxic nature of the sediments. Changes in redox condition significantly affect lability of specific organic compounds.

Thus the fatty acid biomarker study of the three mangrove systems of Cochin estuary could substantiate the bulk parameter approaches effectively to characterise the multiple sources of the organic matter and their biogeochemical transformations. The variations in the organic matter sources and the diagenentic pathways due to the environmental settings of the mangrove systems could also be well documented through this approach. From the study, fatty acid biomarkers are proved to be highly effective to find the relative abundance of algae, zooplankton and bacteria. However, vascular plants (mangrove and terrestrial plants) share common fatty acid markers. Therefore, it is difficult to distinguish between mangrove and terrestrial plants organic matter inputs through the long chain fatty acids. Hence other lipid biomarkers like sterols and terpenoids along with the fatty acids could be more effective to differentiate the terrestrial higher plant sources from the mangrove litter.

List of Publications

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