TRITERPENOIDS AS BIOMARKERS OF MANGROVE ORGANIC MATTER IN COCHIN ESTUARINE SYSTEM

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To my Parents….

Declaration

I hereby declare that the thesis entitled **"Triterpenoids as Biomarkers of Mangrove Organic Matter in Cochin Estuarine System"** 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.

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Ratheesh Kumar C. S.

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PREFACE

Mangrove forests, known as rainforests of the sea are one of the most important coastal ecosystems in the world in terms of primary production and coastal protection. It occupies a large fraction of the tropical coastline, dominating the intertidal zone of diverse environmental settings.These ecosystems are important interface for the carbon cycle in tropical coastal environments. The carbon fixed in mangroves is highly important in the coastal food webs and the litter from mangroves and the subsequent formation of detritus and its tidal export have also profound effect on promoting biodiversity richness. Mangroves produce large amounts of organic matter and nutrients, which support not only fauna and flora of the mangrove system itself, but also adjacent habitats. A better knowledge of the geochemical characteristics is required for the sustainable management of these vulnerable ecosystems.

Estuaries, the important areas of world's coastal zones link the carbon cycle of the oceans to the continents. The riverine transported organic matter can undergo significant biogeochemical modifications and therefore estuaries are regarded as highly active areas in terms of organic matter and nutrient processing. Primary production generates large amounts of organic matter in these transitional systems, of which a significant portion sinks through the water column and is ultimately preserved in sediments. Therefore, defining the sources and composition of organic matter within estuaries is essential to understanding the carbon cycle.

Information about processes controlling the delivery of organic matter to coastal environments and how the signatures of these inputs are reflected in newly deposited sediments is important to our understanding of global biogeochemical cycles. 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 mangrove ecosystems. The application of recent analytical techniques has produced a wealth of new information but has also indicated the gaps in our knowledge on organic matter cycling in these ecosystems. The quantity and quality of organic matter preserved in sediments varies greatly depending on the nature of material delivered to the sediment and on the depositional environment. Due to the complex nature of organic matter in mangrove sediments, bulk sedimentary parameters not completely successful in evaluating the sources. Hence an effective tool for the source characterization of organic matter coastal ecosystems is biomarker approach.

Biomarkers are chemical "signatures" present in environmental samples which provide useful information about sources of organic matter important to freshwater, estuarine and marine ecosystems. Molecular biological markers are natural products that can be assigned to a particular biosynthetic origin. For environmental and geological studies, the most useful molecular biomarkers are organic compounds with high taxonomic specificity and potential for preservation. In other words, the most effective biomarkers have a limited number of well-defined sources; they are recalcitrant against geochemical changes and easily analyzable in environmental samples.

The thesis entitled “Triterpenoids as Biomarkers of Mangrove Organic Matter in Cochin Estuarine System” is an attempt to characterize the organic matter in the surface sediments of mangrove and adjacent estuarine stations with special emphasize on triterpenoid biomarkers. Three mangrove ecosystems and three estuarine stations around Cochin region, southwest coast of India were selected for the present study. The thesis is divided into five chapters. Chapter 1 is the **Introduction** and it deals with the aim and scope of the present study. Chapter 2 is **Materials and Methods**. This chapter deals with the nature and general geographical features of the study area. It also contains the details of the sampling and analytical methodology. The results of general hydrographic parameters are also described here. Chapter 3 is **Geochemistry** and it includes the seasonal and spatial variations of the geochemical parameters in the surface sediments. It deals with the general sedimentary characteristics, mineralogy, elemental composition, phosphorus fractionation and heavy metal geochemistry. Chapter 4 is **Biochemical Composition**. It covers the biochemical composition of organic matter in the surface sediments to examine the quality and quantity of organic matter. Bulk sedimentary parameters such as elemental ratios and stable isotope ratio are also employed for source characterization of organic matter. Chapter 5, **Triterpenoid Biomarkers in Sediments**, characterize the organic matter in the sediments of the mangrove and estuarine ecosystems under study, to assess the possible sources with the help of triterpenoid biomarkers along with other lipid biomarkers. All the biogeochemical characteristics of the two ecosystems under study are summarized at the end of the thesis.

Contents

Chapter 1

GENERAL INTRODUCTION

Coastal ecosystems are widely recognised as biogeochemically active regions, where organic matter inputs from a variety of sources undergo intense biogeochemical processing. These ecosystems play an important role in the global carbon cycle as large fluxes of carbon and carbon-related tracers move between the land, ocean, and atmosphere in these regions. Despite their relatively small areal extent, their carbon sequestration represents a large inventory of organic matter; comparable to global riverine carbon discharges (Gattuso *et al.*, 1998). Coastal ecosystems are characterised by high carbon burial capacity (Duarte *et al.*, 2005) and these are also regarded as “hotspots” in terms of mineralization (Middelburg *et al.*, 2005). Deposition of organic matter and its preservation is greater in coastal sediments than for any other sedimentary reservoir (Romankevich, 1984; Hedges and Kiel, 1995). In marine environment the flux, preservation, and accumulation of organic carbon are governed by important factors such as primary productivity of the surface water, sedimentation rate and supply of terrigenous organic matter from the surrounding continents (Berger *et al.*, 1989; Stein, 1991). Understanding the functioning of these diverse ecosystems and constraining their carbon and nutrient budgets is therefore of prime importance in improving the reliability of global oceanic budgets.

1.1 Biogeochemistry of Coastal Ecosystems

The geochemistry of sediments is controlled by both the composition of the material initially deposited in the sediments and the chemical, biological, or physical processes that affect this material after its deposition. These processes fall within the general category of what is commonly referred to as early diagenesis (Berner, 1980). A key fact that has emerged in the past 20-30 years of research in sediment geochemistry is that the oxidation, or remineralisation of organic matter deposited in sediments is either the direct or the indirect causative agent for many early diagenetic changes. The key role that organic matter remineralisation plays in many early digenetic processes thus led to significant efforts for understanding and quantifying these processes. Historically, there has been more of a tendency to use inorganic geochemical studies to quantify rates of sediment carbon remineralization processes. However, the increasing interest among the researchers to use organic geochemical measurements to examine the rates of these processes has built important links between inorganic and organic geochemical approaches to the study of sediment biogeochemistry.

1.1.1 Phosphorus Fractionation as a Geochemical Signature

More than 30 elements are cycled through the environment by biogeochemical cycles. The term biogeochemical cycles express the interaction among the organic (bio-) and inorganic (geo-) worlds, and focuses on the chemistry (chemical-) and movement (cycles) of chemical elements and compounds. There are six important biogeochemical cycles that transport carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. Coastal sedimentary environments play an important role in the biogeochemical cycling of phosphorus because the conditions in these environments are suitable for the transformation, accumulation and mineralization of nutrients (Babu and Nath, 2005; Hou *et al.*, 2009). Because of their ecotonal location phosphorus cycle in coastal wetlands, estuaries and mangroves have been extensively investigated for several decades (Rozan *et al.*, 2002; Ashraf *et al.*, 2006; Monbet *et al.*, 2007a, 2007b). The chemical behaviour of phosphorus within the sediment can be more efficiently evaluated on the basis of different phosphorus species, instead of total phosphorus content.

The advantage of studying the geochemistry using phosphorus fractionation is that the element is available in various oxidation states (Morton and Edwards, 2005) and the monitoring of phosphorus in various forms can be easily done. An understanding of phosphorus speciation in marine sediments is essential in evaluating response to environmental changes, understanding phosphorus cycling, assessing the bioavailability of this element in coastal environment, as well as the cycles of carbon, sulphur, oxygen and nitrogen (Andrieux and Aminot, 1997; Jensen *et al.*, 1998; Schenau and De Lange, 2001). The majority of studies concerning phosphorus dynamics in wetlands have attributed enrichment, availability and limitation of phosphorus to adsorption effects, reflecting the importance of adsorption effects in phosphorus distribution and composition (Fisher and Acreman, 2004). Phosphorus fractionation in sediments can provide valuable information on the origin phosphorus in sediments, diagenetic transformations of organic phosphorus, the degree of pollution from anthropogenic activities, the bioavailability of phosphorus in sediments and the biogeochemical processes (Schenau and De Lange, 2001). Phosphorus undergo significant digenetic modifications during burial (Fang *et al.*, 2007) and as a result of which various solid phase reservoirs of phosphorus in coastal sediments are considered to result from the interactions of complex biogeochemical processes.

Phosphorus is considered as a key element in global biogeochemical cycles (Sanudo-Wilhelmy *et al.*, 2001). Sediment bound phosphorus constitutes a vast source of potential nutrient for phytoplankton, macrophytes, and benthic algae. Sediment bound phosphorus is significant in shallow systems because of the fact that variable forms of bioavailable phosphorus in the upper sediments can be a major source of P to the water column biota via numerous physically, chemically and biologically mediated processes (Reddy and D'Angelo, 1994). Organic compounds containing phosphorus are found in all living matter. The two major steps of the phosphorus biogeochemical cycle conversion of organic phosphorus to inorganic phosphorus and back to organic form are both bacterially mediated. Conversion of insoluble forms of phosphorus, such as calcium phosphate, $CaHPO_4$, into soluble forms, principally PO_4^{3-}, is also mediated by microorganisms. Organic phosphorus in tissues of dead plants and animals, and in animal waste product is also converted bacterially to phosphates which thus released to the environment is taken up again into plant and animal tissue.

An exact stoichiometric and structural identification and quantification of inorganic phosphorus species are very complicated (Luderitz and Gerlach, 2002) and a common method to estimate the stock of potentially accessible forms is to fractionate phosphorus based on the extractability by leaching reagents of increasing aggressiveness (Golterman, 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). Sequential extraction techniques can also be employed to estimate the phosphorous release potential (Reitzel *et al.*, 2005; Zak *et al.*, 2008) and its diagenetic transformations (Hupfer and Lewandowski, 2005)

1.1.2 Heavy Metal Biogeochemistry

Processes involved in weathering and erosion of geological formations result in transfer of a wide range of metals to coastal lowlands and aquatic environments. Heavy metals are present in marine waters in both dissolved and solid forms and play an important role in many biogeochemical cycles. The concentration and distribution of heavy metals in sediments depends on interrelated geochemical parameters such as pH, Eh, texture, organic carbon etc. Phosphorus has a strong affinity to oxide iron, aluminium and manganese. Reduction and oxidation (redox) state of these metals regulate the dynamics of phosphorus in sediments. The mechanisms of phosphorus mobilization are explained by the reduction of insoluble metal oxides such as Fe(III) to soluble Fe(II) in sediments or water whereby phosphorus bound to Fe(III) or adsorbed to iron complexes is returned to solution when system redox potentials change from aerobic to anaerobic conditions. Therefore, the study of heavy metal concentration in sediments along with phosphorus is a pre-requisite for the geochemical characterization of the aquatic ecosystems.

The major processes controlling the retention of metals in coastal ecosystems are cation exchange, complexation with organics, precipitation as oxides, oxyhydroxides, carbonates and sulphides (Dunbabin and Bowmer, 1992). Major parameters that influence metal behaviour in natural waters are pH (Harter, 1992; Tam and Wong, 1996) and Eh (Harbison, 1986). Precipitation of metals at the sediment-water interface is encouraged by high pH values. Other factors controlling the distribution of metals include the levels of organic matter, geochemical composition of sediments, levels of microbial activities and dynamicity of the overlying water column. Therefore, the heavy metal concentrations and distribution study can be employed in the evaluation of the geochemical process occurring in sedimentary environments.

1.1.3 Bulk Organic Matter Source Indicators

The overall environmental conditions existing in any aquatic system cannot be explained by the application of geochemical parameters such as general sedimentary characteristics, phosphorus fractionation or heavy metal distribution studies. Information about processes controlling the delivery of organic matter to coastal environments and how the signatures of these inputs are reflected in newly deposited sediments is important to our understanding of global biogeochemical cycles. The diagenesis or the mineralization process occurring in sedimentary environments is associated with organic matter and therefore the characterization of organic matter is a prerequisite for biogeochemical studies. Biochemical composition of sedimentary organic matter has been used to gather information on the origin and parameters controlling the diagenetic fate of organic matter (Colombo *et al.*, 1996). The components usually studied under the biochemical composition consist of carbohydrates, proteins, lipids, protein to carbohydrates ratio, lipids to carbohydrate ratio etc.

Biogeochemical processes in marine sediments are intimately linked to processes and conditions in the water column: they are fuelled by deposition of materials from the water column (e.g. labile organic matter) and are affected by the composition of bottom waters (Soetaert *et al.*, 2000; Burdige, 2006). Biogeochemical conditions and processes in coastal sediments differ from those in the water column because of differences in transport processes (molecular versus eddy diffusion and particle settling versus bioturbational mixing), relevant time scales and availability of particulate and dissolved substances. Deposition of labile and reactive materials from the water column provides energy and nutrients for heterotrophic consumers inhabiting marine sediments. The organic material processed is assimilated by heterotrophic organisms (secondary production) or respired and mineralized. The majority of the organic matter deposited is processed by a diverse community of microbes, protozoans and metazoans and only a small part is eventually buried due to sediment accretion (Hedges and Keil, 1995; Middelburg and Meysman, 2007).

In order to distinguish the source of the sedimentary organic matter the application of stable carbon isotope ratio and elemental composition is a common trend in biogeochemical studies. A number of important bulk sediment parameters are available for the evaluation of organic matter sources and its fate within marine sediments including C/N ratios (Ruttenberg and Goñi, 1997; Yamamuro, 2000; Perdue and Koprivnjak, 2007), $\delta^{13}C$ and $\delta^{15}N$ signatures (Lee, 2000; Kerherve *et al.*, 2001; Cloern *et al.*, 2002; Goñi *et al.*, 2003; Alt-Epping *et al.*, 2007; Ramaswamy *et al.*, 2008). The use of these bulk parameters as source indicators is reliant on there being markedly different signatures between the different organic matter sources (Kennedy *et al.*, 2004).

The C/N ratio has been used as an organic matter quality indicator (Huston and Deming, 2002), although the elemental quantification doesn't give any clue to the chemical features of the organic matter and overestimates the nutritionally available nitrogen due to the abundance of nitrogenous compound such as humic material. 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, whereas organic matter from terrestrial vascular plants has C/N ratios of 20 and greater (Ishiwatari and Uzaki,1987; Lehmann *et al.*, 2002). This distinction arises from the absence of cellulose in algae and its abundance in vascular plants. 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). Organic C, N and phosphorus (P) ratios, or C/N/P ratios (Redfield ratio), have also been widely employed to distinguish the origin of sedimentary organic matter as a result of the distinctive C/N/P ratios of terrestrial and marine plants (Redfield *et al.*,1963; Ruttenberg and Goñi, 1997). Selective degradation of organic matter components during early diagenesis, however, has the potential to modify C/N/P ratios of organic matter in sediments (Meyers, 1997).

Stable isotopes have been commonly used to identify origin of organic matter (Dittmar *et al.*, 2001; 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 lighter isotopes. These fractionation processes have proven to be useful in determining source of organic matter in biogeochemical 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 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.

1.2 Mangrove Ecosystems

The coastal lines of the world are bordered by zones of fringing wetlands, such as salt marshes in temperate latitudes and mangroves in the tropics. Mangroves are tropical formations of angiosperm vegetation growing along the coast, lagoon, estuaries and riverbanks. The term mangrove is used to designate halophytic and salt resistant marine tidal forests comprising of trees, shrubs, palms, epiphytes, ground ferns and grasses, which are associated in stands or groves (Premanathan *et al.*, 1999). These widespread marine vascular plants, occupy approximately 1.6×10^5 km^2 along subtropical and tropical coastlines (Alongi, 2002, 2008). Mangrove ecosystems are known to be potentially significant sources of organic matter to adjacent estuaries and coastal waters on a global scale (Jennerjahn and Ittekkot, 2002; Dittmar *et al.*, 2006). These ecosystems play a very dynamic and significant role in the estuarine mouths having a potentially high impact on the carbon budget of the global coastal zone.

A mangrove community is composed of plant species whose special physiological adaptations allow them to survive the deoxygenated soils, variable flooding and salinity stress conditions imposed by the coastal environment. Mangrove plants mostly grow within the sheltered intertidal flat deltaic lands, funnel shaped bays, broad estuarine mouths, shallow or frequently tidal inundated coastal lines (Thom, 1982). They prefer mostly the humid atmosphere (60-90%) with moderate to high annual rainfall, between 1000-3000 mm. The factors like water level or tidal inundation, water and soil salinity, pH, sediment flask, oxygen potential, availability of anions and cations, hydrodynamics, stresses etc. are important in determining the habit and habitat selection, individual species distribution, succession pattern and interspecific competition among these mangroves. The ecosystem is periodically flooded by incoming and outgoing tides, and it has fluctuating salinities and alternating aerobic-anaerobic conditions.

Mangrove forests are effective in sequestering nutrients (Bouillon *et al*., 2008) and enhance water quality in surrounding habitats by reducing eutrophication and turbidity (Valiela and Cole, 2002; Victor *et al*., 2004).These marine vascular plants can influence a variety of above and below-sediment physical and chemical characteristics, and consequently can alter the species composition and trophic structure of benthic communities (Levin et al., 2006; Alongi, 2009). The mangrove ecosystems serve as nursery grounds for many economically important fin and shell-fishes (Machado *et al*., 2004; Manson *et al*., 2005) and provide nesting habitats for a variety of birds (Nagelkerken *et al*., 2008). They also act as major ecosystem engineers by reducing water flow and trapping sediments, which can lead to enhanced densities of deposit feeding fauna (Demopoulos, 2004; Demopoulos and Smith, 2010) as well as limiting coastal erosion, and providing a buffer to tropical storms and tsunamis. They are also efficient traps of sediment particles and thereby reduce coastal erosion (Wafar and Untawale, 2001). Because of the regular tidal flooding and draining in many mangrove forests, the material exchange with adjacent waters can be very efficient. The export of detrital material from mangroves forms the base food in the estuarine and coastal environments (Twilley, 1985; Benner *et al*., 1986), which depends to a large extent, on the geomorphology and tidal characteristics of the ecosystem. Their extensive root systems trap and stabilize sediments, making them important 'sediment sinks' by reducing siltation of waterways and estuaries, improving water quality and protecting reefs from upstream sediment loads.

1.2.1 Distribution of Mangroves

Mangroves occupy large tracts along sheltered coasts, estuaries and in deltas where they are influenced by tides and widely different conditions of salinity and rainfall regimes. They are also found around coastal lagoons, communicating with the sea and where the effect of tides may be weak and the salinity very low. These ecosystems are usually found only in tropical climates, as they need consistently warm conditions for development and survival. They occur approximately in 112 countries and territories (Kathiresan and Bingham, 2001) and are largely confined to the regions between 30° N and 30°S of the equator. Notably, extension beyond this limit are to the north in Bermuda (32° 20' N), Japan (31° 22' N), and to the south in Australia (38° 45' S), New Zealand (38° 03' S) and on the East Coast of South Africa (32° 59' S).

Mangrove distribution is circumglobal with the majority of populations occurring between the latitudes of 30° N and 30°S (Tomlinson, 1986). At one time, 75% of the world's tropical coastlines were dominated by mangroves. Unfortunately, mangrove extent has been significantly reduced due to human activities in the coastal zone. There are two centers 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, and Pacific North and South America) where the number of species is only 8. Thus, New World forests are relatively depauperate compared to Old World forests. The most diverse biogeographical regions are in the Indo-West Pacific (Alongi, 2002). According to estimate made by FAO/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 in the world. Globally, about one third of the mangrove forests have been lost within the past 50 years (Alongi, 2002). Millennium Ecosystem Assessment Report (2005) also states that 35% of the total mangrove area has been lost worldwide in last several decades. The establishment of shrimp farms has been the main cause of mangrove loss in many countries over the past 30 years (Rodriguez, 2001).

1.2.2 Mangrove Ecosystems of India

India has a coastline of 7,516 km with an Exclusive Economic Zone of 2.02 million km^2. Along the Indian coastline, the brackish water areas including marshes, backwaters, mangroves, inter-tidal and sub-tidal measure about 14,16,300 hectares. Indian coast consist of mangroves along the islands, major deltas, estuaries and backwaters of the East Coast. They also exist along the oceanic island groups of the Andaman and Nicobar. The total mangrove area is estimated to be 670,000 hectares. The mangroves along the West Coast of India are dense, comparatively small in area, scattered, less in diversity and less complicated in terms of tidal creek network. It is due to the fact that the coastal zone of the West coast is narrow and steep in slope due to the presence of Western Ghats. Secondly, there is no major west flowing river. Gangetic Sunderbans (418,888 hectares), Andaman-Nicobar Islands (115,000 hectares), Krishna, Cauvery and Godavari deltas and Mahanadi delta are some of the best mangrove formations of India.

Of the Country's total area under the mangrove vegetation, 70% is recorded on the east coast and 12% on the west coast. The bay islands (Andaman and Nicobar) account for 18% of the Country's total mangrove area (Krishnamurthy *et al.*, 1987; Kathiresan and Ravikumar, 1995). The mangroves have a vast existence on the east coast of India due to the nutrient-rich alluvial soil formed by the rivers - Ganga, Brahmaputra, Mahanadhi, Godavari, Krishna and Cauvery and a perennial supply of freshwater along the deltaic coast. But, the deltas with alluvial deposits are almost absent on the west coast of India, only funnel shaped estuaries or backwaters are present (Gopal and Krishnamurthy, 1993).

The deltaic mangroves on the east coast are about 57% (2,738 km^2) of the country's total area of mangroves. The insular mangroves exist in the Bay islands (Andaman and Nicobar) on many tidal estuaries, small rivers, neritic islets and lagoons, accounting for 20% (383 km^2) of total Indian mangroves. However, the extent of mangroves keeps on changing over a period in different states of the east coast and the Bay Islands. The satellite data between 1993 and 1997 revealed a considerable increase in mangrove cover: 31.13% in West Bengal; 25.4% in Bay Islands; and, 12.83% in Orissa and a reduction in mangrove cover in other states: 76.7% in Tamil Nadu; and 20.21% in Andhra Pradesh (Kathiresan, 2000).

According to Forest Survey of India (FSI, 1999), out of 4, 87,100 hectares of mangrove wetlands in India, nearly 56.7% (2, 75,800 hectares) is present along the east coast and 23.5% (1, 14,700 hectares) along the west coast, and the remaining 19.8% (96,600 hectares) is found in the Andaman and Nicobar islands (Selvam, 2003). The Sundarbans of India and Bangladesh put together forms the single largest block of mangroves of the world. It covers an area of about 1 million hectares, of which 60% is located in Bangladesh and the remaning western portion, lies in India (Choudhuri and Choudhary, 1994). Mangroves are under serious threat of degradation; India has lost about 40% of its mangrove cover within this century (Krishnamurthy *et al.*, 1987). Indian Remote Sensing Satellites are being used to estimate the present status and loss, if any in mangrove areas recently (Vijay *et al.,* 2005).

1.2.3 Mangroves of Kerala Coast

The length of Kerala coast is 560 km extending from North to South parallel to the Western Ghats. The higher population density in Kerala coast has resulted in tremendous pressure on the natural ecosystem, particularly the mangroves. The wetlands of Kerala included a large mangrove swamp centuries ago and anthropogenic activities modified the system into settlements, agricultural fields, filtration ponds and prawn culture fields. Vast areas of mangal lands were cleared and reclaimed for urbanization, construction of harbors, ports, prawn farming, coconut plantation and rice fish culture (Gopalan *et al.*, 1983). These reclaimed lands of the coastal areas are underdeveloped and rice culture or brackish water fish and prawn culture are commonly practiced there. Ramachandran *et al.*, (1986) have reported several mangrove species from Kerala coast and have also reported that mangrove habitat in the State has destructed for fuel and wood harvest.

According to one estimate in the recent past Kerala had 70,000 hectares of mangrove, which had diminished to less than 4200 hectares (Mohanan, 1997). Some other estimate indicates the extend of mangrove vegetation to be 1671 hectares at present within a distance of 500m from the coastline. Forest Survey of Inda (FSI, 2003) reported 800 hectares area of mangrove cover in Kerala state, with 300 hectares moderately dense and 500 hectares open mangrove vegetation. A recent study by Radhakrishnan *et al.*, (2006) showed that mangrove vegetation in four northern districts of Kerala- Kasargod, Kannur, Kozhikkode and Malappuram is approximately 3500 hectares, which represents the 83% of mangrove cover in the state. Mangroves are found in small isolated patches along the coast and backwaters. The major concentrations are found in the Vallapattanam river mouth, Kannur district, Puthuvypin at Ernakulam district and Kumarakom (Vembanad lake east bank) at Kottayam district. Certain patches are also found in Kozhikkode districts, Alappuzha, Kollam and Thiruvananthapuram. At Kannamaly and Kumbalangi of Cochin region, mangroves are found in a stretch of around 8 hectares each. Panambukadu 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). Important mangrove species include: *Rhizophora apiculata, Rhizophora mucronata, Bruguiera gymnorrhiza, Avicenia officinalis, Avicennia marina, Acanthus ilicifolius Sonneratia caseolaris, Sonneratia apetala, Excoecaria agallocha* and *Cerebra mangha.*

The Cochin estuarine system includes a system of interconnected lagoons, bays and swamps penetrating the main land and enclosing many islands in between, whose total area amounts to approximately 500 km^2. The major sources of fresh water in the estuary are the two rivers, the Periyar in the northern part and Muvattupuzha in the southern part. In addition, several small tributaries, irrigation channels and innumerable drains contribute to the system. Mangrove ecosystems are found in isolated patches along the banks of this estuary. They cover an area of 2.6 km^2. The total mangrove area in the state of Kerala has decreased from 1000 km^2 a century ago to a mere 17 km^2 (Basha, 1991).

1.2.4 Biogeochemical Structure of Mangroves

Intertidal mangrove ecosystems are an important interface for the carbon cycle in some tropical coastal environments. Due to their high productivity, turnover rates of organic matter and the permanent exchange with terrestrial and marine ecosystems; mangroves are of particular importance for the biogeochemical cycling of carbon and associated elements along tropical continental margins. The sources of primary productivity include mangrove vegetation themselves; algal colonies associated with the mangrove root surfaces, moist forest floor and phytoplankton communities in the associated bay and lagoons. In the tropical mangrove ecosystem, the vegetation and its associated biota play a major role in contributing organic matter.

Sedimentary organic matter is the major reservoir of organic carbon in the global carbon cycle. The litter fall is the most important source of organic carbon in biogeochemical cycles in these ecosystems (Wafar *et al.*, 1997; Clough *et al.*, 2000). Litter from trees and subsurface root growth provide significant inputs of organic carbon to mangrove sediments (Alongi, 1998) and it represents about one third of the net primary production (Alongi *et al.*, 2005). Other important sources providing organic carbon inputs to mangrove ecosystems include: allochthonous riverine or marine material (e.g., sea grasses), autochthonous production by benthic or epiphytic micro- or macroalgae, and local water column production by phytoplankton (Bouillon *et al.*, 2004). The primary food source for aquatic organisms in mangrove dominated estuaries occurs in the form of particulate organic matter derived chiefly from the litter fall. The decomposition of mangrove leaf material occurs primarily through microbial action and the leaching of water soluble compounds. It is through the decomposition process that nutrients and other organic compounds such as lipids are released to estuarine waters and sediments via tidal transport. Mangrove ecosystems play a prominent role as sources of organic matter which may be transferred to adjacent coastal waters through the export of detritus and living organisms (Robertson and Duke, 1990). These tidal forests contribute 11% of the total input of terrestrial carbon into the ocean and 15% of the total carbon accumulating in modern marine sediments (Jennerjahn and Ittekkot, 2002). Despite their small area relative to other habitats, mangroves contribute ~10% of the terrestrial dissolved organic carbon which is exported to the ocean globally (Dittmar *et al.*, 2006).

Mangrove-derived detritus is an important food source for decomposer food webs including many macro invertebrates, such as sesarmid crabs (Grapsidae) that are notable in their ability to consume mangrove litter (Fratini *et al.*, 2000; Cannicci *et al.*, 2008). In many cases considerable input of local or imported algal detritus is consumed by other animal species such as fiddler crabs and various gastropods (Bouillon *et al.*, 2002; Kristensen and Alongi, 2006). Litter handling by the fauna not only affects microbial carbon transformations, but also the amount of organic carbon available for export. Irrespective of the pathways of organic matter consumption and food web structure, all organic matter that is not exported by tidal action enters the sediment where it is consumed, degraded and chemically modified. In mangrove sediments, the degradation of organic matter is mediated by both aerobic respiration and anaerobic sulphate reduction processes involving a variety of electron acceptors (Alongi, 1998). A fraction of mangrove detritus escapes degradation and is permanently buried within the mangrove sediments or exported to adjacent ecosystems through tidal forcing. Organic carbon that escapes microbial degradation is stored in sediments and in some mangrove ecosystems, the organic-rich sediments may extend to several meters depth. Mangrove-derived dissolved organic carbon is also released into the water column and can add to the total organic carbon export. 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). Numerous organic compounds have been characterized from mangrove tissues, including carbohydrates, amino acids, lignin derived phenols, tannins, fatty acids, triterpenoids and n-alkanes. Many of these may, together with stable isotopes, exhibit a strong source signature and are potentially useful tracers of mangrove-derived organic matter.

Mangrove ecosystems play an important role in nutrient cycling and energy flow in tropical coasts (Lacerda *et al.*, 1993; Silva, 1996; Silva and Mozeto, 1997). The nutrients required to maintain the high productivity of these ecosystems are met by inputs from rivers, tides and benthic activities (Jennerjahn and Ittekot, 2002). The phosphorus dynamics in mangrove sediments are closely coupled to the activity of Fe- and sulphate-reducing bacteria, which are the primary microbial decomposers in the normally reduced sediments (Sherman *et al.*, 1998; Kristensen *et al.*, 2000). Phosphorus cycle in tropical mangroves is multifarious because of the periodic flooding of sediment with both fresh and saline waters (Salcedo and Medeiros, 1995).

In natural environments, the associations of metals and their distributions depend on various parameters including redox conditions (Guo et al., 1997) and organic contents (Mounier *et al.*, 2001). Mangrove ecosystems, developing in the intertidal zone of most tropical and subtropical regions, are characterized by major contrasts in redox conditions and high rates of organic carbon accumulations (Huc, 1980). The geochemistry of mangroves influences the metal distribution and its availability in this environment. These intertidal ecosystems are generally water-logged and anoxic. The redox conditions prevailing in the system determine the fate of metals. In mangals, tidal deposition is also important in determining metal distribution (Mackey and Hodkinson, 1995).

1.3 Estuarine Biogeochemistry

Estuaries, which are dynamic, complex and unique systems, are among the most productive marine ecosystems in the world (Chapman and Wang, 2001). These represent a biogeochemically active zone, significantly transforming terrestrial riverine inputs to the coastal zone. Estuaries are commonly described as semi-enclosed bodies of water, situated at the interface between land and ocean, where seawater is measurably diluted by the inflow of freshwater (Hobbie, 2000). Not only do they provide a direct resource for commercially important estuarine species of fishes and shellfish, but they also provide shelter and food resources for commercially important shelf species that spend some of their juvenile stages in estuarine marshes. They are significant for human welfare through their role in transportation, production of food, waste disposal and various recreational pursuits.

Estuarine areas are characterized by high primary production and shallow water depth, both of which allow a relatively large fraction of autochthonous and allochthonous organic matter to reach the sea floor and to be ultimately preserved in anoxic sediments (Hedges and Keil, 1995). Processing of organic matter along the estuarine mixing zone and in tidal creeks has the potential to dramatically alter the quantity, sources, and composition of organic carbon before its export to the coastal zone and leads to a strongly heterotrophic water column, resulting in a high CO_2 efflux toward the atmosphere. Therefore estuarine areas are good environments for studying the origin, pathway and fate of sedimentary organic matter due to the rapid accumulation of fine sediments and consequent sealing of these materials from bacterial remineralization (Hedges and Keil, 1999).

The diversity in the origin of estuarine organic matter depends not only on the biological and geographical contexts, but also on the socio-economic environment through the effects of urbanisation, industrialisation and regional development (Galois *et al.*, 2000). The sources of organic matter within estuarine sediments is important in understanding the roles of terrestrial-or estuarine-derived organic matter as sources of energy and nutrients to coastal systems (Yamamuro, 2000; Goñi *et al.*, 2003) and the potential enhancement of nutrient loads that contribute to eutrophication processes (Yamamuro, 2000). The origin of organic matter in estuarine systems is often diverse, as they receive organic matter from both autochthonous and allochthonous sources, including: phytoplankton (Meyers, 1997), algae (Meziane and Tsuchiya, 2000), bacteria (Dale, 1974), terrestrial vegetation (Mfilinge *et al.*, 2005). The preservation of organic matter in coastal and estuarine sediments is believed to be principally controlled by productivity; sedimentation accumulation rate, bottom water and sediment redox conditions, and sorption as a function of specific surface area of sediments (Hedges and Keil, 1995).

There is great interest in identifying the organic matter sources that drive oxygen demand and nutrient regeneration within shallow coastal systems. Estuaries are generally regarded as highly eutrophic systems as a result of anthropogenic nutrient inputs. The complexity of the physicochemical and biological processes found in these transition zones, pose challenges for investigating the estuarine nutrient dynamics. The identification of P compounds in estuarine sediments and the knowledge of changes in the makeup of the P pool in space and time are highly relevant (Morton and Edwards, 2005; Hou *et al.*, 2009) because of its influence on the productivity (Caraco *et al.*, 1990). There is a conspicuous lack of information about the concentration, turnover rates, transport, and fate of P in the estuarine systems.

Also there are distinct variations from one estuary to another, defying generalization. Since this nutrient element is associated with productivity and the availabity of various P fractions depends on other geochemical parameters it is an important in describing the geochemical conditions of sedimentary environments.

Changes in sediment oxidation/reduction state and pH influence the solubility of both metals and nutrients (Miao *et al.*, 2006). Metals may be present in the estuarine system as dissolved species, as free ions or forming organic complexes with humic and fulvic acids. Additionally, many metals associate readily with particulates and become adsorbed or co-precipitated with carbonates, oxyhydroxides, sulphides and clay minerals. The distribution and accumulation of trace metals is influenced by sediment texture, mineralogical composition, reduction/oxidation state, and adsorption and desorption processes, and physical transport (Buccolieri *et al.*, 2006). Since the distribution of heavy metals in sediments depends on organic matter content and other geochemical variables, it can be used in the geochemical evaluation along with phosphorus fractionantion.

1.4 Biomarker Approach

Variations in productivity, as well as fluctuations in delivery, make it difficult to resolve processes contributing to the storage of organic matter in coastal environments. Moreover, natural organic matter originates from a diverse array of marine and terrigenous materials, each of them lying along continuum of reactivity ranging from more labile components-dominant in the inputs from marine organisms (primary and secondary producers in the water column and benthic organisms)-to less reactive materials from higher plants and soils (Volkman *et al.*, 1987; Keil *et al.*, 1994; Hedges and Keil, 1995). The biochemical composition of organic matter sources varies and the differences in source signatures are not always unique enough to identify components in complex mixtures such as sediments. The C/N ratio is seriously affected by the preferential remineralisation of nitrogen in marine sediments or nitrogen sorption onto clay minerals (Schubert and Calvert, 2001) and $\delta^{13}C$ of total organic carbon values of a mixture of C3 and C4 plants could mimic marine algae (Goñi *et al.*, 1998). Furthermore, both indices cannot provide detailed information about specific organic matter sources. Due to the aforementioned limitations, a detailed study of lipid biomarkers (biomarker approach) enables the recognition of the major sources contributing to the sedimentary organic matter (Wakeham *et al.*, 2002).

Various methods have been employed to characterize organic matter in aquatic environments. Among them, lipid tracers or biomarkers are particularly useful because they can reveal valuable information on organic matter sources at the molecular level (Meyers, 2003). Of the available biomarkers, lipids provide better source characterization than other biochemical classes due to the number of unique biosynthetic pathways organisms use to produce these compounds as well as their relatively high geochemical stability. Source indicators of bulk organic matter are important in identifying the general origins of organic matter, but molecular constituents of sedimentary organic matter pools (biomarkers) provide details of production, delivery and preservation (Meyers, 1997). Previous studies have successfully used this approach to assess sedimentary organic matter sources in coastal areas, estuaries, rivers and lakes (Jaffe *et al.*, 2001; Bianchi *et al.*, 2002; Mead *et al.*, 2005). Sedimentary lipids have been successfully used to infer environmental changes that have impacted their sources (Zimmerman and Canuel, 2000).

The term biomarker molecule has been defined by Meyers (2003) as "compounds that characterize certain biotic sources and that retain their source information after burial in sediments, even after some alteration." This molecular information is more specific and sensitive than bulk elemental and isotopic techniques in characterizing sources of organic matter, and further allows for identification of multiple sources (Meyers, 1997, 2003). Biomarker is defined as "a molecule whose carbon skeleton can unambiguously link to that of a known biological precursor compound" (Killops and Killops, 2005). More generally: "Organic compounds found in sediments which have properties that can be directly related to a known biological precursor". These are organic compounds derived from formerly living organisms and are ubiquitous in sedimentary organic matter. These organic molecules with a high degree of structural complexity are particularly informative and thus suitable for studying geochemical reactions because they provide the possibility of relating a certain product to a specific precursor. Within the stable carbon-carbon skeleton of such compounds are embodied essential information on the habitat, nature and fate of the ancestral flora and fauna which can facilitate the reconstruction of environment of deposition of ancient sediments and petroleum. Despite the various biological and abiotic alterations of the biomolecules in the sediments, some compounds retain their basic skeletal structures and can be used as characteristic molecular markers (Peters *et al.*, 2005). The molecular distribution of biomarkers provides particularly useful information about the source, diagenetic alteration, preservation and historical changes in organic matter (Canuel *et al.*, 1995; Budge and Parrish, 1998; Koch *et al.*, 2003). The presences of sedimentary biomarkers have been reported in rocks back to the Precambrian (Jackson *et al.*, 1986; Brocks *et al.*, 2005). Biomarkers can yield valuable information pertaining to the source organisms, the thermal maturity of the host organic matter and the palaeoenvironmental conditions (of redox, salinity, etc.) in the water column and at the sediment surface which prevailed during deposition (Brocks and Summons, 2003).

Lipid biomarkers are fossil biochemicals detected in the geological record whose basic skeletal features have been sufficiently well-preserved to allow unambiguous links to known, contemporary natural product precursors. Eventhough they represent only a small fraction of the total organic carbon, their diversity and specificity make them useful compounds to study the sources and transformation of organic matter (Azevedo, 2003). Lipids are important biomarkers because they carry a strong carbon-number predominance that is inherited from their biosynthesis (e.g., buildup from acetate), and their homolog distribution can reflect biogenic origin (e.g., marine versus terrestrial vegetation (Simoneit, 1977a, 1977b). Lipids, while not as abundant as other biogeochemical components (e.g. carbohydrates and proteins), have been largely used to assess the supply of autochthonous (marine) and allochthonous (terrestrial) inputs of organic matter in the marine environment.

Higher-plant biomarkers provide a geochemical tool to identify terrestrial organic detritus in global compartments (e.g. atmosphere, sediments, etc.). They have been used to study the export of carbon by rivers to the ocean, and have revealed a surprisingly large pool of refractory land-derived organic matter in marine sediments (Prahl *et al.*, 1994). The most useful biomarker molecules are those that are thermodynamically-stable, with a limited number of well-defined biological sources and which are relatively straightforward to analyze. Certain lipid compound classes fit all these criteria, and polycyclic terpenoids or highly branched hydrocarbon skeletons, such as hopanoids or steroids, are extremely resistant to degradation. Hopanes, steranes and other hydrocarbon biomarkers may survive hundreds of millions of years of burial in sedimentary rocks and in their petroleum products and can even be detected in some late Archean sedimentary rocks (Brocks *et al.*, 1999; Sherman *et al.*, 2007).

1.4.1 Major Classes of Compounds as Biomarkers

Typical chemical fossils include the terpenoids (e.g. pristane, phytane, sterane, hopane); the porphyrins (e.g., chlorophyll), the odd-numbered n-paraffin carbon chains (e.g., C_{25} to C_{37}) the iso- and anteiso- branched carbon chains (e.g., 2-Methyldecane and 3-Methyldecane respectively). The degradation products of cyclic lipids such as terpenoids and steroids thus can yield information of the degradation of source specific biomarkers in sediments. Higher-plant-derived biomarkers are well-recognized constituents of many sedimentary rocks (Tissot and Welte, 1984). Despite the alteration processes, the degradation products still retain their characteristic basic structure and can be used as biomarkers for their biological sources (Tissot and Welte 1984; Simoneit, 1986). Furthermore, the type and degree of the alteration provides mechanistic information about the processes that lead to the formation of the degradation products (Tissot and Welte, 1984; Killops and Killops, 1993). Homologous aliphatic (lipids) and cyclic (terpenoids) compounds are both utilized as biomarkers in sedimentary environments. The homologous aliphatic compounds have strong carbon number predominance and are derived from the epicuticular waxes and related lipids of higher plants. The compounds are primarily n-alkanes (C_{27}, C_{29}, C_{31}, C_{33}), n-alkanols, and n-alkanoic acids both homologous series have dominant (C_{24}, C_{26}, C_{28}, or C_{30}), with lesser amounts of other oxygenated homologous species.

Hydrocarbons

Of the lipid biomarkers n-alkanes with odd chain 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). The presence of phytane, a C_{20} isoprenoid in marine sediments reflect petroleum inputs or contribution from Archaebacteiria (Volkman and Maxwell, 1986). Simple branched alkenes such as 7- and -8 methyl heptadecene are found in many species of cyanobacteria (Han *et al.*, 1968), and in algal mats and lagoonal sediments. Unusual classes of highly branched polyunsaturated alkenes (HBI alkenes) have been recognised in many studies of marine sediments (Gearing *et al.*, 1976). A high abundance of C_{25} HBI alkene originating from diatom was reported in sediments of Peru Upwelling (Volkman *et al.*, 1983).

Long chain Ketones

Distributions of long chain aliphatic (C_{19} to C_{35}) ketones having a carbonyl at the 2nd positions (methyl ketones) have been found in some coastal marine sediments (Volkman *et al.*, 1983). These compounds can be s derived from oxidation of n-alkanes via the intermediate n-alkan-2ols (Cranwell *et al.*, 1987). Very long straight chain (C_{35} to C_{40}) unsaturated methyl and ethyl ketones with trans double bonds are termed alkenones (Volkman *et al.*, 1995). The ratio of the concentrations of the tri- to di- unsaturated C_{37} ketones in sediments were used to estimate palaeotemperature during the time of the sediments deposition (Prahl and Wakeham, 1987; Sikes *et al.*, 1997).

Fatty Acids

Due to their structural diversity, source specificity and relative stability, fatty acids have proved to be useful biomarkers for assessing the sources and fates of organic matter in marine (Boon *et al.*,1999), coastal (Meziane *et al.*, 2002; Copeman and Parrish, 2003), and estuarine systems (Zimmerman and Canuel, 2001; Arzayus and Canuel, 2004; Mfilinge *et al.*, 2005). Numerous studies have used the source information provided by fatty acids to estimate the relative contribution of terrestrial, algal, planktonic and bacterial fatty acids to the total pool in marine sediments (Zimmermann and Canuel, 2001; Camaco-Ibar *et al.*, 2003). Fatty acids with carbon atoms ≥22 are synthesized mostly by vascular plants (Volkmann *et al.*, 1980), and are considered indicative of higher plant markers in sediments (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 (Volkmann *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).

1.4.2 Terpenoids as Organic Matter Biomarkers

Terpenoids are widely distributed in the geosphere (Brassell *et al.*, 1983; Chaffee *et al.*, 1986; Simoneit, 1998). The diterpenoids originate mainly from conifers while the triterpenoids are derived mainly from angiosperms (Simoneit, 1977b, 1986; Sukh Dev, 1989). Diagenetic processes results in the transformation of biosynthesized precursor molecules ("bio-terpenoids") to their diagenetic products, the biomarkers ("geo-terpenoids"). In spite of these diagenetic alterations, the biomarkers still retain the characteristic structures of their precursors and can be assigned to their structural classes. The terpenoids thus are valuable markers for the determination of the biological source of organic material in geological samples (Simoneit, 1986, 1998, 1999).

Several lines of study have employed pentacyclic terpenoids as well-suited tracers for the primary source of organic matter from mangrove due to their stability during sedimentation and diagenesis (Killops and Frewin, 1994; Versteegh *et al.*, 2004; Koch *et al.*, 2005). Pentacyclic triterpenoids are mostly produced by higher plants and constitute a highly diversified family of molecules (Das and Mahato, 1983; Mahato *et al.*, 1992; Mahato and Sen, 1997). Mangrove plants distributed in intertidal tropical and subtropical regions are rich sources of pentacyclic triterpenoids (Hogg and Gillan, 1984; Ghosh *et al.*, 1985; Koch *et al.*, 2003). Triterpenoid alcohols appear to be excellent indicators for higher plants, although their use as quantitative markers can be hindered by degradative loss and conversion to triterpenoid ketones, alkenes and aromatic hydrocarbons (ten Haven and Rullkotter, 1988; Rullkotter *et al.*, 1994). Several studies of mangrove systems in South America (Koch *et al.*, 2003; Boot *et al.*, 2006), Florida (Killops and Frewin, 1994) and Victoria, Australia (Wannigama *et al.*, 1981) revealed that triterpenoid alcohols could be useful markers for identifying organic matter in sediments derived from mangroves. Koch *et al.*, (2003) found that leaves of *Rhizophora mangle* (red mangrove) were characterized by high amounts of β-amyrin, germanicol, taraxerol, and lupeol. *Avicennia germinans* (black mangrove) mainly contained betulin, lupeol, and sitosterol, whereas significant quantities of sitosterol and lupeol were present in *Laguncularia racemosa* (white mangrove). Versteegh *et al.*, (2004) reported that leaves from *Rhizophora* mangle and *Rhizophora racemosa* are extraordinarily rich in taraxerol. Taraxerol was the main component in the mangrove sediments and Koch *et al.*, (2003) proposed this compound as a marker for mangrove-derived organic matter in the mangrove sediments of Brazil. Killops and Ferwin (1994) used β-amyrin and taraxerol and their degradation products to trace organic matter from mangroves (*Rhizophora* mangle) in sediments from Florida Bay and they found that, in *Rhizophora* mangle leaf cuticle, β-amyrin was largely confined to epicuticular wax, while taraxerol appeared to be a cutin component. Volkman *et al.*, (2000) used a dihydroxylated triterpenoid betulin and other triterpenoidal alcohols to show that sediments from the Waden Sea contained organic matter from eroded peats. The predominance of taraxerol among the triterpenoid alcohols in core sediments collected from the Amazon Fan, in connection with Ocean Drilling Project, was attributed to either greater mangrove productivity or erosion of mangrove sediments (Boot *et al.*, 2006). Triterpenoids of higher plant origin are often found in coastal marine sediments and were also reported from the Peru upwelling region (Volkman *et al.*, 1987) as well as deep-sea sediments in Baffin Bay (ten Haven *et al.*, 1992).

Sterols (tetracyclic triterpenoids) and compounds derived from them by diagenetic reactions are ubiquitous in sediments. A number of studies have shown that phytosterols could be used as tracers of various inputs and transformation processes to environments due to their structural diversity, biosynthesis and stability (Volkman, 1986; Mudge and Norris, 1997). 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). Phytosterols like β-sitosterol have recently been identified as biomarker for mangroves (Koch *et al.*, 2003). Some species show a predominance of a single sterol, such as cholesterol in marine eustigmatophytes, 24-methyl-cholesta 5,22E-dien-3β-ol in some diatoms and mixtures of 4-desmethyl and 4-methyl sterols in some species of dianoflegellates (Volkman, 1986). Dinoflegellates are the major source of 4-meythyl sterols in marine systems and the C_{30} sterol 4α 23, 24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol) is often used as a biomarker for dinoflegellates (Volkman *et al.*, 1993). Sterols with fully saturated ring system [5α (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).

1.4.3 Structure and Classification of Terpenoids

Terpenoids are a class of lipids displaying a great diversity of structures and functions, ranging from the small volatile molecules in sex pheromones to the large molecules in natural rubber. Isoprene (2-methylbuta-1, 3-diene), a branched diunsaturated C_5 hydrocarbon, is the building block of a large family of open chain and cyclic isoprenoids and terpenoids. They contain oxygen in various functional groups. The skeleton of terpenoids may differ from strict additivity of isoprene units by the loss or shift of a fragment, generally a methyl group. They are all formally constructed from C_5 isoprene units, the number of which can be used to classify terpenoids. Most of them are multicyclic structures that differ from one another not only in functional groups but also in their basic carbon skeletons. Most naturally occurring terpenoids contain oxygen, commonly in alcohol, aldehyde, ketone and carboxylic acid groups. Terpenoids do not necessarily contain exact multiples of 5 carbons and allowance has to be made for the loss or addition of one or more fragments and possible molecular rearrangements during biosynthesis. In reality terpenoides are biosynthesized from acetate units derived from the primary metabolism of fatty acids, carbohydrates and some amino acids.

Table 1.1 Classification of terpeniods

Classification	Number of isoprene units
Monoterpenoids	2
Sesquiterpenoids	3
Diterpenoids	4
Sesterpenoids	5
Triterpenoids	6
Tetraterpenoids	8
Polyterpeniods	>8

Essential oils of higher plants are enriched in monoterpenes (C_{10}) with two isoprene units. Farnesol, an unsaturated C_{15} alcohol, is an example of a sesquiterpenoid with three isoprene units. The acyclic diterpenoid phytol is probably the most abundant isoprenoid on earth. It occurs esterified to chlorophyll a and some bacteriochlorophylls and is, thus widely distributed in the green pigments of aquatic and subaerial plants. Sesterterpenoiods (C_{25}) are of relatively minor importance except in some methanogenic bacteria (Volkman and Maxwell, 1986). Cyclisation of squalene or its epoxide is the biochemical pathway to the formation of a variety of pentacyclic triterpenoids (C_{30}) consisting of six isoprene units. Steroids are tetracyclic triterpenoids that are also biochemically derived from squalene epoxide cyclisation, but have lost in most cases, upto three methyl groups. Monoterpenoids, nominally containing two isoprene units, are particularly abundant in higher plants and algae. Because of their volatility they are important attractants (e.g. insect pheromones), but are probably best known as components of essential oils of plants (e.g. menthol in peppermint oil).

Some sesquiterpenoids function as essential oils in plants, while others act as fungal antibiotics. The acyclic compound farnesol is widely distributed in nature, being found in many plants and chlorophyll of many bacteria. Mono and dicyclic sesquiterpenoids are common in plants. The most important acyclic diterpenoid is phytol. It forms the part of the chlorophyll-a molecule and is present in many other types of chlorophyll. The saturated analogue of phytol, dihydrophytol (or phytanol), is present in a variety of bacterial glyceride ether lipids. Most diterpenoids are di- and tricyclic compounds, and are especially common in higher plants. In particular, the resins of gymnosperms (eg. conifers) are characterized by diterpenoids such as agathic, abietic, communic and pimaric acids, and alkenes such as kaurene and hibaene. They act as generally protective agents, sealing wounds and discouraging insect and animal attack. Sesterterpenoids are not well represented among the geochemically important terpeniods, other than the highly branched alkenes found in planktonic (Belt *et al.*, 2001) and benthonic (Volkmann *et al.*, 1994) diatoms.

All triterpenoids appear to be derived from the acyclic isoprenoid squalene ($C_{30}H_{50}$) which is a ubiquitous component in organisms (e.g. shark oil, vegetable oils, fungi). Most terpenoids are either pentacyclic or tetracyclic. The latter belong primarily to the important class of compounds, the steroids. The IUPAC system of carbon numbering of the pentacyclic and tetracyclic triterpenoids are shown in figures1.1 and 1.2. Steroids are merely modified triterpenoids and are widespread in both animal and plant kingdoms and many microorganisms. Most C_{30} pentacyclic triterpenoids with a six membered E ring are of higher plant origin, commonly occurring as resin constituents. Three major series can be distinguished among these higher plant triterpenoids: the oleanoid (eg. β amyrin) ursanoid (eg. α-amyrin) and lupanoid (eg.lupeol) in contrast, the hopanoids have a five membered E ring and are often called the bactereohopanoids because they are common components of cell membranes in eubacteria, particularly diploptene, diplopterene, diplopterol, bacteriohopanetetrol (Ourisson *et al.*, 1987; Kannenberg and Poralla, 1999).

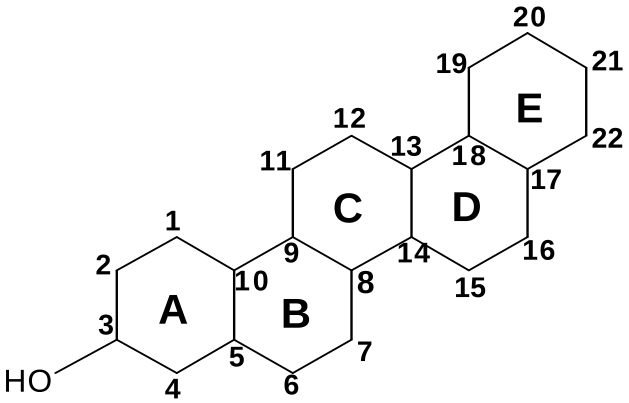

Figure 1.1 Standard numbering and ring naming convention for pentacyclic triterpenoid core.

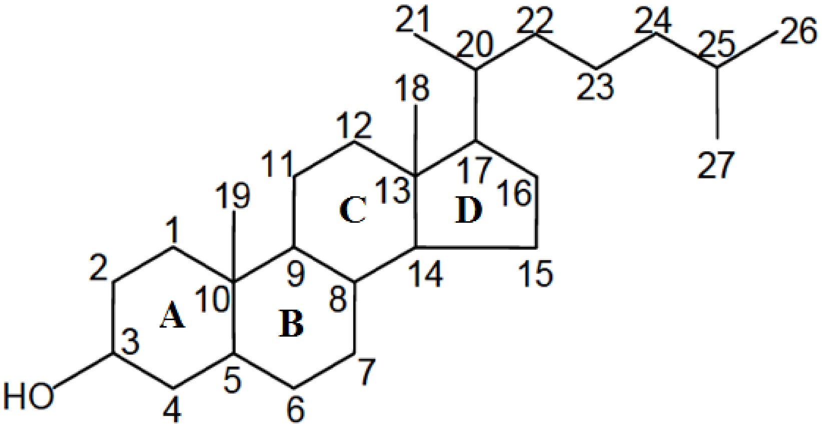

Figure 1.2 Standard numbering and ring naming convention for tetracyclic triterpenoid core.

1.4.4 Biosynthesis of Terpenoids

The major route for terpenoid biosynthesis, the mevalonate pathway, is summarized in figure 1.3. Acetyl CoA is involved in the generation C_6 mevolonate unit, a process that involves reduction by NADPH. Subsequent decarboxyllation during phosphoryllation in the presence of ATP yields the fundamental isoprenoid unit, isopentenyl pyrophosphate (IPP), from which terpenoids are synthesized by enzymatic condensation reactions.

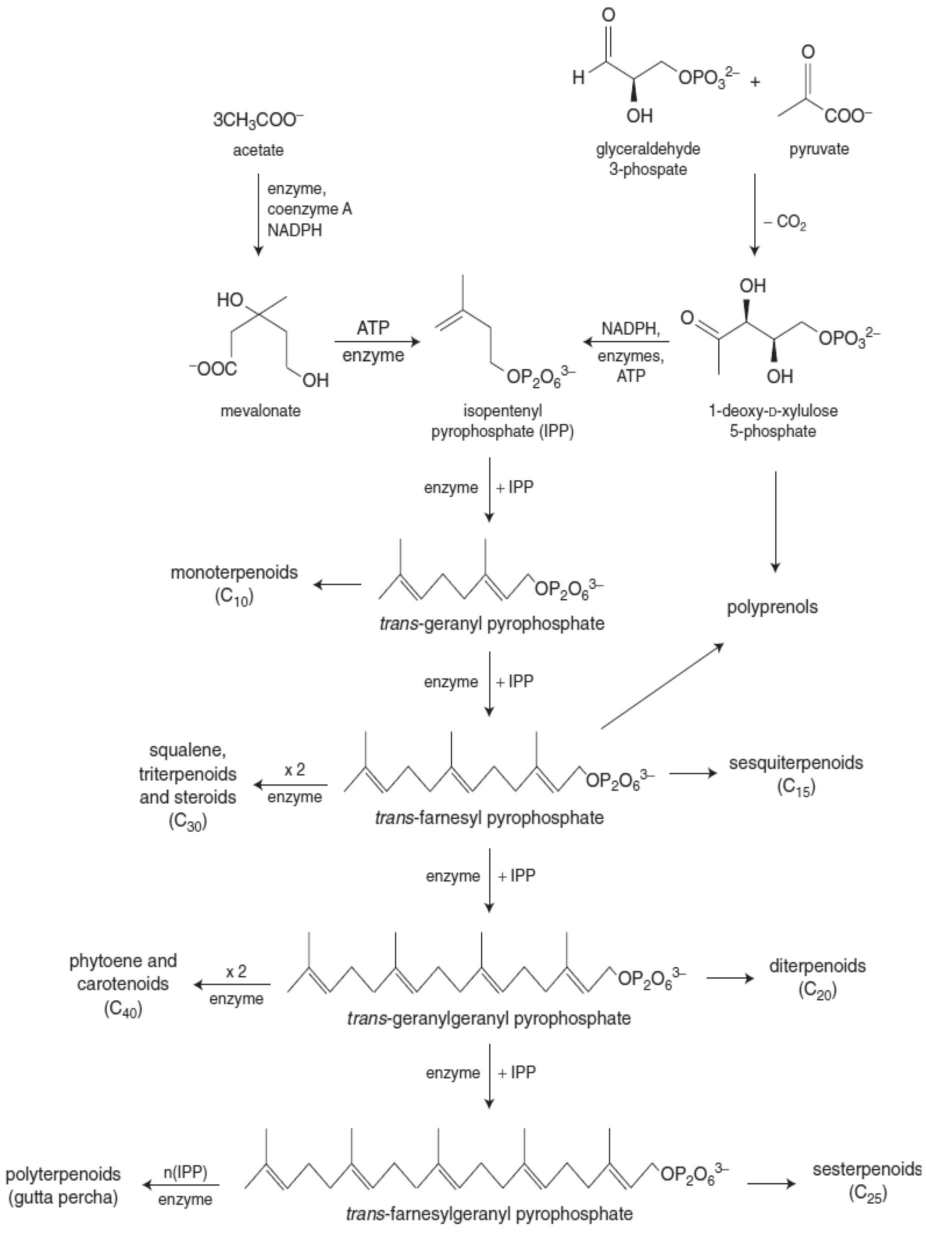

Figure 1.3 Biosynthesis of terpenoids via the mevelonate and deoxyxylulose pathways

A variety of triterpenoids and phytosterols widely distributed in plants are biosynthesized from a common precursor 2, 3-oxidosqualene by the enzyme oxidosqualene cyclases (OSCs). 2, 3-Oxidosqualene therefore locates at the branching point of isoprenoid pathway toward phytosterols or terpenoid biosynthesis (Abe *et al.*, 1993). The cyclization of oxidosqualene into terpenoids and phytsoterols are one of the most fascinating reactions found in nature and their biosynthetic pathway has been shown to be complicated and divergent. The enzymes, which perform these reactions, belong to oxidosqualene cyclases (OSCs) family. In higher plants, OSC family member cycloartenol synthase and lanosterol synthase are responsible for sterol biosynthesis, and other OSCs are involved for triterpenoid synthesis. The cyclization, rearrangement and deprotonation reaction leading to the different product of triterpenoids. Enzymatic cylization of 2, 3-oxidosqualene into sterols proceeds via the so-called "chair-boat-chair" conformation to yield the C-20 protosteryl cation (figure 1.4), which is then converted to cyloartenol or lanosterol. Triterpenoid synthesis, on the other hand, involves cyclization of the corresponding "chair-chair-chair" conformation of the substrate to give the tetracylic dammarenyl cation. This cation may then either be converted to dammarene-like triterpenoids by the OSC dammarenediol synthase (Tansakul *et al.*, 2006) or undergo expansion during the cyclization and rearrangement of 2, 3-oxidosqualene to promote ring expansion and annulation to form lupenyl cation, after D-ring expansion via C-16 migration followed 18β E-ring cylization from dammarenyl cation to form lupeol with direct deprotonation without rearrangement. Additionally, β-amyrin, also originate from dammarenyl cation intermediates, and allow further E-ring expansion via C-21 migration and some rearrangement before deprotonation or by the addition of a hydroxyl group, resulting in a multi products of oleanane class of terpenoids, such as germanicol or taraxerol (Abe *et al.*, 1993; Haralampidis *et al.*, 2002; Xu *et al.*, 2004).

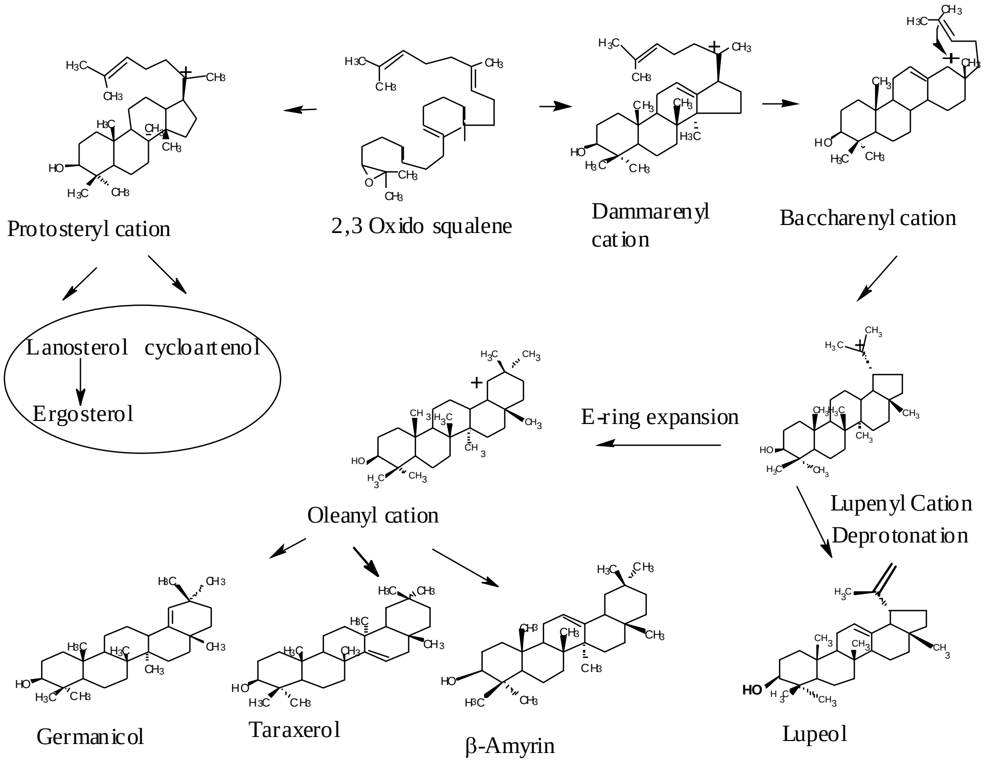

Figure 1.4 Formation of triterpenoids from 2, 3 oxidosqualene

1.5 Aim and Scope of the Present Study

Vascular plants play a prominent role in structuring the flow of organic matter in coastal ecosystems (Wardle *et al.*, 2004). Organic matter in the coastal sediments consist of a mixture of different sources, including locally produced macrophyte material, microphytobenthos and suspended organic matter imported from outside the ecosystem boundaries during tidal inundation. Mangroves are highly productive wetland ecosystems, which play an important role in the biogeochemical cycles of the coastal environment (Jennerjahn and Ittekkot 2002; Feller *et al.*, 2003). These ecosystems have profound influence on the carbon balance of tropical coastal ecosystems. The mangrove detritus and the subsequent mineralized nutrients are exported out of the mangrove ecosystem through tidal flushing.

Despite repeated demonstration of their economic and societal value, these ecosystems are subjected to destruction due to aquaculture, coastal development, altered hydrology, sea-level rise and nutrient enrichment. They may be disappearing more quickly than inland tropical rainforests, with little public notice. According to Duarte *et al.*, (2005), the surface area of mangrove ecosystems worldwide declines by ~2% per year, causing major changes in the coastal carbon cycle. Due to ecological and economic importance as a coastal resource (Kathiresan and Qasim, 2005), it requires more attention to protect these ecosystems. Therefore, biogeochemical characterisation has to be employed as a tool for the conservation and sustainable management of these vulnerable ecosystems.

The biogeochemical functioning of mangrove environments is well constrained for a number of well-studied sites around the world, but our ability to elucidate carbon and nutrient budgets of these ecosystems is limited. The biogeochemistry of mangroves is the least understood one because of their sediment complexity due to the tidal influx of allochthonous organic matter and also the input of local vegetation. 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 sources. Common chemical parameters are insufficient to describe the biogeochemical character of this fragile ecosystem effectively. Bulk geochemical parameters such as biochemical composition, elemental composition and stable carbon isotope ratio are relatively reliable proxies of organic matter origin. Eventhough bulk geochemical parameters such as elemental and isotopic compositions of sedimentary organic matter, have commonly been used to distinguish organic matter from autochthonus versus allochthonous sources, does not explain the chemical nature of the organic matter deposited. The biochemical composition of organic matter sources varies and the differences in source signatures are not always unique enough to identify components in complex mixtures such as sediments. Hence, an effective tool for the source characterization of organic matter in coastal ecosystems is biomarker approach.

Estuarine areas serve as final receptors of natural and anthropogenic organic matter derived from land and carried by river and atmosphere. It is essential to determine the relative contribution of different sources of organic carbon to the biogeochemical cycles in estuarine environments to have a better understanding of their ecological importance. Due to the complexity of organic matter sources in estuaries and the problems associated with making only bulk measurements to constrain them, the application of chemical biomarkers has become widespread in estuarine research (Bianchi and Canuel, 2001).

Pentacyclic and tetracyclic triterpenoids are useful biomarkers for monitoring the preservation and degradation of vascular plant derived organic matter in sediments. Despite their importance as characteristic molecular biomarkers, the presence of steroids and terpenoids in sedimentary organic matter has not been sufficiently investigated. Very little work has so far been reported on the biogeochemical evaluation of sedimentary environments using biomarker approach from India, although such works on mangrove as well as non-mangrove sediments are reported worldwide. Therefore, the analysis of terpenoid biomarkers is a prerequisite for the source characterization of organic matter in the sediments of the study area. The objective of the study was to investigate the sources of organic matter in the surface sediments of the mangrove and estuarine ecosystems around Cochin and also to study the export of mangrove organic matter to the adjacent estuarine system using biomarker approach.

The present work aims at:

- Study of the geochemical characteristics of the systems using phosphorus fractionation mineralogy and heavy metal along with other general sedimentary parameters.
- Assessment of biochemical composition (total lipids, proteins and total carbohydrates), elemental composition and stable carbon isotopic ratio of total organic matter to study the quantity, quality and source characterization of organic matter.
- Extraction, identification and distributional character of triterpenoids present in the surface sediments of mangrove and estuarine ecosystems.
- The use of triterpenoid biomarkers together with other lipid biomarkers such as n-alkanes, n-alcohols to characterize the sources of organic matter in mangrove and estuarine systems.

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Chapter 2

MATERIALS AND METHODS

2.1 Description of the Study Region

Cochin estuary (09º40\ 12\\ – 10º10\46\\N and 76º 09\ 52\\ -76º23\57\\ E) is a part of Vembanad-Kol wetlands and it is the largest estuarine system in the southwest coast of India. This estuary is topographically divisible into two arms; a southern one extending south of barmouth from Cochin to Thanneermukkam and a northern one extending north from Cochin to Azhikode. It is very peculiar and complex in the sense that it lies parallel to the coastline with several islands and small arms. Unlike any other estuaries in the world, six rivers with their tributaries along with several canals bring large volumes of freshwater into the Cochin estuary. It is one of the highly productive estuarine systems (Qasim, 2003) and has been designated as a 'Ramsar site' (No. 1214). This tropical aquatic system is under the profound influence of the monsoon, which contributes to about 71% of the annual rainfall (Jayaprakash, 2002) and accordingly there are three seasonal conditions prevailing viz. monsoon (June-September), post-monsoon (October-January) and pre-monsoon (February-May). Tides at Cochin are of a mixed semi-diurnal type, with the maximum spring tide range of about 1m (Srinivas, 1999), resulting in incomplete flushing. Hydrobiological studies of the estuary (Menon *et al.*, 2000) showed that the high flushing during monsoon completely transforms the estuary into a freshwater habitat. The abundant mangrove vegetation of these regions has been shrinking in area due to land reclamation and developmental activities and also due to pollution by industrial effluents as well as domestic sewage.

The estuary is experiencing increased anthropogenic interventions since the last five decades. Indiscriminate reclamation, urban development, discharge of untreated and partially treated sewage, industrial effluents and siltation are the major threats to this sensitive ecosystem. The Cochin estuary has been shrinking in its area from 315 km^2 in 1912 to 256 km^2 in 1980 (Gopalan *et al.*, 1983). This estuary has been classified under the "Ecologically Sensitive Zone" according to the Environmental Protection Act 1986. Three mangrove systems and three estuarine stations along the northern arm of Cochin estuary were chosen for the present study (Figure 2.1). The estuarine stations were Bolgatty (Station E1), Ponnarimangalam (E2) and Mulavukad (E3). The stations E2 and E3 are away from Cochin bar mouth receiving fresh water input from river Periyar. Station E1 is about 1 km away from Cochin bar mouth and Vallarpadam, a region with small patches of mangroves is located in the confluence of E1. The mangrove station Mangalavanam (M3) is connected to E1 through a narrow canal.

Station 1: Puthuvyppu (M1)

It is a mangrove nursery maintained by the fisheries research unit of Kerala Agriculture University and is located about 100m 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). This is the largest single stretch of mangrove in Kerala and has an area of approximately 101hectares (Geetha *et al.,* 2008). This mangrove area is regularly inundated by a semi diurnal rhythm of Cochin bar mouth and the tidal waters bring in lot of fish seed of commercially valuable species of prawns. However, a lot of developmental pressures, including the proposed gas thermal plant, are threatening the very existence of these mangroves. Heavy developmental activities after the construction of the bridges connecting the mainland are threatening the existence of mangrove patches in the adjoining areas as well.

Station 2: Murikkumpadam (M2)

It is a densely populated fisher-folk settlement. The dominant species in this system are *Acanthus ilicifolius, Rhizophora apiculata, Rhizophora mucronata, Excoecaria agallocha* and two mangrove associates: *Clerodendronica and Acrostica* (Sebastian and Chacko, 2006). Discharge of sewage and disposal of garbage and solid waste are the major sources of pollution here. This station is very close to the Arabian Sea.

These two stations form part of the island called Vypin, which is one of the most densely populated coastal zones. Vypin is the largest single stretch of mangroves found in Kerala. It covers an area of 101 hectares. Vypin Island is well known for its Pokkali fields. Mixed silvi-agri- aquacultural farming is practiced here. Large areas of mangroves have been destroyed for prawn and fish culture. The pressure of the growing population is also a threat to these mangroves.

Station 3: Mangalavanam (M3)

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* species (Subramanian, 2000). This mangrove forest 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.

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 include: *Acanthus ilicifolius, Avicennia officinalis, Avicennia marina, Bruguiera gymnorrhiza, Excoecaria agallocha, Kandelia candel, Lumnitzera recemosa, Rhizophora mucronata, Rhizophora apiculata, Sonneratia caseolaris.* In Mangalvanam, the identified species are: *Acanthus ilicifolius, Avicennia officinalis, Avicennia marina, Bruguiera gymnorrhiza, Kandelia candel, Lumnitzera recemosa, Rhizophora mucronata, Rhizophora apiculata* and *Sonneratia caseolaris.*

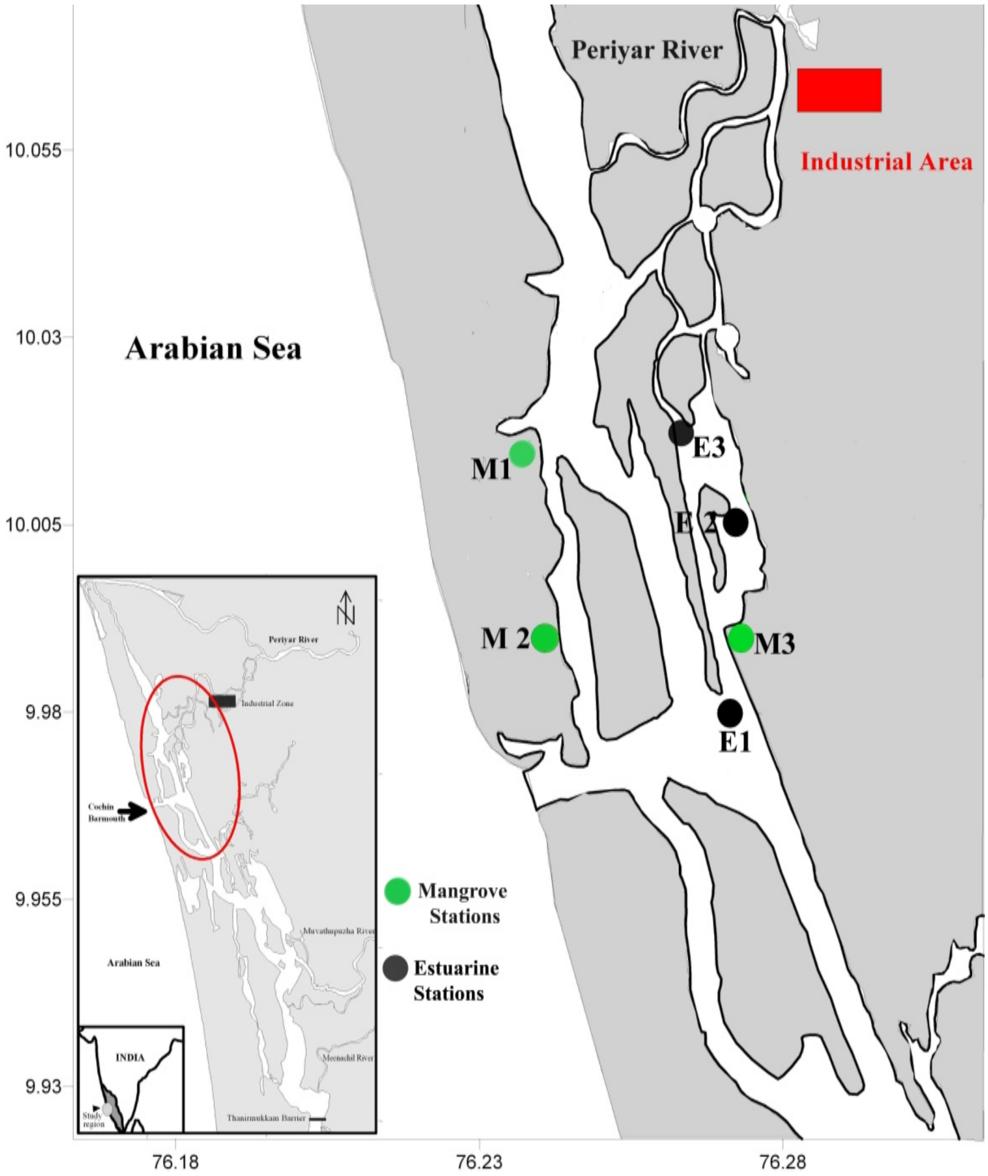

Figure 2.1 Location map of the sampling stations

2.2 Sampling and Analytical Methodology

Samples of water and surface sediments were taken from three mangrove and three estuarine stations during December 2005, April 2006 and July 2006. Collection of samples from mangrove station Mangalavanam (M3) during monsoon could not be carried out due to technical reasons. During high tide, surface water samples were collected from both mangrove and estuarine stations using a clean plastic bucket. Bottom water samples from the estuarine stations were collected using Niskin Sampler. Surface sediment samples were taken from the mangrove stations using a clean plastic spoon. In order to get a true representation of the mangrove ecosystems, sediment samples were collected from three different parts of each system and pooled together for analysis. Van Veen grab (0.042 m^2) was used to collect the surface sediment samples from the estuarine stations. 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 value was reported.

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 Genesys 10UV 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 freeze-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 (1956) 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 analyzed 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 Atomic Absorption Spectrometry (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	Certified Value	Obtained Concentration (n=3)
Co (μg/g)	11.4 ± 2.1	10.67 ± 2.68
Cr (μg/g)	123 ± 1.4	112 ± 0.65
Cu (μg/g)	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 (μg/g)	229 ± 15	189.47 ± 10.75
Ni (μg/g)	55.3 ± 3.6	49.16 ± 2.01
Pb (μg/g)	22.7 ± 3.4	24.9 ± 0.08
Zn (μg/g)	119 ± 12	123.64 ± 2.51

The sequential extraction scheme by Golterman (1996) using chelating agents was employed for estimating different phosphorus fractions (Figure 2.2). 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).

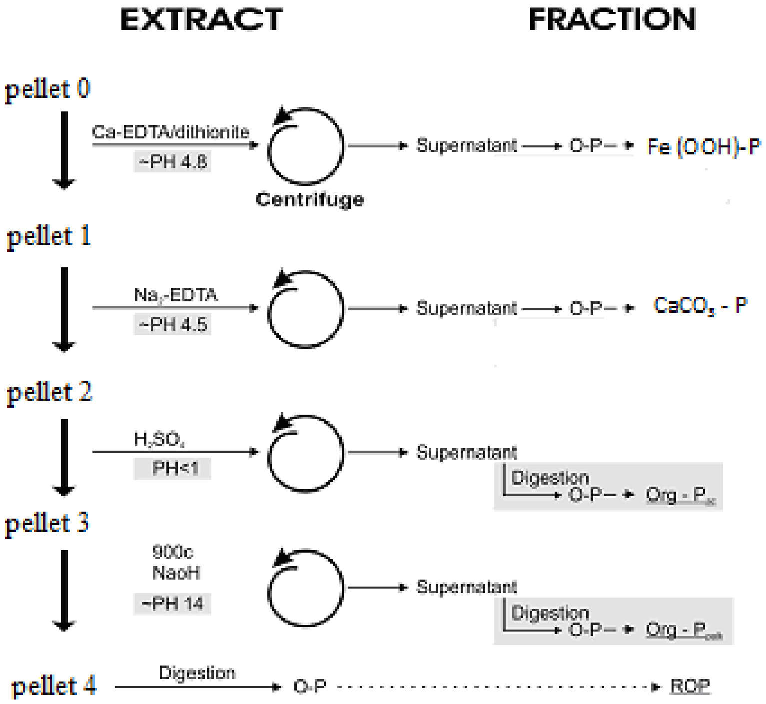

Figure 2.2 Sequential extraction scheme for phosphorus fractionation

2.2.3 Biochemical Composition

Spectrophotometric methods were employed for the determination of biochemical compounds in sediments. 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 a factor of 0.16 (Mayer *et al.*, 1986). Total carbohydrates (CHO) were analyzed 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 value was 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). Tannin and lignin in sediments were extracted using 0.05M NaOH at 60° for 90 minutes and the estimated spectrophotometrically by the sodium tungstate-phosphomolybdic acid method (Nair *et al.*, 1989; APHA, 1995), using tannic acid as the standard. The principle involved is the development of a blue colour on reduction of Folin phenol reagent by the aromatic hydroxyl groups present in tannins and lignins. The effects of Mg and Ca hydroxides and/or bicarbonates present in the seawater were suppressed by the addition of trisodium citrate solution (Nair *et al.*, 1989).

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:

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

2.2.4 Triterpenoid Biomarkers

For the extraction and analysis of triterpenoids, finely milled freeze-dried sediment samples were Soxhlet extracted for 72 h with a mixture of dichloromethane methanol (2:1, v/v) for 72 h (Yi Duan and Lanhua Ma, 2001). Copper foil was placed in the flasks to bind any sulphur present. The solvent extract was filtered and concentrated by use of a rotary evaporator and then under blow-down with dry nitrogen gas. The extracted material was saponified overnight with 6% KOH–methanol at room temperature. Both neutral and fatty acid fractions were successively recovered with dichloromethane, the latter after acidification with concentrated HCl to pH 1. The neutral lipid fraction were partitioned from the alkaline solution into hexane and fractionated into individual compounds classes by column chromatography on silica gel (activated for 3 h at 150 ^{0}C) using n-hexane, dichloromethane and methanol to elute the aliphatic, aromatic and hetero component fractions (Otto and Simoneit, 2001). Excess solvent was removed by vacuum rotary evaporation and fractions were transferred in dichloromethane to vials, dried under high purity nitrogen gas, and stored at 4°C until analysis. Aliquots of the separated fractions were converted to trimethylsilyl derivatives by reaction with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and pyridine for 3 h at 70 °C.

Gas chromatography-mass spectrometry (GC-MS) analyses of the derivatized fractions and separated fractions were performed on a Perkin Elmer model Auto System XL Gas Chromatograph coupled to Perkin Elmer model Turbo Mass Gold Mass spectrometer (5.0.0). Separation was achieved on a fused silica capillary column coated with DB1 (30 m x 0.25 mm id., 0.25 μm film thickness). The GC operating conditions were as follows: temperature hold at 125 °C for 5 min, increase from 125 to 200 °C at a rate of 4°C min^{-1} with an isothermal hold for 5 min, then increase temperature from 200 °C at a rate of 4°C min^{-1} to 260 °C with an isothermal hold for 5 min. In the last stage the temperature increases from 260 °C to 280°C at a rate of 4°C min^{-1} with a final isothermal hold at 280°C for 20 min. Helium was used as carrier gas with a flow rate of 0.5 ml min^{-1}. The sample was injected splitless with the injector temperature at 300 °C. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV ionization energy and scanned from 50 to 500 Dalton. Data were acquired and processed with the Totalchrom workstation software (version 6.2.1). Individual compounds were identified by comparison of mass spectra with literature and library data, comparison with authentic standards and interpretation of mass spectrometric fragmentation patterns. Structural assignments were based on comparison of the gas chromatographic retention times with those of authentic standards and by interpretation of mass spectra or comparison with published mass spectral data. The compounds were identified by their GC retention times and published mass spectra (Philip, 1985). Mass spectral identification was confirmed by comparing the obtained mass spectra with those of authentic standards or mass spectra stored in the NIST MS Library (version-2.0) and NBS library system.

All solvents and silica gel (230-400 mesh) were purchased from Merck ('Suprasolv', Darmstadt, Germany). Gas chromatographic standards for terpenoids and sterols were purchased from Sigma-Aldrich (USA). All materials used (silica gel, glass and cotton wool, anhydrous sodium sulphate, etc.) were soxhlet extracted with methanol: acetone (50:50) overnight and twice with methylene chloride for 24 h, and kept dry (in desiccator) until use. All glassware was cleaned by ultra sonic bath followed by heating at high temperature.

2.2.5 Statistical Analysis

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 ANOVA (stations x seasons). Principal component analysis (SPSS 15.0) was done to find out the factors contributing to different biogeochemical processes occurring in both mangrove and estuarine systems.

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). In mangrove systems, 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 M3, true marine situation could be observed 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 6.5 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 M3 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 mangrove ecosystems

Parameters	M1			M 2			M 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 ($mgCaCO_3/l$)	164	132	144	216	132	132	92	-	68
DO (mgO_2/l)	6.4	3.0	1.4	6.5	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

-Due to technical reasons samples from station M3 during monsoon season could not be collected.

In the case of estuarine stations (Table 2.3), pH of the water varied from 7.01(E2, monsoon) to 8.15 (E2, pre-monsoon) in surface and 7.01(E2, monsoon) to 8.77 (E1, pre-monsoon) in bottom. Salinity of the samples varied from 0.04 to 32.92 psu and 0.04 to 34.02 psu for surface and bottom waters respectively. The highest value for salinity was recorded during post-monsoon at station E1, which is located at the confluence of bar mouth. The observed dissolved oxygen concentrations were in the range 4.90 - 7.68 mg/l for surface waters and 3.59-8.0 mg/l for bottom waters. The maximum value for this parameter was recorded at E2 during monsoon. Alkalinity varied from 18 to 317 (mg $CaCO_3/l$) for surface and 28 to 208 (mg $CaCO_3/l$) for bottom waters. Station E1 recorded the maximum concentration for inorganic nitrite with a variation from 0.34 to 1.26 µmol/l surface waters and 0.32 to 0.95 µmol/l for bottom samples. The observed nitrate content varied from 4.46 to 46.65 µmol/l in surface waters and 5.36 to 91.95 µmol/l in bottom waters. The estimated inorganic phosphate content ranged from 1.97 to 4.77µmol/l (surface) and 1.88 to 4.89 µmol/l (bottom). The concentration of silicate in surface water samples varied from 60.35 to 142.5µmol/l, while in bottom samples it ranged between 59.73 and 133µmol/l. The maximum content for silicate was recorded at E3 (surface) during monsoon. The observed higher silicate content during monsoon season might be attributed to river run off.

Table 2.3 Seasonal variations of hydrographical parameters in estuarine stations.

Parameters	Depth	E 1			E2			E3		
		Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
pH	Surface	8.04	7.44	8.13	8.15	7.01	7.74	7.04	7.03	7.73
	Bottom	8.77	7.26	8.31	7.92	7.01	7.92	7.47	7.15	7.84
Salinity (psu)	Surface	9.12	0.11	32.92	4.52	0.04	30.70	2.35	0.04	21.68
	Bottom	26.37	0.09	34.02	12.19	0.08	32.51	2.98	0.04	28.00
DO(mg/l)	Surface	6.53	6.24	4.90	5.88	7.68	5.22	5.88	6.56	5.55
	Bottom	5.71	6.56	3.59	6.04	8.00	5.22	5.22	7.36	5.55
Alkalinity (mg $CaCO_3$/l)	Surface	60	317	68	36	156	56	18	156	52
	Bottom	100	208	108	54	156	64	28	104	52
Nitrite(µmol/l)	Surface	0.34	1.26	0.55	0.63	0.35	0.79	0.48	0.57	1.18
	Bottom	0.32	0.95	0.73	0.41	0.51	0.77	0.57	0.53	0.75
Nitrate(µmol/l)	Surface	14.26	13.53	6.09	4.46	46.65	6.27	20.5	19.28	10.87
	Bottom	12.64	20.63	11.16	5.36	13.53	9.14	48.2	91.95	18.17
Phosphate (µmol/l)	Surface	4.41	3.58	2.77	3.93	3.24	1.97	2.76	4.77	4.02
	Bottom	1.88	4.89	3.52	3.88	3.33	3.97	3.01	2.84	3.32
Silicate(µmol/l)	Surface	64.58	137.75	95.83	60.35	109.50	85.94	100.35	142.50	115.6
	Bottom	59.73	133.00	78.64	60.12	109.44	84.30	68.31	100.01	98.75

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Chapter 3

GEOCHEMISTRY

3.1 Introduction

Phosphorus fractionation in sediments can be considered as an indexing of various biogeochemical processes. Fractionation is a commonly used method to characterize phosphorus in aquatic sediments and it separates phosphorus into several forms according to solubility and reactivity against various reagents. In the present study, phosphorus fractions in mangrove and estuarine sediments were analyzed to quantify different fractions of phosphorus in the mangrove and adjacent estuarine stations to assess the processes leading to the fractional distribution of phosphorus, thereby evaluating the geochemical setting of the sedimentary environment.

Phosphorus retention and release in mangrove sediments depend on several factors including pH, redox potential, tidal inundation, the nature of the phosphorus compounds supplied to the sediment-water interface, sedimentation rate, bioturbation, diagenetic processes etc. (Ruttenberg and Berner, 1993; Silva and Sampaio, 1998; Schenau and de Lange, 2001). Phosphorus cycling and bioavailability in estuaries depend upon phosphorus speciation (Paludan and Morris, 1999; Andrieux-Loyer and Aminot, 2001). Sequential chemical extraction techniques have been a useful tool to examine these processes (Ruttenberg, 1992; Jensen and Thamdrup, 1993), since the analysis of total sediment phosphorus does not represent an accurate measurement of phosphorus exchangeability and bioavailability for algal growth. Phosphate can be present in association with iron, aluminium and calcium oxides or adsorbed on the surface of minerals and organic materials. The range of conditions such as salinity, pH or redox potential present in estuarine systems determines the relative importance of each P fraction (Lebo, 1991; Paludan and Morris, 1999).

Although many different extraction schemes for sedimentary phosphorus fractions do exist, the 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 rather exhaustive or unique with respect to the targeted compounds (Turner *et al.*, 2005). Sequential extraction techniques are rather laborious, tedious, time consuming and it is subjected to several potential errors including 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 sample volume ($\leq$ 0.5g), are relatively simple to perform, and require only basic laboratory equipment. To avoid disadvantages like the uncertainty in the bioavailable and the nonspecific 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. The Ca-EDTA solution has an advantage over the CDB or NaOH to extract both Fe (OOH) and Fe (OOH)-P with optimal efficiency and EDTA solutions are recommended for inorganic-P extractions without disturbing organic phosphorus (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) 1M Ammonium Chloride	Loosely bound P
	b) 0.05M Ammonium Fluoride	Aluminium bound P
	c) 0.1 N Sodium Hydroxide	Iron Bound P
	d) KCl-$C_6H_8O_6$-EDTA	Occluded P
2. Hieltjes and Lijkema (1980) and Nurnberg (1998)	a)1M Ammonium Chloride	Loosely bound P
	b) 0.11M Sodium bicarbonate and Dithionate	Iron Bound P
	c) 0.1N sodium hydroxide	Aluminium bound P
	d) 0.5N Hydrochloric acid	Calcium bound P
	e) Persulphate digestion of NaOH extraction	Labile organic/Polyphosphate P Refractory organic P
	f) Persulphate digestion of remaining sediment	
3. Psenner *et al.*,(1984) modified by Hupfer et al., (1995)	a) NH_4Cl-at neutral pH	Labile, Loosely bound or adsorbed P
	b) Buffered dithionate	Reductant soluble P, bound to Fe oxides and hydroxides
	c) NaOH	P adsorbed to metal oxides and bound to humic substances
	d) HCl	P bound to carbonates, apatite – P and released by the dissolution of oxides
	e) Digestion	Organic and refractory P

4. SEDEX procedure Ruttenberg, (1992)	a)CDB (citrate-dithionite-bicarbonate)	Labile and oxide- associated P
Modified by Anderson and Delaney, (2000)	b)1M sodium acetate buffered to pH 4	Authigenic CFA
	c)1N Hydrochloric acid	Detrital Apatite P
	d) 50% Magnesium nitrate to sediments, drying in oven at 80^o and ashing at 550^o C,1N HCl	Organic P
5. De Groot and Golterman, (1993)	a) Buffered Ca EDTA/dithionite	Iron bound P
	b) Na_2EDATA	Calcium bound P
	c) H_2SO_4	Acid soluble P
	d) 2M NaOH pH 1 with H_2SO_4	Alkali Soluble P
	e) Digestion	Humic and Fulvic P
		Residual Organic P
6. Jensen and Thamdrup (1993)	a) $MgCl_2$	Loosely sorbed P
	b)Buffered dithionite extraction	Iron bound P
	c) NaOH	Al/Clay mineral and humic bound
	d) HCl Digestion	Calcium bound P
		Refractory organic P
7. Golterman, (1996)	a) Ca-EDTA/dithionite	Iron bound phosphorus (Fe-IP)
	b) Na-EDTA	Calcium bound fraction (Ca-IP)
	c) 0.5M H_2SO_4	Acid soluble organic phosphorus (ASOP)
	d) 2M NaOH at 90°C for 2 hours	Alkali soluble organic phosphorus (Alk-OP)
	e) $K_2S_2O_8$ digestion in acid medium for1 hour	Residual organic phosphorous (ROP)

3.2 Results

The spatial and seasonal variations of different geochemical parameters in the surface sediments of the study region, including texture, mineralogy, major and trace elemental composition are explained in this chapter.

3.2.1 General Sedimentary Characteristics

In mangrove sediments, pH varied from 5.85 to 7.10 (Table 3.2). Marked variation in Eh values from -398 to +12 mV was recorded in sediments and highly negative value for redox potential was observed at station M3. Texture analysis revealed that silt was the major fraction in the mangrove sediments, ranging between 28.51±3.0 and 71.79±6.8% and the highest silt content was recorded at M1 during pre-monsoon. Clay content ranged between 19.07±2.22% and 39.68±2.79% with the maximum content observed at M2 during post-monsoon. By applying Folk, (1974) classification (Figure 3.1), station M1 was silty during premonsoon and changes to sandy mud during monsoon. It was muddy during post-monsoon period. Station M2 did not exhibit any seasonal variations in texture and was muddy in nature. Station M3 displayed sandy mud nature during pre-monsoon and changed to silty in post monsoon.

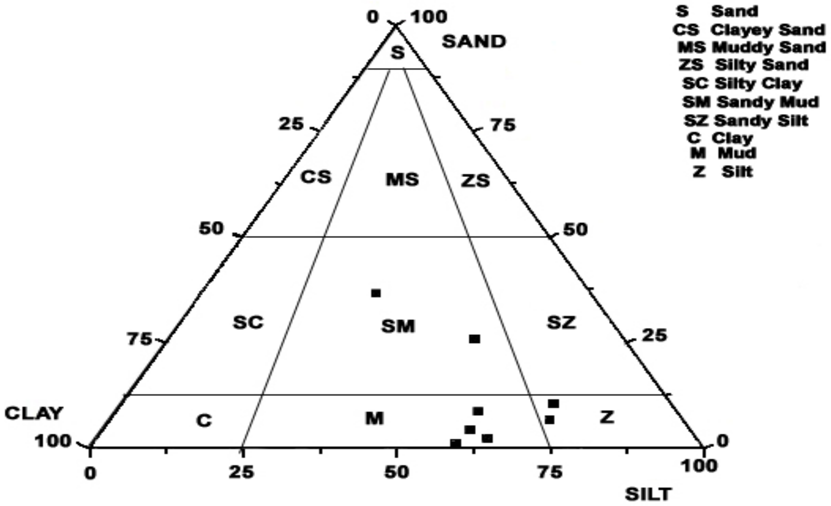


Figure 3.1 Ternary diagram for mangrove sediments. Sediment nomenclature after (Folk, 1974)

However, in the case of estuarine sediments (Table 3.3), pH varied from 6.60 to 7.65 and was maximum at E3 during post-monsoon. The Eh values varied from -260 to -150 with its highest negative value noticed at E3 during post-monsoon. The predominance of the silty sand nature of sediments from E2 and E3 was noticed according to Folk, (1974) classification (Figure 3.2). Station E1 transformed from mud (pre-monsoon) to sandy mud nature (post-monsoon). Sediments from E3 exhibited sandy silt nature during pre-monsoon and transformed to sandy mud during post monsoon.

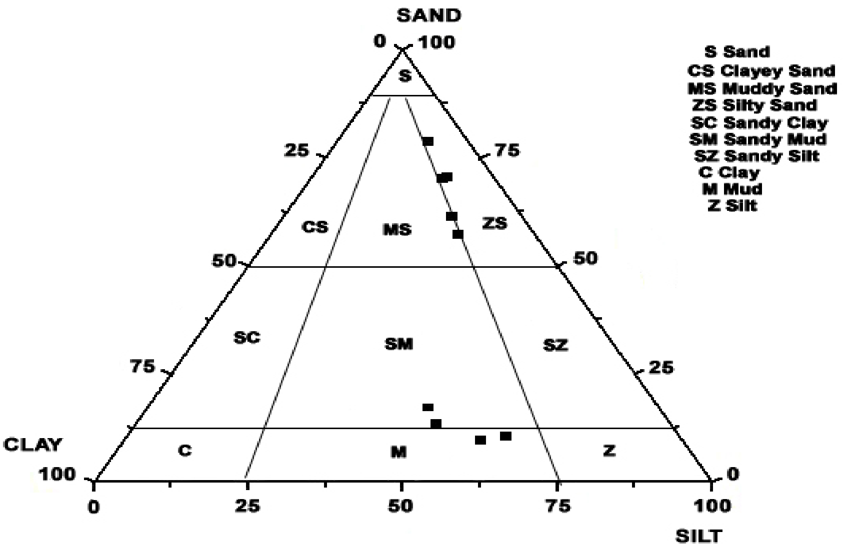


Figure 3.2 Ternary diagram for estuarine sediments. Sediment nomenclature after (Folk, 1974).

CHNS analysis of the mangrove sediments (Table 3.4) showed that they were rich in total carbon. It ranged from 2.91±0.06 to 7.64±0.09% and was observed to be maximum at M3. Generally, post-monsoon season was found to have higher carbon content, except for station M1. Organic carbon content in mangrove sediments exhibited marked variation from 2.7±0.02% (M2, pre-monsoon) to 6.70±0.13% (M3, post monsoon). It constituted 82.63 to 94.59%

of the total carbon and was reported to be higher at M3. Concentration of total nitrogen recorded its highest value at station M1 and it ranged between 0.12±0.05% (M2, pre-monsoon) and 0.28±0.01% (M1, post-monsoon). Total sulphur content in mangrove sediments varied from 0.98±0.012 to 2.58±0.023% and was maximum at station M3.

In estuarine sediments, station E1(post-monsoon) exhibited the maximum content for organic carbon and it varied from 0.68±0.006 to 3.04±0.13% (Table 3.5). Whereas, total carbon content varied from 0.98±0.09 to 3.35±0.07%. CHNS analysis revealed that the total carbon content was higher at all stations during post-monsoon season, a similar trend exhibited by mangrove ecosystems. The concentration of the total N varied from 0.047±0.001% (E3, pre-monsoon) to 0.17±0.008% (E1, monsoon), total S varied from 0.18±0.01% (E2, pre-monsoon) to 1.7±0.01% (E1, pre-monsoon) in the estuarine sediments.

Analysis of variance (ANOVA, two factors without replication) showed that sediment texture did not have any significant seasonal variations in mangrove ecosystems (Table 3.2). Carbon, nitrogen and sulphur did not exhibit any significant spatial and seasonal variations (Table 3.4) in mangrove sediments. ANOVA revealed that in estuarine sediments (Table 3.3), sand and silt exhibited spatial variation ($p= 0.031$ and $p=0.018$ respectively). But clay did not display any spatial or seasonal variation. Total sulphur exhibited spatial variation in estuarine sediments ($p=0.047$; Table 3.5) with its maximum content observed at E1 during pre-monsoon. The seasonal variation of total carbon, nitrogen and total sulphur are depicted in figures 3.3 and 3.4 respectively.

Table 3.2. Seasonal variation of general sedimentary parameters in mangrove sediments.

Parameters	M1			M2			M3			ANOVA P-Value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Spatial	Seasonal
pH	6.20	5.85	7.00	6.46	6.48	7.10	6.65	-	6.60	0.31	0.43
Eh	-98	-16	-10	12	-41	-53	-337	-	-398	0.44	0.18
Sand %	6.73± 1.90	36.60± 4.80	8.87± 2.86	4.34± 1.10	2.32± 0.91	0.94± 0.09	23.59± 2.60	-	10.46± 1.92	0.115	0.160
Silt %	71.79± 6.8	28.51± 3.00	58.92± 4.17	59.97± 2.10	63.78± 6.7	59.38± 6.90	45.56± 3.10	-	70.47± 5.70	0.780	0.428
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	21.97± 3.10	-	19.07± 2.22	0.069	0.620

-Due to technical reasons samples from station M3 during monsoon season could not be collected.

Table 3.3. Seasonal variations of general sedimentary parameters in estuarine sediments.

Parameters	E1			E2			E3			ANOVA P-Value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Spatial	Seasonal
pH	6.60	7.61	7.57	7.17	7.60	7.60	7.31	6.65	7.65	0.37	0.78
Eh	-209	-205	-208	-200	-213	-248	-237	-150	-260	0.29	0.89
Sand%	10.64± 0.91	9.65± 0.93	13.50± 1.27	73.68± 2.47	75.93± 2.52	87.69± 4.75	61.44± 2.84	70.3± 2.27	17.18± 0.21	0.031	0.732
Silt%	61.47± 2.11	57.75± 1.73	48.63± 0.9	9.90± 0.2	16.56± 0.66	4.07± 0.08	27.23± 0.54	21.4± 0.43	45.55± 0.82	0.018	0.993
Clay%	27.90± 0.56	32.59± 0.65	37.87± 1.28	16.42± 0.81	7.51± 0.15	8.24± 0.17	11.34± 0.22	8.32± 0.36	37.27± 0.77	0.113	0.391

Table 3.4. Seasonal variations of carbon, nitrogen and sulphur in mangrove sediments

Parameters	M1			M2			M3			ANOVA P-Value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Spatial	Seasonal
Total Organic Carbon (TOC) %	3.90± 0.01	6.30± 0.13	3.90± 0.03	2.70± 0.02	3.10± 0.03	4.90± 0.05	5.60± 0.06	-	6.70± 0.13	0.14	0.27
Total Carbon %	4.23± 0.11	6.75± 0.07	4.72± 0.04	2.91± 0.06	3.31± 0.05	5.85± 0.11	5.92± 0.09	-	7.64± 0.09	0.20	0.23
Total Nitrogen %	0.19± 0.07	0.28± 0.01	0.17± 0.01	0.12± 0.05	0.14± 0.006	0.21± 0.009	0.22± 0.01	-	0.26± 0.01	0.10	0.38
Total Sulphur (TS) %	1.20± 0.016	1.72± 0.032	0.98± 0.012	1.02± 0.012	1.28± 0.018	1.88± 0.023	2.12± 0.036	-	2.58± 0.023	0.60	0.94
Total Phosphorus %	0.23± 0.006	0.22± 0.005	0.23± 0.006	0.30± 0.007	0.24± 0.006	0.29± 0.007	2.8± 0.019	-	2.87± 0.021	0.43	0.16
Total Fe %	5.83	4.26	4.56	5.76	5.74	5.71	5.62	-	5.74	0.48	0.32
C:P Ratio	18.33	30.32	20.99	9.74	13.87	19.95	2.11	-	2.67		
N:P Ratio	0.82	1.26	0.76	0.40	0.59	0.72	0.08	-	0.09		
TOC:TS Ratio	3.25	3.66	3.98	2.65	2.42	2.61	2.64	-	2.60		
Fe:P Ratio	25.26	19.14	2.28	19.28	24.05	19.47	2.01	-	2.00		

-Due to technical reasons samples from station M3 during monsoon season could not be collected.

Table 3.5. Seasonal variations of carbon, nitrogen and sulphur in estuarine sediments

Parameters	E1			E2			E3			ANOVA P-Value	
	Pre	**Mon**	**Post**	**Pre**	**Mon**	**Post**	**Pre**	**Mon**	**Post**	**Spatial**	**Seasonal**
Total Organic Carbon	2.90±	3.00±	3.04±	0.98±	0.74±	2.85±	0.82±	0.68±	2.10±	0.06	0.40
(TOC) %	0.11	0.11	0.13	0.06	0.04	0.01	0.005	0.006	0.08		
Total carbon%	3.12±	3.26±	3.32±	1.38±	1.08±	3.35±	1.32±	0.98±	2.38±	0.06	0.48
	0.58	0.06	0.06	0.03	0.05	0.07	0.05	0.09	0.50		
Total Nitrogen%	0.148±	0.170±	0.150±	0.050±	0.060±	0.14±	0.047±	0.050±	0.120±	0.08	0.54
	0.04	0.008	0.01	0.009	0.06	0.003	0.001	0.007	0.02		
Total Sulphur (TS) %	1.70±	1.48±	1.50±	0.18±	0.57±	0.25±	0.76±	0.29±	1.41±	0.04	0.71
	0.10	0.20	0.50	0.01	0.004	0.02	0.09	0.002	0.09		
Total Phosphorus %	0.17±	0.18±	0.20±	0.15±	0.08±	0.08±	0.09±	0.09±	0.19±	0.57	0.21
	0.003	0.004	0.005	0.003	0.002	0.002	0.002	0.002	0.004		
Total Fe %	4.76	3.34	4.54	4.03	5.11	3.34	5.21	3.62	3.24		
C:P Ratio	17.58	17.07	14.95	6.38	8.97	34.13	9.66	7.48	11.32		
N:P Ratio	0.9	0.97	0.74	0.33	0.73	1.68	0.55	0.55	0.65		
TOC:TS Ratio	1.71	2.03	2.03	5.44	1.30	11.40	1.08	2.34	1.49		
Fe: P Ratio	28.85	19.01	22.33	26.24	61.94	40.00	61.37	39.82	17.47		

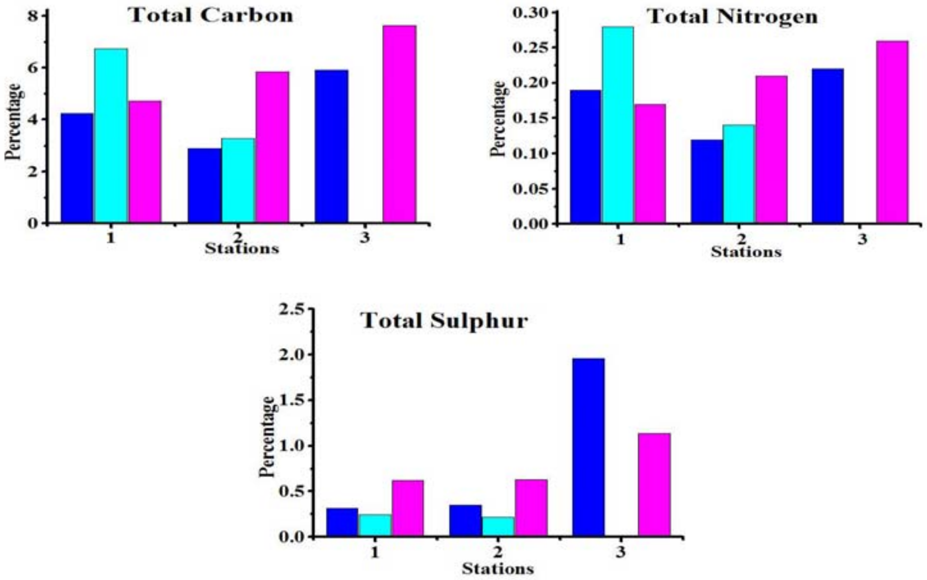


Figure 3.3 Seasonal variation of total carbon, total nitrogen and total sulphur in mangrove sediments

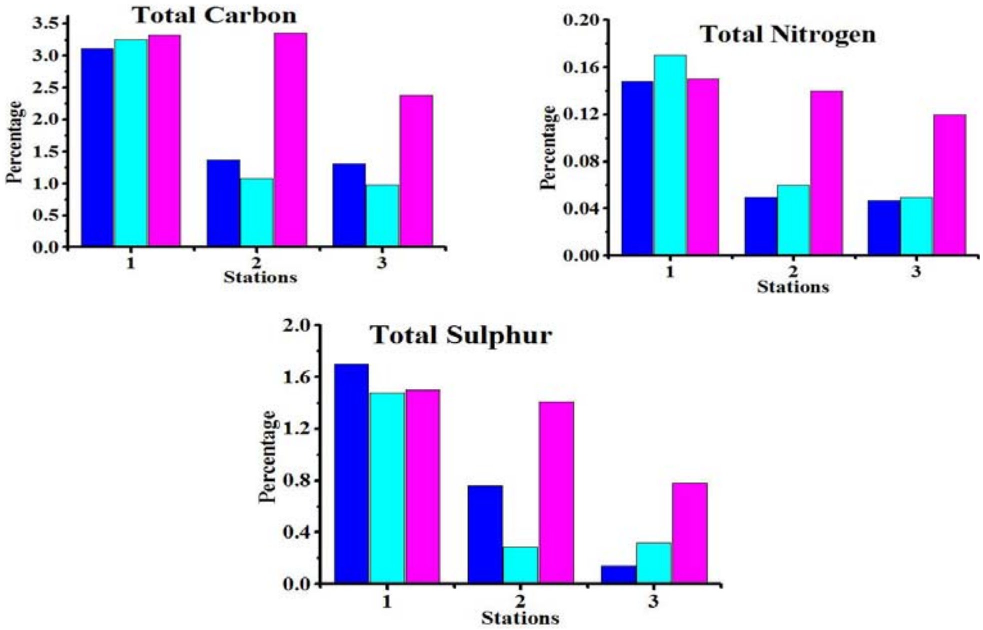


Figure 3.4 Seasonal variation of total carbon, total nitrogen and total sulphur in estuarine sediments.

3.2.2 Phosphorus Fractionation

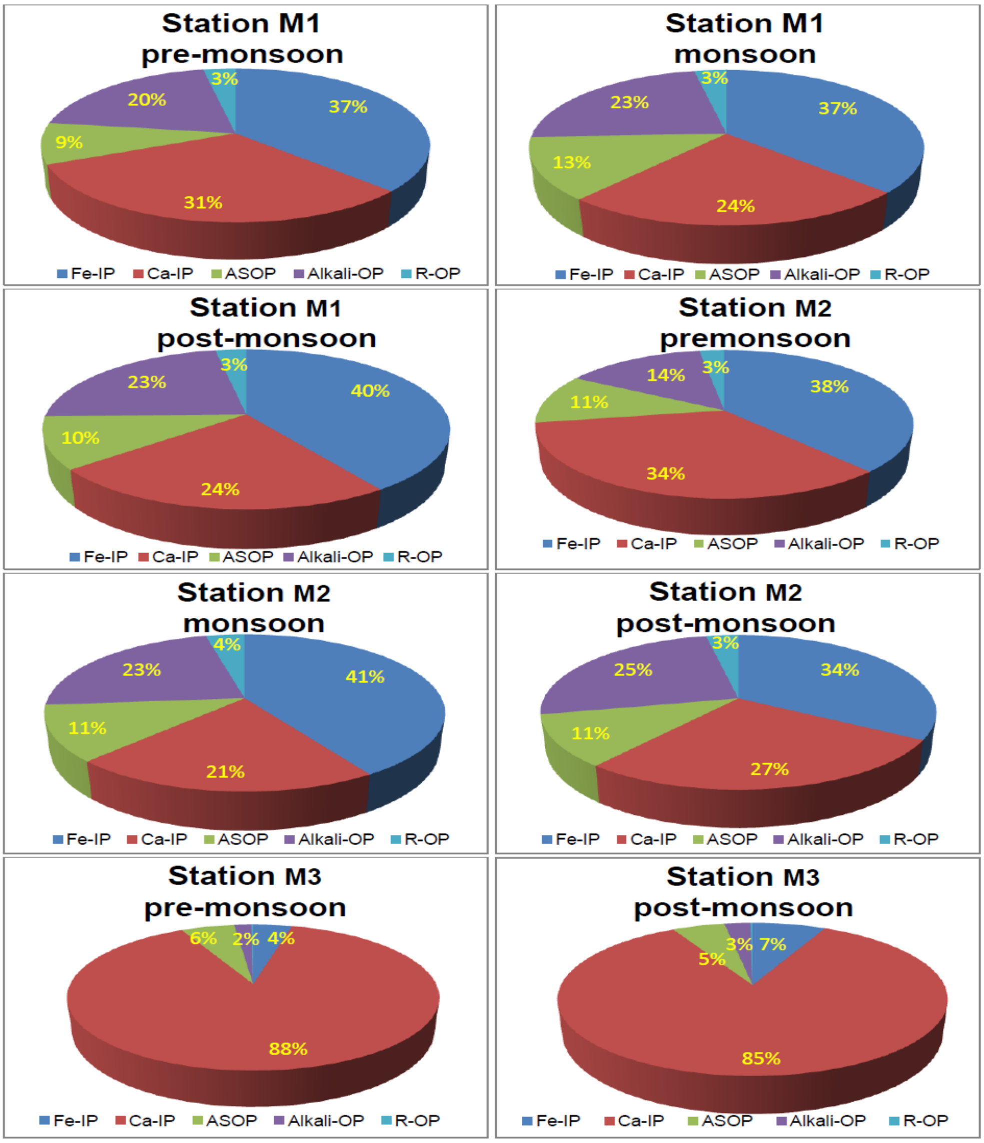
Fractionation of phosphorus in mangrove sediments (Tables 3.6) revealed significant spatial variations for different phosphorus fractions in the mangrove systems under study. Among the bio-available P fractions, iron bound phosphorus (Fe-IP) varied from 825±19 to 2080±31 μg/g among the mangrove systems. The percentage composition of various P-fractions in mangrove sediments is depicted in figure 3.5. Fe-IP was the major fraction in the first two stations accounting for about 38% and 37.67% of the total phosphorus respectively. Whereas, calcium bound phosphorus content (Ca-IP) exhibited wide fluctuations in the mangrove sediments and ranged between 505±28 to 24764±171μg/g. Station M3 was exclusively dominated by Ca-IP contributing to about 87% of the total phosphorus. Acid soluble organic phosphorus (ASOP) varied from 201±3 to 1555±16μg/g and was almost uniformly distributed in all the three systems with a percentage range of 5.5-10.67%. It could be observed that alkali soluble organic phosphorus (Alk-OP) ranged between 428±11 and 738±21 μg/g. This fraction was very low at M3 (2.2%), while it showed similar distribution in other two stations. Residual organic phosphorus (R-OP) was the smallest fraction (0.2-3.3% of total P) and its content varied from 48±1.4 to 92±2.7μg/g.

Total phosphorus calculated as the sum of all fractions, varied from 2226±54 to 28,665±209 μg/g. Comparing to other two stations, M3 showed about 10 fold increases in total phosphorus content. The first two stations did not exhibit any noticeable difference in phosphorus fractions and inorganic fractions constituted to about 65% of total phosphorus. But at M3 inorganic fraction was found to be about 92%. The bioavailable fraction of phosphorus estimated for the mangrove sediments at M1 and M2 were about 75% whereas at M3, this fraction constituted about 98%. Analysis of variance (Table 3.6) revealed 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, ASOP and ROP fractions (p=0.009, 0.02 and 0.01 respectively), which were significantly higher at M3. Fe-IP did not exhibit any significant variation among stations.

Table 3.6. Different fractions of phosphorus in the mangrove sediments (µg/g)

Parameters	M1			M2			M3			ANOVA p-value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Seasonal	Spatial
Fe-IP	856 ±22	825 ±19	909 ±24	1142 ±34	979 ±26	982 ±32	1129 ±36	-	2080 ±31	0.41	0.90
Ca-IP	727 ±21	541 ±13	532 ±17	1019 ±26	505 ±28	803 ±17	24764 ±171	-	24287 ±189	0.43	0.009
ASOP	201 ±3	289 ±7	236 ±5	322 ±8	274 ±10	318 ±6	1555 ±16	-	1499 ±27	0.46	0.02
Alkali-OP	452 ±13	501 ±21	506 ±12	428 ±11	539 ±17	738 ±21	504 ±10	-	735 ±18	0.30	0.70
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	0.10	0.01
Total-P	2308 ±58	2226 ±54	2249 ±56	2987 ±73	2387 ±59	2933 ±71	28000 ±192	-	28665 ±209	0.43	0.16

-Due to technical reasons samples from station M3 during monsoon season could not be collected.


Station M1 pre-monsoon

Station M1 monsoon

Station M1 post-monsoon

Station M2 premonsoon

Station M2 monsoon

Station M2 post-monsoon

Station M3 pre-monsoon

Station M3 post-monsoon

Figure 3.5 Percentage composition of various phosphorous fractions in mangrove sediments.

Table 3.7 Different fractions of phosphorus in the estuarine sediments (μg/g).

Parameters	E1			E2			E3			ANOVA-p value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Seasonal	Spatial
Fe-IP	967	1059	1241	943	249	339	236	671	1117	0.75	0.29
	±24	±28	±34	±20	±7	±8	±6	±12	±32		
Ca-IP	289	295	410	278	249	298	116	44	367	0.12	0.15
	±8	±9	±12	±7	±6	±8	±4	±2	±10		
Acid-OP	148	167	172	143	169	105	96	88	162	0.84	0.37
	±2	±3	±3	±2	±3	±2	±2	±2	±3		
Alkali-OP	180	214	185	150	136	77	396	96	185	0.44	0.44
	±3	±4	±3	±2	±2	±1.5	±8	±2	±3		
R-OP	67	23	25	22	22	15	5	9	25	0.65	0.29
	±1.6	±0.6	±0.7	±0.6	±0.6	±0.2	±0.1	±0.1	±0.6		
Total-P	1650	1757	2033	1536	825	835	849	909	1855	0.57	0.21
	±34	±36	±46	±32	±20	±20	±22	±23	±38		

The variation in concentration of various fractions of phosphorous in estuarine sediments is shown in table 3.7. The percentage composition of various P-fractions in estuarine sediments is depicted in figure 3.6. Iron-bound fraction was found to be the main phosphorous pool in the estuary, accounting for 60%, 44% and 55% of the total phosphorus in stations E1, E2 and E3 respectively. The Fe-IP content ranged between 236±6µg/g (E3, pre-monsoon) and 1241±34µg/g (E1, post-monsoon). The observed variation for Ca-IP concentration was from 44±2µg/g (E3, monsoon) to 410±12µg/g (E1, post-monsoon). Acid soluble organic phosphorous varied from 88±2 µg/g (E3, monsoon) to 172±3 µg/g (E1, post-monsoon). The contribution of ASOP to total phosphorus pool in the estuarine sediments was 9.33, 14 and 10%, for stations E1, E2 and E3 respectively. The percentage of Alk-OP fraction contributed its maximum towards total phosphorous pool at E3 (22.33%). It exhibited similar distribution trend in other two stations and varied from 77±1.5 to 396±8µg/g. Compared to mangrove systems, in estuarine sediments also, R-OP was the smallest fraction. Its content ranged between 5±0.1µg/g (E3, pre-monsoon) and 67±1.6µg/g (E1, pre-monsoon) and constituted about 1.0 -2.33% of total phosphorus pool.

Total phosphorus content in the estuarine sediments varied from 825±20 to 2033±46µg/g. Compared to the other two stations, E1 exhibited the highest total phosphorus content. First two stations did not display any noticeable difference in concentration of phosphorus fractions and the contribution of inorganic fractions to total phosphorus was about 78% and 72% respectively. But at E3 contribution of inorganic fraction was about 67% of the total phosphorus. Fe-IP was found to be the most abundant fraction in all stations except at E3 (pre-monsoon), where alkali-OP was the major fraction. The estimated bioavailable fraction of phosphorus in the sediments of E1 and E2 were about 87.33% and 86% respectively, whereas at E3, it constituted about 77%. Analysis of variance (ANOVA) revealed no significant spatial and seasonal variations for any of the phosphorus fractions in the estuarine sediments. It was observed that concentration of all the P fractions were higher in mangroves than estuarine sediments.

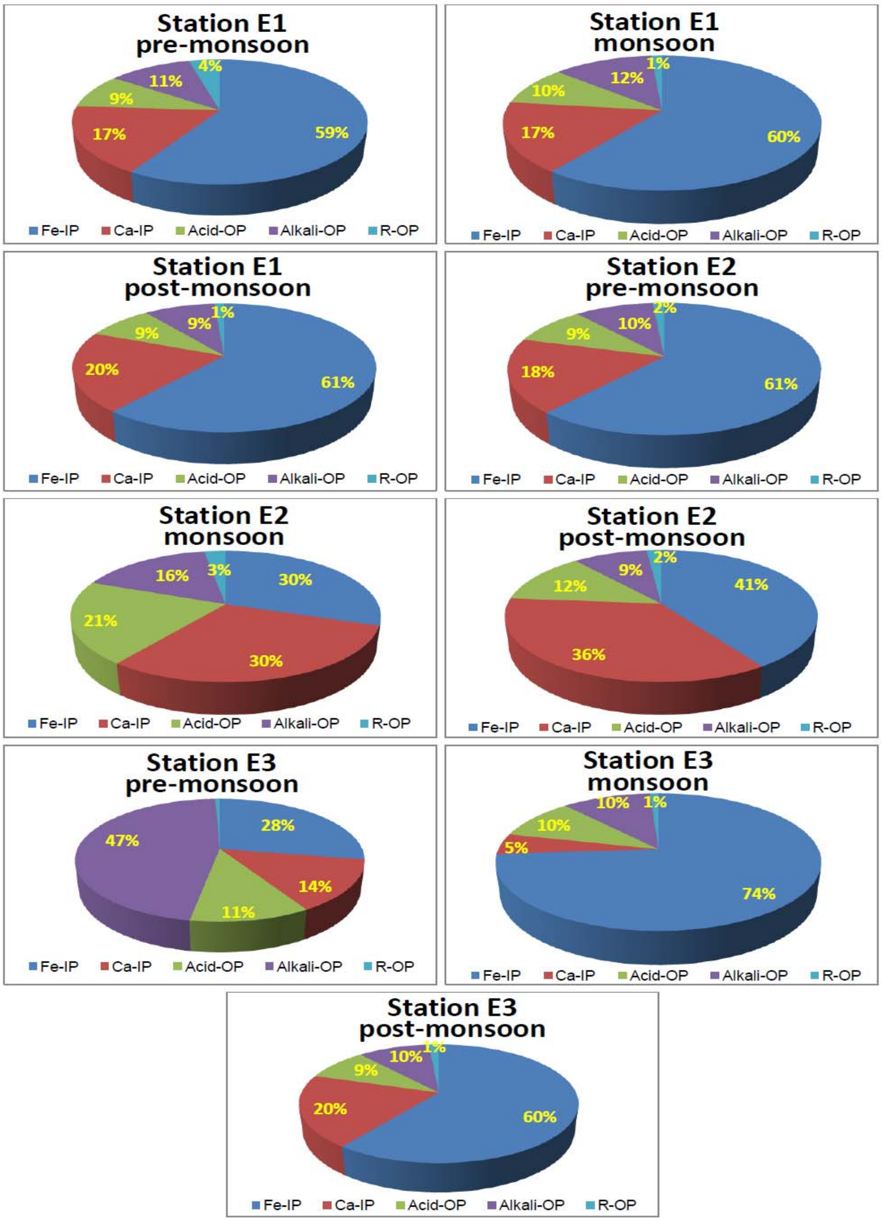


Figure 3.6 Percentage composition of various phosphorous fractions in estuarine sediments.

3.2.3 Mineralogy

X-ray diffraction (XRD) is an important tool in mineralogy for identifying, quantifying and characterizing minerals in complex mineral assemblages (Moore and Reynolds, 1997). Powder X-ray diffraction analyses of the representative sediment samples of the study region showed that perovskite and sodalite were the dominant minerals in M1 and M2 respectively. Presence of monetite, a rare calcium phosphate mineral, was observed at M3 along with silica (Figure 3.7). But in the case of estuarine sediments (Figure 3.8), XRD revealed the presence of quartz and monetite as abundant minerals at E1 and E2. The presence of quartz, monetite and the aluminosilicate clay mineral kaolinite was reported at E3.

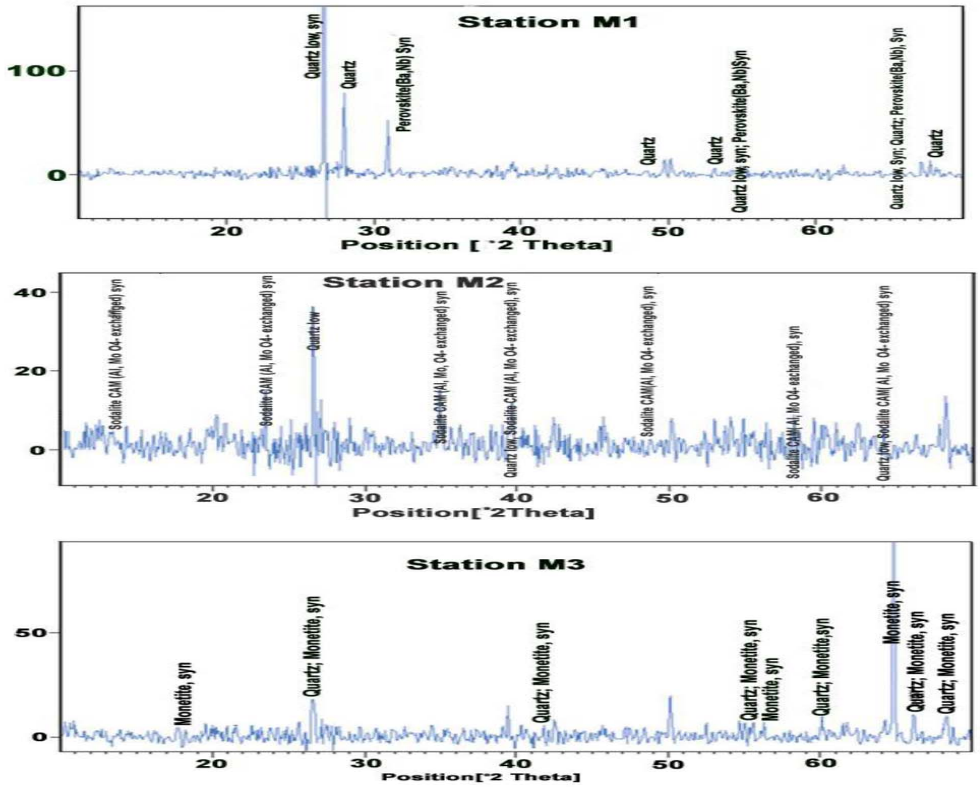


Figure 3.7 X-Ray Diffraction spectra of the mangrove sediments.

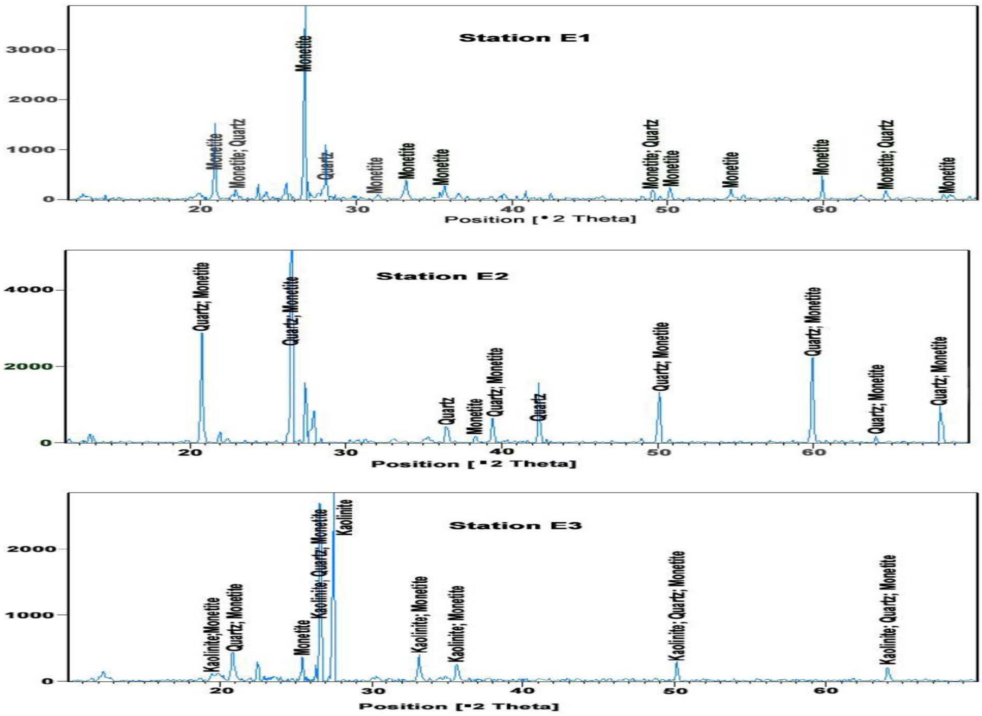


Figure 3.8 X-Ray Diffraction spectra of the estuarine sediments

3.2.4 Major Element Composition

Since the major elements are conservative in nature and unlikely to be affected by diagenesis, only the post-monsoon samples were analyzed to access their background levels. X-Ray Fluorescence (XRF) analysis showed that silicon was the major element in all the three stations, followed by aluminium and iron in the case of mangroves (Table 3.8). XRF analysis of mangrove sediments revealed very high contents of calcium and phosphorus at M3 compared to the other two stations. The SEM-EDS analysis of the sediment sample of M3 (Figure 3.9) also showed high mass percentages of calcium (9.79%) and phosphorus (5.33%). Sulphur content was also significantly higher at station M3. Aluminium and iron exhibited lower concentration at M3 compared to the other two stations. Thermal gravimetry analysis-TGA (Table

3.8) analysis showed an average moisture content of 7.47% and the average weight loss after 900° C was about 25.30% in mangrove sediments. The XRF analysis of the estuarine sediments (Table 3.8) also revealed the similar trend with higher abundance of the major elements silicon followed by aluminium and iron at all stations. SEM- EDS analysis of mangrove sediments (Figure 3.9) also revealed that of all the major elements silicon was the major portion followed by aluminium and iron. In the estuarine sediments, TGA (Table 3.8) revealed an average moisture content of about 6.88% and the average weight loss after 900° was about 22.77%.

Table 3.8 Major elemental composition and TGA results (weight %) of the sediments.

Compound	M 1	M 2	M 3	E 1	E 2	E 3
SiO_2	40.03	39.57	35.06	36.5	67.8	36.34
TiO_2	0.748	0.82	0.552	0.88	0.58	0.83
Al_2O_3	16.63	17.94	13.04	18.29	13.25	17.81
MnO	0.033	0.04	0.034	0.03	0.02	0.03
Fe_2O_3	7.99	8.96	4.86	8.43	3.38	7.87
CaO	1.434	0.807	7.278	0.89	1.55	0.95
MgO	2.901	2.799	1.947	2.96	1.67	3.15
Na_2O	3.25	2.325	2.302	3.16	2.44	3.79
K_2O	1.547	1.316	0.885	1.21	2.01	1.2
P_2O_5	0.63	0.622	6.76	0.63	0.43	0.59
SO_3	0.096	0.158	1.25	3.77	1.3	3.7
Cr_2O_3	0.034	0.033	0.02	0.32	0.13	0.81
CuO	0.009	0.007	0.01	0.008	0.002	0.001
NiO	0.01	0.01	0	0.03	0.01	0
Rb_2O	0.006	0.006	0.003	0.004	0.002	0.001
SrO	0.029	0.016	0.054	0.068	0.021	0.034
ZnO	0.018	0.012	0.044	0.048	0.052	0.021
ZrO_2	0.013	0.01	0.012	0.018	0.014	0.010
Loss on ignition at 110° C	7.4	7.67	7.34	6.84	6.64	6.42
Loss on ignition at 900° C	24.61	24.63	26.66	22.64	22.78	22.9

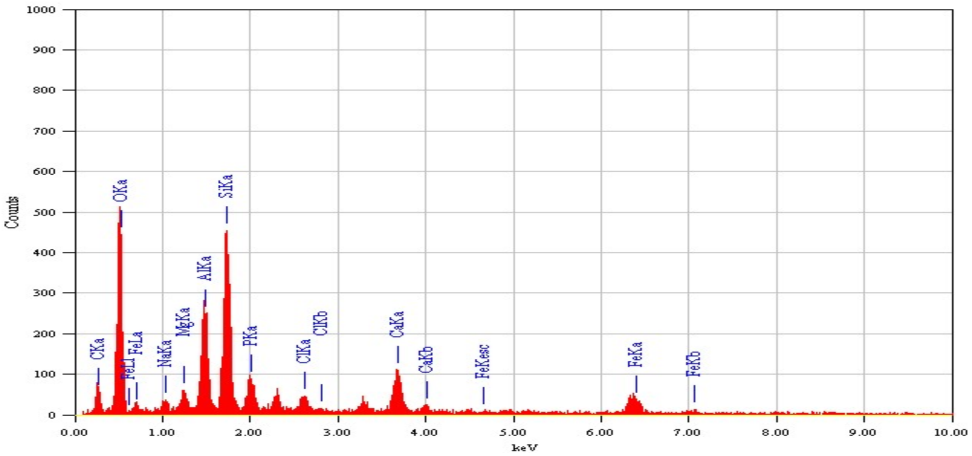


Figure 3.9 SEM-EDS spectrum of mangrove sediment (Station M3).

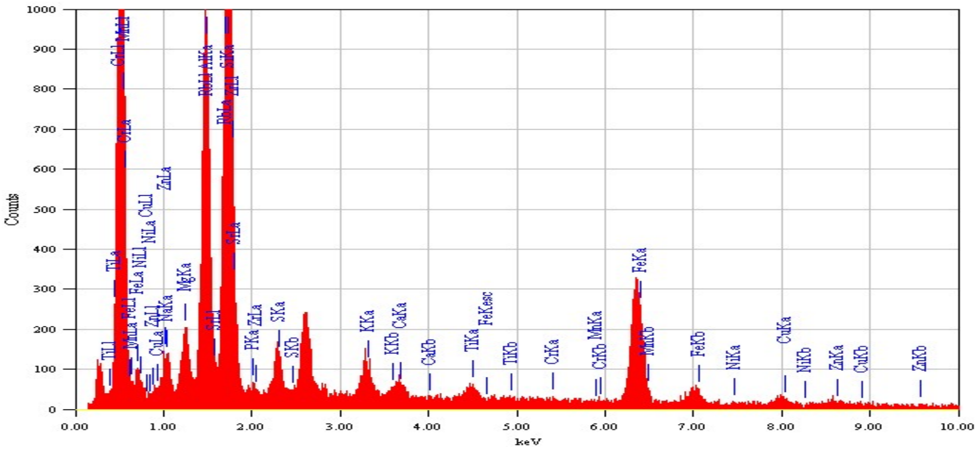


Figure 3.10 SEM-EDS spectrum of estuarine sediment (Station E1)

3.3 Discussion

Geochemical characteristics of mangrove sediments depend on various parameters such as organic matter content, water table fluctuation, bioturbation (Clark *et al.*, 1998), vegetation species (Nickerson and Thibodeau, 1985; Kryger and Lee, 1996) and ground-water movement (Baltzer, 1982; Baltzer, *et al.*, 1995). Analysis of the general sedimentary parameters showed the predominance of a fine substratum and silt was the major fraction in all the seasons in the mangrove sediments. High redox potential indicates that the sediments of both estuarine and mangrove systems were generally anoxic in nature and station M3 was found to be highly reducing. The reducing condition in the sediment of station M3 might be attributed to the high organic matter content. The texture of the sediments has a significant role on the physicochemical processes as well as on the species diversity of the depositional environment (Badarudeen *et al.*, 1996).

Total organic carbon: total sulphur (TOC: TS) ratio gives a qualitative indication of the redox status of the environment of deposition, when TS concentrations are high (Raiswell *et al.*, 1987). TOC: TS ratios > 5 are considered as oxic sediment with oxygenated bottom water, TOC: TS = 1.5-5 indicates sediments deposited under periodic anoxia and TOC: TS < 1.5 represents anoxic sediment with anoxic water. In mangrove sediments, TOC: TS ratio varied from 2.42 to 3.98 (Table 3.4) and in estuarine system it ranged between 1.08 and 11.4 (Table 3.5) respectively. The TOC: TS values in the study region can be included in the second category suggesting that the sediments undergo sulphate reduction below an oxygenated water column (Hedges and Keil, 1995).

Continental aquatic environments are often dominated by fine-grained sediments, which have an important role due to the properties that their fine grain-size and chemistry impart to them (Hillier, 1995). As a result of chemical and physical processes (e.g. ion exchange and fixation) that they may undergo during cycles of transport and temporary deposition, clay minerals may act as important vehicles and buffers of environmental pollutants, which tend to be strongly sorbed to clay minerals surfaces (Förstner *et al.*, 1990). Mineralogical fingerprints were 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 M1 and M2 respectively. Perovskite is a calcium titanium oxide mineral species composed of calcium titanate, with the chemical formula $CaTiO_3$. Sodalite is a sodium aluminium silicate chloride mineral, Na_4Al_3 $(SiO_4)_3Cl$. Monetite, an anhydrous calcium phosphate mineral ($CaHPO_4$) was reported at M3 and all estuarine stations. Kaolinite [$Al_2Si_2O_5(OH)_4$] was detected at estuarine sediments collected from station E3. It is the most important clay mineral of the two-layer group, also referred to as the 7-Ångstrom clay minerals which consist of interlinked tetrahedron-octahedron units. This mineral develops under warm and humid conditions, by chemical weathering of feldspars in tropical soil.

Station M3 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 resulted in the accumulation of bird guano and dead remains of birds, a rich source of phosphorus. 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 is also reported evidence for highly anoxic condition prevailing in this unique ecosystem. Authigenic filamented pyrites have been reported at Mangalavanam (Rosily, 2002), presence of which can be regarded as an indicator of highly anoxic conditions (Jeng and Huh, 2001). 5α-Cholestan-3β-ol, 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 (Onac and Veres, 2003). The presence of monetite indicates the complexity of the ecosystem as it is a thermodynamically metastable calcium phosphate mineral (Effler, 1987). The presence of monetite validates the presence of Ca-IP at station M3. Major elemental analyses using XRF and SEM-EDS revealed high mass percentages of calcium and phosphorus at M3, which was about 10 times higher than that at the other two mangrove stations. This could be taken as a confirmation of the existence of the rare mineral monetite. The high contents of calcium and phosphorus in estuary and the anoxic condition favour the formation of monetite in estuary. Another possibility of its presence in estuarine stations might be attributed to the tidal export of this mineral from mangrove station M3.

Nutrient elements in the study region did not exhibit any seasonal trend in mangrove (Table 3.4) and estuarine (Table 3.5) sediments. In both systems, no correlation (Tables 3.10 and 3.11) was observed for CNS composition with texture indicating the absence of any granulometric dependence. The positive correlation of sediment carbon with total nitrogen indicated a common source of organic matter to sediments in both mangrove and estuarine systems. These elements also showed significant negative correlations with the redox potential, in the case of mangrove sediments, pointing towards the preservation of organic matter in anoxic condition.

Stoichiometric ratios of nutrients are utilized to determine the origin and transformation of organic matter (Yamamuro, 2000). By mass, Redfield (1958) predicts a C: P ratio of 7 for algal material, while Hecky *et al.*, (1993) indicated that wide range of C: P and N: P ratios in aquatic sediments can still be considered to follow Redfield ratio (C: P= 28 to 56 and N: P=4 to 9). In the present investigation, the C: P ratio varied from 2.11 to 30.32 (Table 3.4), in the mangrove systems and was very low at station M3. But in the case of estuarine sediments, C: P ratio ranged between 6.38 and 34.13 (Table 3.5). The lower values for this ratio indicated enrichment of phosphorous in sediments of both estuarine and mangrove systems. The observed N: P ratio in mangrove sediments revealed considerable variations among stations and ranged between 0.08 and 1.26 (Table 3.4). But in estuarine system, it varied from 0.33 to 1.68 (Table 3.5). It was very low at M3, indicating high enrichment of phosphorus at this station. The lower N: P ratios also suggest that there might be higher benthic nitrogen recycling, and denitrification, and the benthic release of nitrogen could play a role in sustaining the productivity of the system (Renjith *et al.*, 2010). The C: P and N: P ratios did not exhibit any seasonal variations and were far below the Redfield ratio in mangrove and estuarine systems. Hence it supports phosphorus enrichment at all stations of the study area.

The Fe: P ratio is considered to be a measure of free sorption sites for phosphate ions on iron oxyhydroxide surfaces and the total Fe: P ratio above 15 (w: w) is enough for Fe to control the benthic flux of P from sediment to oxic water (Jensen *et al.*, 1992). Rydin and Brunberg, (1998) also described the formation of an effective oxic barrier with the above Fe: P ratio. The Fe: P ratio varied from 2 to 25.26 (Table 3.4) and 17.47 to 61.94 (Table 3.5) in mangrove and estuarine systems respectively. This ratio exhibited values greater than 15 in mangrove and estuarine systems indicated high Fe content in surface sediments to bind P in the estuarine system.

The earlier reported research works based on phosphorus fractionation in sediments of different aquatic systems are presented in Table 3.9. Fractionation of phosphorus in the mangrove sediments showed that station M3 is abnormally enriched with Ca-IP. The presence of monetite at M3 supports the enrichment of Ca-IP at this station. The diagenesis of sediments containing bird or bat guano can also lead to the crystallization of apatite and other P-minerals (Tiessen *et al.*, 1996). The high mass percentages of calcium and phosphorus by XRF and SEM-EDS analysis confirm the phosphorus enrichment at M3.

Table 3.9 Important works on phosphorus fractionation in the sediments of different aquatic systems.

Sl.No.	Aquatic system and refence	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)	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. The changes in the exchangeable P together with the marked regional variation in the calcium bound P exemplified the complex variability of phosphorous in this estuary.
3	Cauvery estuary and Pichavaram mangroves (Ramanathan, 1999)	Ramanathan *et al.*, (1996)	In this estuary, P was mainly associated with Al-P followed by Ca-P and Fe-P suggesting origin by natural weathering. In the mangroves Al-P was dominant in the outer channels and in the interior channel increases in the Fe-P, organic P and Total P. P is widely distributed in all fractions of the sediments. In the estuary P was higher in the coarse sand and silt fractions as compared to fine fractions suggesting the anthropogenic input to the Cauvery estuary. In the mangroves the P was generally abundant in the coarser fractions in comparison to finer fractions in the outer channels and reverse trend was noticed in the interior channels.
4	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. These results suggest a rapid mineralization of org-P and readsorption of organic P on Fe (OOH) during the sediments resuspension.
5	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 Ca bound P (2.76 – 37.25%) and Ca bound P (1.49 – 33.50%) were the other dominant forms.

6	Mangrove ecosystems around Cochin (Shaly, 2003)	Golterman (1996) modification by Pardo *et al.,* (1998)	Total P in the surficial sediments was in the range between 480.72 and 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%)
7	Japan Sea (Cha *et al.*, 2005)	Ruttenberg (1990) modified by Rao (1994)	The total P ranged 13.0 -35.5 μmol/g. The concentration of detrital-P was very low (0.7-2.6) accounting less than 105(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.
8	Eastern Arabian Sea (Babu and Nath, 2005)	SEDEX procedure Ruttenberg (1992) modified by Anderson and Delaney (2000)	Total P ranged between 920 and 2496 ppm, while it was 1070 – 1632 ppm in off Cochin region. P-org was lower in shelf (6%) and relatively high in deep sea sediments (12-17%). In sediments overlain by OMZ, P-org proportion is relatively high in the SE Arabian Sea (8-13%), which has less P-org than the deep sea sediments.
9	Cochin estuary (Renjith *et al.*, 2010)	Golterman (1996), with modification of Diaz-Espejo *et al.*, (1999)	Iron-bound inorganic phosphorus (5.04–474.24 μg/g), calcium-bound inorganic phosphorus (11.16–826.09 μg/g), acid soluble organic phosphorus (22.22–365.86 μg/g). Among the non-bio available fraction alkali soluble organic fraction was the major one (51.92–1,002.45 μg/g). Residual organic phosphorus was comparatively smaller fraction ranging between 29.27 and 279.83 μg/g. Total phosphorus in the surface sediments ranged between 319.54 and 2,938.83 μg/g.
10	Present study	Golterman, (1996)	Mangrove (μg/g) Estuary (μg/g) Fe-IP (825± 19 to 2080±31) (263±6 to 1241±34) Ca-IP (502± 28 to 24764±171) (44 ±2 to 410±12) ASOP (201± 3 to 1555± 16) (88±2 to 172±3) Alk-OP (428± 11 to 738± 21) (77±1.5 to 396±8) ROP (48±1.4 to 92±2.7) (5±0.1 to 67±1.6) T P (2226±54 to 28665±460) (825 ±20 to 2033 ±46)

Iron- and calcium-bound inorganic fractions and acid soluble organic fractions of phosphorus are generally considered to be bioavailable (Diaz-Espejo *et al.*, 1999). Bioavailable fractions of phosphorus at stations M1 and M2 were about 75%, whereas 98% of the total phosphorus was bioavailable at station M3. In estuary, the bioavailble fraction constituted 87.33%, 86% and 77 % of total sedimentary phosphorus. Since the bulk of the total phosphorus is bioavailable, these mangrove and estuarine sediments have the potential to act as source of phosphorus to the overlying waters.

Correlation analysis confirmed the significant interrelations among the bioavailable fractions. In estuarine sediments (Table 3.11), the calcium bound phosphorous exhibited significant positive correlation with total carbon, organic carbon and acid soluble organic phosphorous. Acid soluble organic P was well correlated with Ca-IP in the case of estuarine sediments. In mangrove systems, highly significant negative correlation (Table 3.10) of Ca-IP with Eh and its highly significant positive correlation with sulphur, the redox indicator also suggested that there is preferential accumulation of Ca-IP when the system is highly reducing. Fe-IP displayed highly significant positive correlations with other two bioavailable fractions (Ca-IP and ASOP) and Ca-IP exhibited highly significant positive correlations with ASOP. The P-fractions available for biological uptake are all well correlated indicating a common biogeochemical balance.

The main inorganic forms of P are the fraction associated with Al, Fe and Mn oxides and hydroxides. Phosphorus and iron are usually strongly associated in sediments, P being adsorbed onto iron compounds with the formation of iron phosphate complexes. The amount of FeOOH is therefore one of the factors controlling P release from sediment. Fe-IP, which is exchangeable between the particulate and dissolved phases through sorption processes, was the major fraction in estuarine sediments except at station E3. Alkali-OP was the major fraction at station E3 during pre-monsoon (47% of the total phosphorus).

Table 3.10 Pearson correlation matrix for various phosphorous fractions in mangrove sediments (n=8).

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

*Correlation is significant at 0.05 level
** Correlation is significant at 0.01 level

Table 3.11 Pearson correlation matrix for various phosphorous fractions in estuarine sediments (n=9)

	pH	Eh	Sand	Silt	Clay	Total C	Organic C	Total N	Total S	Fe	Fe-IP	Ca-IP	Acid-OP	Alkali-OP	R-OP
pH	1														
Eh	-0.64	1													
Sand	-0.03	0.04	1												
Silt	-0.11	0.03	-0.98 (**)	1											
Clay	0.21	-0.13	-0.96 (**)	0.88 (*)	1										
Total C	0.23	-0.33	-0.85 (*)	0.79 (*)	0.87 (*)	1									
Org.C	0.13	-0.33	-0.79 (*)	0.74 (*)	0.81 (*)	0.97 (**)	1								
Total N	0.25	-0.27	-0.84 (*)	0.78 (*)	0.86 (**)	0.99 (**)	0.94 (**)	1							
Total S	0.05	-0.14	-0.89 (*)	0.85 (*)	0.86 (**)	0.95 (**)	0.93 (**)	0.94 (*)	1						
Fe	-0.45	-0.18	-0.06	0.13	-0.04	-0.16	-0.08	-0.27	-0.08	1					
Fe-IP	-0.04	0.13	-0.30	0.30	0.27	0.56	0.55	0.53	0.58	-0.36	1				
Ca-IP	0.56	-0.51	-0.43	0.34	0.51	0.71 (*)	0.72 (*)	0.70 (*)	0.68	-0.26	0.59	1			
Acid-OP	0.45	-0.19	-0.20	0.20	0.19	0.39	0.38	0.43	0.41	-0.48	0.57	0.79 (*)	1		
Alkali-OP	0.10	-0.30	0.03	0.05	-0.12	-0.11	-0.14	-0.21	-0.26	0.38	-0.11	-0.17	-0.07	1	
R-OP	-0.40	-0.01	-0.45	0.55	0.29	0.50	0.60	0.48	0.63	0.21	0.44	0.43	0.46	-0.14	1

*Correlation is significant at 0.05 level
** Correlation is significant at 0.01 level

In the case of mangroves, Fe-IP was the major fraction at stations M1 and M2. 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 (Silva and Mozeto, 1997; Caraco *et al.*, 1989). Generally, the release of this phosphate fraction from the sediment is controlled by sulphate reduction (Caraco *et al.*, 1989) and is considered more bioavailable under the redox (Eh) variations observed in the mangrove sediments (Silva and Mozeto 1997; Caraco *et al.*, 1989). Sulphide produced from sulphate reduction may reduce the iron-oxides and thus promote a release of iron-bound phosphorus (Jensen *et al.*, 1995; Howarth *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) 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 $Fe(OH)_3$. Microbial degradation of organic matter consumes oxygen, especially in summer, and further releases Fe-bound P. Degradation of organic P compounds also releases phosphate (PO_4-P), making it available to bacteria and algae.

The involvement of iron in the dynamic equilibrium between the sediment and water as explained above had 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 by 15 (by weight) while Caraco *et al.*, (1993) suggested that this ratio should be above 10 to regulate phosphorus release. The higher values of Fe: P ratios in the sediments justify the higher concentration of Fe-IP at mangrove stations M1 and M2. The Fe: P ratio was higher than 15 at all estuarine stations except at E1during monsoon and post-monsoon and E3 during post-monsoon, pointed towards the higher concentration of Fe-IP fraction. The Fe: P ratio was very low at M3, resulting in lower percentage of iron bound phosphorus in the system. Periodic fluctuations in the redox potential of this system might also result in lower Fe-IP content at M3. Microbially induced changes in pH and redox potential affect the ability of sediments to retain inorganic P (Roden and Edmonds, 1997; Gachter and Muller, 2003).

Salinity plays a role in the seasonal variations of P fractions as it controls the flocculation and sedimentation mechanisms in estuarine environments, changing the availability of elements (Kautsky, 1998). During the monsoon season, when the system changes to a freshwater condition, Ca-IP is found to be lower. It might be due to the lower availability of solid calcium carbonates, which is governed by salinity and pH (Huanxin *et al.*, 1994; Zwolsman, 1994). In estuarine sediments, Ca-IP, referring to the fraction that is fixed in sediments and may be lost into deep sediments through the burial process, exhibited significant positive correlation with total carbon, organic carbon and total nitrogen (Table 3.11). Carbonate-adsorbed P has been identified as an important and potentially dominant phase of P in shallow-water tropical carbonate-rich sediments, due to the high adsorption capacity of carbonates (Short *et al.*, 1990; Millero *et al.*, 2001). The higher concentration of calcium-bound P in the estuarine sediments (E2, during post-monsoon) might be interpreted by the favourable accumulation of calcium carbonate under higher salinity and pH conditions (Huanxin *et al.*, 1994). Calcium bound phosphorus dominate in mangrove sediments (Silva and Mozeto, 1997), due to its stability under redox (Eh) variations observed in the mangroves (Nriagu, 1976; Silva *et al.*, 1998).

The slight acidic pH of the sediments at M3 provides high stability to Ca-IP (Silva and Sampaino, 1998). The increase in Ca-IP at all stations of mangrove (during pre-monsoon) and estuary (post-monsoon) might be due to 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 phosphorus combined with calcium under high salinity acts as a principal mechanism for its retention.

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 positive correlation with total sulphur in mangrove sediments indicated that highly reducing environment is favourable for retention of ASOP in sediments similar to Ca-IP.

Alk-OP generally constitutes humic phosphate and phytate phosphate (Golterman *et al.*, 1998; Golterman, 2001). Phytate and phytic acid (inositol hexaphosphate) is organic phosphate widely spread in plants (Hess, 1975), soils (Stevenson, 1982) and aquatic sediments (De Groot and Golterman, 1993; Dvorakova, 1998). Phosphorus associated with humic acids has been considered either to be an integral part of humic acids or as a phosphate metal/organic matter complex (Stevens and Stewart, 1982). Irrespective of the higher content of total organic matter, the labile fraction was very low in the mangrove sediments, signaling to the dead organic matter accumulation (Joseph *et al.*, 2008). This might result in the higher concentration of Alk-OP in mangroves, which is non-bioavailable. The abnormal higher content for Alk-OP at E3 could be attributed to river runoff of humic-associated P and the lower salinity (2.35 psu- Table 2.3; Chapter 2) which might have prevented the flocculation in the upper reaches.

Organic phosphorus is a complex fraction, the exact nature of which is not precisely known (Reitzel *et al.*, 2007). As a result of 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; Andersen *et al.*, 2001). The lower content of this fraction in sediments of both mangrove and estuarine stations might be attributed to diagenesis. Degradation of organic P compounds also releases phosphate (PO_4-P), making it available to bacteria and algae. Residual organic-P was the smallest fraction estimated in sediments from both mangrove and estuarine stations. As a result of the diagenetic reorganization of P within sediments, organic phosphorous concentrations usually decrease with time as it is ultimately transformed to bioavailable forms. Bacteria are generally considered to be the catalysts that accelerate the solubilization of P (Gatcher and Meyer, 1993) and the processes of anoxic mineralization of phytate (Golterman *et al.*, 1998) could release organic P buried during monsoon season. Generally, organic bound phosphorus accounted for 6 to 19% of total in coastal sediments (Hirata, 1985). The high percentage of organic bound phosphorus at station M1 and

M2 indicated that mineralization of phosphorus was less, whereas at M3, active mineralization was occurring. In the case of mangrove station M3, C: P ratio was very low and it is reported that mineralization of organic phosphorus and C: P ratio is intensively related (Reddy and Delaune, 2008). Also, organic phosphorus mineralization is high under anaerobic condition than aerobic conditions (Bridgam *et al.*, 1998). Very high redox potential at M3 results in higher mineralization and subsequently lower concentration of organic phosphorus. The low percentages of organic bound phosphorus in estuary could be attributed to mineralization process in reducing condition. The variations in the phosphorus content in the three mangrove systems could also be favored by the difference in local vegetation (Alongi, 1989). Organic matter associated with *Avicennia* dominated 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* dominated sediments either microbial conversion is negligible or the organic components are more refractory (Alongi, 1989). Station M3 is dominated by *Avicennia* species and the high content of inorganic phosphorus in this system could be deduced to be a signal of higher levels of diagenetic activity.

3.4 Geochemistry of Heavy Metals in Sediments

Transformations during early diagenesis and changing redox state characterize the chemical forms of metals in sediments. The major changes that occur in redox conditions between oxic waters and anoxic sediments have profound influences on the speciation and bioavailability of many heavy metals. The adsorption and desorption of heavy metals in wetland soils depend on one or a combination of sediment properties including pH, cation exchange capacity, organic matter, clay content, redox potential, salinity, iron and manganese oxides, presence of other metals, the quantity of heavy metals accumulated, the alternating aerobic and anaerobic conditions among others (Lacerda *et al.*, 1993; Tam and Wong, 1995). The concentration and distribution of heavy metals in sediments therefore depends on interrelated geochemical parameters such as pH, Eh, texture, organic carbon etc. Mangrove sediments are anaerobic and reduced, as well as being rich in sulphide and organic matter and the sheltered slack water conditions allows the deposition of fine particles normally enriched with heavy metals. Phosphorus has a strong affinity to oxide iron, aluminum and manganese. Reduction and oxidation (redox) of these metals regulate the dynamics of phosphorus in sediments. The mechanisms of phosphorus mobilization are explained by the reductions of insoluble metal oxides such as Fe (III) to soluble Fe (II) in sediments or water whereby phosphorus bound to Fe (III) or adsorbed to iron complexes is returned to solution when system redox potentials change from aerobic to anaerobic conditions. Therefore, the study of heavy metal concentration in sediments along with phosphorus is a pre-requisite for the geochemical characterization of the aquatic ecosystems.

Mangroves favour the retention of water-borne heavy metals (Silva *et al.*, 1990; Tam and Wong, 2000) and the subsequent oxidation of sulphides between tides allows metal mobilization and bioavailability (Clark *et al.*, 1998). 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 the detritus food chain in adjacent coastal waters (Murray, 1985; Silva, 1988). 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). Estuaries, the complex and dynamic environments, which form the part of coastal system, are a major reservoir of trace metals, both of anthropogenic and natural origins (Bryan *et al.*, 1980; Langston, 1982). They are well known to act as filters that prevent compounds transported by rivers to reach the sea. Sedimentation, favoured by flocculation of the fine particles carried by fresh water, constitutes one of the main processes responsible for trapping material in the estuarine system. Flocculation and coagulation occur as soon as the salinity increases in the upper part of the estuary. Furthermore, the hydrodynamic conditions generated by tides and the existence of salt wedges are also favourable to the deposition and upward transport of sediments containing heavy metals along the bottom boundary layer.

3.4.1 Accumulation of heavy metals in sediments

The heavy metal concentrations in surface sediments of mangrove ecosystems (Table 3.12) ranged: 4.26-5.83 % for Fe, 1.23 -1.78 % for Mg, 210.5 - 315.3μg/g for Mn, 101.3-455.68 μg/g for Zn, 53.30 - 90.22 μg/g for Cr, 30.60 - 69.35 μg/g for Ni, 19.5-39.50 μg/g for Pb, 23.97-39.12 μg/g for Cu, 12.82-23.08 μg/g for Co and 0.06-0.22 μg/g for Cd.

The variation in the metal content in estuarine sediments (Table 3.13) was Fe (3.24-5.21 %), Mg (0.06 - 1.58 %), Zn (110.68 - 741.93 μg/g), Mn

(22.03 - 252.93 μg/g), Cr (4.38 - 89.38 μg/g), Ni (4.40 - 56.45 μg/g), Cu (0.95 - 41.80 μg/g), Pb (ND - 28.0 μg/g), Co (4.80 - 21.58 μg/g), Cd (0.05 - 10.50 μg/g), dry weight of the sediment. Mn content exceeded Zn and Cu exceeded Pb in mangrove sediments, but reverse trend observed in the estuarine system.

In mangrove sediments, ANOVA (Table 3.12) revealed that cadmium exhibited significant spatial variation ($p=0.04$) and it exhibited higher content at M3. Co and Cr exhibited significant spatial variation ($p=0.05$ and $p=0.016$ respectively) and their content was lower at M3 compared to other two stations. Copper, iron, magnesium, manganese and lead did not show any significant spatial and seasonal variations. But nickel displayed significant spatial variation ($p=0.01$) and its content was lower at M3. Zinc showed significant spatial variation ($p=0.02$), and recorded higher concentration at M3.

In the case of estuarine sediments, ANOVA (Table 3.13) established significant seasonal variation for Cd ($p=0.047$). Co and Cr also showed similar distributional trends ($p=0.05$ and $p=0.02$ respectively). No spatial and temporal variations were exhibited by Cu, whereas Fe displayed significant seasonal variation ($p=0.019$). Manganese showed significant seasonal variation ($p=0.01$) with its maximum content reported at E2 during post-monsoon. In general, concentration of magnesium recorded higher concentration during pre-monsoon season ($p=0.007$). Ni showed no spatial variations, but was high during pre-monsoon ($p=0.01$). Zn content was significantly high ($p=0.01$) during pre-monsoon.

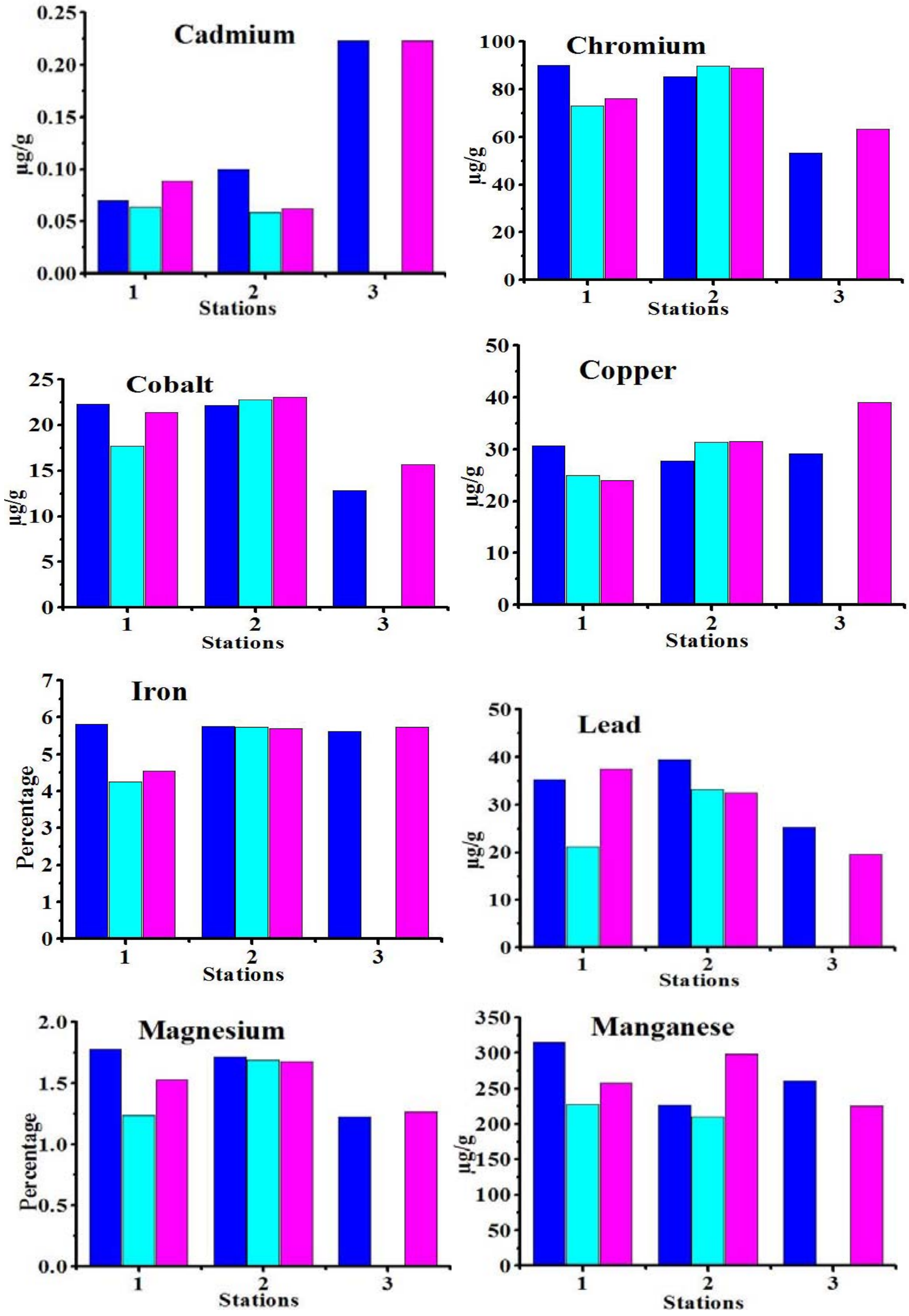
Table 3.12 Concentration of various heavy metals in mangrove sediments. Results of ANOVA are also shown.

Parameters	M 1			M 2			M 3			ANOVA-p value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Spatial	Seasonal
Cd (µg/g)	0.07	0.06	0.09	0.10	0.06	0.06	0.22	-	0.22	0.04	0.32
Co (µg/g)	22.30	17.73	21.42	22.20	22.80	23.08	12.82	-	15.70	0.005	0.25
Cr (µg/g)	90.22	73.18	76.08	85.30	89.77	89.05	53.30	-	63.27	0.016	0.46
Cu (µg/g)	30.75	24.98	23.97	27.80	31.38	31.58	29.17	-	39.12	0.76	0.46
Fe (%)	5.83	4.26	4.56	5.76	5.74	5.71	5.62	-	5.74	0.48	0.32
Mg (%)	1.78	1.24	1.53	1.72	1.69	1.68	1.23	-	1.27	0.10	0.22
Mn (µg/g)	315.30	227.80	257.80	227.00	210.50	299.10	261.00	-	225.95	0.28	0.18
Ni (µg/g)	55.50	55.43	54.75	68.80	69.35	65.57	30.60	-	40.25	0.01	0.52
Pb (µg/g)	35.25	21.25	37.50	39.50	33.25	32.50	25.25	-	19.50	0.60	0.20
Zn (µg/g)	128.80	101.30	111.30	116.00	112.60	132.60	315.10	-	455.68	0.002	0.38

-Due to technical reasons samples from station M3 during monsoon season could not be collected.

Table 3.13 Concentration of various heavy metals in estuarine sediments. Results of ANOVA are also shown.

Parameters	E 1			E2			E 3			ANOVA p- value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Spatial	Seasonal
Cd (µg/g)	9.38	0.05	5.10	7.88	3.40	10.50	9.03	2.45	1.83	0.48	0.047
Co (µg/g)	19.43	4.80	8.68	20.75	5.35	17.28	21.58	12.45	7.65	0.60	0.05
Cr (µg/g)	84.18	4.38	23.30	70.48	7.90	52.55	89.38	27.58	17.95	0.84	0.02
Cu (µg/g)	41.80	0.95	13.03	13.03	3.53	28.43	38.85	19.70	10.88	0.79	0.24
Fe (%)	4.76	3.34	4.54	4.03	5.11	3.34	5.21	3.62	3.24	0.82	0.019
Mg (%)	1.16	0.06	0.29	1.58	0.09	0.79	1.48	0.26	0.16	0.32	0.007
Mn (µg/g)	236.05	22.03	58.80	241.68	31.60	252.93	190.00	167.30	142.93	0.55	0.01
Ni (µg/g)	54.23	5.38	16.40	56.45	4.40	38.03	54.65	22.05	15.60	0.68	0.01
Pb (µg/g)	13.50	ND	12.00	28.00	5.75	22.25	22.50	21.00	11.25	0.28	0.24
Zn (µg/g)	741.93	110.68	331.93	645.05	211.93	630.68	658.18	227.55	156.30	0.54	0.01


Cadmium
µg/g
0.00
0.05
0.10
0.15
0.20
0.25
Stations
1
2
3
Chromium
µg/g
0
20
40
60
80
100
Stations
1
2
3
Cobalt
µg/g
0
5
10
15
20
25
Stations
1
2
3
Copper
µg/g
0
10
20
30
40
50
Stations
1
2
3
Iron
Percentage
0
1
2
3
4
5
6
7
Stations
1
2
3
Lead
µg/g
0
10
20
30
40
50
Stations
1
2
3
Magnesium
Percentage
0.0
0.5
1.0
1.5
2.0
Stations
1
2
3
Manganese
µg/g
0
50
100
150
200
250
300
350
Stations
1
2
3

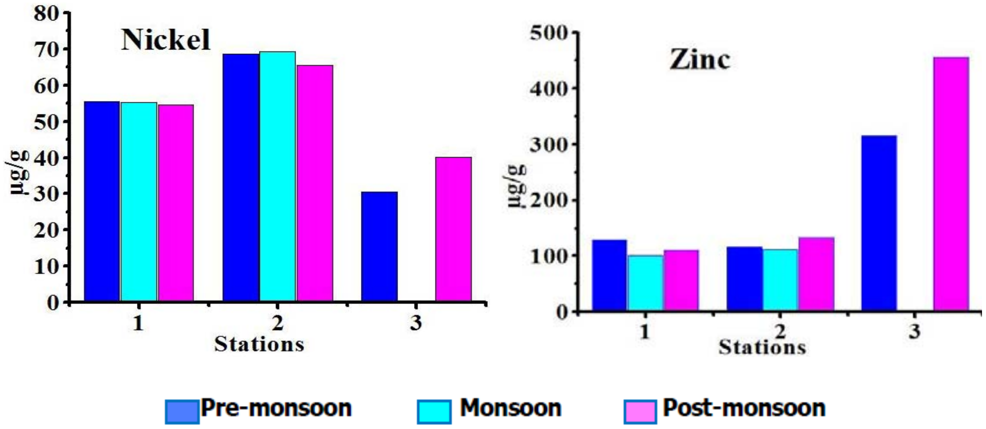
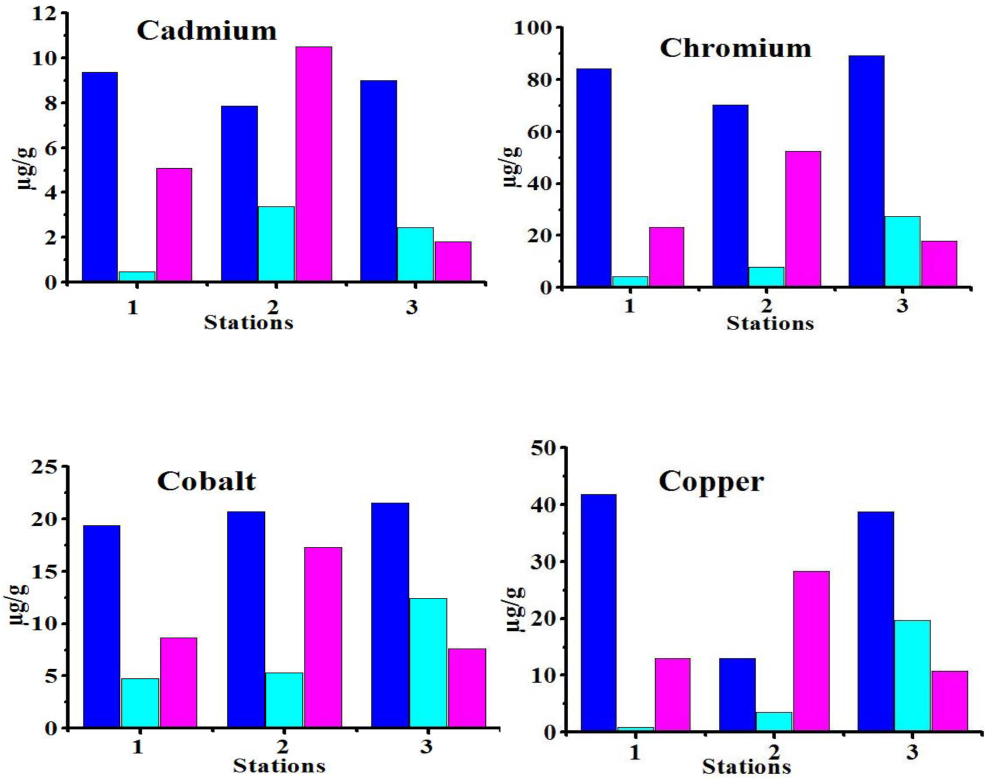


Figure 3.11 Seasonal variation of various heavy metals in mangrove sediments.



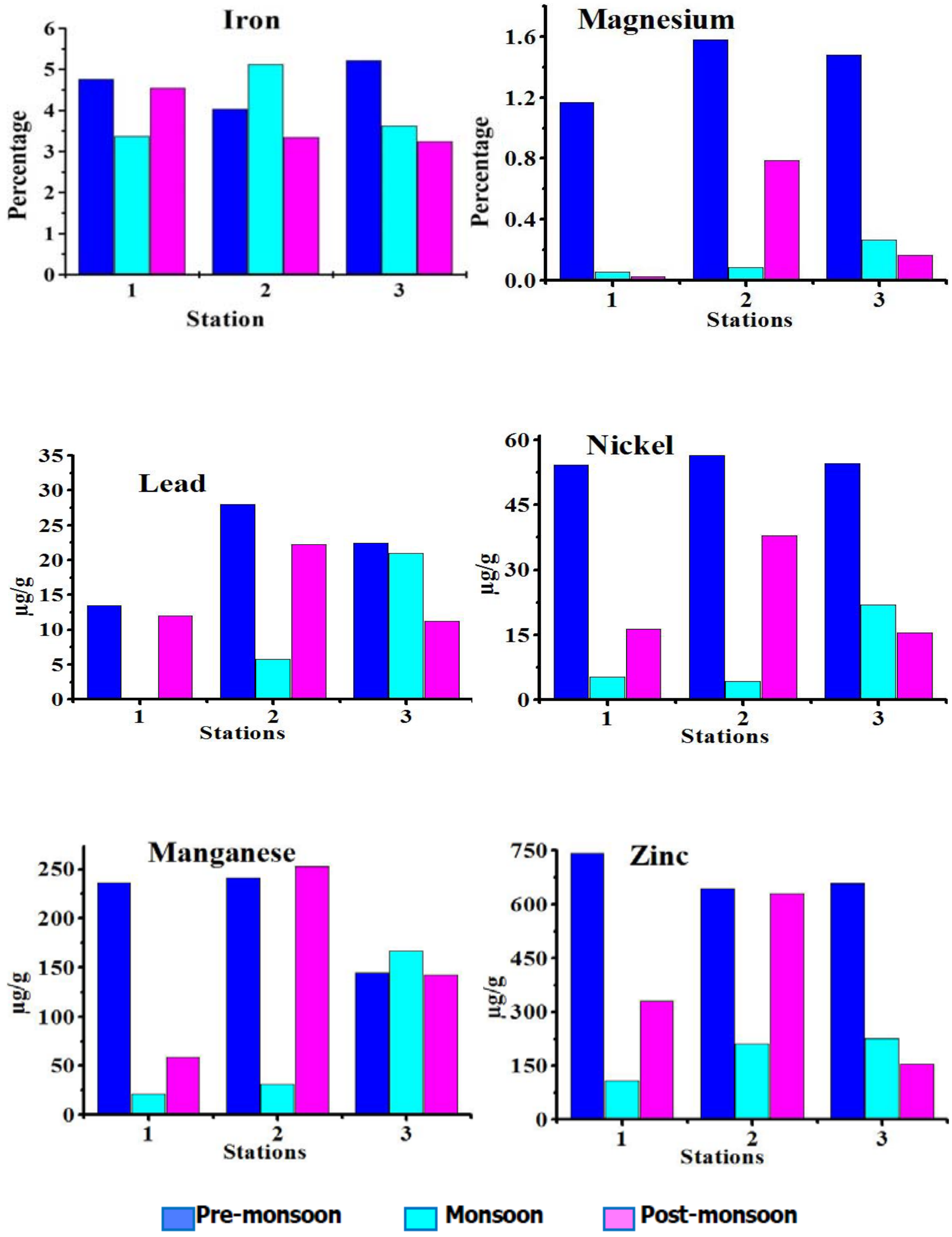


Figure 3.12 Seasonal variation of various heavy metals in estuarine sediments.

3.5 Discussion

The heavy metal content in the sediments of the study region was comparable with other reported works (Table 3.14). 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). Transformations during early diagenesis and changing redox state characterize the chemical forms of metals in aquatic sediments. The redox potential of the sediment can affect metal trapping directly through a change in the oxidation state of the metal itself, or indirectly through a change in the oxidation state of ions that can form complexes with the metal. The mangrove mud banks can be considered as a massive, sub-oxic bed reactor (Aller, 1998), the repetitive redox cycling may induce dissolution of some forms of heavy metals and explain their lower concentrations in mangrove sediments as compared to the estuarine system. The redox state of the sediment controls the solubility, or bioavailability of heavy metals (Kehew, 2001).

Table 3.14. Concentration of heavy metals in sedimentary environments of various parts of the world.

Location	Heavy Metals										Reference
	Cd (µ g/g)	Co (µ g/g)	Cr (µ g/g)	Cu (µ g/g)	Fe (%)	Mg (%)	Mn (µ g/g)	Ni (µ g/g)	Pb (µ g/g)	Zn (µ g/g)	
Cochin estuary	0.8-1.8	-	-	5.0-61	4.4-29.1	-	15-640	-	-	116-1385	Paul and Pillai,1983
Cochin estuary	0.3-8.4	1.0-25	4.0-100	1.0-50	2.8-20.2	-	7-170	-	3.0-50	14.0-259	Nair,1992
Mangroves of Panama	6.6-10	-	10.0-23.3	4.0-56.3	0.17-0.98	-		27.3-91.8	32.5-78.2	10.9-105	Guzman and Jimenez,1992
Cauvery Estuary	-	-	6.0-85	2.0-46	0.58-2.93	1.20-1.70	264-1800	-	12.0-47	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	-	-	0.20-192.0	Jayasree and Nair,1995
Veli Mangroves	-	20.0-25.0	-	23.0-61.0	0.83-1.95	-	60-320	-	16.0-87.0	44.0-80.0	Badarudeen,1997
Kochi Mangroves	-	44-122	-	12.0-69	1.56-6.78	-	60-171	-	20.0-49	15.0-83.0	Badarudeen,1997
Pichavaram mangrove	2.0-10.0	19.0-54.0	89-186	20-81	2.34-4.69	0.41-0.52	385-1248	38-86	6.0-17	50-130	Ramanathan et *al.*, 1999
Vellar estuary	4.0-9.0	14-36	28-43	4.0-20	2.03-3.10	0.39-0.57	86-130	15-31	4.0-8.0	71.0-122	Ramanathan et al., 1999
Mangroves sediments of UAE	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 sediments	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	-	-	1.0-85	BDL – 67	0.12-3.93	-	57-751	6.0-75	7.0-107	1.0-246	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	15.0-121	5.4-53.2	1.41-6.18	-	141-337	16.0-66.5	19.3-71.3	92.0-1266	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
Mangroves of Kochi	0.062-0.223	12.82-23.08	53.30 - 90.22	23.97-39.12	4.26-5.83	1.23 - 1.78	210.5 - 315.35	30.60 - 69.35	30.60 - 69.35	101.3-455.68	Present study
Cochin estuary	0.05 – 10.50	4.80 - 21.58	4.38 - 89.38	0.95 – 41.80	1.34-5.21	0.06 - 1.58	22.03 - 252.93	4.40 - 56.45	ND - 28.0	110.68 - 741.93	Present study

BDL- below detection limit, ND-not detected

Pearson correlation matrix for mangrove (Table 3.15) and estuarine (Table 3.16) systems revealed differences in interrelationships existing among the geochemical parameters in these ecosystems. In mangroves, sand exhibited highly significant negative correlation with Mg and silt. Clay fraction exhibited highly significant negative correlations with the metals such as Cd and Zn, but showed significant positive correlation with Ni. Highly significant negative correlations of total C, organic C and total N with Mg and Pb were recorded in mangrove sediments. Total P displayed highly significant positive correlations with Cd and Zn and highly significant negative correlations with Co, Cr and Ni in mangrove sediments. Clays have high specific surface area and can directly trap heavy metals, but they also may act as a substrate for organic matter flocculation (Keil *et al.*, 1994) that in turn adsorbs metals. The metal scavenging ability of sediments increases as the particle size decreases (Unnikrishnan and Nair, 2004; Casey *et al.*, 2007). The inverse relationship of the metals Cd and Zn with texture in the mangrove sediments pointed toward the anthropogenic enrichment of these metals in the mangrove systems. In estuary the metal-metal interrelationships were statistically significant except the relationships of Mn. The absence of any significant correlation of the heavy metals with texture, organic carbon, total nitrogen and total sulphur in the estuarine sediments also reflected that their input is not through the weathering processes.

Table 3.15 Pearson correlation matrix for the sedimentary variables of mangrove stations (n=8)

	Sand	Silt	Clay	pH	Eh	Total C	Organic C	Total N	Total P	Total S	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Sand	1.00																			
Silt	-0.86 (*)	1.00																		
Clay	-0.16	-0.32	1.00																	
pH	-0.59	0.39	0.17	1.00																
Eh	-0.18	-0.17	0.81 (*)	-0.10	1.00															
Total C	0.48	-0.32	-0.22	0.06	-0.55	1.00														
Organic C	0.61	-0.43	-0.25	-0.05	-0.56	0.99 (**)	1.00													
Total N	0.43	-0.17	-0.43	-0.03	-0.74 (*)	0.95 (**)	0.94 (**)	1.00												
Total P	0.25	0.04	-0.73 (*)	0.14	-0.97 (**)	0.53	0.56	0.71 (*)	1.00											
Total S	0.26	-0.10	-0.57	0.35	-0.83 (*)	0.42	0.45	0.51	0.89 (*)	1.00										
Cd	0.23	0.06	-0.73 (*)	0.16	-0.93 (**)	0.45	0.49	0.62	0.98 (*)	0.89 (*)	1.00									
Co	-0.69	0.42	0.62	0.16	0.81 (*)	-0.60	-0.68	-0.69	-0.87 (*)	-0.82 (*)	-0.86 (*)	1.00								
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 (**)	1.00							
Cu	-0.36	0.60	-0.50	0.14	-0.69	0.35	0.28	0.57	0.58	0.28	0.49	-0.20	-0.12	1.00						
Fe	-0.66	0.71 (*)	-0.30	0.24	-0.36	-0.30	-0.37	-0.06	0.29	0.21	0.26	0.12	0.20	0.69	1.00					
Mg	-0.80 (*)	0.59	0.37	0.19	0.61	-0.76 (*)	-0.85 (*)	-0.75 (*)	-0.69	-0.61	-0.66	0.91 (**)	0.91 (**)	-0.06	0.45	1.00				
Mn	-0.23	0.25	-0.14	0.26	0.03	-0.02	-0.10	-0.07	-0.16	0.07	-0.18	0.23	0.25	-0.03	0.21	0.37	1.00			
Ni	-0.52	0.18	0.79 (*)	0.00	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	1.00		
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	0.20	0.87 (*)	0.28	0.65	1.00	
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	1.00

*Correlation is significant at 0.05 level

** Correlation is significant at 0.01 level

Table 3.16 Pearson correlation matrix for the sedimentary variables of estuarine stations (n=9)

	pH	Eh	Sand	Silt	Clay	Total C	Org.C	Total N	Total S	Total P	Pb	Cd	Ni	Cu	Cr	Zn	Fe	Mn	Mg	Co
pH	1																			
Eh	-0.69	1																		
Sand	-0.29	0.41	1																	
Silt	0.22	-0.39	-0.98 (**)	1																
Clay	0.37	-0.41	-0.96 (**)	0.90 (**)	1															
Total C	0.33	-0.34	-0.90 (**)	0.87 (*)	0.88 (*)	1														
Org.C	0.26	-0.28	-0.84 (*)	0.81 (*)	0.83 (*)	0.97 (**)	1													
Total N	0.28	-0.3	-0.86 (*)	0.83 (*)	0.85 (*)	0.96 (**)	0.95 (**)	1												
Total S	0.20	-0.3	-0.91 (**)	0.90 (**)	0.87 (*)	0.95 (**)	0.94 (**)	0.95 (**)	1											
Total P	0.03	-0.36	-0.57	0.57	0.54	0.53	0.62	0.47	0.58	1										
Pb	0.01	-0.23	-0.25	0.22	0.28	0.05	0.02	-0.08	0.07	0.29	1									
Cd	0.15	-0.36	-0.51	0.47	0.54	0.31	0.30	0.18	0.32	0.57	0.85 (*)	1								
Ni	0.03	-0.33	-0.43	0.43	0.39	0.22	0.20	0.08	0.26	0.56	0.88 (*)	0.93 (*)	1							
Cu	0.02	-0.34	-0.49	0.48	0.46	0.30	0.32	0.18	0.28	0.67	0.78 (*)	0.90 (*)	0.90 (*)	1						
Cr	0.03	-0.31	-0.42	0.43	0.38	0.20	0.19	0.06	0.22	0.59	0.85 (*)	0.93 (**)	0.99 (**)	0.93 (**)	1					
Zn	0.13	-0.4	-0.54	0.52	0.52	0.32	0.31	0.18	0.35	0.63	0.83 (*)	0.98 (**)	0.97 (**)	0.91 (**)	0.97 (**)	1				
Fe	0.03	-0.33	-0.46	0.47	0.41	0.25	0.25	0.11	0.27	0.58	0.82 (*)	0.92 (**)	0.98 (**)	0.93 (**)	0.99	0.96 (**)	1			
Mn	0.08	-0.37	-0.25	0.21	0.28	0.21	0.22	0.13	0.26	0.46	0.58	0.57	0.59	0.52	0.55	0.6	0.53	1		
Mg	0.06	-0.34	-0.34	0.34	0.33	0.11	0.08	-0.02	0.15	0.49	0.87 (*)	0.90 (**)	0.98 (**)	0.84 (*)	0.97 (**)	0.94 (**)	0.95 (**)	0.54	1	
Co	0.03	-0.35	-0.45	0.46	0.41	0.22	0.19	0.08	0.26	0.51	0.89 (*)	0.92 (**)	0.99 (**)	0.9 (**)	0.97 (**)	0.96 (**)	0.98 (**)	0.57	0.95 (**)	1

*Correlation is significant at 0.05 level
** Correlation is significant at 0.01 level

3.5.1 Enrichment factor (EF)

Normalization was attempted to differentiate between the metals originating from human activity and those from natural weathering processes. It is a powerful tool for the regional comparison of trace metals content in sediments and can be applied to determine enrichment factors for the studied metals with respect to crustal average (Nolting *et al.*, 1999). Since Al, Fe, and grain size all tend to co-vary, the use of a single normalizer can often represent several underlying geochemical relationships. In this work, enrichment factors were computed by normalizing with Fe (Blomqvist *et al.*, 1992). Iron is conservative during diagenesis (Berner, 1980) and its geochemistry is similar to that of many trace metals both in oxic and anoxic environments. Natural concentrations of Fe in sediments are more uniform than Al and beyond the influence of humans, justify its use as a normalizer (Daskalakis and O'Connor, 1995). An EF value less than 1.5 suggests that the trace 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 trace metal is delivered from non-crustal materials or non-natural weathering processes and that the trace metals are provided by other sources (Feng *et al.*, 2004).

The enrichment factor was calculated for each metal, using iron as normalizing element using the equation EF = (metal/Fe) sediment/ (metal/Fe) 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.

The values of enrichment factor for mangrove sediments (Table 3.17) indicated a minor enrichment for Pb and Zn and no enrichment for other metals. The EF values for Pb in mangrove systems ranged between 0.78 and 1.88 with its highest value being recorded at M1 during post-monsoon. The highest EF value for Zn was reported from M3 and the values for enrichment factor varied from 0.94 to 3.82 respectively.

The analysis of the EF values of the estuary (Table 3.17) revealed that Pb exhibited a minor enrichment at all stations. Cadmium exhibited extremely severe enrichment in all estuarine stations with values varying from 5.7 to 505.57. Moderately severe enrichment for Zn (EF, 3.98 -10.39) at all the estuarine stations was noticed in the present study with its highest value was recorded at E1.

Table 3.17 Enrichment factor for heavy metals in sediments.

Metal	M1			M2			M3			E 1			E 2			E 3		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	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	5.7	505.57	299.69	342.62	478.47	297.83	229.79	225.42	263.94
Co	0.26	0.28	0.32	0.26	0.27	0.27	0.15	-	0.18	0.24	0.38	0.27	0.24	0.35	0.35	0.52	0.42	0.28
Cr	0.79	0.87	0.85	0.75	0.79	0.79	0.48	-	0.56	0.17	0.77	0.9	0.27	0.8	0.89	0.86	0.74	0.87
Cu	0.54	0.6	0.53	0.49	0.55	0.56	0.53	-	0.69	0.07	0.86	0.89	0.24	0.86	0.33	1.23	0.89	0.76
Mg	0.87	0.83	0.96	0.86	0.84	0.84	0.63	-	0.63	0.14	0.55	0.70	0.16	0.68	1.12	0.47	0.39	0.81
Mn	0.29	0.29	0.3	0.21	0.2	0.28	0.25	-	0.21	0.09	0.21	0.27	0.11	0.41	0.32	0.55	0.62	0.2
Ni	0.64	0.88	0.81	0.8	0.81	0.77	0.37	-	0.47	0.27	0.72	0.76	0.2	0.76	0.94	0.91	0.85	0.7
Pb	1.38	1.14	1.88	1.57	1.32	1.3	1.03	-	0.78	0.48	1.78	0.65	0.87	1.52	1.59	2.95	2.08	0.99
Zn	1.06	1.15	1.17	0.97	0.94	1.12	2.7	-	3.82	3.98	10.39	7.49	6.74	9.08	7.7	6.74	6.08	6.08

-Due to technical reasons samples from station M3 during monsoon season could not be collected.

3.5.2 Geoaccumulation index (Igeo)

The geoaccumulation Index (Igeo) introduced by Muller (1979) was used to assess metal pollution in sediments according to the equation Igeo = Log_2 (Cn/1.5Bn), where Cn = measured concentration of heavy metal in the mangrove sediment, Bn =geochemical background value in average shale (Wedepohl, 1995) 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 trace 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).

In order to determine the extent of pollution in sediment and better estimate anthropogenic inputs using the heavy metals load in sediment, the geoaccumulation index can be employed (Ridgway and Shimmield, 2002). Igeo values estimated for sediments of the study region are depicted in table 3.18. All metals except Zn(>1.0, M3) exhibited lower values for Igeo, indicating the sediments of mangrove ecosystems are unpolluted to moderately polluted as a result of anthropogenic activities. The estimated Igeo values of estuarine sediments revealed that Cd exhibited very strongly polluted condition at all stations. Zn exhibited moderately polluted condition and the highest value for Igeo was reported at E1 and all other metals displayed unpolluted nature. 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 the sediments collected from station M3.

Table 3.18 Geoaccumulation index for heavy metals in sediments.

Metal	M 1			M 2			M3			E 1			E 2			E3		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
Cd	-6.2	-6.3	-5.8	-5.7	-6.4	-6.3	-4.5	-	-4.5	0.15	6.82	7.7	6.24	7.87	7.45	5.77	5.35	7.65
Co	-2.2	-2.5	-2.3	-2.2	-2.2	-2.1	-3	-	-2.7	-4.4	-3.6	-2.4	-4.3	-2.6	-2.3	-3	-3.7	-2.2
Cr	-0.6	-0.9	-0.8	-0.7	-0.6	-0.6	-1.3	-	-1.1	-5	-2.5	-0.7	-4.1	-1.4	-0.9	-2.3	-2.9	-0.6
Cu	-1.1	-1.4	-1.5	-1.3	-1.1	-1.1	-1.2	-	-0.8	-6.2	-2.4	-0.7	-4.3	-1.3	-2.4	-1.8	-2.6	-0.8
Fe	-0.2	-0.7	-0.6	-0.3	-0.3	-0.3	-0.3	-	-0.3	-2.4	-2.2	-0.5	-2.2	-1	-0.8	-2.1	-2.5	-0.4
Mg	-0.4	-1	-0.7	-0.5	-0.5	-0.5	-1	-	-0.9	-5.2	-3	-1	-4.8	-1.6	-0.6	-3.2	-3.8	-0.7
Mn	-2	-2.5	-2.3	-2.5	-2.6	-2.1	-2.3	-	-2.5	-5.9	-4.4	-2.4	-5.3	-2.3	-2.4	-2.9	-3.2	-2.8
Ni	-0.9	-0.9	-0.9	-0.6	-0.6	-0.6	-1.7	-	-1.3	-4.2	-2.6	-0.9	-4.5	-1.4	-0.9	-2.2	-2.7	-0.9
Pb	0.23	-0.5	0.32	0.4	0.15	0.12	-0.3	-	-0.6	-3.4	-1.3	-1.2	-2.4	-0.4	-0.1	-0.5	-1.4	-0.4
Zn	-0.2	-0.5	-0.4	-0.3	-0.3	-0.1	1.14	-	1.68	-0.4	1.22	2.38	0.57	2.15	2.18	0.68	0.13	2.21

-Due to technical reasons samples from station M3 during monsoon season could not be collected.

Zinc is a very mobile element under oxidizing and acidic conditions; in alkaline and reducing environments, however, Zn substantially decreases in mobility due to its affinity for S and the tendency to form sulphide phases (Thornton, 1983; Alloway, 1990a). In the present study, highly significant negative correlation of Zn with Eh and positive correlation with sulphur support the retention under anoxic conditions, in mangrove sediments. 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.

Strong negative correlation of cadmium with clay and Eh and its positive correlation with total S in mangrove sediments suggested the anthropogenic origin and its accumulation under anoxic condition. Adsorption and desorption of Cd is highly variable depending on the type of colloid and local pH-Eh conditions. It can also form complexes with Cl or can be adsorbed in larger quantities by organic matter or Fe oxyhydroxides than by smectitic clays, despite the latter's high cation exchange capacity (Alloway, 1990b). 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). Cr does not form sulphide minerals and found to be immobilized in refractory organic compounds in mangrove sediments (Lacerda *et al.*, 1991).

The lack of a clear enrichment of other metals except Pb and Zn in the mangrove sediments 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-Dıaz and Morse, 1992). In estuarine system, total S showed no significant correlations with the heavy metals. Under anaerobic conditions, active sulphide co-precipitation rapidly removes many of the trace metals from the dissolved phase (Balistrieri et al., 1994; Clark *et al.*, 1998; Schlieker et al., 2001). The redox sensitive elements Fe and Mn did not exhibit any correlation with the sedimentary parameters in mangrove systems. But in the case of estuary, Fe displayed highly significant positive correlations with all other metals except Mn. The poor association of Mn with other metals suggests that Mn-oxide may be only a minor host phase for these elements in both environments.

3.5.3 Principal Component Analysis

Principal Component Analysis (PCA) was employed to discover and interpret relationships between the various sedimentary variables and also to deduce the geochemical processes in these ecosystems. Varimax orthogonal rotation was applied in order to identify the variables that are more significant for each factor based on the significance of their correlations that are expressed as factor loadings (Buckley *et al.*, 1995; Davis, 2002). PCA analysis of mangrove sediments (Table 3.19) showed that five components accounting for a total of 97.41% variance. First component accounted for about 39.86 % of the total variance was characterized by very high positive loadings on clay, Eh, Co, Cr and Ni and strong negative loadings on total phosphorous, total sulphur, Cd and Zn. This component reflected the redox process controlling the heavy metal distribution in sediments. Diagenesis, a redox process, largely mediated by sedimentary microorganisms and the suitable indicators to this are the redox element sulphur, organic carbon and nitrogen. Component 2 accounted for 24.13% of total variance, was mainly characterized by high loadings on carbon and nitrogen and negative loadings on Pb. This could be attributed to the flocculation and sedimentation of the organic matter. Variables included in component 3 accounted for 19.18% of the total variance and exhibited high positive loadings on silt and Fe and negative loadings on sand. This component likely to represented the granulometric factor. Fourth factor is the pH effect, which seems to be negligible in the geochemical distribution as there is very less pH variation in the study region.

Table 3.19 Factor loadings of sedimentary variables for mangrove stations

Sedimentary variables	Components				
	1	2	3	4	5
pH	-0.101	-2.90E-02	0.229	0.951	0.14
Eh	0.847	-0.401	-0.338	5.20E-02	1.94E-03
Sand	-0.322	0.35	-0.752	-0.445	-6.15E-02
Silt	4.01E-02	-0.221	0.898	0.145	0.158
Clay	0.764	-8.21E-02	-0.344	0.401	-0.231
Total Carbon	-0.27	0.945	-0.146	8.77E-02	4.80E-02
Organic Carbon	-0.334	0.908	-0.288	5.55E-03	-1.37E-02
Total Nitrogen	-0.423	0.899	7.06E-02	-5.87E-02	-9.75E-03
Total Phosphorus	-0.903	0.343	0.212	3.23E-02	-1.34E-01
Total Sulphur	-0.926	0.152	-2.72E-02	2.77E-01	8.32E-02
Cd	-0.932	0.236	0.173	6.69E-02	-1.51E-01
Co	0.865	-0.369	2.55E-01	1.64E-01	1.28E-01
Cr	0.881	-0.319	0.309	-2.22E-02	1.51E-01
Cu	-0.257	0.447	8.45E-01	-5.24E-02	-9.71E-02
Fe	-0.187	-0.228	0.861	4.94E-02	5.86E-02
Mg	0.621	-0.576	4.52E-01	1.15E-01	2.44E-01
Mn	5.58E-02	-4.34E-02	0.116	1.23E-01	9.75E-01
Ni	0.918	-0.299	1.31E-01	9.02E-02	-1.78E-01
Pb	0.439	-0.797	0.129	3.12E-01	1.81E-01
Zn	-0.777	0.466	3.84E-01	1.42E-02	-1.43E-01
Eigen value	10.97	4.34	1.703	1.391	1.076
% of variance	39.86	24.13	19.18	7.74	6.5

Extraction method: principal component analysis
Rotation method: varimax with Kaiser Normalization

Table 3.20 Factor loadings of sedimentary variables for estuarine stations.

Sedimentary variables	**Components**		
	1	**2**	**3**
pH	-3.37E-02	0.184	0.924
Eh	-0.281	-0.23	-0.841
Sand	-0.3	-0.907	-0.145
Silt	0.298	0.891	9.43E-02
Clay	0.283	0.874	0.216
Total Carbon	7.22E-02	0.968	0.143
Organic Carbon	6.84E-02	0.959	6.91E-02
Total Nitrogen	-6.59E-02	0.974	0.112
Total Phosphorus	0.518	0.558	-2.61E-02
Total Sulphur	0.111	0.971	4.81E-02
Cd	0.924	0.24	0.106
Co	0.971	0.146	4.32E-02
Cr	0.98	0.134	1.46E-02
Cu	0.626	0.906	-3.97E-02
Fe	0.586	0.962	1.67E-02
Mg	0.648	0.969	8.13E-02
Mn	0.129	0.856	0.185
Ni	0.979	0.145	3.48E-02
Pb	0.905	-4.88E-02	4.62E-02
Zn	0.95	0.253	0.106
Eigen value	11.07	5.16	1.47
% of variance	45.6	34.12	8.74

Extraction method: principal component analysis
Rotation method: Varimax with Kaiser Normalization

In estuarine sediments (Table 3.20), PCA revealed that three factors ascribed almost 88.46 % of the total variance of the system. Factor 1 accounted for 45.60 % of the total variance and had significant positive loadings on all heavy metals analyzed and totals P. It showed no loadings on the other sedimentary parameters and this could be attributed to anthropogenic factor. The factor 2 is characterized by significant positive loadings on silt, clay, total carbon, organic carbon, total nitrogen, total sulphur, Fe, Mn and Mg and it accounted for 34.12 % of the total variance. This seemed to be the granulometric factor and sorption/desorption effect on the fine-grained minerals and organic matter. The influence of redox potential and pH changes on these metals in sediment could be deduced from factor 3 which scored 8.74 % of the total variance.

3.6 Conclusions

Texture analysis revealed the predominance of fine substratum and silt was the major fraction in the sediments of mangrove and estuarine stations. The observed Eh values pointed towards the anoxic nature of the sediments in the study region. TOC : TS ratio revealed the sediment deposition process in a periodically fluctuating anoxic condition. The reducing condition prevailing in the study region had an important role in the distribution various phosphorous fractions and heavy metal distribution. X-ray Diffraction analysis of the sediments revealed the presence of monetite, an anhydrous calcium phosphate mineral ($CaHPO_4$) at mangrove station M3. XRD analysis showed that perovskite and sodalite were the dominant minerals at M1 and M2 respectively. The high mass percentages of calcium and phosphorus by XRF and SEM-EDS analysis confirmed the existence of calcium phosphate mineral at M3. In estuarine system XRD revealed the presence of quartz and monetite as abundant minerals in the sediments from E1 and E2. In addition to quartz and monetite, E3 exhibited the presence of aluminosilicate clay mineral kaolinite. X-Ray Fluorescence analysis showed that silicon was the major element in all the three stations, followed by aluminium and iron in the case of mangroves. The XRF analysis of the estuarine sediments also revealed the similar trend with higher abundance of the major elements silicon followed by aluminium and iron at all stations.

Phosphorus fractionation study indicated that the both mangrove and estuarine systems act as a sink of phosphorus, primarily as Fe-IP and Ca-IP fractions. Organic phosphorous exhibited lower content in sediments due to diagenesis. Internal loading in these systems acts as a source of phosphorus to the adjacent coastal waters. Stoichiometric ratios of elements (C: P, N: P and Fe: P) confirmed phosphorus enrichment at all stations of the both mangrove and estuarine systems. Higher Fe: P ratio in mangrove and estuarine systems indicated high Fe content in surface sediments to bind P in the estuarine system pointing towards phosphorous accumulation. The very high silt + clay content in these systems might favor the higher retention of phosphorus. In the case of mangroves, the first two stations behave identically and Fe-IP was the major fraction in these stations. Station M3 is unique because of the accumulation of bird guano in this bird sanctuary and it resulted in about 10 fold increase in total phosphorus content. This station is exclusively dominated by Ca-IP and rare mineral, monetite was detected in the system.

The overall order in the concentration of heavy metals in mangrove sediments was, Fe >Mg >Mn >Zn> Cr >Ni>Pb> Cu >Co> Cd. In estuary, the variation in the metal content displayed the trend: Fe >Mg > Zn >Mn >Cr >Ni >Cu >Pb >Co >Cd. The enrichment factor calculated, for heavy metals indicated a minor enrichment for lead and zinc and no enrichment for all other metals in mangrove sediments. The geoaccumulation index values revealed that sediments of the mangrove ecosystem are unpolluted to moderately polluted. The analysis of the EF values for the metals in estuarine sediments revealed that Cd and Zn exhibited extremely severe enrichment and moderately severe enrichment respectively and a minor enrichment for Pb. The geoaccumulation index values supported the accumulation of the trace metals such as Cd and Pb in estuarine sediments. The observations suggest that the mangrove ecosystems are relatively unpolluted and may be acting as a sink for heavy metals. But in the estuarine sediments accumulation of toxic trace metals was noticed. The element associations obtained by correlation coefficient and principal component score could indicate origin of heavy metals in the sediments of the study area and the factors controlling its distribution. The influence of granulometry on the distribution of trace metals in the sediments was not observed, signaling towards the anthropogenic contribution of toxic trace metals. The association of phosphorus with calcium and the redox sensitive heavy metal Fe, had a profound influence on the distribution of phosphorous in the sediments of the study region. Thus, the combined use of phosphorus fractionation and heavy metal distribution studies alongwith other geochemical variables was useful in evaluating the geochemical condition of the sedimentary environments.

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Chapter 4

BIOCHEMICAL COMPOSITION

4.1 Introduction

The overall geochemical processes prevailing in any aquatic system cannot be explained merely by the application of geochemical parameters such as general sedimentary characteristics, phosphorus fractionation or heavy metal distribution studies. Redox status of the sedimentary environments is directly linked with the diagenesis, the process of organic matter remineralisation. Both autochthonous as well as allochthonous organic compounds resistant to degradation and those survive diagenesis are stored in sedimentary environments depending on the redox state existing in the aquatic systems. Therefore, the assessment of the quantity and quality of organic matter, whether labile or refractory, is a prerequisite for explaining diagenetic processes. Biochemical composition of sedimentary organic matter has been used to gather information on the origin and parameters controlling the diagenetic fate of organic matter (Colombo *et al.*, 1996). Thus it is an important tool employed for the evaluation of the quantity and quality of organic matter and can provide a clear insight into the biogeochemical characterization of the sedimentary environments.

4.1.1 Quality of Sedimentary Organic Matter

Mangroves can act either as a sink for organic matter accumulation, or as reservoirs able to fertilize adjacent coastal areas through organic and inorganic nutrient export (Jannerjahn and Ittekkot, 2002; Dittmar *et al.*, 2006). The sources of organic matter in mangrove sediments are characterized 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 detritus and terrigenous inputs (Bouillon *et al.*, 2004). 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 utilization, transformation, accumulation and export (Viollier *et al.*, 2003). The labile fraction of organic matter consists mainly of simple and/or combined molecules (i.e. biopolymers); it includes carbohydrates, lipids and proteins, 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 labile portion of organic matter could be suitable to assess the trophic status of coastal marine systems (Dell'Anno *et al.*, 2002; Pusceddu *et al.*, 2003). Refractory organic compounds such as humic and fulvic acids, structural carbohydrates and 'black' carbon account generally for most of the sedimentary organic matter and are easily accumulated in marine sediments (Middelburg *et al.*, 1999; Zegouagh *et al.*, 1999).

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 more labile compounds (Downs and Lorezen, 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 the sediments (Cowie and Hedges, 1992; Danovaro *et al.*, 1999; Fiordelmondo and Pusceddu, 2004). 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 help of biochemical indices like concentration of protein, carbohydrate, lipids, protein/carbohydrate ratio and lipid/carbohydrate ratio (Dell'Anno *et al.*, 2000; Dell'Anno *et al.*, 2002). Eventhough, 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 quality and quantity of organic matter in surface sediments of the mangrove and adjacent estuarine stations in terms of the biochemical composition and thereby to identify the major biogeochemical pathways. The combinations of elemental and carbon isotopic composition can also be used to distinguish sources of organic matter in sediments and in settling particles. Therefore, along with biochemical composition, bulk parameters like elemental composition and $\delta^{13}C$ of the total organic matter were also used to characterize the sources of organic matter in the study region.

4.2 Results

The spatial and seasonal variations of organic matter, proteins, total carbohydrates and lipids in the surface sediments of mangrove and estuarine systems under study are described in tables 4.1 and 4.2 and figures 4.1 and 4.2. The results of biopolymeric carbon, elemental composition and $\delta^{13}C$ of total organic matter are also depicted in tables 4.1 and 4.2. The spatial and seasonal variation of C/N, PRT/CHO, LPD/CHO, tannin and lignin are also represented in figures 4.3 and 4.4.

4.2.1 Total Organic Matter (TOM)

Total organic matter in mangrove sediments ranged from 4.64±0.03% (M2, pre-monsoon) to 11.52±0.22% (M3, post-monsoon). Station M1exhibited a variation in TOM from 6.70±0.05% (post-monsoon) to 10.84±0.22% (monsoon). TOM content ranged between 4.64±0.03% (pre-monsoon) and 8.43±0.09% (post-monsoon) at M2. In sediments collected from M3, the estimated concentration for TOM content varied from 9.63±0.10% (pre-monsoon) to 11.52±0.22% (post-monsoon).

In the case of estuarine sediments, TOM varied from 1.17±0.01% (E3, monsoon) to 5.23±0.23% (E1, post-monsoon). It recorded a variation in concentration from 4.99±0.19% (pre-monsoon) to 5.23±0.23% (post-monsoon) at E1. In the sediments from E2, it ranged between 1.27±0.07% (monsoon) and 4.90±0.02% (post-monsoon). The reported TOM concentration at E3 varied from 1.17±0.01 % (monsoon) to 3.61±0.14% (post-monsoon).

4.2.2 Proteins (PRT)

Protein concentrations varied from 702±8.4 μg/g (M2, pre-monsoon) to 4608±17.7μg/g (M3, post-monsoon) in the mangrove sediments. At station M1, PRT concentrations ranged between 887±13.3μg/g (pre-monsoon) and 1527±16.8μg/g (post-monsoon). The observed variation in PRT content at M2 was from 702±8.4μg/g (pre-monsoon) to 1325±13.25μg/g (post-monsoon). The estimated protein content ranged between 887±14.2 μg/g (pre-monsoon) and 4608±17.7 μg/g (post-monsoon) at M3.

In estuarine sediments, the protein content recorded values ranging between 205±13μg/g (E3, monsoon) and 1924±102 μg/g (E1, post-monsoon) in the estuarine sediments. PRT content recorded a variation from 1025±14 μg/g (monsoon) to 1924±102μg/g (post-monsoon) at E1. The reported values for the concentration of protein in the sediments from E2 exhibited a variation from 270±36 μg/g (pre-monsoon) to 694±24 μg/g (post-monsoon). Whereas at E3, it ranged between 205±13 μg/g (monsoon) and 1252±128μg/g (post-monsoon).

The quantity of protein nitrogen estimated by multiplying the protein content with the factor 0.16 (Mayer *et al.*, 1986), for mangrove and estuarine sediments varied from 112 to 737μg/g and from 32.8 to 307.84μg/g respectively.

4.2.3 Total Carbohydrates (CHO)

Total carbohydrates in mangrove sediments ranged between 505±10.1 μg/g (M3, pre-monsoon) and 2458±49.2μg/g (M2, post-monsoon). At M1, CHO showed a variation from 779±9.3μg/g (monsoon) to 1790±23.3μg/g (post-monsoon). At M2, CHO varied from 653±8.5μg/g (monsoon) to 2458±49.2μg/g (post-monsoon). Total carbohydrates in sediments from M3 ranged from 505±10.1μg/g (pre-monsoon) to 1716±68.64μg/g (post-monsoon).

The range for total carbohydrate content in the estuarine sediments was from 250±12μg/g (E2, pre-monsoon) to 1229±76μg/g (E2, monsoon). The total carbohydrate concentration at E1 varied from 594±74μg/g (monsoon) to 1013±136μg/g (pre-monsoon). Station E2 exhibited CHO content ranging between 250±12μg/g and 1229 ±76μg/g. The estimated CHO content at E3 recorded a variation from 351±63μg/g (monsoon) to 1171±141μg/g (post-monsoon).

4.2.4 Lipids (LPD)

Total lipid content in mangrove sediments exhibited a variation from 804±34.5μg/g (M2, monsoon) to 6816±602.2μg/g (M3, post-monsoon) in mangrove sediments. The concentration of LPD at M1 ranged between 2244±102.4μg/g (post-monsoon) and 2789±155.8 μg/g (pre-monsoon). Station M2 recorded a LPD content varying from 804±34.5 μg/g (monsoon) to 1620±86.2 μg/g (post-monsoon). The sediments from M3, exhibited a total lipid concentration ranging between 5931±359.3μg/g (pre-monsoon) and 6816±602.2 μg/g (post-monsoon).

Total lipids exhibited a marked variation from 312±18μg/g (E2, pre-monsoon) to 2815±256μg/g (E1, monsoon) in estuarine system. At El, lipid content ranged between 2326 ±178μg/g (post-monsoon) and 2815±256μg/g (monsoon). Lipid content at E2 recorded a variation from 312±48μg/g (pre-monsoon) to 1255 ±24μg/g (monsoon). The estimated LPD concentration in the sediments collected from E3 ranged between 698±13μg/g (pre-monsoon) and 1539 ±124μg/g (post-monsoon).

Table 4.1. Seasonal variations of biochemical components in mangrove sediments. Results of ANOVA are also shown.

Parameters	M1			M2			M3			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	0.22	0.20
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	0.28	0.007
Labile organic matter (µg/g)	4719	4539	5561	3236	2327	5403	7323	-	13140	0.54	0.23
BPC(µg/g)	2944	2767	3147	1895	1291	2848	5085	-	8057	0.11	0.16
Organic Carbon %	3.90± 0.01	6.30± 0.13	3.90± 0.03	2.70± 0.02	3.10± 0.03	4.90± 0.05	5.60± 0.06	-	6.70± 0.13	0.14	0.27
Total Organic matter (TOM) %	6.72± 0.02	10.84± 0.22	6.70± 0.05	4.64± 0.03	5.33± 0.05	8.43± 0.09	9.63± 0.10	-	11.52± 0.22	0.17	0.25
Tannin and Lignin (µg/g)	1740± 18.2	1981± 21.3	1080± 14.6	748± 10.8	982± 12.2	794± 11.2	1221± 15.3	-	2352± 26.8	0.59	0.84
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		
C/N ratio	20.53	22.5	22.94	22.5	22.14	23.33	24.45	-	25.76		
$\delta^{13}C$ ‰	-26.28± 0.19	-26.40± 0.19	-26.46± 0.33	-25.28± 0.11	-25.48± 0.09	-25.53± 0.07	-26.57± 0.11	-	-26.71± 0.13		

-Due to technical reasons samples from station M3 during monsoon season could not be collected.

Table 4.2. Seasonal variations of sedimentary parameters in the surface sediments of estuarine system. Results of ANOVA are also shown.

Parameters	E1			E2			E3			ANOVA P-Value	
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Spatial	Seasonal
Total Lipids (μg/g)	2375 ± 210	2815 ± 256	2326 ± 178	312 ± 48	1255 ± 24	595 ± 64	698 ± 13	719 ± 24	1539 ± 124	0.013	0.442
Protein (μg/g)	1724 ± 264	1025 ± 14	1924 ± 102	270 ± 36	331 ± 16	694 ± 24	673 ± 51	205 ± 13	1252 ± 128	0.009	0.037
Total Carbohydrates (μg/g)	1013 ± 136	594 ± 74	923 ± 38	250 ± 12	1229 ± 76	250 ± 18	513 ± 12	351± 63	1171 ± 141	0.822	0.901
Labile organic matter (μg/g)	5112	4434	5173	832	2815	1539	1884	1275	3962	0.05	0.59
BPC (μg/g)	3031	2851	3056	466	1595	886	1058	780	2237	0.03	0.582
Organic Carbon%	2.90± 0.11	3.00± 0.11	3.04± 0.13	0.98± 0.06	0.74± 0.04	2.85± 0.01	0.82± 0.005	0.68± 0.006	2.10± 0.08	0.069	0.402
Total Organic matter %	4.99± 0.19	5.16± 0.19	5.23± 0.23	1.66± 0.10	1.27± 0.07	4.90± 0.02	1.41± 0.08	1.17± 0.010	3.61± 0.14	0.066	0.442
Tannin and Lignin (μg/g)	75.39± 1.2	120.45± 3.8	114.09± 2.4	33.86± 0.83	59.51± 0.91	94.02± 1.24	51.20± 0.81	73.40± 1.13	127.34± 3.1	0.025	0.078
PRT/CHO	1.7	1.72	2.08	1.08	0.27	2.77	1.31	0.58	1.07		
LPD/CHO	2.34	4.74	2.52	1.25	1.02	2.38	1.36	2.05	1.31		
C/N ratio	19.59	17.65	20.27	19.60	12.33	20.36	17.45	13.60	17.50		
δ^{13}C ‰	-24.28 ± 0.08	-24.18 ± 0.06	-24.22 ± 0.12	-24.73± 0.11	-25.31± 0.13	-25.27± 0.09	-24.04± 0.17	-24.48± 0.11	-24.12± 0.21		

4.2.5 Biopolymeric Carbon (BPC)

The biopolymeric carbon fraction of the sedimentary organic carbon in mangrove sediments ranged between 1291μg C/g (M2, monsoon) and 8057μg C/g (M3, post-monsoon). BPC fraction at station M1 varied from 2767μg C/g (monsoon) to 3147μgC/g (post-monsoon). At station M2, it ranged between 1291(monsoon) to 2848 μg C/g (post-monsoon). The estimated BPC fraction at station M3 varied from 5085(pre-monsoon) to 8057μg C/g (post-monsoon).

The biopolymeric carbon fraction of sedimentary organic carbon in estuary ranged from 466 μg C/g to 3056μg C/g. Sediments collected from E1 exhibited BPC varying from 2851μg C/g (monsoon) to 3056μg C/g (post-monsoon). The variation in biopolymeric fraction at E2 was from 466 μg C/g (pre-monsoon) to 1595μg C/g (monsoon). At E3, the observed BPC fraction was in the range between 780μg C/g (monsoon) and 2237μg C/g (post-monsoon).

4.2.6 Tannin and Lignin

Tannin and lignin content in mangrove sediments exhibited variation from 748±10.8 to 2352±26.8μg/g with an average of 1362μg/g. Estuarine sediments exhibited an average tannin and lignin content of 83.25μg/g and it ranged from 33.86±0.83 to 127.34±3.1μg/g.

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

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 compounds. The $\delta^{13}C$ of total organic matter in mangrove sediments ranged between -26.71±0.13 and -25.28±0.11‰. Station M3 was observed to be more depleted with ^{13}C whereas station M2 recorded comparative enrichment. The observed variation in $\delta^{13}C$ values of the estuarine sediments was from -25.31± 0.13‰ (E2, monsoon) to -24.04±0.17 ‰ (E2, pre-monsoon).

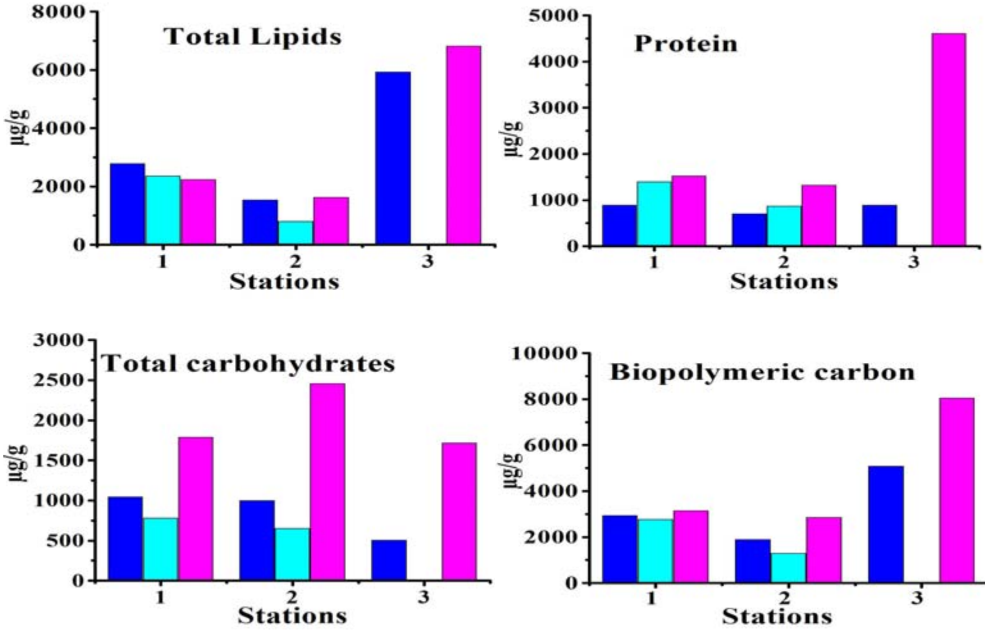

Figure 4.1. Seasonal variation of biochemical components in the mangrove sediments.

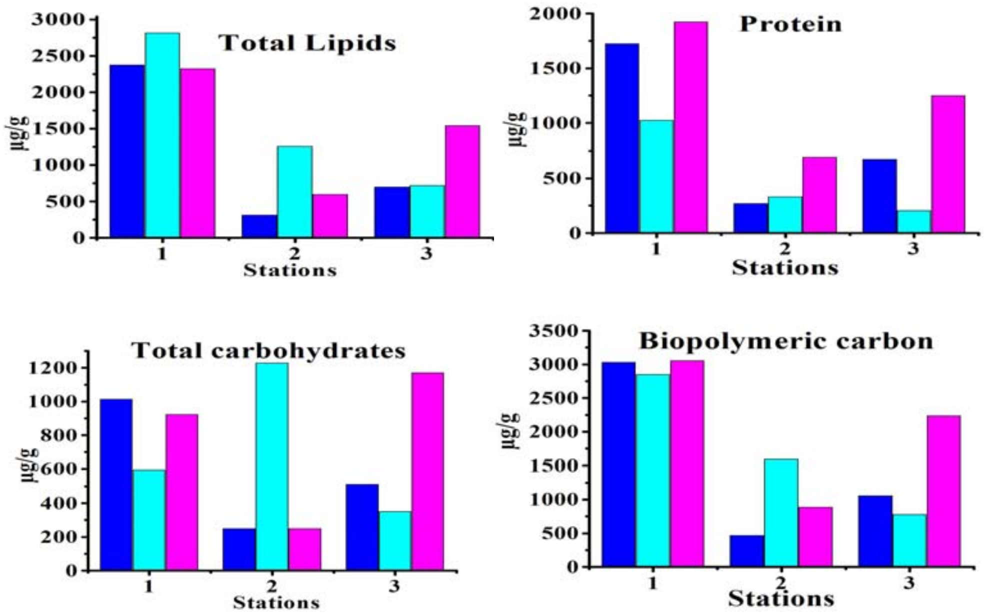

Figure 4.2 Seasonal variation of biochemical components in the estuarine sediments.

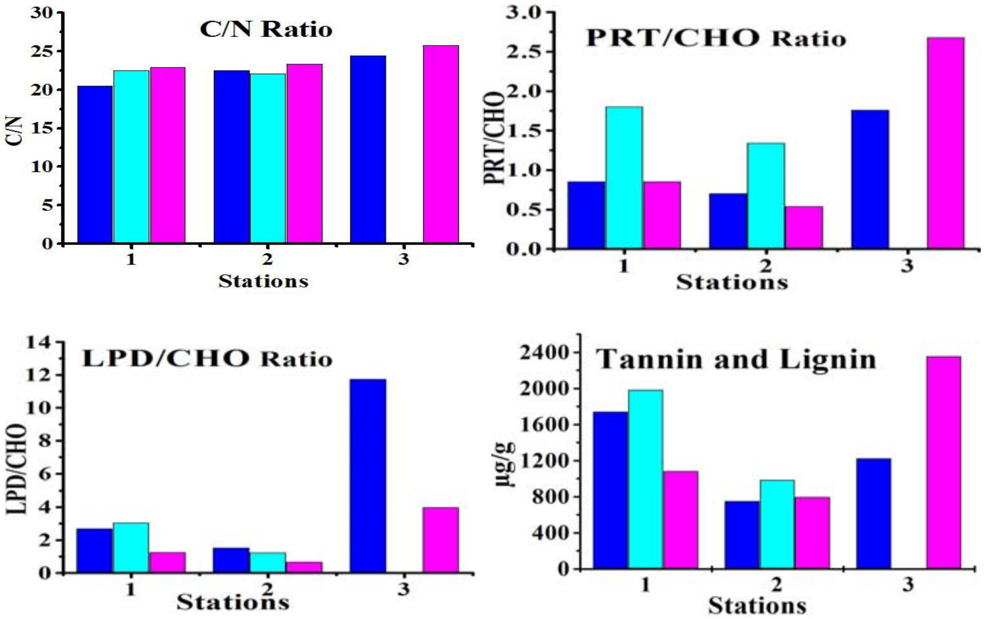

Figure 4.3. Seasonal variation of C/N, PRT/CHO LPD/CHO, tannin and lignin in mangrove sediments.

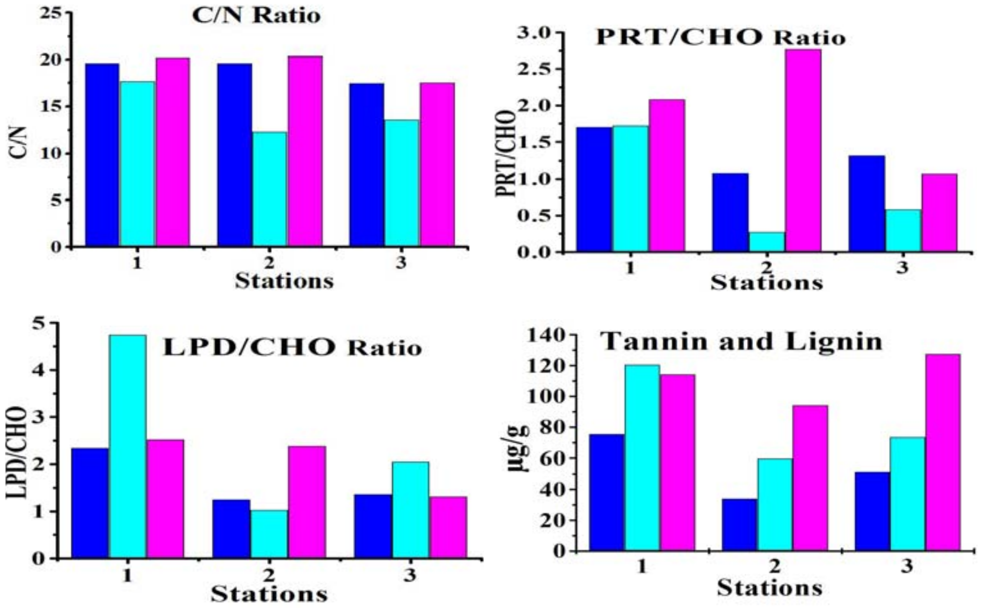

Figure 4.4 Seasonal variation of C/N, PRT/CHO, LPD/CHO, tannin and lignin in estuarine sediments.

4.3 Discussion

The concentration of the biochemical constituents was higher in sediments of mangrove (Table 4.1) than in estuary (Table 4.2). Some important research works based biochemical composition of sediments from different aquatic environments is shown in table 4.3. The biochemical composition of sedimentary organic matter in the study region showed a dominance of lipids followed by proteins and carbohydrates. Biochemical composition of sedimentary organic matter seems to be quite different from other coastal systems, which is usually characterized by dominance of proteins and carbohydrates over lipids (Meyer-Reil, 1983; Sargent *et al.*, 1983; Fabiano and Danovaro, 1994). The high concentrations of sedimentary lipids, proteins and carbohydrates recorded in the study area could be related to the morphodynamic, hydrological and physicochemical 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 M3 might be due to its preservation under highly anoxic conditions.

Table 4.3 Comparison between the biochemical compositions of different aquatic environments.

Sl. No	Area	Lipids (µg/g)	Proteins (µg/g)	Carbo-hydrates (µg/g)	References
1	Baltic Sea	-	3800-7700	400-4000	Meyer-Reil,1983
2	Gulf of Gascogne	220	1850	2440	Khripounoff *et al.*, 1985
3	W-Mediterranean	10-660	500-2600	900-4200	Fichez,1991
4	Ionian and Aegean Seas	50-190	70-160	1200-2500	Danovaro *et al.*,1993
5	Ligurian Sea (Prelo)	300-3600	50-1600	300-3600	Danovaro *et al.*, 1994
6	Tyrrhenian Sea	10-20	300-1700	300-1900	Fabiano and Danovaro, 1994
7	Ligurian Sea (Zoagli)	20-210	20-70	130-670	Fabiano *et al.*,1995
8	Ligurian Sea	90-630	20-300	300-5300	Danovaro and Fabiano,1995
9	Lower St.Lawrence Estuary	820-1470	110-400	7580-10700	Colombo *et al.*,1996
10	Marsala Lagoon	300-4500	2200-12100	800-70500	Pusceddu *et al.*,1999
11	Mediterranean Sea	350	350	1600	Albertelli *et al.*,1999
12	South Pacific	7200	5750	5800	Neira *et al.*, 2001
13	Intertidal flat sediments of Galician Coast (NW Spain)	50-1483	400-4016	32-675	Cividanes *et al.*,2002
14	Mundaka Estuary	300-5000	0.00-16700	200-5700	Cotano and Villate, 2006
15	Western Continental Shelf of India	-	90-1020	1080-9880	Jacob *et al.*,2008
16	Eastern Continental Shelf of India	-	170-550	1280-4430	Jacob *et al.*, 2008
17	Mangroves of Cochin	-	200-1240	1550-7860	Geetha *et al.*,2008
18	Cochin Estuary	312-2815	205-1924	250-1229	Present study
19	Mangroves of Cochin	804-6816	702-4608	505-2458	Present study

In the present investigation, all the biochemical components in estuarine sediments showed higher concentration during post-monsoon, except CHO, which was lower during monsoon. Station M3 recorded comparatively higher concentrations of biochemical compounds. Labile organic matter, the sum of these three biochemical compounds, varied from 2327 to 13140 µg/g in the mangrove sediments. On annual basis, lipids were dominant class among labile organic compounds, followed by protein and carbohydrates. LPD contribution to the labile organic matter pool in these sediments at stations M1, M2 and M3 was 50.48%, 37.33% and 66.43% respectively of labile organic matter. PRT contribution to the labile organic matter pool at stations M1, M2 and M3 were 25.70%, 27.86% and 23.59% respectively of labile organic matter. CHO contribution to the total organic matter pool at stations M1, M2 and M3 were 23.81%, 34.81% and 9.98% respectively of labile organic matter.

In estuarine system also, lipids were the dominant class among labile organic compounds, followed by protein and carbohydrates. Of the biochemical components, the contribution of lipid to the labile organic matter pool at E1, E2 and E3 were 51.66%, 40.33% and 44% respectively. Total protein consisted of 31.33%, 29.66% and 28% of the labile organic matter pool at stations E1, E2 and E3 respectively. In the estuarine sediments, the total carbohydrates contributed to 17%, 30% and 28% of the labile organic matter pool at stations E1, E2 and E3 respectively.

Analysis of variance (Table 4.1) revealed that lipids exhibit significant spatial variations (p=0.0003) with higher values at M3, while protein did not display any variation. In mangroves, carbohydrates recorded significant seasonal variation (p=0.007) with higher concentrations were reported during post-monsoon season at all stations. Lipids showed significant spatial variations (p=0.013, Table 4.2) in estuarine stations, while protein showed significant spatial (p=0.009) and seasonal (p=0.037) variations. In estuarine system tannin and lignin content recorded significant spatial variation (p=0.025) and exhibited the maximum content at E3. Spatial as well as temporal variations were absent for carbohydrate in the estuarine ecosystem.

Sedimentary protein concentrations reflect the productivity of marine ecosystems 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 polyhydroxyllated compounds ranging in size from 5-6 carbon sugars to large biopolymers (starch, 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. Lipids in sediments are derived not only from aquatic biota but also from higher plant wax. 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 dominance of lipids and protein over carbohydrates indicated the nutritive value as the freshness of the labile organic matter in the sediments of the study region.

Labile organic matter (LOM), the sum of the concentration of biochemical components (carbohydrates, proteins and lipids) in mangrove sediments ranged from 2327 to 13140μg/g. The high concentration of LOM at station might be due to its almost closed nature resulting in restricted tidal activity that consequently aided the retention of organic matter. But in the case of estuarine sediments, it varied from 832 to 5173μg/g. Irrespective of the higher content of total organic matter, the labile organic matter was low in both mangrove and estuarine sediments. The contribution of labile organic matter to total organic matter pool in mangrove sediments ranged from 4.16-11.45% whereas in estuarine sediments it ranged from 3.14-22.17%. Hence in both sediments a large fraction of total organic matter is represented by refractory material or is uncharacterized. The refractory fraction was comparatively higher in mangrove sediments.

The sum of PRT, CHO and LPD carbon is referred to as biopolymeric carbon (BPC) (Fichez, 1991; Fabiano *et al.*, 1995). PRT, CHO and LPD concentrations were converted to their carbon equivalents by using the following conversion factors: 0.49, 0.40 and 0.75 g of C/g, respectively (Fabiano and Danovaro, 1994). In the present study, BPC exhibited no significant variations in mangroves, while only spatial variation was noticed for estuarine stations which might be attributed to the changes in organic matter deposition in sediments associated with the strong river discharge in upper reaches of the estuary. Tenore and Hanson (1980) suggested that about 5-15% of sedimentary detritus, depending on the environmental characteristics is available at any time for benthic consumers. BPC exhibited its higher concentration at M3 in the case of mangroves and it accounted for 4.4 to 12.09% of total organic carbon. But in estuary it constituted about 3 to 21.5% of total organic carbon and station E1 recorded the highest content for BPC.

Total nitrogen concentrations of mangrove sediments were higher than that of estuarine sediments. Labile nitrogen, which is widely considered as the major limiting factor for deposit feeders was 5.94% of the total nitrogen in mangrove sediments and 12.94% in estuarine sediments. Hence, the sedimentary nitrogen was more refractory in mangroves than in estuaries. Nitrogen occurring in sedimentary organic matter mainly derives from living organisms. Proteins and peptides, the most abundant nitrogen containing substances in sedimentary organic matter, 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 (Nguyen and Harvey, 2001., Knicker and Hatcher, 2001., Zang *et al.*, 2001). It must be noted that labile total organic matter, total nitrogen and biochemical components were found in higher concentrations in the sediments of the study region, eventhough the labile to total organic matter is less in percentage.

Protein to carbohydrate ratio (PRT:CHO) is used as an index to determine the origin of material present in sediments and to distinguish between the presence of fresh materials and age of sedimentary organic matter (Danovaro *et al.*,1993; Cividanes *et al.*, 2002). Dominance of carbohydrates and lower PRT: CHO ratio (< 1) is a typical feature of detrital heterotrophic environments (Danovaro, 1996). 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 potentially limiting factor for the benthic consumers (Fabiano *et al.*,1995). This ratio varies from <0.1 in oligotrophic deep sea sediments from a depth 500-2400m in the Eastern Mediterranean Sea (Danovaro *et al.*, 1993) to >10 in coastal Antarctic sediments (Pusceddu *et al.*, 2000). On the other hand, low PRT: CHO ratios suggest the presence of aged organic matter (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 Eastern Mediterranean Sea sediments [Danovaro *et al.*, (1993)] to higher than 10 in coastal Antarctic sediments (Pusccedu, 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.*, 2003).

PRT: CHO ratio 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 mangrove sediments ranged between 0.54 (M2, post-monsoon) and 2.68 (M3, post-monsoon). Higher PRT: CHO at M3 (>1) indicated the presence of fresh organic matter. Stations M1and M2 showed PRT: CHO ratios greater than one (>1) only during monsoon season. During pre-monsoon and post monsoon, the lower ratios indicated the presence of aged or less degradable organic matter. However, in estuarine sediments, PRT: CHO ratio ranged between 0.27 (E2, monsoon) and 2.77 (E2, post-monsoon). PRT: CHO ratio >1 was reported at E1 (during all seasons), at E2 and E3 (except monsoon).The higher PRT: CHO ratio in estuarine sediments pointed towards newly generated detritus.

The PRT: CHO ratio was generally higher in estuarine sediments than in mangrove sediments. Estuaries are generally subjected to strong hydrodynamic conditions such as tidal action, mixing and freshwater discharge and hence it is more dynamic compared to mangroves, where there is very low water exchange. The higher PRT: CHO ratios in estuarine sediments when compared to mangroves indicated that in the former there is low dead organic matter accumulation, probably due to the strong hydrodynamic condition of estuaries. On the contrary, the low hydrodynamic condition in mangroves favours the accumulation of sedimentary organic matter.

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 contents in the sediments (Grémare *et al.*, 1997; Fabiano and Pusceddu, 1998; Grémare *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 enzymatic ally hydrolysable amino acids or protein contents (Grémare, *et al.*, 1997; Cartes *et al.*, 2002; Grémare *et al.*, 2002). LPD: CHO ratio in mangrove sediments ranged from 0.66 (M2, post-monsoon) to 11.74 (M3, pre-monsoon). But the ratio varied from 1.02 (E2, monsoon) to 4.74 (E1, monsoon) in estuarine sediments. The mangrove sediments showed higher concentrations of lipids and consequently high LPD: CHO ratio than that of estuarine sediments. Mangroves are generally considered as promoters of estuarine production. The high LPD: CHO ratio, which is an index of energy character, supports this fact. The estimated LPD: CHO ratio was very high at station M3. High LPD: CHO ratios were observed at the mangrove and estuarine systems pointed towards the high quality of labile organic matter to support benthic fauna.

The quantification of the phenolic compounds such as tannin and lignin in sediments provide information on the input of land-derived organic detritus in marine systems. Their estimation in sediments would enable us to determine directly the relationship between allochthonous and autochthonous organic matter. The occurrence of higher tannin and lignin content in the sediments of the study region could be attributed to mangrove input since these are components of higher plants including mangroves.

The tannins and lignins are high molecular weight polycyclic aromatic compounds widely distributed throughout the plant kingdom (Schnitzer and Khan, 1972; Finar, 1976; Field and Lettinga, 1987). Lignin is a nitrogen-free co-polymer of various phenyl propenyl alcohols that is present in vascular plants. Because of the exclusive association with higher plants, lignin is usually considered as a specific tracer of terrestrial plant remains. Mangrove species exhibit a typical vascular-plant lignin signature, with great variations between leaves and wood, the latter being richer in lignin oxidation product.

Tannins occur in plant leaves, roots, wood, bark, fruits and buds (Kraus *et al.*, 2003), and are estimated to be the fourth most abundant compound types produced by vascular plant tissue after cellulose, hemicellulose and lignin (Hernes and Hedges, 2000). Tannins in vascular plants occur as two types, condensed and hydrolysable. In mangrove species, tannin is an abundant component as high as 20% dry weight (Benner *et al.*, 1990), which prevents damage from herbivory (Zucker, 1993; Coley and Barone, 1996; Hernes *et al.*, 2001). In addition to having a biomarker potential, tannin greatly contributes to the properties of bulk organic matter including colour, astringency and reactivity [Lin *et al.*, (2006)]. Hernes *et al.*, (2001) found that green leaves of *Rhizophora* mangle may contain more than 6% tannins, being third in abundance after polysaccharides (21%) and amino acids (9%). Condensed tannin consists of 80% procyanidin and 20% prodelphinidin. The latter, with its higher degree of hydroxylation, appears to be more labile than procyanidin tannin. Leaching, which induces an increase in polymerization of condensed tannin, is an important mechanism for tannin removal from leaves.

Stoichiometric ratios of nutrients are utilized to determine the origin and transformation of organic matter based on the generalization that organic matter derived from marine plankton has atomic C /N ratios ranging between 4 and 10 while mangrove plants have C /N ratios of 20 and above (Prahl et al., 1994; Jennerjahn and Ittekkot, 1997; Yamamuro, 2000).The range of C /N values are typically lower for aquatic organic matter than for terrestrial organic matter (Meyers and Lallier-Verges,1999) and the ranges were 6.0-9.0 for planktonic organisms and 20.0-100.0 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 (Müller, 1997). In shallow coastal ecosystems, most of the organic carbon and nitrogen produced by microphytobenthos and macroalgae rather than phytoplankton (Barranguet *et al.*, 1996; Lucas *et al.*, 2000).

The C /N ratios in the mangrove sediments ranged between 20.53 and 25.76, indicating a higher plant input. However, in the estuarine sediments, reported values of C/N ratio varied from 12.33 to 20.36, which are intermediate values characteristic for autochthonous and terrestrial input of organic matter (Muri *et al.*, 2004). The C /N ratios in the sediments of the study area were comparable with similar aquatic environments (Verma and Subramanian, 2002; Bouillon *et al.*, 2004). Using C /N =13 and C /N=16 for the marine and terrestrial end members, respectively (Colombo *et al.*, 1995), it could be deduced that organic matter in the estuarine system are of mixed origin with a major contribution from vascular plants.

In mangroves, leaf litter fall have been implicated as significant source of organic matter (Kristensen *et al.*, 1995). The addition of bacterial biomass to decaying leaf material may have reduced observed C/N ratios (Rice, 1982; Rice and Hanson, 1984), as rapid declines in C /N ratios have been reported when bacterial colonisation is optimised, eg. through crab processing of mangrove leaf litter (Werry and Lee, 2005), a pathway common in sub-tropical Indo-west-Pacific mangroves. The grazing of mangrove leaf litter has been proposed as an important link in coastal and estuarine food web dynamics (Lee, 1997, 1998). The lower C /N ratios observed within the sediments of the study area, indicated bacterial activity and the degradation of vascular plant detritus.

Stable carbon isotope signatures ($\delta^{13}C$) of the various carbon inputs are often different and therefore can be used as powerful tracers of carbon sources in various ecosystems (Dehairs *et al.*, 2000; Yamamuro, 2000; Dittmar *et al.*, 2001; Bouillon *et al.*, 2003, 2004; Goni *et al.*, 2006; Hu *et al.*, 2006). Enzymatic and diffusional fractionation processes lead to discrimination against ^{13}C during photosynthesis that varies between C3, C4 and Crasculacean Acid Metabolism (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 in C4 plants 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 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).

In the present investigation, the observed $\delta^{13}C$ values for mangrove sediments pointed towards the vascular plant input (mangroves- C3 woody plants). Comparatively more enriched value for $\delta^{13}C$ was recorded at station M2 and more depleted values at station M1 and M3. Generally, more enriched values in the low organic carbon sites, and more depleted values in organic rich sediments were observed. From the observed relationships, it can be assumed that due to the almost closed nature of station M3, much of the mangrove area rarely inundated because of the low tidal flushing and act as retention sites where mangrove carbon is a significant contributor to the sediment organic carbon pool. Whereas, the sediments from station M2 reflect a balance between inputs (possibly including microphytobenthos) and carbon imported from the water column during high tide. The more depleted $\delta^{13}C$ values reported for the estuarine sediments indicated a major contribution of organic matter from the vascular plants. Earlier reported $\delta^{13}C$ values of various compartments in different aquatic ecosystems are presented in table 4.4. Thus, from the bulk parameter approach, it could be seen that organic matter in the mangrove systems are of higher plant origin and in estuary a mixed origin of organic matter involving contributions from both marine and terrestrial vascular plants.

Table 4.4 δ^{13}C values of different compartments of aquatic ecosystem.

Sl.No	Study Region	Sample and δ^{13}C value (‰)	References
1	Sound estuary, Plum Island	Sediments -19.4 to -22.9	Deegen and Garritt,1997
2	Galle,South-West Sri Lanka	Leaves of *Rhizophora apiculata* -31.5 ± 1.4	Bouillon *et al.*, 2003
3	Pambala, South-West Sri Lanka	*Rhizophora mucronata* -31.3 ± 0.9	Bouillon *et al.*, 2003
4	Coringa wild life sanctuary, Andhra Pradesh	Mangrove leaves -28.7 ± 1.1	Bouillon *et al.,* 2003
5	Gazi bay, Kenya	Mangrove sediments -26.5 to -22.1 Seagrass beds -25.5 to -16.0 *Avicennia marina* -30.4 ± 1.3 to -31.2 ± 0.9 *Rhizophora mucronata* -29.0 ± 0.6 to -29.8 ± 0.8	Bouillon *et al.*, 2004
6	Roebuck Bay, Dampier	Leaves of *Rhizophora* sp. -25.92 ± 0.68 Leaves *of Avicenna marina* -27.49 ± 0.40 Coarse POM -26.43 ± 0.21 Medium POM -24.91 ± 0.16 Fine POM -23.72 ± 0.37 Macro algae green -11.04 ± 0.86 Macrao algae brown -13.81 ± 0.69 Planktons -5.51 ± 0.11 to-19.35±0.10	Compton *et al.*, 2008
7.	Mangrove and estuarine system, Kochi	Mangrove sediments -26.71±0.13 to -25.28±0.11‰. Estuarine sediments -25.31± 0.13 to -24.04±0.17 ‰.	Present study

Correlation analysis of mangrove sediments (Table 4.5) showed that BPC had highly significant positive relations with total carbon, organic carbon, total nitrogen, total lipids and proteins. Total lipids showed highly significant positive correlations with organic carbon, total sulphur. Proteins also showed significant positive correlations with total carbon and organic carbon. Significant positive correlations for tannin and lignin with organic carbon and total nitrogen was observed in mangrove systems. Texture had no significant positive correlation with any of the parameters in the mangrove sediments. Total carbohydrates were also not correlated with any sedimentary parameters. The higher concentration of carbohydrates during post monsoon season in mangrove sediments pointed towards the possibility of input of vascular plant materials, especially mangrove litter.

A clear difference was noticed in estuary, where all biochemical parameters except carbohydrates showed significant positive correlation with texture, total carbon, organic carbon, total nitrogen and total sulphur (Table 4.6). The absence of any significant correlations of carbohydrates with other sedimentary parameters also suggest different origin and diagenetic pathways for carbohydrates.

Table 4.5 Correlation between sedimentary parameters in mangrove sediments. (n=8)

Parameters	Sand	Silt	Clay	Total Carbon	Organic Carbon	Total Nitrogen	Total Sulphur	Total Lipids	Protein	CHO	BPC	Tannin and lignin
Sand	1	-0.86 (**)	-0.16	0.55	0.65	0.70	0.25	0.34	0.02	-0.46	0.20	0.50
Silt	-0.86 (**)	1	-0.32	-0.32	-0.40	-0.46	-0.09	0.04	0.28	0.36	0.16	-0.05
Clay	-0.16	-0.32	1	-0.35	-0.39	-0.33	-0.57	-0.83 (*)	-0.47	0.25	-0.73 (*)	-0.64
Total Carbon	0.55	-0.32	-0.35	1	0.98 (**)	0.95 (**)	0.48	0.69	0.70	0.29	0.77 (*)	0.69
Organic Carbon	0.65	-0.40	-0.39	0.99 (**)	1	0.97 (**)	0.50	0.72 (*)	0.64	0.13	0.76 (*)	0.73 (*)
Total Nitrogen	0.70	-0.46	-0.33	0.95 (**)	0.97 (**)	1	0.33	0.58	0.54	0.11	0.62	0.77 (*)
Total Sulphur	0.26	-0.09	-0.57	0.48	0.50	0.33	1	0.82 (*)	0.27	-0.07	0.69	0.12
Total Lipids	0.34	0.04	-0.83 (*)	0.69	0.72 (*)	0.58	0.82 (*)	1	0.66	-0.04	0.95 (**)	0.62
Protein	0.03	0.28	-0.47	0.70 (*)	0.64	0.54	0.27	0.66	1	0.40	0.85 (**)	0.69
CHO	-0.47	0.36	0.26	0.29	0.13	0.11	-0.07	-0.04	0.40	1	0.21	-0.09
BPC(μg/g)	0.21	0.16	-0.74 (*)	0.77 (*)	0.76 (*)	0.62	0.69	0.95 (**)	0.85 (**)	0.21	1	0.66
Tannin and lignin	0.50	-0.06	0-.65	0.69	0.74 (*)	0.77 (*)	0.12	0.62	0.69	-0.09	0.66	1

*Correlation significant at 0.05 level

** Correlation significant at 0.01 level

Table 4.6 Correlation between sedimentary parameters in estuarine sediments. (n=9)

Parameters	Sand	Silt	Clay	Organic Carbon	Total Carbon	Total Nitrogen	Total Sulphur	Total Lipids	Protein	CHO	BPC	Tannin and Lignin
Sand	1	-0.97 (**)	-0.93 (**)	-0.65	-0.59	-0.69 (*)	-0.97 (**)	-0.89 (**)	-0.84 (**)	-0.53	-0.92 (**)	-0.66
Silt	-0.97 (**)	1	0.83 (**)	0.59	0.53	0.65	0.97 (**)	0.91 (**)	0.80 (**)	0.53	0.92 (**)	0.58
Clay	-0.93 (**)	0.83 (**)	1	0.67 (*)	0.63	0.69 (*)	0.87 (**)	0.78 (*)	0.83 (**)	0.47	0.83 (**)	0.72 (*)
Organic Carbon	-0.65	0.59	0.67 (*)	1	0.99 (**)	0.98 (**)	0.67 (*)	0.71 (*)	0.80 (**)	0.16	0.73 (*)	0.73 (*)
Total carbon	-0.59	0.53	0.63	0.99 (**)	1	0.97 (**)	0.62	0.66	0.77 (*)	0.11	0.68 (*)	0.71 (*)
Total Nitrogen	-0.69 (*)	0.65	0.69 (*)	0.98 (**)	0.97 (**)	1	0.71 (*)	0.78 (*)	0.77 (*)	0.24	0.79 (*)	0.79 (*)
Total Sulphur	-0.97 (**)	0.96 (**)	0.87 (**)	0.66 (*)	0.61	0.71 (*)	1	0.91 (**)	0.89 (**)	0.65	0.96 (**)	0.64
Total Lipids	-0.89 (**)	0.91 (**)	0.78 (*)	0.71 (*)	0.66	0.78 (*)	0.91 (**)	1	0.77 (*)	0.55	0.97 (**)	0.65
Protein	-0.85 (**)	0.80 (**)	0.83 (**)	0.79 (**)	0.77 (*)	0.77 (*)	0.89 (**)	0.77 (*)	1	0.51	0.88 (**)	0.61
CHO	-0.53	0.53	0.47	0.16	0.11	0.24	0.65	0.55	0.51	1	0.66	0.34
BPC	-0.92 (**)	0.92 (**)	0.83 (**)	0.73 (*)	0.68 (*)	0.78 (*)	0.96 (**)	0.97 (**)	0.88 (**)	0.66	1	0.67 (*)
Tannin and lignin	-0.66	0.58	0.72 (*)	0.73 (*)	0.71 (*)	0.79 (*)	0.64	0.66	.618	0.34	0.66 (*)	1

*Correlation significant at 0.05 level
** Correlation significant at 0.01 level

The quality and quantity of sedimentary organic matter in aquatic systems is mainly controlled by the biogeochemical processes taking place in the system and Principal Component Analysis (PCA) was employed 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, which can be more easily interpreted (Manly, 1997; Panigrahy *et al*., 1999; Spencer, 2002). The parameters for the PCA were selected in such a way that the component of the analysis can give indication of the significance of the 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, 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 mangrove sediments revealed three components without significant differences accounted for a total of 92.84% variance (Table. 4.7 and Figure. 4.5). First component accounted for 35.77% of the total variance and showed very high positive loadings on total carbon, organic carbon, total nitrogen, carbohydrates, proteins and BPC. But this component had no significant loadings on sediment texture and total sulphur, one of the major redox indicators. Hence, the first factor seemed to be autochthonous input including the mangrove litter and this could be the main source of organic matter in mangrove sediments.

Component 2 exhibited high negative loadings on clay and positive loadings on sulphur, lipids and BPC accounted for 34.84% of total variance. It also displayed a statistically significant negative loading on carbohydrate, pointing towards the diagenetic pathway. The low negative loading pattern of 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 reverse is true under oxic conditions (Harvey *et al.*, 1995). Thus the anaerobic diagenetic processes account for the second major biogeochemical process in these ecosystems.

Component 3 accounted for 22.23% of total variance and displayed 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 seemed that third component was the geochemical processes other than diagenesis, which includes siltation and sorption/desorption.

Principal component analysis of estuarine sediments displayed only one component accounting for 89.37% variance (Table 4.7 and Figure 4.6). It exhibited high positive loadings on all the sedimentary parameters except sand, which showed high negative loading. The high positive loadings for redox indicators such as total sulphur, total and organic carbon and total nitrogen indicated that the major process that can operate in the system was the diagenesis.

Table 4.7 Factor loadings for various biochemical components in sediments.

Sedimentary Variables	**Components**			
	Mangrove			**Estuary**
	1	**2**	**3**	**1**
Sand	0.224	0.179	0.937	-0.970
Silt	-0.165	0.238	-0.943	0.947
Clay	0.011	-0.948	0.064	0.905
Total Carbon	0.934	0.237	0.246	0.993
Organic Carbon	0.879	0.28	0.379	0.977
Total Nitrogen	0.839	0.466	0.175	0.979
Total Sulphur	0.230	0.751	0.163	0.986
Lipids	0.408	0.901	0.098	0.936
Proteins	0.693	0.64	-0.146	0.896
Carbohydrates	0.664	-0.307	-0.611	0.664
BPC	0.607	0.784	-0.081	0.982
Percentage of Variance	35.77%	34.84%	22.23%	89.37%

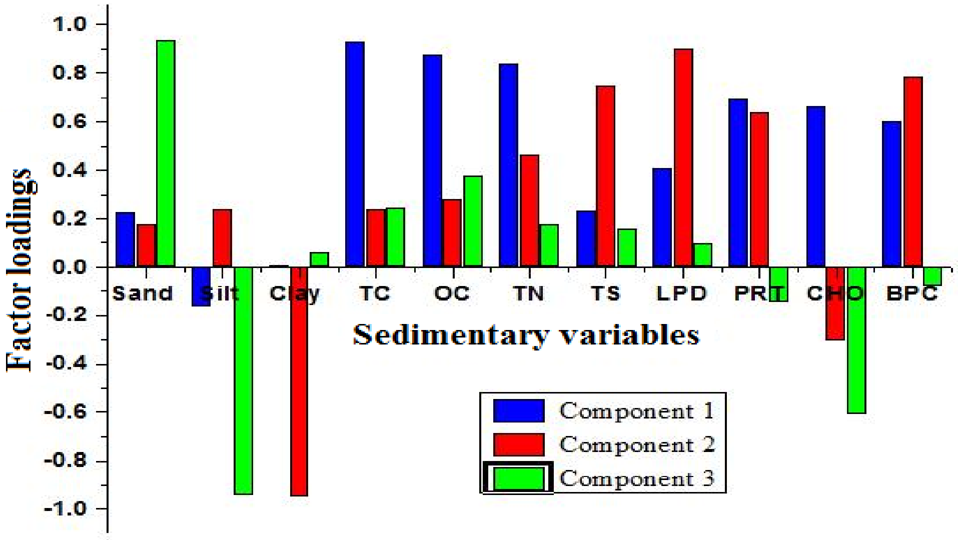

Figure 4.5 Loading pattern of various biogeochemical parameters for the different components in PCA analysis of mangrove system.

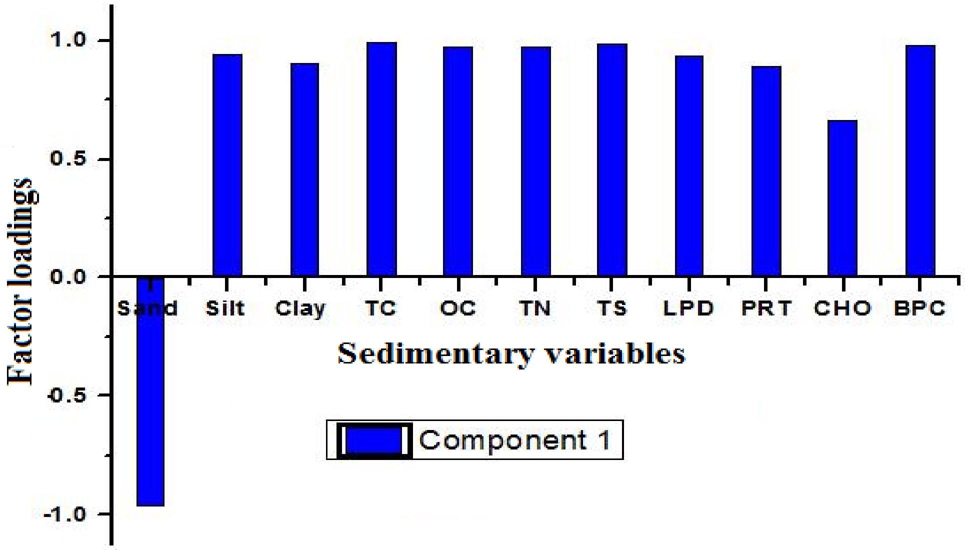

Figure 4.6 Loading pattern of various biogeochemical parameters for the different components in PCA analysis of estuarine system.

4.4 Conclusion

Sedimentary organic matter was higher in mangroves than in their adjoining estuary. However the percentage ratio of the labile to total organic matter indicated that most of the deposited organic matter is refractory in both environments. The refractory fraction was higher in mangrove sediments when compared to estuarine sediments. This discrepancy between the high amounts of organic matter and its low nutritional value suggests that these ecosystems behave as a detrital trap and organic matter tends to accumulate.

Biochemical composition of sedimentary organic matter provided evidence for high nutritional value, due to the dominance of lipids and protein fractions over carbohydrates in the study region. All the biochemical components, in general, recorded higher concentrations during post monsoon and lower concentrations during pre-monsoon seasons, except total carbohydrates, which was lower during monsoon. In mangrove systems, station M3 recorded comparatively higher concentrations of all the analyzed biochemical components. Carbohydrates exhibited significantly higher values during post-monsoon season in mangroves. The higher PRT: CHO ratios in estuarine sediments compared to mangroves indicated that in the former there is low dead organic matter accumulation, probably due to the strong hydrodynamic condition prevailing in the estuary. On the contrary, the low hydrodynamic condition in mangroves favours the accumulation of sedimentary organic matter. The lower PRT: CHO ratios (<1) at M1 and M2 (pre monsoon and post monsoon) indicated the presence of aged or less degradable organic matter ie., terrestrial or vascular plant input. The higher LPD: CHO ratios estimated for mangrove and estuarine systems indicated high quality of labile organic matter to support benthic fauna. The higher tannin and lignin content in the sediments of the study region confirmed the mangrove input these are typical components of higher plants including mangroves.

The observed C /N ratios in the mangrove ecosystems indicated a vascular plant input to the total organic matter pool. However, in the estuarine sediments, the C /N ratio estimated were intermediate values characteristic for autochthonous and terrestrial input of organic matter. The observed $\delta^{13}C$ values for mangrove sediments pointed towards the higher plant input (mangroves- C3 woody plants). Stable carbon isotope ratio analysis revealed comparatively more enriched values at station M2 and more depleted values at stations M1 and M3 indicating vascular plant input to the organic matter pool. Whereas, the sediments from station M2 reflected a balance between inputs (possibly including microphytobenthos) and carbon imported from the water column during high tide. The more depleted $\delta^{13}C$ values reported for the estuarine sediments indicated a major contribution of organic matter from vascular plants. Thus, from the bulk parameter approach, it could be seen that organic matter in the mangrove systems are of higher plant origin and in estuary, a mixed origin of organic matter involving input from both marine and terrestrial vascular plants.

The application of bulk parameters such as biochemical composition, elemental and stable carbon isotope ratios was useful in the assessment of the quality and quantity of sedimentary organic matter and it revealed the fact that the biogeochemistry of sediments was very complex and cannot be explained effectively by these methods. Further studies like organic matter biomarkers are essential to understand the origin of the organic matter in the mangrove and estuarine sediments of the study region.

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Chapter 5

TRITERPENOID BIOMARKERS IN SEDIMENTS

5.1 Introduction

Triterpenoid biomarkers are the oxygenated natural products and their derivatives formed from the reductive and oxidative modification of their precursors. The oleananes, ursanes, fernanes, lupanes, and their derivatives belong to this class of compounds and are widely distributed in many varieties of higher plant species (Jacob *et al.*, 2007; Simoneit, 2008). Triterpenoids constitute the major proportions of non saponifiable lipids, and these compounds have been detected in cuticular waxes of mangrove and other plant species (Wannigama *et al.*, 1981; Ghosh *et al.*, 1985; Koch *et al.*, 2003). The estimation of lipid composition of mangroves can provide insight into the sources and accumulation rates of sedimentary organic matter, since lipid comprises a significant proportion of carbon output from mangroves (Wannigama *et al.*, 1981; Hogg and Gillan, 1984). Nonsaponifiable lipids basically denote simple lipid fractions except for fatty acids (saponifiable lipids) after alkaline hydrolysis of the total lipids, and contain sterols, terpenoids, long-chain alcohols and alkanes. They represent a more stable lipid fraction than the saponifiable lipid fraction, and their peculiar resistance to microbial degradation has been considered to be a relatively important factor in controlling the diagenetic pathways (Killops and Frewin, 1994; Koch *et al.*, 2005).

The biochemical composition of organic matter sources varies and differences in source signatures are not always unique enough to identify components in complex mixtures such as sediments. Because of the limitations in using elemental composition, stable isotope composition and biochemical composition for source characterisation of sedimentary organic matter, the biomarker approach was employed. Pentacyclic triterpenoids have been widely used as biomarkers to trace sources of organic matter in sedimentary environments (Cranwell, 1984; Grimalt *et al.*, 1991; Simoneit, 2002; Jacob *et al.*, 2005). Triterpenoids with a single hydroxyl group such as α- and β- amyrin, germanicol and taraxerol are usually employed as terrestrial plant biomarkers. Despite the diagenetic alteration, the triterpenoid biomarkers retain the characteristic structures of their precursors and can be assigned to their structural classes. Their tendency to resist biodegradation and occurrence in sediments suggest the potential application as specific higher plant derived biomarkers (Otto *et al.*, 2002; Jacob *et al.*, 2007; Simoneit, 2008). Eventhough these classes of compounds exhibit high potentialities for reconstructing past depositional environments, neither these compounds nor their digenetic transformation products are frequently used for the organic geochemical characterization of sediments of the coastal ecosystems in our country. The analysis of triterpenoids along with other lipid compounds is a prerequisite for the interpretation of biomarker signals in the sediments of the study region.

5.2 Results

The results of the GC-MS analysis of the surface sediments of the mangroves and estuarine systems are furnished in Table 5.1 and 5.2. The chromatograms for the hexane and dichloromethane fractions extracted from the sediment samples are also displayed below in figures (5.1 - 5.14). The presence of n-alkanes with carbon number ranging between C16 to C34 was observed in both estuarine and mangrove systems. The high relative abundance of odd carbon n-alkanes was observed for both mangrove and estuarine systems. The presence of fatty alcohols with carbon number ranging between C24 and C30 were also detected in mangrove and estuarine stations. The occurence of pentacylclic triterpenoids of oleanane, ursane and lupane skeleton α-amyrin, β-amyrin, taraxerol, germanicol, lupeol and their degradation products were also noticed in both systems. The important tetracyclic triterpenoids such as cholesterol, stigmasterol, β-sitosterol and their degradation products were also detected in the surface sediments of the study region.

Table 5.1 Results of the GC-MS analysis of hexane fraction.

Sl.No.	Compound name	M W	Composition	Retention time (mts)	Stations					
					Mangroves			Estuary		
					M1	M2	M3	E1	E2	E3
1	pyrene	202	$C_{16}H_{10}$	22.39	-	-	-	+	+	-
2	8-heptadecene	238	$C_{17}H_{34}$	22.59	+	+	+	+	+	+
3	n-octadecane	254	$C_{18}H_{38}$	26.55	+	+	+	+	+	+
4	n-eicosane	282	$C_{20}H_{42}$	27.86	+	-	-	-	+	+
5	n-heneicosane	296	$C_{21}H_{44}$	29.75	+	+	+	+	+	+
6	n-tetracosane	338	$C_{24}H_{50}$	34.46	-	+	+	+	+	+
7	n-pentacosane	352	$C_{25}H_{52}$	37.45	+	+	+	+	+	+
8	n-hexacosane	366	$C_{26}H_{54}$	39.77	-	-	+	+	+	-
9	n-heptacosane	380	$C_{27}H_{56}$	42.32	+	+	+	+	+	+
10	n-octacosane	394	$C_{28}H_{58}$	44.30	+	-	+	+	+	+
11	n-triacontane	422	$C_{30}H_{62}$	48.89	+	-	-	+	+	-
12	n-hentriacontane	436	$C_{31}H_{64}$	53.79	+	+	+	+	+	+
13	n-dotriacontane	450	$C_{32}H_{66}$	54.88	-	+	-	+	+	+
14	n-tetratriacontane	478	$C_{34}H_{70}$	56.36	+	-	-	+	+	-

+ detected
-not detected

Table 5.2 Results of the GC-MS analysis of dichloromethane fraction

Sl.No.	Compound name	MW	Composition	Retention time (mts)	Stations					
					Mangroves			Estuary		
					M1	M2	M3	E1	E2	E3
1	glycerol-TMS ether	308	$C_{12}H_{32}O_3Si_3$	28.16	+	+	+	+	+	+
2	des-A-lupane	330	$C_{24}H_{42}$	32.08	+	-	+	-	-	-
3	2-pentacosanone	366	$C_{25}H_{50}O$	33.85	+	+	+	+	+	-
4	2-heptacosanone	394	$C_{27}H_{54}O$	36.52	+	+	+	+	+	+
5	n-docosanol-TMS ether	398	$C_{25}H_{54}OSi$	38.53	+	-	+	+	+	-
6	n-tetracosanol-TMS ether	426	$C_{27}H_{58}OSi$	40.57	+	+	+	+	-	-
7	n-hexacosanol-TMS ether	454	$C_{29}H_{62}OSi$	42.21	+	+	+	+	+	+
8	n-octacosanol-TMS ether	482	$C_{31}H_{66}OSi$	44.17	+	+	+	+	+	+
9	n-triacontanol	510	$C_{33}H_{70}OSi$	45.53	+	+	+	+	+	-
10	Cholesta 3,5 diene-7-one	382	$C_{27}H_{42}O$	46.78	+	+	-	+	-	-
11	taraxer-14-ene	410	$C_{30}H_{50}$	47.25	-	-	+	-	-	-
12	olean-12-ene	410	$C_{30}H_{50}$	48.53	+	+	+	+	+	+
13	urs-12-ene	410	$C_{30}H_{50}$	50.13	-	-	+	-	-	-
14	stigmast-4-en -3-one	412	$C_{29}H_{48}O$	50.51	+	+	-	-	-	+
15	β-amyrenone	424	$C_{30}H_{48}O$	51.49	+	+	+	+	-	-
16	lup-20(29)-en-3-one	424	$C_{30}H_{48}O$	52.12	-	+	-	-	-	-
17	taraxerone	424	$C_{30}H_{48}O$	53.13	+	+	+	+	+	+
18	friedelan-3-one	426	$C_{30}H_{50}O$	54.48	+	+	+	+	+	+
19	cholesterol- TMS ether	458	$C_{30}H_{54}OSi$	56.13	+	+	+	+	+	+
20	coprostanol-TMS ether	460	$C_{30}H_{56}OSi$	56.53	-	+	-	-	-	-
21	campesterol-TMS ether	472	$C_{31}H_{56}OSi$	56.87	+	+	+	+	-	-
22	stigmasterol-TMS ether	484	$C_{32}H_{56}OSi$	58.18	+	+	+	+	+	+
23	β-sitosterol-TMS ether	486	$C_{32}H_{58}OSi$	61.15	+	+	+	+	+	+
24	germanicol -TMS ether	498	$C_{33}H_{58}OSi$	62.55	+	+	+	+	+	-
25	β-amyrin-TMS ether	498	$C_{33}H_{58}OSi$	65.08	+	+	+	+	+	+
26	α-amyrin-TMS ether	498	$C_{33}H_{58}OSi$	66.13	+	+	+	+	-	+
27	taraxerol-TMS ether	498	$C_{33}H_{58}OSi$	69.48	+	+	+	+	-	-
28	lupeol –TMS ether	498	$C_{33}H_{58}OSi$	70.89	+	+	+	+	+	+
29	betulin-TMS ether	586	$C_{36}H_{66}O_2Si_2$	72.05	+	+	+	+	-	-

+ detected

-not detected

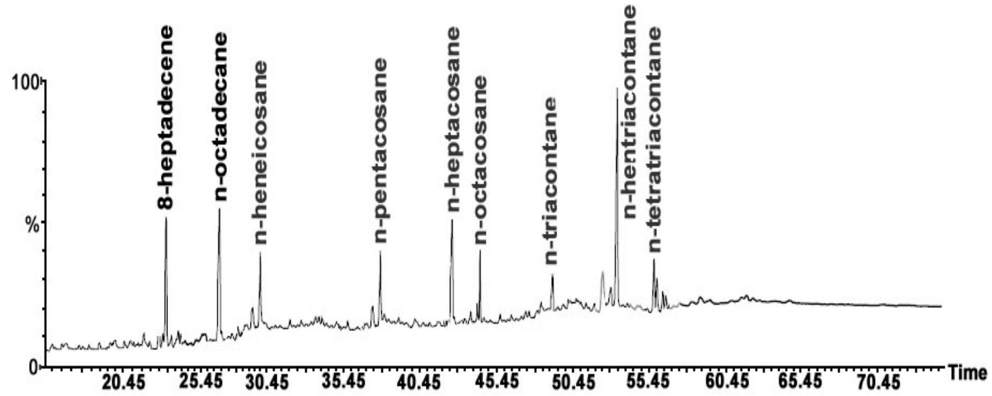


Figure 5.1 Chromatogram for hexane fraction of Puthuvypin (M1)

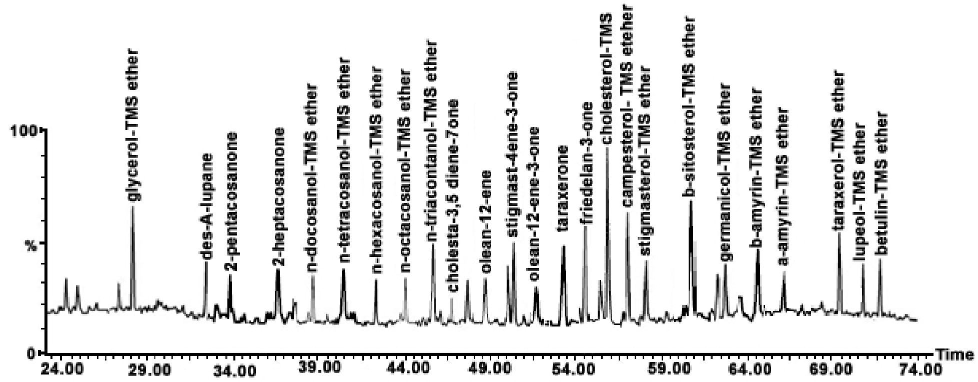


Figure 5.2 Chromatogram for dichloromethane fraction of Puthuvypin (M1)

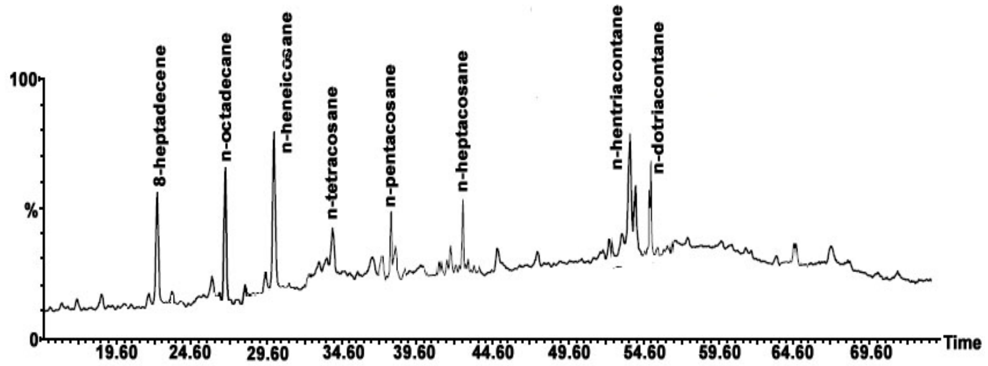


Figure 5.3 Chromatogram for hexane fraction of Murikkumpadam (M2)

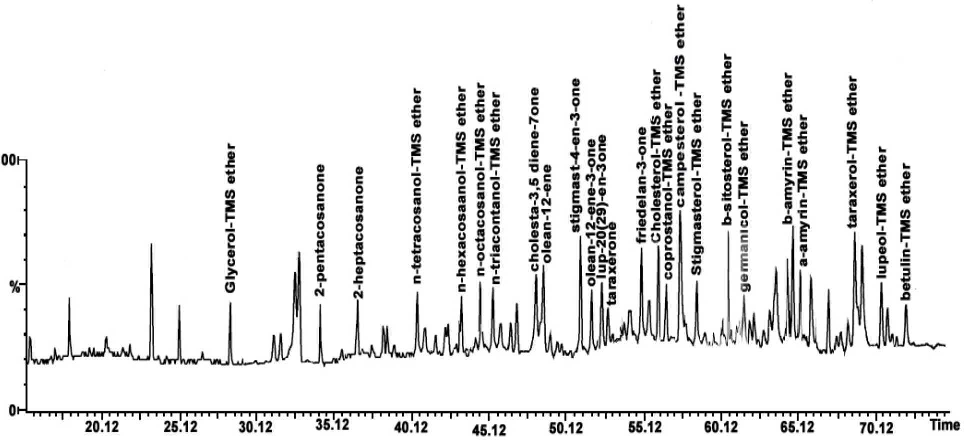


Figure 5.4 Chromatogram for dichloromethane fraction of Murikkumpadam (M2)

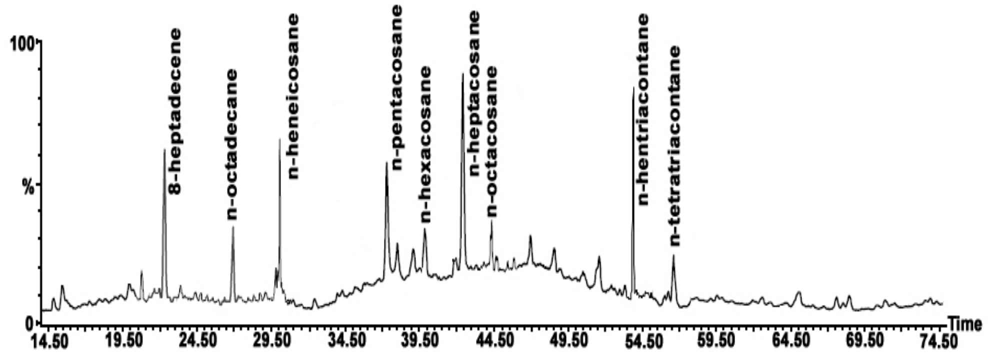


Figure 5.5 Chromatogram for hexane fraction of Mangalavanam (M3)

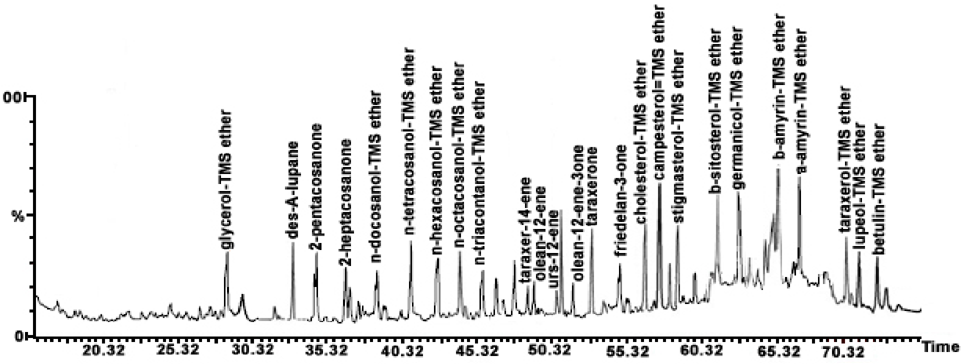


Figure 5.6 Chromatogram for dichloromethane fraction of Mangalavanam (M3)

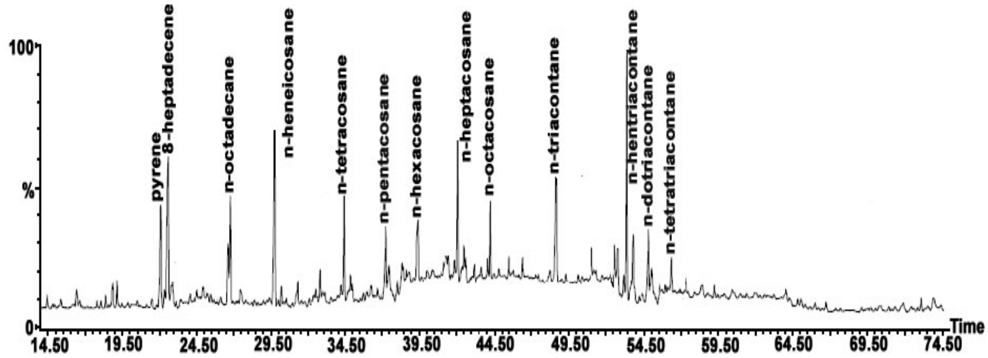


Figure 5.7 Chromatogram for hexane fraction of Bogatty (E1)

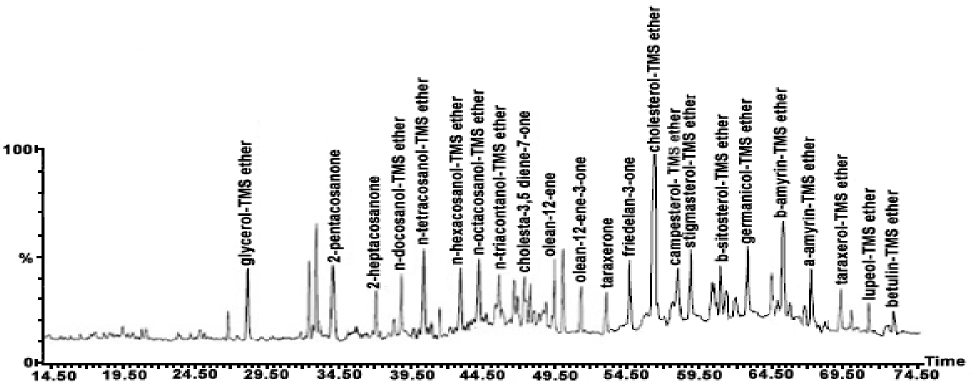


Figure 5.8 Chromatogram for dichloromethane fraction of Bogatty (E1)

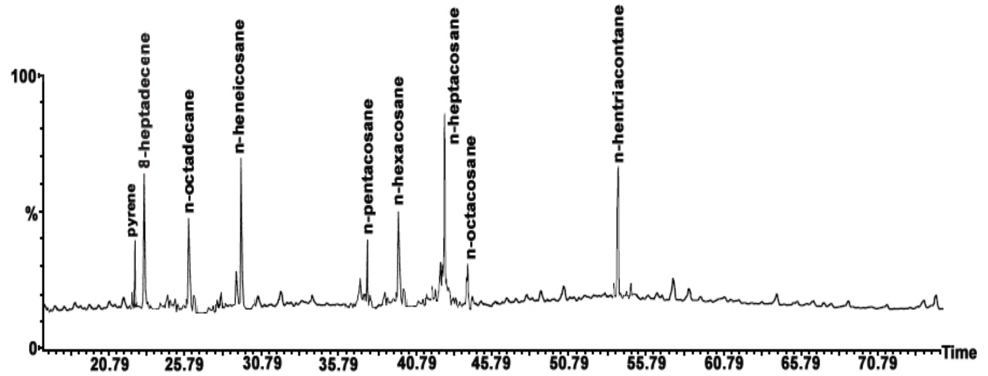


Figure 5.9 Chromatogram for hexane fraction of Ponnarimangalam (E2)

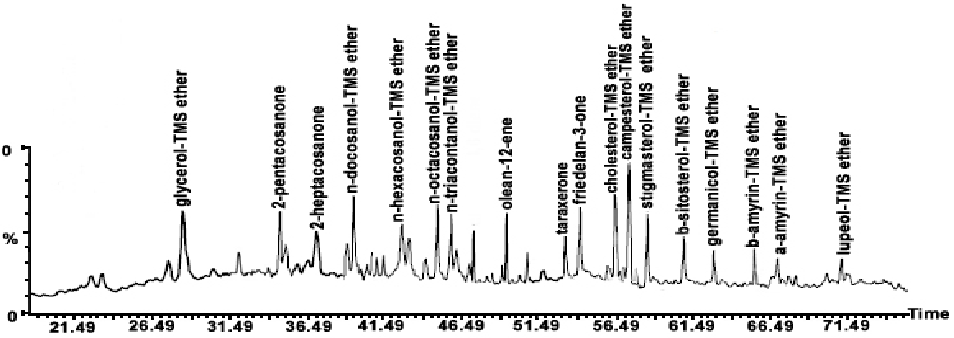


Figure 5.10 Chromatogram for dichloromethane fraction of Ponnarimangalam (E2)

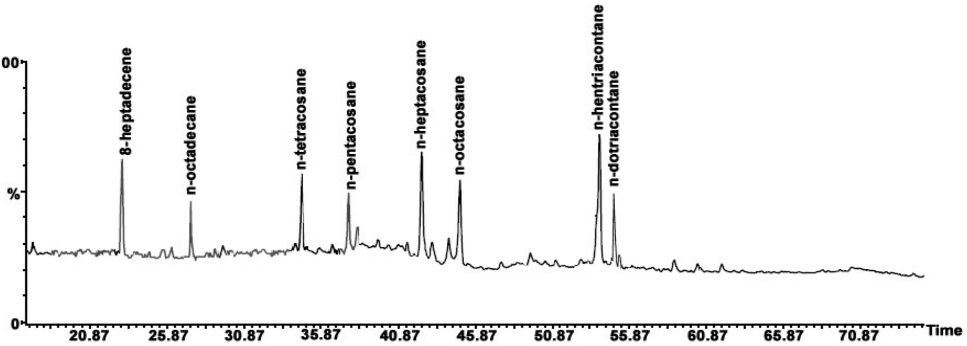


Figure 5.11 Chromatogram for hexane fraction of Mulavukad (E3)

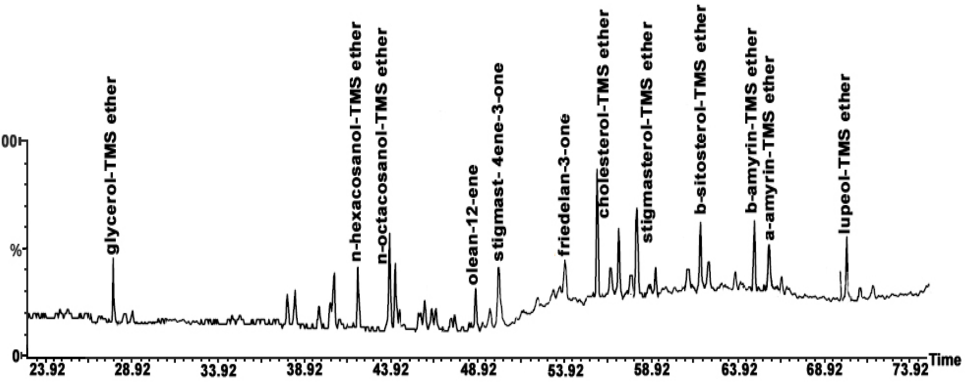


Figure 5.12 Chromatogram for dichloromethane fraction of Mulavukad (E3)

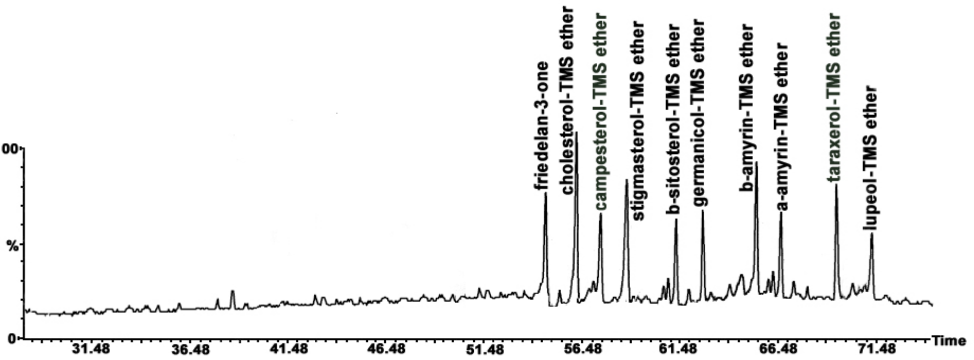


Figure 5.13 Chromatogram for the triterpenoid standards

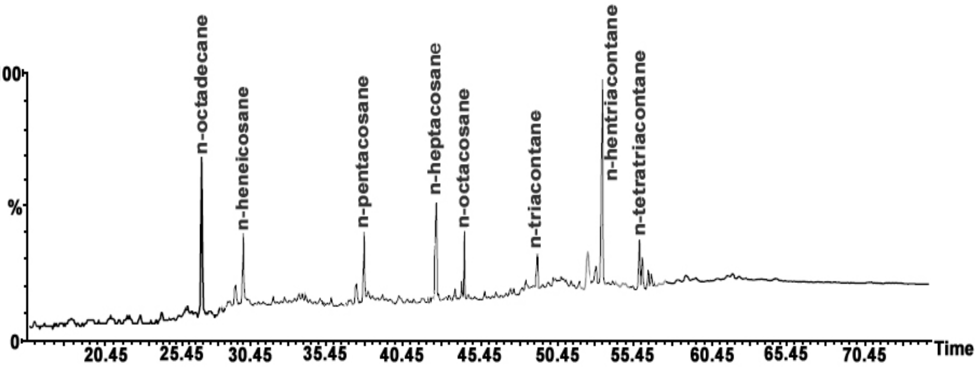


Figure 5.14 Chromatogram for the hydrocarbon standards

5.3 Discussion

5.3.1 Hydrocarbons

Straight chain alkanes (n-alkanes) are common biomarkers found in sedimentary organic matter (Sikes *et al.*, 2009). They are known to be biosynthesized by a wide variety of both marine and terrestrial plants. The presence of n-alkanes in aquatic sediments has been widely used as a proxy to identify possible sources of organic matter, i.e., to distinguish between inputs from terrestrial higher plants and aquatic primary producers (Schubert and Stein, 1997). In higher plants, n-alkanes are derived from epicuticular waxes, have long chain lengths (C25-C35), and an odd over even chain length predominance (Eglinton and Hamilton, 1967). They usually found in the waxy coating of stems, leaves, flowers, or fruit, cover the range to at least n-dohexacontane (Eglinton *et al.*, 1962; Capella *et al.*, 1963; Eglinton and Hamilton, 1967). Because alkanes are less readily assimilated than other food substances and are chemically less reactive than most biological compounds, they are preferentially preserved relative to other organic materials in sedimentary environments. The marked structural and distributional resemblances between certain alkanes in rocks and compounds in organisms have led to the widespread use of alkanes as molecular or chemical fossils (Belsky *et al.*, 1965; Eglinton and Calvin, 1967; Oró and Nooner, 1967).

n-Alkanes exhibit characteristic mass spectra showing a monotonic series of C_nH_{2n+1} ion which decrease in abundance with increasing m/z value. It could be observed from GC-MS analysis that sediment samples from the study area was dominated by a series of n-alkanes ranging from C16 to C34 which reach a maximum at tetratriacontane ($C_{34}H_{70}$). Detection of n-alkanes being performed based on GC retention characteristics of authentic standards and comparison of the mass spectra with published data (Gutierrez *et al.*, 2008; NIST MS Library). Their mass spectral characteristics are summarized in table 5.3.

The detection of 8-heptadecene (C17) was based on the comparison of mass spectrometric fragmentation scheme provided in NIST MS library with prominent ions at m/z 55, 69, 238. n-Alkanes with odd chain such as n C15, n C17 and n C19 are indicative of algal inputs (Han *et al.*, 1968) and are often accompanied in higher abundance by the corresponding alkenes. Thus the occurrence of n-heptadecene in the sediments of study region could be attributed to the contribution from autochthonous algal detritus. It has been found as the major component of algal species recognized in the lagoons (Han and Calvin, 1969; Blumer *et al.*, 1971) and of cyanobacteria (Paoletti *et al.*, 1976; Philip *et al.*, 1978) found in algal mats (Cardoso *et al.*, 1976; Boon *et al.*, 1983).

Table 5.3 Characteristic mass fragments of various hydrocarbons detected in sediments.

Sl.No.	Compound	Molecular formula	MW	Characteristic mass fragments
1	pyrene	$C_{16}H_{10}$	202	200, 201, 202, 203
2	8-heptadecene	$C_{17}H_{34}$	238	55, 69, 238
3	n-octadecane	$C_{18}H_{38}$	254	57, 71, 352
4	n-eicosane	$C_{20}H_{42}$	282	57, 71, 85, 282
5	n-heneicosane	$C_{21}H_{44}$	296	57, 71, 85, 296
6	n-tetracosane	$C_{24}H_{50}$	338	57, 71, 85, 338
7	n-pentacosane	$C_{25}H_{52}$	352	57, 71, 85, 352
8	n-hexacosane	$C_{26}H_{54}$	366	57, 71, 85, 366
9	n-heptacosane	$C_{27}H_{56}$	380	57, 71, 85, 380
10	n-octacosane	$C_{28}H_{58}$	394	57, 71, 85, 394
11	n-triacontane	$C_{30}H_{62}$	422	57, 71, 85, 422
12	n-hentriacontane	$C_{31}H_{64}$	436	57, 71, 85, 436
13	n-dotriacontane	$C_{32}H_{66}$	450	57, 71, 85, 450
14	n-tetratriacontane	$C_{34}H_{70}$	478	57, 71, 85, 478

Among the hydrocarbons, pyrene (C16) was identified by comparison of the mass spectra with published data and the characteristic mass fragments have prominent m/z values at 200, 201, 202, 203 (NIST MS Library). The sediments from estuarine station E1 and E2 exhibited the occurrence of polycyclic aromatic hydrocarbon (PAH) - pyrene. Pyrene is listed as a priority pollutant by the U.S. Environmental Protection Agency and a useful model compound to study higher-molecular-weight PAH behaviour in sediments. This group of nonionic hydrophobic organic contaminants ubiquitously present in coastal areas and formed from anthropogenic activities such as:

fossil fuel burning, the release of uncombusted petroleum products, and creosote wood treatment (Gschwend and Hites, 1981; Wakeham *et al.*, 1980a; 1980b). PAHs are especially problematic because they exhibit toxicity and mutagenicity at very low concentrations and have a high tendency to bind to natural organic matter (Simpson *et al.*, 2005). Its low water solubility and strong affinity for organic matter (Sims and Overcash, 1983; Mackay *et al.*, 1992) contribute to the persistence in sediments.

The sediment samples from the mangroves exhibited high relative abundance for n-alkanes ranging between C23 and C34 revealing odd carbon predominance. In estuarine sediments also odd carbon chain predominance could be distinguished. Long-chain n-alkanes (between C25 and C35), that are characteristic components of epicuticular waxes of mangrove leaf surfaces, can be used as tracers of higher plant remains (Dodd *et al.*, 1998; Rafii *et al.*, 1996; Versteegh *et al.*, 2004). Unusually high concentration of C28 n-alkane in *Avicennia* and *Rhizophora* from French Guiana and the predominance of C31 in plants of these genera in West Africa was also reported [Rafii *et al.*, (1996); Dodd *et al.*, (1998)]. The abundance of C31 relative to C28 *n*-alkanes in the sediment extracts of the study area confirmed the use of odd over even carbon predominance of n-alkanes distribution as indicator of terrestrial organic matter contributions to geological materials (Pancost and Boot, 2004; Jansen *et al.*, 2006; Chikaraishi and Naraoka, 2007). Thus the occurrence of long chain n-alkanes with odd carbon predominance in sediments of the study region could be attributed to mangrove input.

The C-max of the most abundant n-alkane can also be used as an indicator of relative source input (Simoneit, 2002). An even C-max at 16 or 18 is not common for sediments and only a few microbial organisms produce direct inputs of predominantly even carbon chain n-alkanes (Davis 1968; Bird and Lynch, 1974). The odd predominance of n-alkanes is probably due to the early diagenetic defunctionalization of even-numbered alcohols, acids, and esters (Tissot and Welte, 1984). The absence of shorter chain n-alkanes in the present study could be attributed to the fact that they are preferentially removed from sediments during decomposition processes. They are presumed to be more suitable substrates for microorganisms than longer chain homologues due to higher solubility of the former, which probably aids their metabolism by microorganisms (Johns *et al.*, 1978). Longer chain terrestrial homologues may be associated with more refractory biopolymers (McCaffrey *et al.*, 1991), resulting in low susceptibility to degradation.

5.3.2 n-Alkan-2-ones

Ketones are one of the most common and important organic compounds in modern sediments (Wenchuan et al., 1999). The important n-alkan-2-ones detected in the sediments of the present study include those with carbon number C25 and C27 (Table 5.2). These compounds were identified by comparison of the mass spectral data from NIST MS library. The characteristic mass fragments of 2-pentacosanone m/z at 55, 57, 58, 59, 71, 366 and 2-heptacosasone displayed the following mass fragments- 55, 57, 58, 59, 71, 394. n-Alkan-2-ones were thought to be formed by microbial β-oxidation of n-alkanes via the intermediate alkan-2-ol (Cranwell *et al.*, 1987; Rieley *et al.*, 1991). Hernandez *et al.*, (2001) reported the occurrence of n-alkan-2-ones in the dominant plants of South Florida, including freshwater wetland vegetation, seagrasses and mangroves, and suggested these as direct sources of n-alkan-2-ones to sediments. Hence, its presence in the sediments indicated a higher plant input to total organic matter. The most abundant compounds of this class include particularly 2-pentacosanone (C25) and 2-heptacosanone (C27), which are transformation products of terrigenous lipids (Volkman *et al.*, 1983).

Two possible origins of these ketones have to be considered. Firstly, long-chain n-alkanes may be oxidised to the corresponding 2- alkanones as has been suggested by Cranwell (1988). Secondly, 2-alkanones may be formed during β-oxidation of long-chain fatty acids by decarboxylation of intermediate 3-oxo fatty acids.

5.3.3 Fatty Alcohols

Fatty alcohols can generally be classified into three categories; short chain, long chain and branched (-iso and -anteiso) compounds based on their main sources. Short chain (≤C20) fatty alcohols are derived from marine organisms including plankton and microalgae, while long chain fatty alcohols (>C20) are associated with terrigenous inputs, particularly terrestrial plants. Short chain compounds predominantly originate from marine organisms but it is also reported that these compounds are derived from unspecified terrigenous sources (Mudge and Seguel, 1999; Seguel *et al.*, 2001). However, ≤C20 compounds are assumed to predominantly have marine sources such as phytoplankton, zooplankton and bacteria (Mudge and Norris, 1997; Treignier *et al.*, 2006). According to Volkman *et al.*, (1999), zooplankton has been identified as a major contributor of fatty alcohols in marine sediments. Moreover, short chain fatty alcohols are believed to derive from fresh water organisms (Triegnier et *al.*, 2006). Despite there being less attention focused on fatty alcohols as biomarkers, they have been largely accepted as a reliable indicator to assess the marine, terrigenous and bacterial contribution to aquatic sediments (Logan *et al.*, 2001; Mudge and Duce, 2005; Treignier *et al.*, 2006). The n-alcohols are most likely derived from waxes of terrestrial higher plants (Gagosian *et al.*, 1981).

Fatty alcohols were detected as trimethyl silyl derivatives and their identification was being performed by comparison of the obtained mass spectra with NIST MS library and published mass spectral data (Philip, 1985).

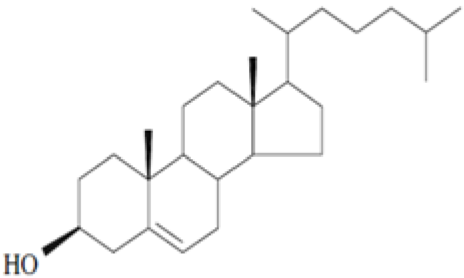
The characteristic mass fragments for fatty alcohols TMS ethers were as follows: glycerol (73, 103, 118, 147, 206, 218); n-docosanol (75, 83, 97, 103, 383); n-tetracosanol (75, 83, 103, 411, 412); n-hexacosanol (75, 83, 103, 440, 441); n-octacosanol (75, 83, 97, 103, 467, 469); n-triacontanol (75, 83, 97,103, 495, 497). The important fatty alcohols detected in the sediments of the study area ranged between C12 and C30 with even over odd carbon predominance. Long-chain n-alkanols (>C20) with a strong even to odd predominance are common constituents of epicuticular higher plant waxes. Their presence in marine sediments has been attributed to inputs from terrestrial vegetation (Eglinton and Hamilton, 1967; Prahl and Pinto, 1987) and therefore, in the present study their occurrence might be attributed to mangrove leaf litter.

5.3.4 Tetracyclic Triterpenoids

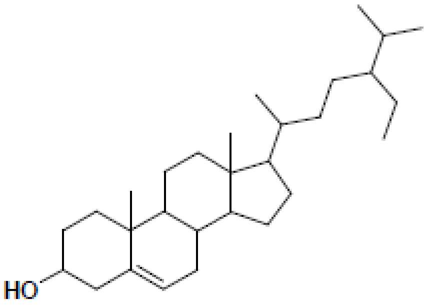
Tetracyclic triterpenoids (steroids) and the compounds derived from them by digenetic reactions are ubiquitous in sediments. Sterols in particular have attracted much attention and have been exploited as source markers for marine particulate and sedimentary organic matter (Nash *et al.*, 2005; Volkman, 2005). Their broad diversity, biosynthetic specificity, and good stability toward diagenetic reworking in marine sediments make sterols excellent indicators for sedimentary contributions of their specific biological sources (Volkman, 1986; Hudson *et al.*, 2001; Nash *et al.*, 2005). Sterols represent a group of geolipid markers that can differentiate between allochthonous, autochthonous and anthropogenic lipid carbon sources in aquatic environments. Steroid compounds are very often used in organic geochemical studies as indicators of input sources and of conditions during diagenesis (Mackenzie *et al.*, 1982; Robinson *et al.*, 1984; Volkman, 1986). Steroid diagenesis and microbial alteration has been described for recent lacustrine and marine sediments (Mackenzie *et al.*, 1982; Volkman, 1986). Important organic geochemical studies based on lipid biomarkers involving triterpenoids in different parts of the world are depicted table 5.4.

Steroids together with terpenoids are typical examples of biomarkers because they contain a high degree of structural information that is retained in the carbon skeleton after sedimentation (Poynter and Eglinton, 1991; Peters *et al.*, 2005) and also provides a chemotaxonomic link between the sedimentary organic matter and the precursor organisms in the biosphere. Sterols are ubiquitous components of cellular membranes in eukaryotic organisms-including phytoplankton, zooplankton and higher plants in which they are known to improve the mechanical properties (eg. fluidity) of the phospholipid bilayers, while prokaryotic organisms (eg. bacteria) do not generally biosynthesize these compounds (Volkman, 1986). The 24-ethyl sterols have been often considered of terrigenous origin, since they are common lipids in higher plant waxes. Many micro algae synthesize C29 sterols and a number of examples are now known where the major sterol is 24-ethylcholesterol or 24-ethylcholesta-5, 22E-dien-3β-ol (Barret *et al.*, 1995), both of which are commonly associated with higher plants (Volkmann, 1986).

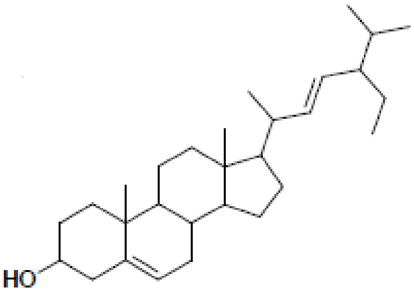
Terrestrial organic matter input can be recognized by the presence of phytosterols in sediments, which are the predominant sterols produced by vascular plants. Although there are more than 40 different phytosterols, the most abundant ones found in vascular plants are β-sitosterol, stigmasterol and campesterol (Tikkanen, 2005; Méjanelle and Laureillard, 2008; Santos *et al.*, 2008). According to Puglisi *et al.*, (2003), these sterols can also be located in seeds, with β-sitosterol being the principal compound.



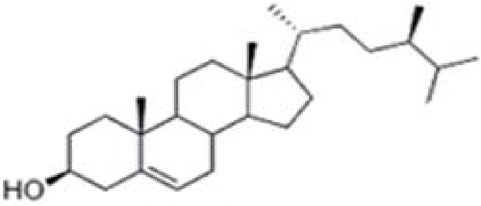
cholesterol



β -sitosterol



stigmasterol



campesterol

The presence of cholesterol was detected as trimethylsilyl ether in all estuarine as well as mangrove stations. The important diagnostic peaks are 458, 368, 329, 255, and 129. Of all the sterol compounds, cholesterol is the most abundant and ubiquitous one in the environment, which is due to it having a variety of sources (Froehner *et al.*, 2008; Pratt *et al.*, 2008). Whilst cholesterol is the main animal sterol (Puglisi *et al.*, 2003), it is also produced by other organisms including diatom, microbial communities, macrophytes, algae, phytoplankton and zooplankton (Logan *et al.*, 2001; Reeves and Patton, 2001; Azevedo, 2003). Thus, the use of cholesterol on its own as a biomarker for organic matter is limited.

Cholesterol content in mangrove species *Bruguiera gymnorrhiza* and *Acanthus ilicifolius* has already been reported (Misra *et al.,* 1984; Hogg and Gillan, 1984). The abundance of *Acanthus* vegetation has been reported in the study area (Subramanian, 2000). In a study by Narayanan, (2006) also reported the presence of cholesterol in the mangrove sediments of Mangalavanam and Vypin region. In the mangrove sediments, the source of cholesterol might be attributed to autochthonous origin, because it is a component of mangrove and is a major faunal dietary transformation product (Narayanan, 2006).

The presence of β-sitosterol (24- ethylcholest-5-en-3β-ol) is taken to be the evidence for contribution from land plants in marine samples. Steroids such as β-sitosterol are known to be abundant in higher land plants (Rieley *et al.*, 1991; Barrett *et al.*, 1995; Meyers, 1997; Sinsabaugh *et al.*, 1997). β-sitosterol was identified in pine wood (Otto and Simoneit, 2002) and in prairie grassland soils (Otto *et al.*, 2005) and this C27 sterol can be considered as a vascular plant marker. It constitutes above 60-75% of the total sterols in different mangrove species (Hogg and Gillan, 1984; Misra *et al.,* 1984; Ghosh *et al.,* 1985). It could also be noted that β-sitosterol degrades more efficiently in the sediments than other sterols such as cholesterol (Koch *et al.*, 2005). β-sitosterol have recently been employed as biomarker for mangroves (Koch *et al.*, 2003). This triterpenoid compound was identified in the sediments of both mangrove and estuarine stations of the study area. It was identified by the GC retention characteristics of the authentic standard as well as mass spectral data. The mass spectrum of the TMS ether of β-sitosterol exhibited prominent m/z values at 486, 396, 381, 357, 356, 129 etc (Brookes *et al.*, 1968). The occurrence of sterols including β-sitosterol could be treated as vascular plant input and in the present study, the source of this sterol in the sediments could be attributed to mangrove detritus (Narayanan, 2006).

Stigmasterol (24-ethylcholesta-5, 22 (E)-dien-3β-ol) is also a mangrove sterol. The presence of this triterpenoid compound was reported in *Avicennia* species (Mahera *et al.*, 2011) and in *Rhizophora* mangle leaves (Killops and Frewin, 1994). The presence of stigmasterol was detected in the surface sediments of both mangrove and estuarine stations. This compound was identified by comparison of GC retention characteristics of the authentic standard and mass spectral data. The diagnostic peaks are at m/z values of 484, 394, 355, 255, 139, 129, 83, 69, 57 (Brookes *et al.,* 1968). The C-24 alkylated sterols are found in marine microorganisms as well as terrestrial plants but the C-24 stereochemistry may be different (Volkman, 1986).

Campesterol (24-methylcholest-5-en-3β-ol) was identified from GC retention characteristics and mass spectral characteristics of authentic standard and published data. The diagnostically significant mass spectral peaks are at m/z values 75,129, 213, 255, 261, 343, 367, 382, 457, 472 etc. (Harvey *et al.*, 1988). This sterol was quantified in mangrove species such as *Acanthus ilicifolius* and *Avicennia marina* (Hogg and Gillan, 1984). Eventhough the abundant species in the mangroves of Cochin region is *Avicennia officinalis* (Sunilkumar and Antony, 1994; Subramanian, 2000), the occurrence of *Acanthus ilicifolius* was found in small patches around the study area. The presence of this sterol was detected in all the mangrove stations, but was detected only at E1 in the case of estuarine sediments. Campesterol and its 24 epimer are not much reported in marine samples. The source of C_{28} sterols is less specific since they are relatively abundant in both algae and terrestrial higher plants. This typical mangrove sterol has already been reported in mangrove sediments of Cochin

(Narayanan, 2006). Its presence in sediments might be attributed to mangrove litter input.

5β-Coprostanol (5β-cholestan-3β-ol) is a C27 stanol formed from the biohydrogenation of cholesterol (cholest-5en-3β-ol) in the gut of higher animals and birds. The compound was identified in sediment extract by the comparison of the mass spectrometric fragments (figure 5.15) described in NIST MS library. Grimalt *et al.*, (1990) proposed the general scheme for its production via a ketone intermediate (figure 5.16). This compound has frequently been used as a biomarker for the presence of human fecal matter in the environment. In the present study, coprostanol was detected only in sediments from station M2 and its source was assigned to anthropogenic (fecal input), because of the huge population density and poor sanitation facilities in this coastal area.

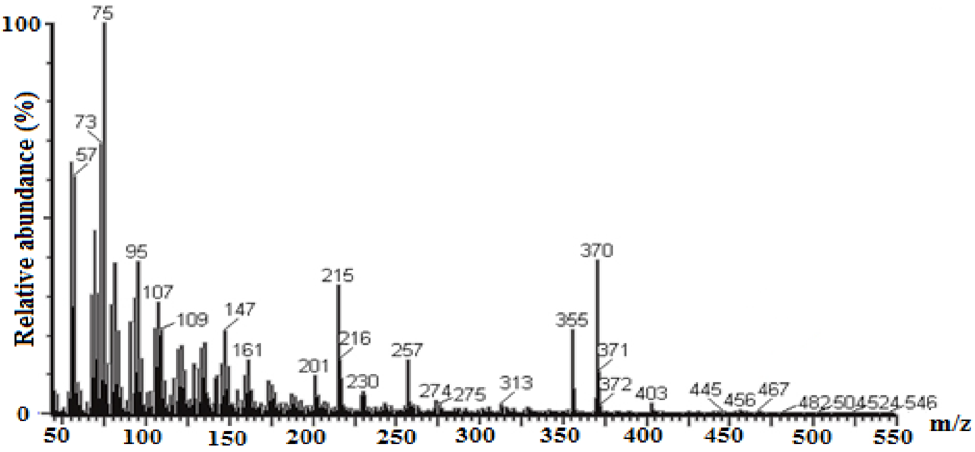


Figure 5.15 Mass spectrum of coprostanol-trimethyl silyl ether

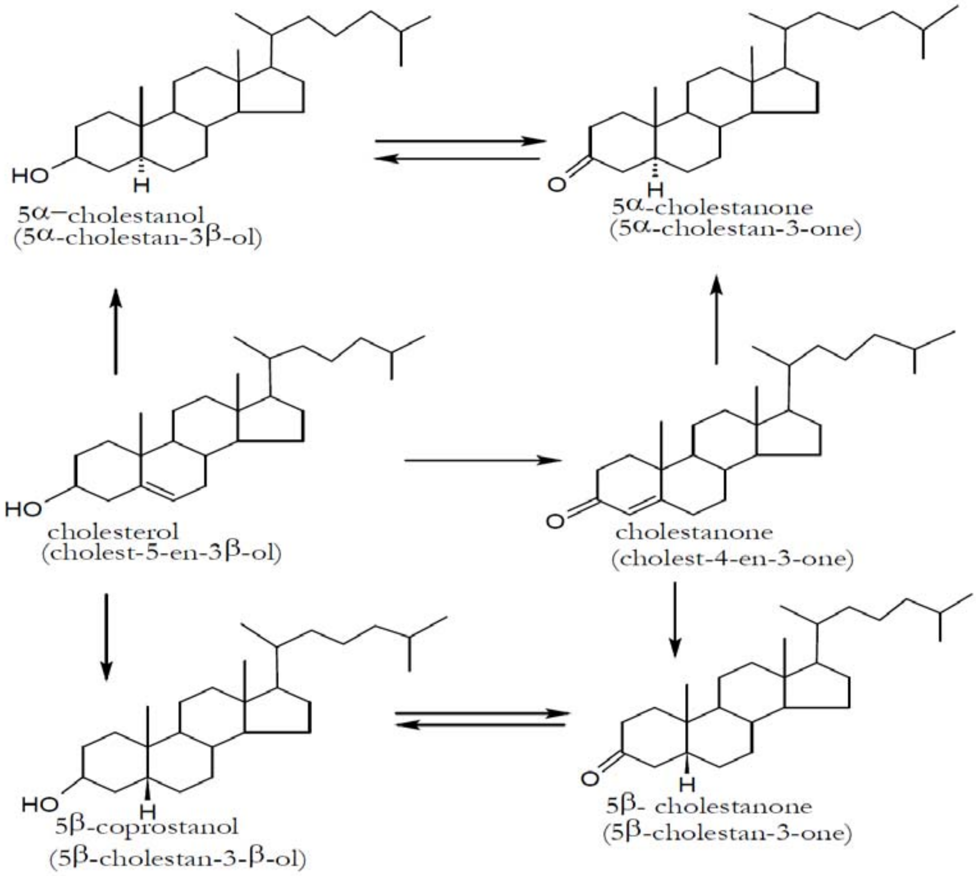
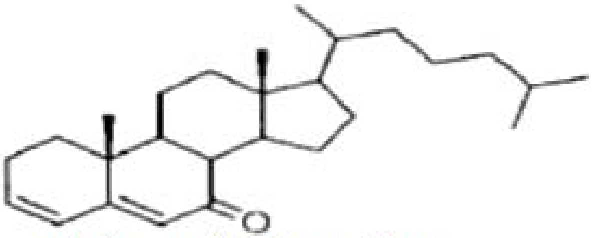


Figure 5.16 General scheme for the formation of coprostanol

Steroidal ketones are often found in marine environments and their distributions reflect early stage oxidation of the sterols present (Gagosian and Smith, 1979). Their occurrence in marine sediments also point towards direct inputs from microalgae such as the dianoflegellates. Δ^5 unsaturated sterols can be converted to Δ^{4-3}-one stenones in sediments by microbial processes similar to those operating in the rumen (Parmentier and Eyssen, 1974). Reduction of these unsaturated steroidal ketones can then give rise to both 5α (H) and 5β (H)-stanols. The important steroidal ketones detected in the sediment samples include cholesta-3, 5-dien- 7-one (C27) and stigmast-4-en -3-one (C29). The former was reported in stations M1, M2 and E1 and the latter was found in the sediments from M1, M2 and E3. These compounds were identified by comparison of the obtained mass spectra with the published data (NIST MS Library). These compounds could be readily recognized by a base peak at m/z 124 in the mass spectrum. Cholesta-3,5diene-7one exhibited characteristic mass fragments at 124 134,159,161,174, 187, 269, 280, 367, 382 etc. Fragmentation pattern of stigmast-4-en-3-one revealed the characteristic mass fragments at 124, 229, 289, 370 and 412. Steroidal ketones in geological samples may arise from direct input of living organisms and/or from microbial/chemical degradation of sterols (Gagosian *et al.*, 1982, Nishimura, 1982; Mermoud *et al.*, 1984). As 5β-homologs have not been detected in biological precursors of sedimentary organic matter (de Leeuw and Baas, 1986), direct inheritance from living organisms is indicated by the nearly exclusive presence of compounds with a 5α configuration, as are found in the sediment samples of the study area. Oxidation products of biogenic lipids such as cholesta-3, 5-dien-7-one, is a product of the oxidative degradation of cholesterol (Meyers and Ishiwatari, 1993).



cholesta- 3,5-diene-7one

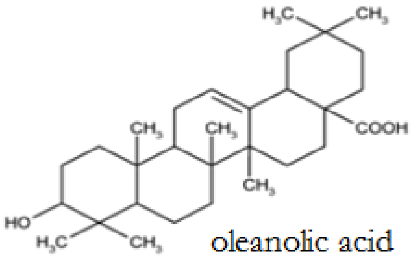
Table 5.4 Some important studies based on lipid biomarkers involving triterpenoids in different parts of the world.

Sl. No.	Biomarker compounds detected and Sample analysed	Study Area	References
1	Triterpenoids in sediments	Peru upwelling	Volkman *et al.*, 1987
2	Triterpenoid and steroid hydrocarbons, sterols and steroid ketones in sediments	Bransfield Strait, Antarctica	Brault and. Simoneit,1988
3	Triterpenoids in Deep Sea sediments	Baffin Bay	ten Haven *et al.*, 1992b
4	Pentacyclic triterpenols, Δ^5 sterols in leaves, sea grass rhizomes and sediments	Everglades and Florida, USA	Killops and Frewin, 1994
5	*n*-alkane (odd-carbon predominance),triterpenoids such as α-amyrin, β-amyrin, lupeol, taraxerol, taraxerone and friedelin and sterols in sediments	Swinn plate Back barrier area near Spiekeroog Island in the northwest German Wadden Sea	Volkman *et al.*, 2000
6	n-alkanes (C16 to C35 with odd carbon predominance), diterpenoids, steroids and pentacyclic triterpenoids of the oleanane, ursane, lupane, and friedelin classes in Fossil conifer species and sediments.	Eocene Zeitz formation, Germany	Otto and Simoneit, 2001.
7	Pentacyclic triterpenoids include: β-amyrin, germanicol, taraxerol, lupeol, betulin in Mangrove leaves, sediment, and excrement from the mangrove crab Ucides cordatus.	Coastal areas of the Bragança peninsula in North Brazil.	Koch *et al.*, 2003
8	Long-chain n-alkanes,triterpenoids such as friedelin, β-amyrenone and α-amyrenone in sediments	Lake Albano, central Italy	Hanisch *et al.*, 2003
9	Aliphatic hydrocarbons, alcohols and fatty acids, sterols in core sediment samples	Macaé, Rio de Janeiro, Brazil	Zink *et al.*, 2004
10	β-sitosterol,taraxerol,α-amyrin, germanicol, lupeol and betulin. Leaves of Rhizophora mangle, Avicennia germinans and Laguncularia racemosa incubated with surface sediment.	Belem (Para) and Sao Luis (Maranhao), Brazil	Koch *etal.*,2005
11	Aliphatic lipids, steroids, diterpenoids and polar triterpenoids of the oleanane, lupane, ursane and friedelane classes. Conifer and angiosperm macrofossils and the clay sediment.	Miocene Clarkia Formation, Idaho, USA	Otto *et al.*, 2005
12	Sterols in Mangrove sediments	Mangroves of Vypin and Mangalavanam, Kochi, Kerala	Narayanan, 2006
13	n-Alkanols, steroids and triterpenoids in sediments	Mesopotamian marshes of Iraq	Rushdi *et al.*, 2006
14	Triterpenoids of the oleanane (28-norolean-17-en-3-one, β -amyrin), ursane (α -amyrin) and lupane (lupeol, betulin) series, as well as steroids (α-sitosterol and stigmasterol). Leaf lipids of two angiosperms plant species, sediments and fossil fuels.	Ibadan, Nigeria	Sonibare and Sojinu, 2009
15	Pentacyclic and tetracyclic triterpenoids, n-alkanes, n-alkan-2-ones and fatty alcohols . Mangrove and estuarine sediments	Mangrove and estuarine stations around Kochi, Kerala	Present study

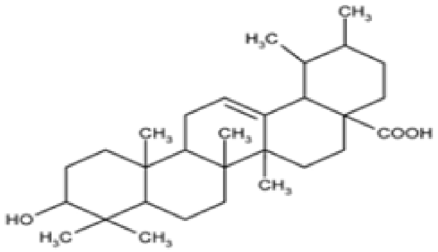
5.3.5 Pentacyclic Triterpenoids

Pentacyclic triterpenoids have frequently been used to characterize sources of sedimentary organic matter as they are common constituents of plants. A variety of pentacyclic triterpenoids with oleanane, ursane, tarexerane, lupane and friedelane skeleton have been identified in higher plants but not in marine organisms. The major triterpenoid alcohols identified in the sediment extract were β-amyrin, α-amyrin, lupeol , and betulin. These triterpenoids can serve as precursors for many biomarkers found in geological samples. In sediments, the most frequently encountered members of these terregeneous biological markers are those having β-amyrin (oleanane) and α-amyrin (ursane) skeleton (Brassell and Eglinton, 1986).

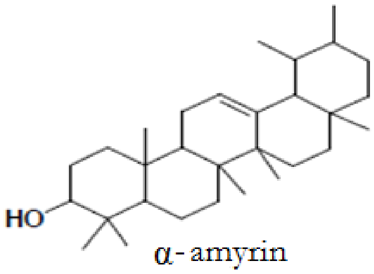
Amyrins are abundant naturally occurring two isomeric pentacyclic triterpenoids. α-Amyrin has the 29-carbon at the 20th position. β-amyrin has the 29th carbon shifted to the 19 position. Alpha amyrin is a precursor of ursolic acid that is obtained by converting the CH_3 in 28 to COOH. The presence of pentacyclic triterpenoids α-amyrin and β-amyrin were detected in sediment samples collected from both mangrove and estuarine stations. Identification of these compounds was performed by GC retention characteristics of the authentic standards and mass spectral data. The mass spectrum of β-amyrin is depicted in figure 5.17. These compounds were employed as biomarkers of organic matter derived from mangroves, in various sedimentary environments (Killops and Frewin, 1994; Koch *et al.*, 2003). Presence of these compounds in estuarine sediments pointed towards the export of higher plant organic matter from mangrove ecosystems to estuarine environment.



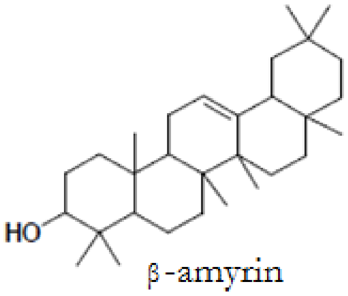
oleanolic acid



ursolic acid



α- amyrin



β -amyrin

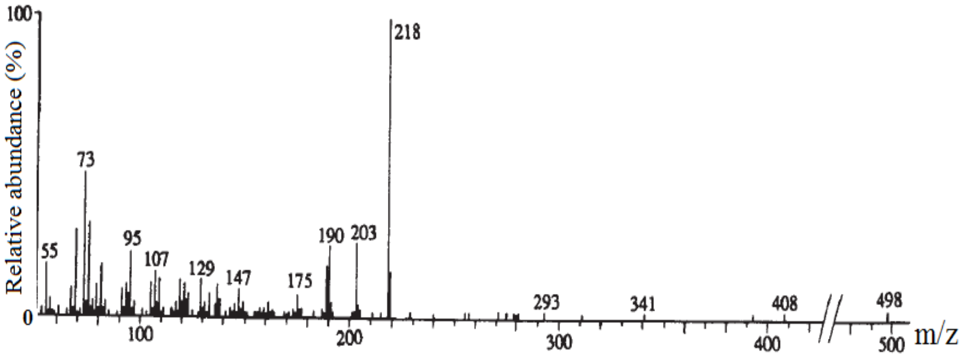


Figure 5.17 Mass Spectrum of β-amyrin (olean-12-en-3β-ol) as TMS ether.

Germanicol, an important pentacyclic triterpenoid of the oleanane series was found in all mangrove stations but was not detected at station E3. GC-MS analysis provided characteristic mass fragments for the standard silylated compound of germanicol having prominent m/z values at 498,483, 393, 369, 279, 231, 204, 189 etc. Figure 5.18 provides the important mass fragments of the silylated compound. This triterpenoid compound was reported as a biomarker for organic matter derived from *Rhizophora* mangle (Koch *et al*., 2003; Killops and Frewin, 1994). The presence of this compound in sediments can therefore be regarded as vascular plant input especially from mangrove vegetation.

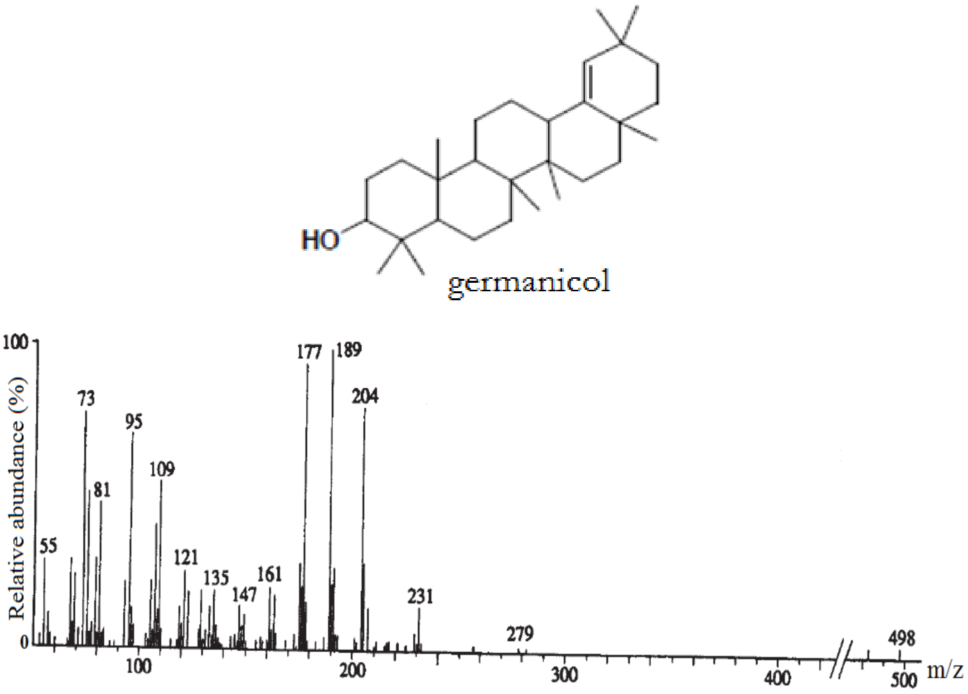
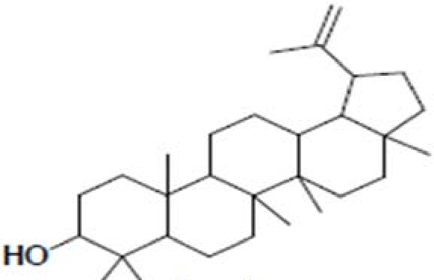
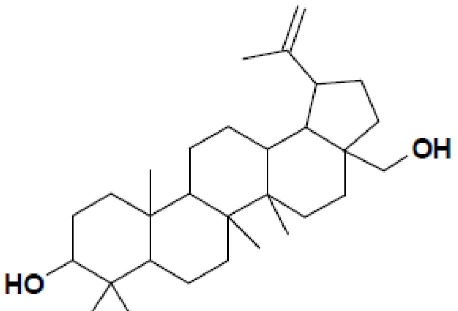


Figure 5.18 Mass Spectrum of germanicol (olean-18-en-3β-ol) as TMS ether

Lupeol, a biomarker for terrestrial higher plant was detected at all stations of the study area indicating angiosperm input to the total organic matter. It was identified using the retention characteristics of the silylated standard compound exhibiting characteristic m/z values at 498, 483, 408, 369, 279, 231, 218, 203, and 189 (Figure 5.19). This compound has been reported in the leaves of *Avicenia marina* (Wannigama *et al.*, 1981). The important pentacyclic triterpenoid diol of the lupane series, betulin was found in all the stations of the study area except at E3. The identification of the compound was achieved by comparison of mass spectrometric fragmentation pattern with published spectra (Philip, 1985). The mass spectra of the silylated compound display abundant fragments at m/z 109,135,189, 203, 229, 279, 293, 337, 367, 393, 483, 496, etc. Its presence was reported in earlier studies of the mangrove species *Rhizophora mucronata* (Ghosh *et al.*, 1985) and *Avicennia germinans* (Koch *et al.*, 2003). The presence of the major triterpenoid in estuarine and mangrove sediment samples provide a convenient marker for higher plants. The occurence of the dihydroxy triterpenoid betulin in estuarine sediment E1 provide strong supporting evidence for a mangrove origin. Betulin was not detected in the estuarine sediments from E2 and E3, possibly due to a greater chemical lability of this compound with two functional groups (Koch *et al.*, 2003).



lupeol



betulin

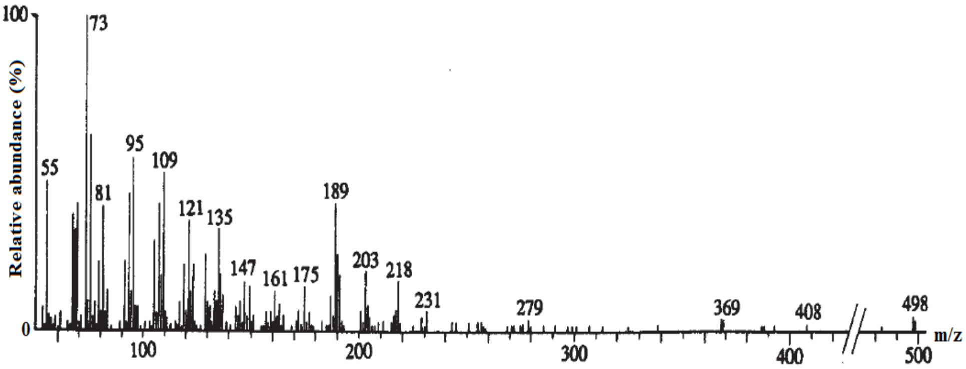
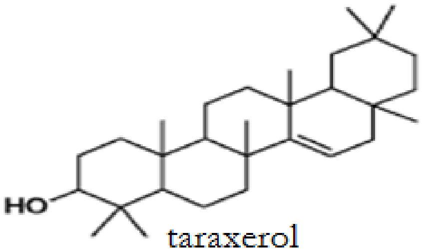


Figure 5.19 Mass Spectrum of lupeol (lup-20(29)-en-3β-ol) as TMS ether

The presence of the pentacyclic triterpenoid taraxerol was detected in the sediments of all stations of the study area except at E3. The identification of this compound was performed with GC retention characteristics of authentic standard and the mass spectral data of the silylated compound exhibiting prominent m/z values at 498, 408, 393, 369, 279, 204, 189, 161 (Figure 5.20). This marker compound has been identified in a wide variety of higher plants (Vilegas *et al.*, 1997; Cordeiro *et al.*, 1999; Setzer *et al.*, 2000). Taraxerol is regarded as a useful marker for mangrove-derived lipids in the sediments. Although taraxerol is of minor importance in the epicuticular waxes of *Rhizophora* species (Ghosh *et al.*, 1985; Rafii *et al.*, 1996; Dodd *et al.*, 1998), it has been reported to dominate the free and bound lipid composition inside *Rhizophora* mangle leaves (Killops and Frewin, 1994), and it accounts for 2.3% of the free non-polar compounds of leaves and stems of the same species (Williams, 1999). High taraxerol levels in sediments have been found associated with mangrove deposits (Middelburg *et al.*, 1993; Johns *et al.*, 1994). Since taraxerol is from the plant inside, rather than from its surface, it will be transported to the sediment largely in plant fragments such as leaves and leaf fragments rather than as an evaporated, wind-transported surface wax.



taraxerol

Taraxerol is found to be more resistant to microbial degradation compared to other oleanane type triterpenoids such as β-amyrin or germanicol, which makes this compound more ideal as a tracer of mangrove-derived organic matter (Killops and Frewin, 1994; Versteegh *et al.*, 2004; Koch *et al.*, 2005). Better preservation of taraxerol in sedimentary environments might be due to the presence of 13α-methyl group that hinder microbial enzyme processes (Koch *et al.*, 2005). In the leaf cuticle of *Rhizophora* mangle, β-amyrin was largely confined to epicuticular wax, while taraxerol appeared to be a cutin component (Killops and Frewin, 1994). Extraordinarily high taraxerol content was reported in the leaves of *Rhizophora* mangle, especially *Rhizophora racemosa* [Versteegh *et al.*, (2004)]. This triterpenoid compound was the main component in the mangrove sediments collected from the mangrove dominated region of Brazil and was proposed as a marker for mangrove-derived organic matter [Koch *et al.*, (2003)]. The predominance of taraxerol in the core sediment collected from Amazon Fan could be attributed to greater mangrove productivity or erosion of mangrove sediments [Boot *et al.*, (2006)].

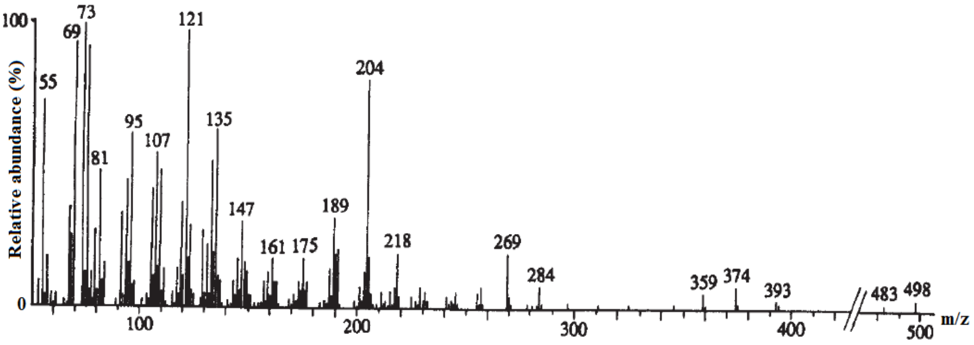
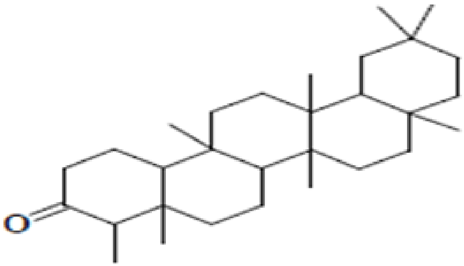


Figure 5.20 Mass Spectrum of taraxerol (taraxer-14-ene-3β-ol) as TMS ether.

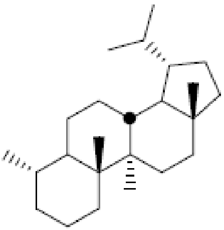
Friedelan-3-one (friedelin) was identified using the GC retention characteristics of the standard compound as well as the mass spectrometric fragmentation pattern. The characteristic mass fragments of this compound exhibited prominent m/z values at 69, 81, 96,109,125, 205, 273,302,411, 426 [Budzikiewicz *et al.*, (1963); Bardy, *et al.*, (2009)]. Its occurrence was noticed in all stations of the present study. Friedelin, the triterpenoid biomarker was reported in fluvial and lacustrine sediments and its origin could be attributed to bark and leaves of numerous angiosperms (Corbet *et al.*, 1980; Logan and Eglinton, 1994; Jaffe *et al.*, 1996; Otto and Simoneit, 2001). Therefore its presence in sediments of the study region indicated mangrove input to the sedimentary organic matter.



friedelin

Early diagenetic degradation products of triterpenoids including des-A-triterpenoid hydrocarbons, triterpenes and triterpenoid ketones were also present in the sediments of the study region. These compounds are thought to be produced by microbial/photochemical processes from their precursors (ten Haven and Rullkotter, 1988; Trendel *et al.*, 1989; ten Haven *et al.*, 1992a, 1992b). Reductive alteration generally yields the parent compound skeleton with various isomerizations of chiral centers and, in some cases, loss of carbon due to decarboxylation and other reactions. Under aerobic conditions, the transformation of plant-derived triterpenoids often involves oxidation, dehydration, hydrolysis, decarboxylation, ring opening, and aromatization reactions (Simoneit, 1998). Oxidative alteration occurs mainly by successive ring aromatization that usually commence from a ring that has a functional group (eg. OH, C=C, C=O; typically on ring-A) by direct dehydrogenation, dehydration, ring rearrangement, or ring opening and subsequent loss. These alterations can occur during transport, by diagenesis in sedimentary environments, or by thermal transformation processes (Versteegh *et al.*, 2004).

The Compound 10β-des-A-lupane (C24) was identified by comparison with published mass spectra (Corbet, 1980; Philip, 1985; Trendel *et al.*, 1989; Woolhouse *et al.*, 1992). Des-A-lupane, has been considered as product of photochemical or photomimetic degradation at ring-A, commencing with the oxygen functionality at C-3 of certain triterpenoids from higher plants (Corbet *et al.*, 1980; Simoneit, 1986). Des-A-triterpenoid hydrocarbons have been observed in petroleum and various sediments and are thought to be originated from photochemically and/or, more likely, microbially- mediated oxidative loss of the A-ring from 3-oxygenated pentacyclic triterpenoids as in the case of α and β amyrin (Corbet *et al.*, 1980; Trendel *et al.*, 1989). Des-A-triterpenoids have also been suggested to be formed through diagenetic transformations of their parent triterpenoids (Corbet *et al.*, 1980; Jaffé and Hausmann, 1995; Jaffé *et al.*, 1996, 2006; Yanes *et al.*, 2006). The presence of these compounds not only testifies to the input of angiospermous organic matter but also tends to confirm the loss of ring A as a common transformation pathway of higher plant triterpenoids (Corbet *et al.*, 1980; Trendel *et al.*, 1989).

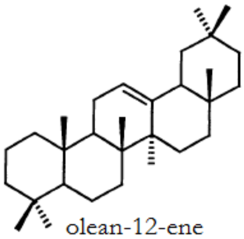


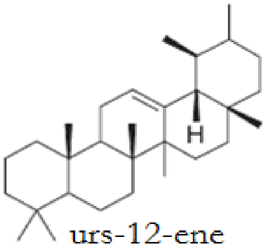
des-A-lupane

The almost uniqueness of des-A-lupane in the specified samples can result from its preferential formation or peculiar resistance to diagenetic transformations. In contrast to the most common higher plant pentacyclic triterpenes (for example, α- and β-amyrins), biomolecules with a lupane structure often own an external double bond on the isopropyl group. Des-A lupane was identified by comparison of the mass spectra with published data (Logan and Eglinton, 1994; Philip, 1985; Trendel *et al.*, 1989; Woolhouse *et al.*, 1992). The mass spectrum of the compound exhibited prominent ions at m/z 163, 149, 191, 177, 206, 287, 217, 315, 330. The detection of various byproducts of biological triterpenoid (des-A triterpene) in the sediment samples provided an insight into origin and dynamics of these compounds in the sedimentary environment. Des-A triterpenes and aromatic derivatives of triterpenes are both produced in anoxic or at least dysaerobic environments and their respective dynamics depend on early digenetic transformation pathways (Jacob *et al.*, 2007). Triterpanes of the oleanane, ursane and lupane series reported in crude oils and sediments are believed to be derived from oxygenated triterpenoids in angiosperms (Rullkötter *et al.*, 1994; Otto and Simoneit, 2001; Otto and Wilde, 2001).

Olean-12-ene ($C_{30}H_{50}$) with a molecular mass of 410 was tentatively identified in all the stations both in mangroves and estuaries. The characteristic mass fragments of this compound have prominent ions with m/z values at 410, 395, 218, 203, 191, 189, 177 [Karliner and Djerassi, 1966; Kashirtsev *et al.*, (2008)]. The mass spectrum of olean-12-ene and urs-12-ene are represented in figures 5.21 and 5.22. The base peak of olean-12-ene and urs-12-ene is derived from the D/E-rings by cleavage through ring C, which involves a retro-Diels-Alder reaction. The fragment at m/z 191 is derived from the A/B-rings (Karliner and Djerassi, 1966). The mass spectrum of olean-12-ene shows a more intense m/z 203 fragment (35%) than the mass spectrum of urs-12-ene (16%), a phenomenon also observed in the mass spectra of olean-12-ene and urs-12-ene, respectively Karliner and Djerassi, (1966). In the present investigation urs-12-ene was detected only at mangrove station M3 and it was tentatively identified by the comparison of the mass spectra with published data. Characteristic mass fragments have m/z values at 410, 395, 218, 203, 191, 189, 177 etc. as described by Karliner and Djerassi, (1966).

It is already reported that olean-12-ene and urs-12-ene are mainly alteration products of β- and α-amyrin respectively [ten Haven e*t al.*, (1992b)]. Oleanene is thought to be derived from pentacyclic triterpenoidal alcohols such as β-amyrin, which is in certain angiosperms (ten Haven and Rullkötter, 1988; Moldowan *et al.*, 1994). The occurrence of triterpenes, especially the defunctionalized, unsaturated and monoaromatic oleanane or ursane, are found mostly in the Dicotylodonea, angiosperm dicots. Only a few gymnosperm species containing these compounds are known (Otto *et al.*, 2005; Stefanova *et al.*, 2005). The early diagenetic transformation of higher plant triterpenes preferentially leads to aromatic or des-A triterpenes rather than to unsaturated or saturated pentacyclic compounds (Jacob *et al.*, 2007).


olean-12-ene


urs-12-ene

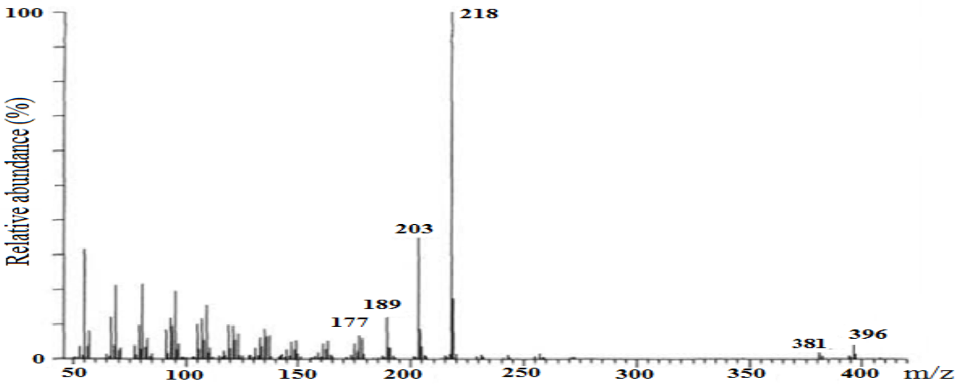


Figure 5.21 Mass spectrum of olean-12-ene

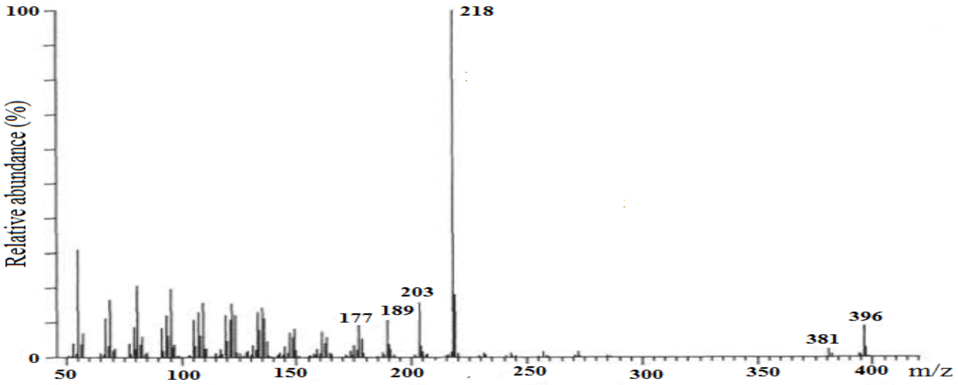


Figure 5.22 Mass spectrum of urs-12-ene

In sedimentary environments, diagenetic transformations of the pentacyclic triterpenoids take place (ten Haven and Rullkotter, 1988; Ekweozor and Telenaes, 1990; Rullkotter *et al.*, 1994) and the important processes are represented in Figure 5.24. Diagenetic reactions leading to the conversion of taraxerene into olean-12-ene in the Baffin Bay sediments was assessed by ten Haven and Rullkötter, (1988). Taraxeroid skeleton may be converted to the oleanoid facile acid- catalysed isomerisation of taraxerol to β-amyrin, or taraxer-14-ene to olean-12-ene (ten Haven and Rullkötter, 1988). Thus, the presence of taraxer-14-ene in sediments of mangrove station M3 might be attributed to mangrove origin. The fact that taraxer-14-ene was not detected in other stations could be due to its rapid transformation to diagenetic intermediates ie. isomerisation of taraxer-14-ene to olean-12-ene (Figure 5.24). The compound was identified by comparison with published mass spectral data (NIST MS Library) and the mass spectrum of the compound is shown in figure 5.23. The presence of taraxer-14-ene in sediments could be indicative that the mangrove derived organic matter is prone to early diagenetic transformations (Neto *et al.*, 2006).

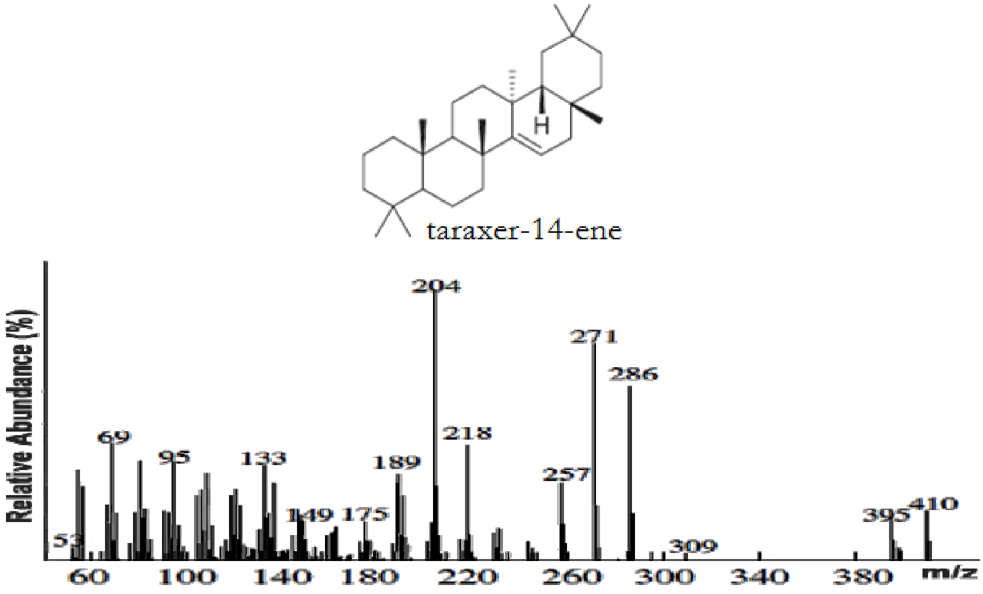


Figure 5.23 Mass spectrum of taraxer-14-ene

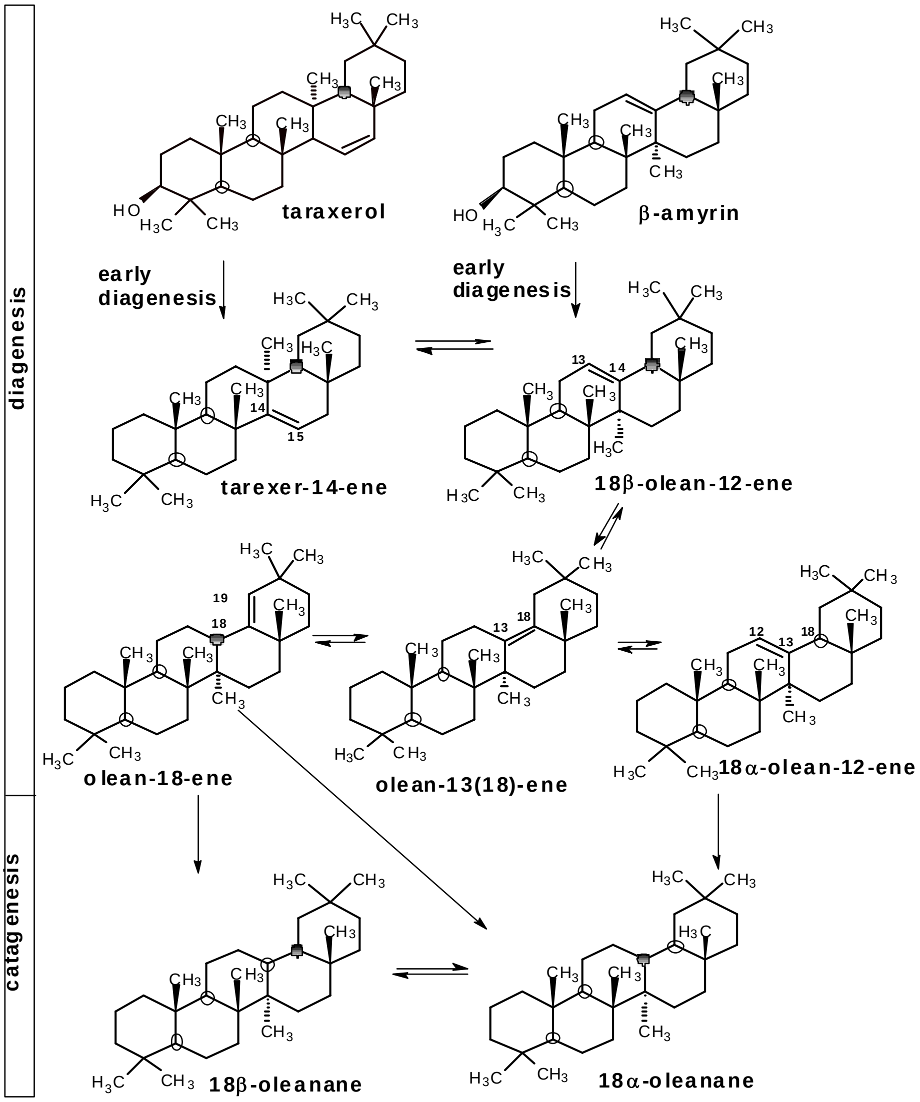
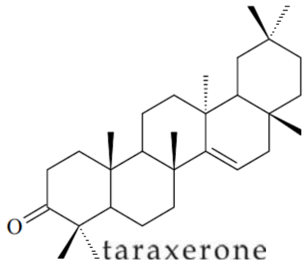


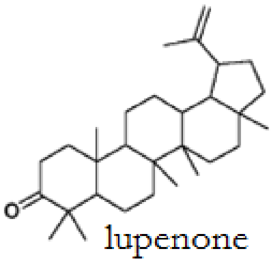
Figure 5.24 Formation of olean-18-ene and 18α-olean-12-ene from higher plant triterpenoids (ten Haven and Rullkotter, 1988; Ekweozor and Telenaes, 1990; Rullkotter *et al.*, 1994).

Among the triterpenoid ketones detected in the present investigation, taraxerone was found in the sediments of all the stations except at E3. The compound was identified tentatively by the comparison of mass spectrometric fragmentation pattern with published spectra [Kashirtsev *et al.*, (2008), NIST MS Library]. The mass spectrum displayed intense fragments at m/z 69, 95,109, 189, 191, 203, 205, 273, 409, 424 (Figure 5.25). Taraxerone was estimated in mangrove leaves and epicuticular waxes (Killops and Frewin, 1994; Simoneit *et al.*, 2009).

The occurrence of lupenone (lup-20(29)-en-3-one) was observed only at mangrove station M2. Lupenone, a component of higher plant bark, was identified by comparison of mass spectra with published data. The characteristic fragment ions have m/z values at 189, 205, 218, 313, 409, 424 [Bardy *et al.*, (2009)]. Lupenone was identified as a component in the leaves of *Bruigueira gymnorrhiza* (Basyuni *et al.*, 2007). Their occurrence in sediments could be attributed to vascular plant input.



taraxerone



lupenone

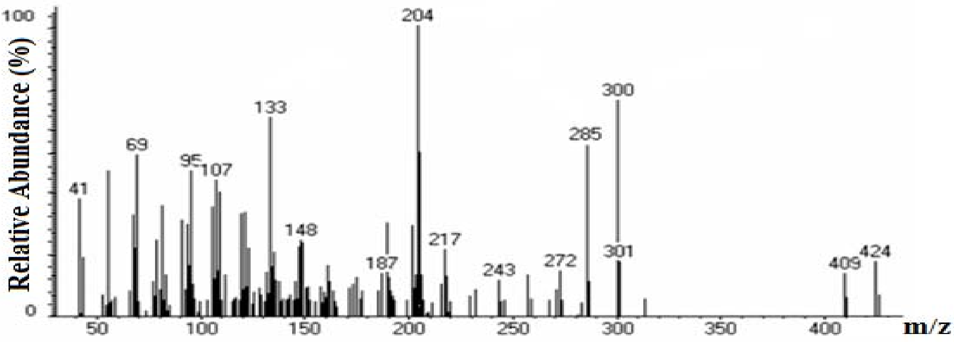


Figure 5.25 Mass spectrum of taraxer-14-ene-3-one (taraxerone)

Olean-12-en-3-one (β-amyrenone) was tentatively identified in the present study by the characteristic mass fragments of this compound 424, 409, 218, 203, 189, 147, 107, 73, 55 etc. [Hemmers *et al.*, (1989)]. β-Amyrenone and α-amyrenone are frequent constituents of higher plants, notably in epicuticular waxes and in the tree bark of angiosperms (Hemmers et al., 1989; Corbet *et al.*, 1980; Tulloch, 1982; Otto and Simoneit, 2001). β-Amyrenone and α-amyrenone could have been formed by oxidation of the corresponding β-amyrin and α-amyrin (Corbet *et al.*, 1980). Derivatives from β-amyrin are predominant over derivatives from α-amyrin due to the fact that this alcohol could be degraded more preferentially than α-amyrin (Dastillung, 1976; Corbet, 1980). Indeed, the double bond in β-amyrin is less hindered than in α-amyrin. So functionalization and the subsequent evolution of derivatives of β-amyrin would be easier than those of α-amyrin. This explanation could be corroborated by the fact that only derivatives of β-amyrin are encountered in crude oils of the Mahakam Delta (Garrigues, 1985). Another explanation could be a higher abundance of β-amyrin in higher plants in comparison to α -amyrin (De Mayo, 1959).

The predominant long-chain aliphatic lipids, tetracyclic triterpenoids (phytosterols) and pentacyclic triterpenoids indicated a major input of vascular plant wax lipids into the organic and mineral horizons of the analyzed mangrove sediments. The identified individual aliphatic lipid compound classes such as n-alkanes, fatty alcohols, and triterpenoids are common constituents of leaf waxes of higher plants and their presence in the mangrove sediments provides information regarding the source of these compounds to sedimentary environments. The export of organic matter from mangrove systems to estuarine stations can be evident from the presence of the typical mangrove biomarker molecules such as tarexerol. Thus tritererpenoid biomarkers typical for angiosperms (oleananes, ursanes, lupanes) allowed the assignment of sedimentary organic matter to the plant group and to assess contributions of angiosperms to the sediment samples. Their presence is often used as a diagnostic tool for terrestrial organic matter supply, usually in a qualitative manner, although higher plant biomarkers have also been used to quantify terrestrial versus algal and or microbial organic matter (Peters and Moldowan, 1993). Hence, in the present study, the contribution of the mangrove vegetation cover has been inferred from the distribution of long-chain n-alkanes (Cranwell, 1982; Reiley *et al.*, 1991; Meyers, 1997), friedelin and β-amyrenone (Corbet *et al.*, 1980; Jaffe *et al.*, 1996; Otto and Simoneit, 2001).

Mangrove ecosystems are regarded as significant sources of organic matter to adjacent estuaries and coastal waters on a global scale (Jennerjahn and Ittekkot, 2002; Dittmar *et al.*, 2006). Mangrove inputs to the estuarine environment was clearly inferred from the biomarker analysis of the sediments by the presence of the various penta and tetra cyclic triterpenoids, their byproducts, long chain hydrocarbons and fatty alcohols. The biomarker compounds detected in the estuarine stations could be attributed to vascular plant input especially from mangrove vegetation cover, since the mangrove ecosystems located in the confluence of estuarine stations export organic detritus through tidal action.

5.4 Conclusions

The study of the distribution of the lipid biomarkers such as such as n-alkanes, n-alkanols, n-alkan 2-ones, triterpenoids and their degradation products was useful for the source characterization of organic matter in the sediments of the study area. Long chain n-alkanes with carbon number ranging between C16 and C34 having odd over even carbon predominance was detected in the sediments of the study region, indicating angiosperm input to sedimentary organic matter. As mangroves are tropical formations of angiosperm vegetation growing along the coast, lagoon, estuaries and riverbanks, the source of long chain n-alkanes could be attributed to mangrove detritus. The occurrence of n-heptadecene (C17) in the sediments could be attributed to algal detritus. The presence of the polycyclic aromatic hydrocarbon pyrene (C16) in sediments collected from estuarine station E1 and E2 pointed towards anthropogenic input to sediments.

The important n-alkan-2-ones detected in the sediments of the present study include those with carbon number C25 and C27. A direct source of n-alkan-2-ones from mangroves to sediments could not be neglected since these compounds are constituents in dominant plants, including freshwater wetland vegetation, sea grasses and mangroves. Other possibility for its origin might have been the process of β-oxidation of n-alkanes or β-oxidation of fatty acids.

The important fatty alcohols detected in the sediments of the study area ranged between C12 and C30 with even over odd carbon predominance. Long-chain n-alkanols (>C20) with a strong even to odd predominance are common constituents of epicuticular higher plant waxes. Thus their sources in sediments could be attributed to mangrove detritus.

The presence of stigmastreol, β-sistosterol and campesterol in the sediment samples might be attributed to vascular plant input. The presence of coprostanol was detected at station M2 and its source was assigned to anthropogenic (fecal input), because of the huge population density and poor sanitation facilities in this coastal area. Cholesterol was detected in the sediments of all the stations. Some sterols (eg. cholesterol) are widely distributed in the biological system, which makes them of limited value for assessing sources of organic matter in sediments. The important steroidal ketones detected in the sediment samples include cholesta-3, 5-dien- 7-one (C27) and stigmast-4-en-3-one (C29). Their occurrence in marine sediments pointed towards direct inputs from microalgae such as the dianoflegellates and their distributions reflect early stage oxidation of the sterols present.

The occurrence of pentacylclic triterpenoids of oleanane, ursane and lupane skeleton α- amyrin, β-amyrin, taraxerol, germanicol, friedelin, lupeol, betulin and their degradation products in the sediments of both mangrove and estuarine systems, testified to the angiosperm input to the organic matter. The presence of taraxerol in sediments indicated increased mangrove input due to either higher mangrove productivity or increased erosion of mangrove deposits. The occurrence of taraxerol along with other biomarker molecules in the more marine-influenced sediments of the estuary pointed towards the export of mangrove detritus through tidal flushing, since this compound is an exclusive marker compound for mangrove input.

Early diagenetic degradation products of triterpenoids including des-A-triterpenoid hydrocarbons (des-A lupane), triterpenes (olean-12-ene, urs-12-ene, taraxer-14-ene) and triterpenoid ketones (taraxerone, β-amyrenone, lupenone) were also present in the sediments of the study region which were produced by microbial/photochemical processes from their precursors. Des-A-lupane, a product of photochemical or photomimetic degradation at ring-A, commencing with the oxygen functionality at C-3 of certain triterpenoids from higher plants was detected in the sediments. Olean-12-ene, 3-oxygenated pentacyclic triterpene could be regarded not only as a molecular marker of terrigenous contribution to the organic matter but also their occurrence in sediments suggested microbial processes (diagenesis) operating in the sedimentary environment.

Thus the detection of triterpenoids and a wide variety of variety of lipid biomarkers highlighted the large contribution of mangrove organic matter to the sediments. The mangrove input of organic matter to the estuarine environment was clearly inferred from the biomarker analysis of the sediments. The biomarker compounds detected in the estuarine stations could be attributed to vascular plant input especially from mangrove vegetation cover, since the mangrove ecosystems located in the confluence of estuarine stations export organic detritus through tidal action. A higher plant origin for these biomarker compounds in sediments was supported by the C/N ratios and stable carbon isotope composition.

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SUMMARY

Vegetated coastal ecosystems are critical components of global ocean carbon and nutrient budgets. Mangroves are highly productive tropical coastal ecosystems which have a potentially high impact on the carbon budget of the tropical and global coastal zone. The carbon dynamics in mangrove ecosystems has been the subject of numerous studies during the past decades, but we are still far from having an integrated view of the overall ecosystem functioning in terms of organic matter processing. The biogeochemistry of mangroves is the least understood one because of their sediment complexity due to the tidal influx of allochthonous organic matter and also the input of local vegetation. The present study is an attempt to characterize the organic matter in mangrove and adjacent estuarine ecosystems, based on elemental composition, stable isotope analysis, biochemical composition and biomarker approach.

Three mangrove ecosystems and three estuarine stations in the northern arm of Cochin estuary were chosen for the present study. Station M1, Puthuvyppu is a mangrove nursery maintained by the fisheries research unit of Kerala Agriculture University and is located about 100m away from the estuarine front. Station M2, Murikkumpadam is a densely populated fisher-folk settlement. These two stations form the part of the island called Vypin, which is one of the most densely populated coastal zones. Station M3, 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 canal is the only source for tidal propagation. In the present study, the three estuarine

stations along the northern arm of Cochin estuary were: Bolgatty (Station E1), Ponnarimangalam (E2) and Mulavukad (E3). The stations E2 and E3 are away from Cochin bar mouth receiving fresh water input from river Periyar. Station E1 is about 1 km away from Cochin bar mouth and Vallarpadam, a region with small patches of mangroves is located in the confluence of E1. The mangrove station Mangalavanam (M1) is connected to E1 through a narrow canal.

Samples of water and surface sediments were collected from the mangrove and estuarine stations during December 2005, April 2006 and July 2006. Among the general sedimentary parameters analyzed texture revealed the predominance of fine substratum and silt was the major fraction in the sediments of both mangrove and estuarine stations. The observed Eh values pointed towards the anoxic nature of the sediments in the study region. TOC: TS ratio revealed the sediment deposition process in a periodically fluctuating anoxic condition. The reducing condition prevailing in the study region had an important role in the distribution various phosphorous fractions and heavy metal distribution. X-ray Diffraction analysis of the sediments revealed the presence of monetite, an anhydrous calcium phosphate mineral ($CaHPO_4$) at mangrove station M3. XRD analysis showed that perovskite and sodalite were the dominant minerals at M1 and M2 respectively. The high mass percentages of calcium and phosphorus by XRF and SEM-EDS analysis confirmed the existence of calcium phosphate mineral at M3. In estuarine system XRD revealed the presence of quartz and monetite as abundant minerals in the sediments from E1 and E2. In addition to quartz and monetite, E3 exhibited the presence of kaolinite. X-Ray Fluorescence analysis showed that silicon was the major element in all the three stations, followed by aluminium and iron in the case of mangroves. The XRF analysis of the estuarine sediments also revealed the similar trend with higher abundance of the major elements silicon followed by aluminium and iron at all stations.

Phosphorus fractionation study indicated that the both mangrove and estuarine systems act as a sink of phosphorus, primarily as Fe-IP and Ca-IP fractions. Internal loading in these systems acts as a source of phosphorus to the adjacent coastal waters. Stoichiometric ratios of elements supported phosphorus enrichment at all stations of the both mangrove and estuarine systems. The very high silt + clay content in these systems might favor the higher retention of phosphorus. In the case of mangroves, the first two stations behave identically and Fe-IP was the major fraction in these stations. Station M3 is unique because of the accumulation of bird guano in this bird sanctuary and it resulted in about 10 fold increase in total phosphorus content. This station is exclusively dominated by Ca-IP and rare mineral, monetite was detected in the system.

The overall order in the concentration of heavy metals in mangrove sediments was, Fe >Mg >Mn >Zn> Cr >Ni>Pb> Cu >Co> Cd. In estuary, the variation in the metal content displayed the trend: Fe >Mg > Zn >Mn >Cr >Ni >Cu >Pb >Co >Cd**.** The enrichment factor calculated, for heavy metals indicated a minor enrichment for lead and zinc and no enrichment for all other metals in mangrove sediments. The geo accumulation index estimated for the present study exhibited very low values in the case of almost all metals except Zn, indicating sediments of the mangrove ecosystem are unpolluted to moderately polluted. The analysis of the EF values of the metals in estuarine sediments revealed that Cd and Zn exhibited extremely severe enrichment and moderately severe enrichment respectively and a minor enrichment for Pb. The geoaccumulation index values supported the accumulation of the trace metals such as Cd and Pb in estuarine sediments. The observations suggest that the mangrove ecosystems are relatively unpolluted and may be acting as a sink for heavy metals but the estuarine sediments are under the threat of severe accumulation of the toxic trace metals. The association of phosphorus with the major element calcium and the redox sensitive heavy metal Fe had profound influence on the distribution of P in the sediments of the study region. Thus, the combined use of phosphorus fractionation and heavy metal distribution studies along with other geochemical variables was useful in evaluating the geochemical condition of the sedimentary environments.

Bulk geochemical parameters such as biochemical composition, elemental composition and stable carbon isotope ratio are relatively reliable proxies of organic matter origin. Sedimentary organic matter was higher in mangroves than in their adjoining estuary. However, the percentage ratio of the labile to total organic matter indicated that most of the deposited organic matter is refractory in both environments. The refractory fraction was higher in mangrove sediments when compared to estuarine sediments. This discrepancy between the high amounts of organic matter and its low nutritional value 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 components, in general, recorded higher concentrations during post monsoon and lower concentrations during pre-monsoon seasons, except total carbohydrates, which was lower during monsoon. In mangrove systems, station M3 recorded comparatively higher concentrations of all the analyzed biochemical components. Carbohydrates exhibited significantly higher values during post-monsoon season in mangroves. The higher PRT: CHO ratios in estuarine sediments compared to mangroves indicated that in the former there is low dead organic matter accumulation, probably due to the strong hydrodynamic condition of estuaries. On the contrary, the low hydrodynamic condition in mangroves favours the accumulation of sedimentary organic matter. The lower PRT: CHO ratios (<1) at M1 and M2 (pre monsoon and post monsoon) indicated the presence of aged or less degradable organic matter ie., terrestrial or vascular plant input. The higher tannin and lignin content in the sediments also confirmed the mangrove input to total organic matter pool, since these are typical components of higher plants including mangroves. The higher LPD: CHO ratios observed at the mangrove and estuarine systems indicated high quality of labile organic matter to support benthic fauna. The observed C/N ratios in the mangrove ecosystems indicated a vascular plant input to the total organic matter pool. However, in the estuarine sediments, the C/N ratio estimated were intermediate values characteristic for autochthonous and terrestrial input of organic matter. Stable carbon isotope ratio analysis revealed comparatively more enriched values at station M2 and more depleted values at stations M1 and M3 indicating vascular plant input to the organic matter pool. The more depleted $\delta^{13}C$ values reported for the estuarine sediments indicated that a major contribution of organic matter was from the terrestrial higher plants. Thus, from the bulk parameter approach, it could be seen that organic matter in the mangrove systems are of higher plant origin and in estuary, a mixed origin of organic matter involving input from both marine and terrestrial plants. The application of bulk parameters was useful in the assessment of the quality and quantity of sedimentary organic matter and it revealed the fact that the biogeochemistry of sediments was very complex and cannot be explained effectively by these methods. Further studies like organic matter biomarkers is essential to understand the origin of the organic matter in the mangrove and estuarine sediments of the study region.

The study of the distribution of the lipid biomarkers such as such as n-alkanes, n-alkanols, n-alkan 2-ones, triterpenoids and their degradation products was useful for the source characterization of organic matter in the sediments of the study area. Long chain n-alkanes with carbon number ranging between C16 and C34 having odd over even carbon predominance were detected in the sediments of the study region, indicating angiosperm input to sedimentary organic matter. As mangroves are tropical formations of angiosperm vegetation growing along the coast, lagoon, estuaries and riverbanks, the source of long chain n-alkanes could be attributed to mangrove detritus. The occurrence of 8-heptadecene (C17) in the sediments could be attributed to algal detritus. The presence of the polycyclic aromatic hydrocarbon pyrene, in sediments collected from estuarine stations E1 and E2 pointed towards anthropogenic input to sediments. The important n-alkan-2-ones detected in the sediments of the present study include those with carbon number C25 and C27. A direct source of n-alkan-2-ones from mangroves to sediments could not be neglected since these compounds are constituents in dominant plants, including freshwater wetland vegetation, sea grasses and mangroves. Other possibility for its origin might have been the process of β-oxidation of n-alkanes or β-oxidation of fatty acids. The important fatty alcohols detected in the sediments of the study area ranged between C12 and C30 with even over odd carbon predominance. Long-chain n-alkanols (>C20) with a strong even to odd predominance are common constituents of epicuticular higher plant waxes. Thus their sources in sediments could be attributed to mangrove detritus.

Terrestrial organic matter input can be recognized by the presence of phytosterols in sediments, which are the predominant sterols produced by vascular plants. Thus the occurrence of stigmastreol, β-sistosterol and campesterol in the sediment samples might be attributed to vascular plant input. The presence of coprostanol was detected at station M2 and its source was assigned to anthropogenic (fecal input), because of the huge population density and poor sanitation facilities in this coastal area. Cholesterol was detected in the sediments of all the stations. The important steroidal ketones detected in the sediment samples include cholesta-3, 5-dien- 7-one (C27) and stigmast-4-en-3-one (C29). Their occurrence in marine sediments pointed towards direct inputs from microalgae such as the dinoflegellates and their distributions reflect early stage oxidation of the sterols present.

The occurrence of pentacyclic triterpenoids of oleanane, ursane and lupane skeleton such as α-amyrin, β-amyrin, taraxerol, germanicol, friedelin, lupeol, betulin and their degradation products in the sediments of both mangrove and estuarine systems, testified to the angiosperm input to the organic matter. The presence of taraxerol in sediments indicated increased mangrove input due to either higher mangrove productivity or increased erosion of mangrove deposits. The occurrence of taraxerol along with other biomarker molecules in the more marine-influenced sediments of the estuary pointed towards the export of mangrove detritus through tidal flushing, since this compound is an exclusive marker compound for mangrove input.

Early diagenetic degradation products of triterpenoids including des-A-triterpenoid hydrocarbons (des-A lupane), triterpenes (taraxer-14-ene, olean-12-ene, urs-12-ene) and triterpenoid ketones (β-amyrenone, taraxerone) were also present in the sediments of the study region which were produced by microbial/photochemical processes from their precursors. Des-A-lupane, a product of photochemical or photomimetic degradation at ring-A, commencing with the oxygen functionality at C-3 of certain triterpenoids from higher plants was detected in the sediments. Olean-12-ene, a 3-oxygenated pentacyclic triterpene could be regarded not only as a molecular marker of terrigenous contribution to the organic matter but also their occurrence of in sediments suggested microbial processes (diagenesis) operating in the sedimentary environment.

Thus, the presence of triterpenoids alongwith a wide variety of lipid biomarkers highlighted the contribution of mangrove organic matter to the sediments. Mangrove inputs to the estuarine environment were clearly inferred from the biomarker analysis of the sediments. The biomarker compounds detected in the estuarine stations could be attributed to terrestrial higher plant input especially from mangrove vegetation cover, since the mangrove ecosystems located in the confluence of estuarine stations export organic detritus through tidal action. A higher plant origin for these biomarker compounds was supported by the C/N ratio and stable carbon isotope composition.

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