Source Characterization of Sedimentary Organic Matter in a Tropical Estuary, Southwest Coast of India: A Biomarker Approach

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Certificate

This is to certify that the thesis entitled "***Source Characterization of Sedimentary Organic Matter in a Tropical Estuary, Southwest Coast of India: A Biomarker Approach***" is an authentic record of the research work carried out by Mr. Gireesh Kumar. T.R, under my supervision and guidance at the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Kochi-682016, in partial fulfillment of the requirements for Ph.D degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any university.

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Declaration

I hereby declare that the thesis entitled " **Source Characterization of Sedimentary Organic Matter in a Tropical Estuary, Southwest Coast of India: A Biomarker Approach"** is an authentic record of the research work carried out by me under the guidance and supervision of Dr. N. Chandramohanakumar, Professor, 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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Gireesh Kumar T.R.

Dedicated to

my father, my inspiration, my role model……

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Preface

Estuaries are highly dynamic and complex transitional environments in which the input, exchange and processing of organic matter and nutrient sources are similarly complex. Estuarine fluxes of materials are controlled not only by physical forcing by river discharge, tidal currents, resuspension, and sedimentation, but also by the often high rates of biological and abiological degradation, alteration, and remineralisation that take place there. The organic matter and nutrient sources play a key role in understanding the ecosystem functioning as a whole and connectivity of estuaries with riverine and coastal marine habitats. The living and nonliving (detritus) sources of organic matter to estuaries can be divided into allochthonous and autochthonous pools – sources produced outside and within the boundaries of the estuary proper, respectively.

Knowledge of source, fate and reactivity of organic matter are critical for understanding the role of estuaries in global biogeochemical cycles. The basic source information can be derived by using a combination of total organic carbon concentration with other additional elemental information. Bulk isotopic ratios of carbon and nitrogen provide complementary information to elemental ratios and are widely used to identify organic matter sources in estuaries. The biochemical descriptors such as chlorophyll pigments and organic biopolymers (proteins, carbohydrates and lipids) are also utilised to assess the quality, benthic trophic status as well as the natural and human induced pressures in estuarine and coastal environments. The bulk organic matter techniques are only effective in determining the relative contribution of autochthonous and allochthonous sources of organic matter due to the overlapping of elemental and isotopic compositions of various end member sources.

The source, fate and diagentic pathway of sedimentary organic matter in estuaries are difficult to delineate due to the complexity of organic matter sources, intensive physical mixing and biological processes. A combination of bulk organic matter techniques and molecular biomarkers are found to be successful in explaining organic matter dynamics in estuaries. The basic requirement for these multi-proxy approaches are (i) sources have significantly differing characteristics, (ii) there are a sufficient number of tracers to delineate all sources and (iii) organic matter degradation and processing have little, similar or predictable effects on end member characteristics.

Although there have been abundant researches that have attempted to tackle difficulties related to the source and fate of organic matter in estuarine systems, our understanding remains limited or rather inconsistent regarding the Indian estuaries. Cochin estuary is the largest among many extensive estuarine systems along the southwest coast of India. It supports as much biological productivity and diversity as tropical rain forests. In this study, we have used a combination of bulk geochemical parameters and different group of molecular biomarkers to define organic matter sources and thereby identifying various biogeochemical processes acting along the salinity gradient of the Cochin estuary.

The thesis is divided into 8 chapters. Chapter 1 provides a general background on the application of bulk organic matter and molecular biomarkers in estuarine ecological studies. It also deals with the aim and scope of the present study. Chapter 2 provides a brief account of salient features of the study area and short description of different sampling points in the estuary. A detailed description of analytical methodology adopted for the study also appended in this chapter.

Chapter 3 discusses the basic redox conditions prevailing in the estuarine system considering the spatio-temporal changes in hydrodynamic conditions. The hypothesis is tested by employing different phosphorous fractions and trace metals. Chapter 4 details the quality, benthic trophic status and sources of sedimentary organic matter. The quality and benthic tropic status is assessed using the biochemical descriptors and chlorophyll pigments. This chapter also discusses the basic sources of organic matter inferred from bulk organic matter techniques.

Chapter 5 covers n-alkanes, the simple and abundant class of molecular biomarker compound, its spatial variations and major geochemical processes is identified using principal component analysis. Chapter 6 describes the spatial variations of fatty acid biomarkers in estuarine sediments and chapter 7 verifies the efficacy of identified neutral compounds as molecular biomarkers. Chapter 8 provides summary and conclusions of the study.

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List of Abbreviations

TAR	Terrestrial to Aquatic Ratio
CPI	Carbon Preference Index
ACL	Average Chain Length
Mon	Monsoon
Post	Post Monsoon
Pre	Pre Monsoon
OC	Organic Carbon
TN	Total Nitrogen
TS	Total Sulphur
TP	Total Phosphorous
TOM	Total Organic Matter
Fe(OOH) ≈P	Iron Bound Phosphorous
$CaCO_3$ ≈P	Calcium bound Phosphorous
Acid-OP	Acid Soluble Organic Phosphorous
Alkali-OP	Alkali Soluble Organic Phosphorous
ROP	Residual Organic Phosphorous
Chl-*a*	Chlorophyll *a*
Pheo	Pheopigments
PRT	Proteins
CHO	Carbohydrates
LIP	Lipids
BSA	Bovine Serum Albumin
BPC	Biopolymeric Carbon
FAME	Fatty Acid Methyl Ester
GC-MS	Gas Chromatography-Mass Spectrometer
BSTFA	N,O-Bis(trimethylsilyl)trifluroacetamide
ANOVA	Analysis of Variance
SPSS	Statistical Package for Social Sciences

PCA	Principal Component Analysis
BAP	Bioavailable Phosphorous
CAM	Crassulacean Acid Metabolism
F	Relative Contribution of Terrestrial Derived Organic Matter
HMW	High Molecular Weight
LMW	Low Molecular Weight
TAlk	Terrestrial Derived n-Alkanes
AAlk	Autogenous n-Alkanes
SFA	Saturated Fatty Acids
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
Br-FA	Branched Fatty Acids
BFA	Bacterial Derived Fatty Acids

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Chapter 1

INTRODUCTION

Contents

1.1 General Introduction

Estuarine systems are intermediate transition zones linking freshwater and marine systems which receives, stores and mixes inflows of both freshwater and salt water (Pritchard, 1967; Attrill and Rundle, 2002; Wolanski, 2007). They are highly productive ecosystems, performing important role in various ecological and biological functions (Dolbeth et al., 2007). Every estuary is unique in terms of their morphological features, climatic settings, tidal incursion and chemical processes, however all estuaries support diverse flora and fauna (Flemer and Champ, 2006). As estuaries are ecotone areas between marine and freshwater habitats, they are characterised by unique combination of physical, chemical and biological features (Telesh and Khlebovich, 2010).

Estuaries are locations of biggest cities in the world. Consequently, estuarine processes are of great importance from geochemical, recreational, economic and ecological point of view (Zwolsman, 1994). Over the past three decades there has been rapid increase in human population growth along the coastal regions of the world. Currently 61% of the world population resides along the coastal margin (Alongi et al., 1998) and by 2025, it is predicted that 75% of the world population will live in the coastal regions (Hinrichsen, 1998). This increased population exploit the estuarine area for food, transport, commerce and settlement. On a global basis, more than half of our coastlines currently suffer from the pressures of development and the impacts are likely to intensify as population in the coastal zone continue to rise. These impacts of demographic changes clearly had detrimental effects on the overall biogeochemical cycling in estuaries (Howarth et al., 2000; 2002).

Rivers carry materials derived from its catchments to estuaries. Nutrient levels have increased in rivers and estuaries, resulting in harmful algal blooms and subsequent reduction in water column oxygen levels (Boyes and Elliot, 2006; Elliot et al., 2007; Wang et al., 2007; Brodie et al., 2011). Extensive urbanisation and industrialisation have resulted in high concentration of both organic and inorganic contaminants in estuarine ecosystems. The changes in water shed hydrology, water diversions and damming of rivers have altered the magnitude and temporal patterns of freshwater flow and sediment discharge to the estuaries. As a result, many of the commercially important fish and shell fish species have been over exploited and the introduced species have resulted in alterations of habitats, loss of native species, and reduction in commercially important species (De et al., 1994; Sinha et al., 1996; Anderson et al., 2002; Martin et al., 2013).

Salinity, the main environmental factor, which plays a decisive role in estuarine processes, defines structural and functional characteristics of aquatic biota in estuaries. The sharp gradients in estuarine master variables such as salinity, pH, dissolved oxygen and particle concentration induce pronounced biogeochemical reactivity in estuaries which include sorption, flocculation and redox cycling of chemical elements (De Jonge et al., 2002; Telesh and Khlebovich, 2010).

Biogeochemical cycle is a pathway by which chemical elements or molecules move thorough both biotic (biosphere) and abiotic (lithosphere, atmosphere and hydrosphere) compartments of earth. Biogeochemistry links processes that control the fate of sediments, nutrients, and organic matter as well as trace metals and organic contaminants. A greater knowledge of biogeochemical cycling in estuaries, which involves the transformation, fate and transport of chemical substance, is critical in understanding the effects of these environmental alterations from regional to global context (Bianchi, 2007). Biogeochemical and physical processes play in regulating the chemistry and biology of estuaries (Bianchi et al., 1999; Hobbie, 2000). Therefore on a global basis, estuarine sediments are important sites for studying and evaluating fluxes, cycling and storage of the chemical elements.

Better understanding of biogeochemical cycle of carbon in estuaries can provide relevant information regarding the local, regional and global carbon cycle. As organic matter is a principle fuel for biogeochemical reactions, it is intimately associated with the fluxes and cycling of other chemical elements in estuarine sediments. The geochemistry of estuarine sediments are controlled by the nature of organic and inorganic materials initially deposited in sediments and the physical, chemical and biological processes that affect the materials after deposition. Those processes fall in the general category of what is commonly referred as early diagenesis (Berner, 1980). The key role of organic matter in early diagenetic processes has led to significant efforts for understanding and quantifying its remineralisation processes.

Estuarine sediments are impacted by wide range of physical and biological forcing that occur over a broad range of spatial and temporal scales (Burdige, 2011). The nature and extend of such forcing coined with biological processes (largely microbial mediated) is directly associated with remineralisation of sedimentary organic matter which in turn has significant impact on biogeochemical cycling of carbon, nitrogen, phosphorus, sulphur and many trace metals including manganese and iron (Burdige, 2011). Hence, the composition of sedimentary organic matter can be used as a tool to determine biogeochemistry of estuarine sediments. The organic matter found in sediments is a function of nature of organic matter reaching the sediments and the rate at which different type of organic matter are degraded by diagenesis pathway. The reaction sequence of this pathway depends on the availability of various terminal electron acceptors (Bianchi, 2007; Burdige, 2011). Estuaries are ideal systems to study such sedimentary processes as a gradient in the concentration of the most common terminal electron acceptors *viz.* molecular oxygen, nitrate, Fe, Mn and sulphate, exist naturally (Zimmerman and Benner, 1994). Through a full accounting of the end products of these aerobic and anaerobic processes, we may gain understanding of various biochemical and geochemical processes.

Estuarine systems are characterised by rate of carbon fixation driven by diverse range of primary producers due to the availability of light and enrichment of inorganic nutrients. In addition to this, estuarine systems are characterised by shallow water depth and often with high benthic primary productivity (Bianchi and Canuel, 2011). Therefore the autochthonous organic matter sources include phytoplankton, macroalgae and benthic primary producers. Rivers carry significant amount of terrestrial derived organic matter into the estuaries. The allochthonous sources include terrestrial plant and animal tissues, organic matter from mangroves and other upland sources and also the soil derived organic matter.

Humans have settled in areas that are in close proximity to estuarine habitats due to the essential functions and values that estuaries provide (Wolanski et al., 2004). Estuaries are essential as means of transport. They provide numerous forms of recreation, preferred for urban development and support commercial shipping and fishing (Flemer and Champ, 2006). Thus the anthropogenic organic matter include sewage, pharmaceutical compounds, organic pollutants and other organic matter derived from industrial and urban activities. Photooxidation, autooxidation, heterotrophic processes and bacterial biomass contribute additionally to the organic matter pools within these systems (McCallister et al., 2006). Therefore, determining the relative contributions of organic matter fuelling various biogeochemical processes in sediments remain a significant challenge due to the wide diversity of organic matter and also due to the continuum of reactivity ranging from more reactive compounds dominant in autochthonous materials to less reactive terrigenous materials (Keil et al., 1994; Hedges and Keil, 1995; Hedges et al., 1997; Hu et al., 2012, 2013).

The composition and fate of organic matter can be used as a tool to unravel the various biochemical and geochemical processes acting in estuarine systems because of its strong coupling with cycles of biogeochemically reactive elements. The composition of organic matter thus acts as a “recorder” of various biogeochemical events. Bulk measurements like elemental and isotopic ratios and molecular biomarkers have been used to identify specific source of organic matter in sediments. Quantitative biomarker analysis is an invaluable tool and used by the environmental chemists to explain environmental processes. Elemental and isotopic compositions of carbon and nitrogen of sedimentary organic matter have been widely used to distinguish organic matter from different sources (Andrews et al., 1998; Gireeshkumar et al., 2013). This could only differentiate the relative importance of two end member organic matter sources – autochthonous and allochthonous (Middelburg et al., 1997; Bianchi and Canuel, 2011). Organisms produce specific organic compounds with geochemical stability through unique biosynthetic pathways. These compounds can be used as molecular biomarkers for source characterisation (Bianchi and Canuel, 2011).

1.2 Molecular Biomarker Approach

The term molecular biomarkers have been defined as "compounds that characterise certain biotic sources and that retain their source information after burial in sediments, even after some alteration" (Meyers, 2003). Killops and Killops, 2005 defined biomarkers as "molecule whose carbon skeleton can unambiguously link to that of a known biological precursor". These organic compounds have often a high degree of structural complexity and are particularly informative. Hence, these organic compounds are suitable for studying biogeochemical processes. They provide the possibility of relating certain products to specific precursors and the certain skeleton of such compounds embodied essential information in the habitat, nature and fate of parent flora and fauna.

The use of molecular biomarker analysis in organic geochemistry arose from the overlapping interest of petrochemistry, geology and ecology through the time between current production of organic matter to its deposition, diagenesis and ultimate transformation to petroleum (Panetta and Gélinas, 2009). Alkanes, fatty acids, amino acids, lignin pigments, carbohydrates, terpenoids etc. were identified in a number of studies to address the source of organic matter (Volkman et al., 2007; Freese et al., 2008; Hu et al., 2009; Yang et al., 2010; He et al., 2010; Aneeshkumar and Sujatha, 2012; Hu et al., 2012; Hu et al., 2013) and its diagenetic transformations in the environment (Ding and Sun, 2005; Chen et al., 2008; Carreira et al., 2011). The quantitative analysis of target biomarker classes and the calculation of relative abundance of individual groups to the total mass of quantified biomarker lead to the high level understanding of sources of organic matter and its degradation pathway (Panetta and Gélinas, 2009).

Molecular biomarker analysis enables the identification and quantification of numerous individual compounds in a single analysis and the cumulative information obtained would intuitively result in a powerful interpretation of system under investigation (Oros and Simoneit, 2000; Panetta and Gélinas, 2009). A number of simple, robust and effective biomarker based indices were developed to get more advanced understanding of carbon dynamics in aquatic systems. They include terrestrial to aquatic ratio (TAR), carbon preference index (CPI), average chain length (ACL), alkenone paleothermometer, stanol to sterol ratio etc. The multivariate statistical tools such as principal component analysis (Yunker et al., 2005; Xu et al., 2006) and cluster analyses (Dittmar et al., 2007) are capable of sifting and sorting complex biomarker data sets to easily decipher organic matter sources as well as diagenetic processes (Panetta and Gélinas, 2009). It must be pointed out that the complexity of organic matter sources and their involvement in various biogeochemical processes warrants the application of multiple biomarkers (multi-proxy) approach as individual biomarker may be subjected to limitations.

1.3 Lipid Compounds as Molecular Biomarkers

Lipids include a broad group of substances produced by organisms that are effectively insoluble in water but extractable by organic solvents like chloroform, hexane, toluene, acetone etc. Lipid compounds include fatty acids, hydrocarbons, terpenoids, steroids and phospholipids which possess biological functions such as energy storage, structural components of cell membranes and important signalling molecules. Lipid biomarker have been extensively used as proxies to characterise the sources and diagenetic state of organic matter in aquatic ecosystems because of their source specificity and higher resistance to bacterial degradation in comparison with other class of organic compounds (Duan, 2000; Jeng et al., 2003; Volkman, 2006; Tesi et al., 2007; Mead and Goni, 2008; Mejanelle and Laureillard, 2008; Dunn et al., 2008; Hu et al., 2013).

A wide variety of different lipid compounds have been found in estuarine sediments attesting to the diversity of biosynthetic pathways employed by aquatic organisms. Most of these compounds have distinctive structures and can be used as biomarkers (molecular markers) for the source characterisation of sedimentary organic matter (Volkman, 2006). Phytoplankton produce compounds such as alkanes (C_{17}, C_{19} and C_{21}), alkenes, highly branched isoprenoids as well as distinctive sterols and unsaturated fatty acids, enabling the recognition of micro-algal organic matter (Hu et al., 2012, 2013).

The input of terrestrial organic matter to estuarine environment can be recognised from lipids of higher plant origin, such as long chain fatty acids, fatty alcohols, C_{29} sterols and pentacyclic triterpenoids (Volkman, 2006; Koch et al., 2003). Bacteria synthesise a diverse range of compounds, such as odd and branched chain fatty acids and hydroxy fatty acids, hopanoids and isoprenoids many of which are particularly stable; for instance, those that contain ether bond (Dunn et al., 2008; Bianchi and Canuel, 2011). The hopanes and steranes can be effectively used to identify petroleum contamination (Hu et al., 2013).

1.4 Aim and Scope of the Study

Cochin estuary forms a complex system of shallow estuarine network running parallel to the coastal line of Kerala, India. The salinity gradient of Cochin backwaters supports diverse species of flora and fauna, according to their tolerance for saline environment. This highly productive tropical estuary acts as a nursery ground for many species; estuarine and marine fin fishes, molluscs and crustaceans. The low lying swamps and tidal creeks dominated by sparse patches of mangroves provide shelter to juveniles of many important species (Menon et al., 2000).

The intensity of research effort expended in elucidating the physical, chemical and biological characteristics of Cochin estuary during the last four decades is indicative of the economic and social importance enjoyed by it. A number of studies have focused on the general physical, chemical and biological aspects of Cochin estuary. There are many data sets available on nutrients and heavy metal contaminants of Cochin estuary. The ecological studies in the Cochin estuary were pioneered by Sankaranarayanan and Qasim, 1969 and Sankaranarayanan and Panampunnayil, 1979. Investigations that carried out in the last four decades have pointed out unfavourable changes in the hydrodynamics (Madhupratap et al., 1977; Joseph and Kurup, 1990; Balachandran, 2001; Revichandarn et al., 2012) and hydrobiological conditions (Qasim, 1980; Lakshmanan et al., 1982;

Menon et al., 2000; Joseph and Ouseph, 2009) of the Cochin estuary. The concentrations of dissolved, particulate and sedimentary metals are well documented (Ouseph, 1992; Balachandran et al., 2005; Deepulal et al., 2012, Selvam et al., 2012). Analysis of the literature showed that the sources of organic matter and its association with other biogeochemical processes were poorly studied in this highly dynamic ecosystem.

A combination of bulk indices (elemental stoichiometry and bulk isotopic composition) and molecular biomarkers have been widely adopted to characterise the sources and diagenetic fate of organic matter in estuarine systems around the world. However, in tropical estuaries, especially in Indian estuaries, our understanding remains limited or sometimes inconsistent. As a case study, we intend to unravel the sources of sedimentary organic matter in the surface sediments of Cochin estuary. The Cochin estuary is among the largest in India and is of interest beyond the sub-continent by being among the better known tropical estuaries so that scientific results may generic.

The objectives of the present study are as follows:

- To find the major biogeochemical characteristics of the Cochin estuary using P speciation and trace metal distribution.
- To assess the nature and quality of bulk sedimentary organic matter and the benthic trophic status of the estuary.
- To explore the spatio-temporal distributional dynamics of carbon and nitrogen isotopes in sediments from the Cochin estuary and to calculate relative contribution of terrestrial derived organic matter in sediments.

- To identify the sources of sedimentary organic matter using multi-proxy approach- using the qualitative and quantitative lipid biomarker data.
- To identify a set of molecular biomarker proxies which enable the proper identification of different organic matter sources in surface sediments.

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Chapter 2

MATERIALS AND METHODS

2.1 Description of Study Area

Cochin estuary is a tropical micro tidal positive estuarine system with two permanent openings to the Arabian Sea and sustains high biological production (Qasim, 2003). The estuary is topographically divided into two arms; a southern one extending towards south of barmouth from Cochin to Thanneermukkom and a northern one extending towards north from Cochin to Azhikode. The estuary is generally wide (0.8–1.5 km) and deep (4–13 m) towards the south, but narrow (0.05–0.5 km) and shallow (0.5–2.5 m) towards the north (Balachandran et al., 2008). The Cochin estuary is under the profound influence of tropical monsoon, which contributes to about 71 % of the annual rainfall (Qasim, 2003) and accordingly there are three seasonal conditions prevailing *viz.* monsoon (June–September), post monsoon (October–January) and pre monsoon (February–May). The Cochin estuarine system sustained rich bio-resources in the pristine condition, but has undergone ecological degradation due to increased industrialisation and urbanisation (Arun, 1998; Martin et al., 2010). The estuary supports diverse species of flora and fauna depending on the capacity to tolerate oligohaline, mesohaline and marine conditions. Low lying swamps and tidal creeks, dominated by mangrove patches are nutrient rich environments and they acts as breeding grounds for many economically important fishes, molluscs and crustaceans (Menon et al., 2000).

Like the most major estuarine systems worldwide, the Cochin estuary has been increasingly impacted by anthropogenic activities like intertidal land reclamation, effluent discharges, expansion for harbour development, dredging activities and urbanisation (Gopalan et al., 1983; Menon et al., 2000). During the last two decades, the estuary also received a high influx of anthropogenic nutrients, heavy metals and organic matter from increased agricultural activities, domestic sewage inputs, industrial effluents and marine fish farming (Thomson, 2002; Qasim, 2003; Balachandran et al., 2005). This estuary houses the second largest port in the south west coast of India and it has three dredged channel maintained at a depth of 10-17m. One approach channel having 10km length and 500m width, and two inner channels Ernakulam channel (5km length and 250-500m width) and Mattanchery channel (3km length and 170 to 250m width), situated on either side of the Willingdon Island (Menon et al., 2000). The first phase of Kochi International Transshipment Container Terminal (ICTT) was commissioned on February, 2011 and it is a transshipment facility of Cochin port.

2.2 Sampling

The southern part and the northern part of the estuary show distinct physico-chemical and biological conditions. The southern part which covers a significant portion of the estuary was therefore selected for the preliminary assessment of sources of sedimentary organic matter. Sampling was carried out from 14 (Table 2.1 and Fig. 2.1) stations from the southern arm of the Cochin estuary. The stations selected were broadly classified into two zones depending upon the nature and intensity of anthropogenic activities. Zone I includes stations S1 to S7, situated around the Cochin harbour region and this area is impacted by shipping, industrial and municipal activities. Zone II consists of stations from S8 to S14 and this area is impacted by agricultural activities. All 14 stations were used for the assessment of redox condition and quality of sedimentary organic matter, while a representative 9 stations were selected for the source characterisation study. Sediments and water samples were collected from 14 locations in the southern arm of Cochin estuary during July 2007, November 2007 and February 2008 representing monsoon, post monsoon and pre monsoon seasons respectively. Water samples were collected using a Niskin sampler (Hydrobios 5L) and sediment samples were collected using a Van Veen grab (0.042 m^2).

Table 2.1 Details of sampling sites selected for the study.

Stations	Depth (m)	Station Name	Lattitude	Longitude
S1	14	Barmouth	9^0 58’ 29’’	$76^0$14’ 37’’
S2	2	Bolgatty	$9^0$58’34’’	$76^0$16’00’’
S3	3.5	Oil Jetty	$9^0$57’42’’	76^0 15’47’’
S4	6	Sulphur Jetty	$9^0$57’46’’	$76^0$16’39’’
S5	3	Fisheries Harbour	$9^0$56’30’’	$76^0$15’56’’
S6	6.5	Shipyard	$9^0$56’49’’	$76^0$17’13’’
S7	8	Thevara	$9^0$55’26’’	$76^0$17’52’’
S8	4	Kumbalam	$9^0$53’33’’	$76^0$18’20’’
S9	3.5	Poothotta	$9^0$52’47’’	$76^0$20’12’’
S10	3	Panavalli	$9^0$52’47’’	$76^0$20’12’’
S11	2.5	Perumbalam	$9^0$52’0’’	$76^0$20’34’’
S12	2	Murinja Puzha	$9^0$50’20’’	$76^0$20’40’’
S13	2	Ittipuzha	$9^0$50’22’’	$76^0$22’40’’
S14	2.5	Thannermukkom bund	$9^0$46’35’’	$76^0$22’15’’

Stations selected for the organic matter source characterisation are represented in bold italics

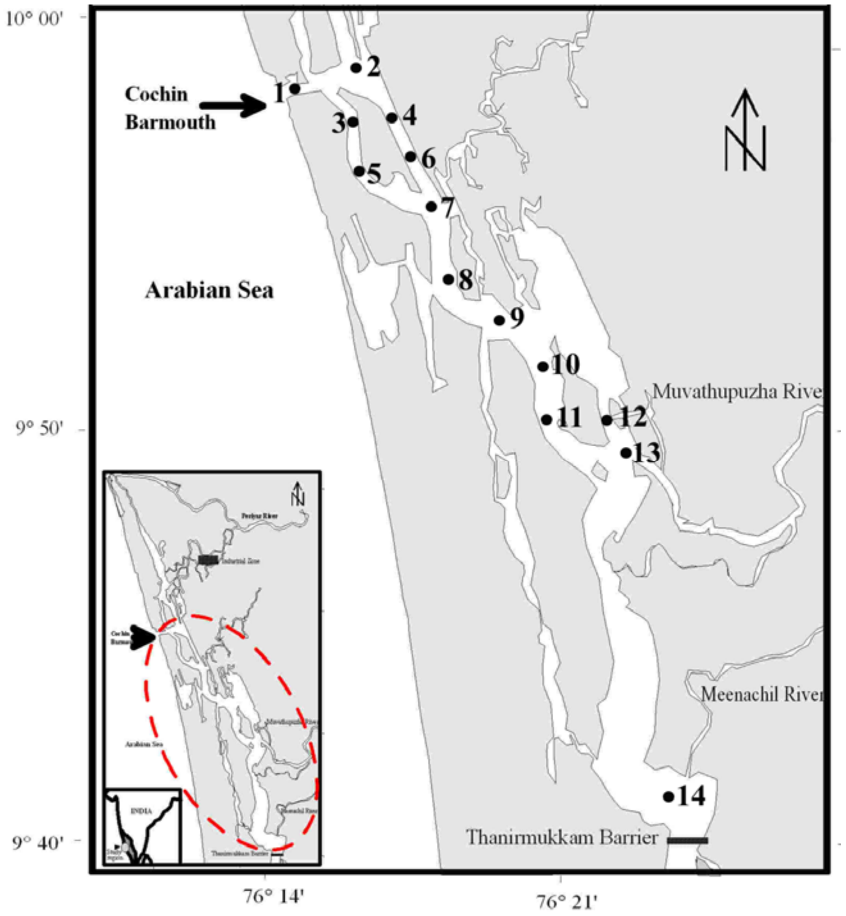

Fig. 2.1 Map of the study area showing locations of sampling sites

2.3 Analytical Methodology

2.3.1 General Water and Sediment Characteristics

Salinity of the water samples was estimated by Mohr- Knudsen method. Water samples were analysed for dissolved inorganic phosphate (PO_4^{3-}) following standard procedure (Grasshoff et al., 1983). Redox potential of the fresh wet sediment was measured using Zobell's solution for the calibration of the electrodes. The textural characteristics of the sediments were determined by pipette analysis after removing the inorganic carbonates using 10% HCl and organic matter using H_2O_2 (Folk, 1980). Total nitrogen (TN) and Total sulphur (TS) were determined using Vario EL III CHN-S Analyser. Organic carbon (OC) was also determined using CHN-S analyser. The analyses were preceded by treatment of samples with 1M HCl to remove carbonates. The process was repeated two/three times in order to ensure the complete removal of carbonates, washed with Milli-Q water to remove salts and finally freeze dried. Samples were run with blank cups in order to correct the carbon and nitrogen associated with tin/silver cups. Acetanilide standards were used to calibrate the elemental analyser. The detection limits for OC and TN are 0.07 and 0.01 respectively. The amount of total organic matter (TOM) is obtained by multiplying the OC values with 1.80 (Muller et al., 1986).

Trace metals in the sediment were estimated using Flame Atomic Absorption Spectrophotometer (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 2.2).

Table 2.2 Analysis of standard reference material (BCSS-1) for heavy metals

Metal	Certified Value	Observed Concentration (n=3)
Co ($\mu g g^{-1}$)	11.4 ± 2.1	10.67 ± 2.68
Cr ($\mu g g^{-1}$)	123 ± 1.4	112 ± 0.65
Cu ($\mu g g^{-1}$)	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 ($\mu g g^{-1}$)	229 ± 15	189.47 ± 10.75
Ni ($\mu g g^{-1}$)	55.3 ± 3.6	49.16 ± 2.01
Pb ($\mu g g^{-1}$)	22.7 ± 3.4	24.9 ± 0.08
Zn ($\mu g g^{-1}$)	119 ± 12	123.64 ± 2.51

2.3.2 Phosphorous Fractionation

The sequential extraction scheme by Golterman (1996) using chelating agents was employed for estimating different P fractions. Compared with other methods, chelating agents allow a specific extraction of inorganic phosphorus with less destruction of organic phosphorus (Golterman, 1996). Iron-bound P (Fe(OOH)≈P) was extracted with buffered Ca-EDTA/dithionite and calcium bound fraction ($CaCO_3$≈P) subsequently with Na-EDTA. The Na-EDTA extraction step was repeated twice to ensure the complete dissolution of $CaCO_3$≈P. In the next step, acid soluble organic P (Acid-OP) was extracted with H_2SO_4 and then alkali soluble organic P (Alkali-OP) with 2 M NaOH at 90^0C for 2 hrs. Residual organic phosphorus (ROP) was measured after digestion with $K_2S_2O_8$ for 1 hr in acid medium. All the extractions were carried out under mild continuous shaking, and the results are expressed on the dry weight basis. The chelating agents and the target phases are shown in Table. 2.3.

Table 2.3 Extraction scheme for the speciation of phosphorus in surface sediments (Golterman, 1996).

Step	Extractant	Form of phosphorus extracted
I	Ca-EDTA/dithionite(pH≈9)	Iron-bound phosphorus (Fe(OOH)≈P)
II	Na-EDTA (pH≈4.5)	Calcium bound P fraction ($CaCO_3$≈P)
III	0.5M H_2SO_4	Acid soluble organic phosphorus (Acid-OP)
IV	2M NaOH at 90^0 C for 2 hrs	Alkali soluble organic phosphorus (Alkali-OP)
V	Digestion with $K_2S_2O_8$ for 1 hr in acid medium	Residual organic phosphorus (ROP)

2.3.3 Biochemical Composition

Chlorophyll *a* (Chl-*a*) and Pheopigments (Pheo) were then measured according to the method of Lorenzen (1967). Pigments were extracted with

90% acetone (24 hrs in the dark at 4^0C). After centrifugation, the supernatant was used to determine functional Chl-*a* and acidified with 0.1 N HCl to estimate the amounts of Pheo (Plante-Cuny, 1974). The phytopigment concentrations were defined as the sum of Chl-*a* and Pheo concentrations.

Total protein (PRT) analysis was conducted following extraction with NaOH (1M, 2hrs at 80^0C) and determined according to Hartree (1972) modified by Rice (1982) to compensate for phenol interference using bovine serum albumin (BSA) as the standard. Total carbohydrates (CHO) were extracted with H_2SO_4 (2M, 2hrs at 100^0C), analysed according to Gerchacov and Hatcher (1972) and expressed in glucose equivalents. Total lipids (LIP) were extracted from 1 g of freeze-dried homogenised sediment samples at 60^0C in a water bath for 30 min, using 30 ml chloroform: methanol (2:1 v/v) (Bligh and Dyer, 1959) and analysed by the method of Barnes and Blackstock (1973). LIP concentrations were expressed as cholesterol equivalents. Blanks for each analysis were performed with pre-combusted sediments at 450-480^0C for 4 hrs. All analyses were carried out in triplicate. PRT, CHO and LIP concentrations were converted to carbon equivalents assuming a conversion factor of 0.49, 0.40 and 0.75 mg, respectively (Fabiano and Danovaro, 1994). The sum of protein, lipid and carbohydrate carbon equivalents was reported as the biopolymeric carbon (BPC) and used as a reliable estimate of the labile fraction of organic matter (Fabiano et al., 1995).

The algal contribution to BPC (CChl-*a*) was calculated as the percentage of Chl-*a* to BPC concentrations, after converting Chl-*a* concentrations into carbon equivalents using a mean value of 40 $mgCmg^{-1}$ Chl-*a* (Pusceddu et al., 1999). The C to Chl-*a* ratio may vary from 10 to greater than 100 and the use of a constant conversion factor may involve errors in the calculation (de Jonge, 1980). However, using this mean value we can compare our data with literature information from other coastal areas (e.g. Pusceddu et al., 1999; 2009; 2011). Also, the protein to carbohydrate ratio (PRT: CHO) was calculated and used as indicators of the status of biochemical degradation processes (Galois et al., 2000).

The $\delta^{13}C$ and $\delta^{15}N$ analyses were carried out using Flash EA 1112 interfaced with IRMS (DELTA V PLUS, Thermo Electron Corporation) at National Institute of Oceanography, Goa, India. Samples were run with blank cups using urea as standard. All analyses were carried out in triplicates and mean of the three measurements were reported.

Stable isotopic ratio values are reported in ‰ notation.

$$\delta\ (‰) = \{(R_{sample} - R_{reference}) / R_{reference}\} \times 1000 \quad \text{(Equation 2.1)}$$

where δ ‰ stands for $\delta^{13}C$ or $\delta^{15}N$, and R_{sample} and $R_{reference}$ are the isotopic ratios of the sample and reference, respectively. For carbon the reference is Peedee belemnite (PDB) and for N, it is air.

2.3.4 Extraction of Molecular Biomarkers

The method described by Harvey (1994) was selected for the present study. Freeze dried sediment samples were soxhlet extracted for 72 hrs with a mixture of dichloromethane: methanol (2:1 ratio). The extracts were combined and evaporated to dryness using rotary evaporation. The extracted residue was subjected to mild alkaline hydrolysis using 6% w/v KOH in methanol and refluxed for 4 hrs at 70^0C. The fraction containing neutral organic biomarkers were recovered with n-hexane, which was evaporated and kept for glass column chromatography. The remaining aqueous layer containing the fatty acid salts was acidified to pH 2 by adding 6M HCl and the fatty acids were extracted with dichloromethane.

The polar lipid fraction containing the fatty acids was evaporated to dryness using rotary evaporation and treated with 10 ml of 12% BF_3in methanol (Sigma Aldrich), while heating at 70°C for 1hr to form the fatty acid methyl esters (FAMEs). The FAMEs were subsequently partitioned from the reaction solution into10ml of hexane. The hexane layer was evaporated to dryness and the extract was then re-dissolved in n-hexane for gas chromatographic analysis. The neutral fraction obtained by mild alkaline hydrolysis of the total lipid extract was separated into individual compound classes on silica gel viz. saturated hydrocarbons, aromatic hydrocarbons and polar compounds (Otto and Simoneit, 2001). The fractions were eluted with hexane, hexane: dichloromethane (1:1), dichloromethane and methanol, respectively, and dried under nitrogen.

2.3.5 Gas Chromatographic Analysis of Molecular Biomarkers

FAME analysis was carried out by Gas Chromatography-Mass Spectrometry (GC-MS) using a Perkin Elmer Clarus GC 620, with MS detector equipped with a non-polar HP ultra-double-fused silica capillary column (30 m, 0.32mm internal diameter, 0.25µm film thickness). Operating conditions were as follows: ion source of electron voltage 70eV kept at 200°C. Spectra were scanned from 50 to 600 m/z with a scan time of 1.50 s. Initially the temperature was increased from 50°C to 200°C at a rate of 2°C per min and held at 200°C for 5 min. Then the temperature was again increased from 200°C to 280°C at a rate of 10°C per min and held at

280°C for 10min. The detector was held at 290°C and helium was used as carrier gas. Full data acquisition was obtained with the use of MS turbo mass version 5.3.2. Quantification was achieved by calibration of FAMEs standards supplied by Sigma Aldrich (Supelco 37 Component FAME Mix, 18919-1AMP). Sample FAMEs were also injected under the above mentioned conditions and their concentrations were determined from the calibration plot.

The neutral fractions eluted by dichloromethane were converted to trimethylsilyl derivatives by reaction with N, O-Bis(trimethylsilyl)trifluroacetamide (BSTFA) and pyridine for 3 hrs at 70°C. Analysis was carried out by GC-MS using a Perkin Elmer Clarus GC 620, with MS detector equipped with a non-polar HP ultra-double-fused silica capillary column (30 m, 0.32 mm internal diameter, 0.25 µm film thickness). Operating conditions were as follows: ion source of electron voltage 70eV kept at 200°C. Spectra were scanned from 50 to 600 m/z with a scan time of 1.50s. Initially the temperature was increased from 50°C to 220°C at a rate of 10°C per min and held at 220°C for 5 min. Then the temperature was again increased from 220°C to 290°C at a rate of 1°C per min and held at 290°C for 10 min. The detector was held at 290°C and helium was used as carrier gas. Full data acquisition was obtained with the use of MS turbo mass version 5.3.2. Individual compounds were identified by comparison of mass spectra with literature and library data, retention time of authentic standards and interpretation of mass spectrometric fragmentation patterns (Philp, 1985; Logan and Eglinton, 1994).

The hydrocarbon fraction eluted with n-hexane using silica gel column was evaporated to 1ml under ultrahigh purity N_2 prior to concentration determination on a Gas Chromatograph equipped with Flame Ionisation Detector (GC-FID) with a 30m×0.25mm i.d. DB-5 column, 0.25 µm film thickness. Oven temperature was held at 50°C for 5 min and then increased to 300°C at a rate of 3°C per min and held for 5 min. The injector temperature was kept at 260°C and the detector temperature was maintained at 325°C. Nitrogen was used as carrier gas with a flow rate of 2ml per min. Identification of individual compounds was achieved by comparison of GC retention times with those of standard compounds. Quantification was made based on the calibration with authentic standards (C_7-C_{40}) Sigma Aldrich).

2.3.6 Statistical Analysis of Data

One way analysis of variance (ANOVA), correlation analysis and factor analysis were done using 'Statistical Package for Social Sciences (SPSS), version-13'. Spatial and seasonal variations were assessed by ANOVA with season and space (stations) as source of variation. The log (x+1) transformation were used to obtained normal distribution of the data set and normality was checked using criteria of Webster, 2001. Factor analysis were used to extract information from the most meaningful parameters which better described the overall data, while afford data reduction with minimal loss of information (Katsaounos et al., 2007) In factor analysis, principal component analysis (PCA) and varimax rotation methods were used for extraction and deriving principal components (PC) respectively. Factor loading were considered significant if they were >0.50.

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Chapter 3

TRACE METALS AND PHOSPHOROUS SPECIATION

Contents

3.1 Geochemistry of the Cochin Estuary

Cochin estuary is a tropical micro-tidal monsoonal estuary having a highly complex geometry and bathymetry. The fluvial discharge of Cochin estuary is influenced by freshwater discharge from two rivers, Periyar and Muvattupuzha, as well as the tidal intrusion from the Arabian Sea. Seasonal salinity fluctuation is more or less governed by river discharge. The river discharge is maximum during monsoon (1346 m^3s^{-1}), moderate in post-monsoon (205 m^3s^{-1}) and minimum (142 m^3s^{-1}) in pre monsoon period (Revichandarn et al., 2012). Salinity values remain close to zero over the surface layer of the estuary during the monsoon. The complete freshening of the estuary takes place during the peak monsoon period. During the monsoon season, the estuary is more dynamic and the pH is found to be less than 7. Post monsoon is a typical transition period. In post monsoon, river discharge gradually decreases and tidal influence gains momentum and the estuarine condition changes to partially mixed type. The productivity is high during this season. In pre monsoon, river discharge is in minimum and the seawater influence is maximum, the estuary is well mixed and homogeneity exists in water. Estuary acquires more or less a stable condition during pre-monsoon season (Menon et al., 2000).

The spatial and seasonal variations in hydrographical properties of water including redox potential, salinity, pH, temperature, dissolved oxygen and particles in suspension influence the biogeochemical cycling of nutrients, trace metals and organic matter in the Cochin estuary. The low salinity and oxygenated water column conditions favours the formation of Fe and Mn hydroxides, whereas the geochemical formation of calcite is low. Trace metals and nutrients are either co-precipitated or adsorbed onto oxides or hydroxides of Fe/Mn (Deepulal et al., 2012). The high energy condition due to the enhanced flow may result in the accumulation of coarser particles in surface sediments. The coarse grained highly permeable sediment allows the dissolved oxygen penetration to the surface sediment keeping it oxic and metastable. Nutrients and trace metals released by anaerobic organic matter degradation diffuse to the oxic layer in the sediments, where it forms oxides or hydroxides and get precipitated. This mechanism effectively sorb nutrients and trace metals, restricting the mobilisation of these elements into the water column during the monsoon period in the entire portion of the estuary. Also these mechanisms operate throughout the year in the fresh water end of the estuary (Martin, 2009).

Increase in salinity during the post monsoon and pre monsoon seasons in Cochin estuary are accompanied by gradients in other variables including pH, dissolved oxygen, type and concentration of suspended particles and organic matter. Clay particles can be negatively charged by isomorphous substitution during the formation of clay minerals. The surface charge of the organic matter depends on the pH, as the negatively charged clay particles move from freshwater to saline water, free cations neutralise the negatively charged surfaces, allowing molecular force of attraction to dominate when the particles are brought close enough. They flocculate leading to the increased deposition in high salinity region. The muddy impermeable sediment towards the high saline region allows only a minimum advective flow of oxygen which favours anaerobic redox processes. The major redox reactions in sediments are organic matter remineralisation and other reactions involving inorganic substances (Burdige, 2011). Microorganisms catalyze these redox reactions that combine most efficient electron donor with most efficient electron acceptor. The most common electron donor is organic matter, molecular oxygen act as the most preferred electron acceptor and if the supply of oxygen is terminated the microbial processes switch to facultative followed by anaerobic respiration which use nitrate, Mn^{4+}, Fe^{3+}, sulphate and finally carbon dioxide producing molecular nitrogen(N_2), ammonium (NH_4^+), Fe^{2+}, Mn^{2+}, H_2S and CH_4. The reductive dissolution of the (oxyhydr)oxides of Fe/Mn releases Fe^{2+} and Mn^{2+} which in turn reacts with sulphide formed from sulphate reduction to form Fe/Mn sulphides (Du Laing, 2011). The adsorbed or co-precipitated trace metals and nutrients releases to the pore and water column which enhances the water column productivity. Carbonates are biogenically and geochemically formed in the high salinity region and directly precipitate metals and nutrients. When the pH decreases below a certain limit, calcification occurs, which results in the mobilisation of trace metals and nutrients.

Sulphates are ubiquitous in oxic estuarine environment and flow into estuary along with sea water or they can be released within sediments during organic matter remineralisation. Reduction of sulphates in anaerobic conditions leads to the formation of sulphides. Microbial communities initiate sulphate reduction of redox potential below -150mV (Mansfeldt, 2004) or -

220mV (Ross, 1989). When the medium turns saturated with sulphides, they serve as a sink for many discharged trace metals in sediments (Salomons et al., 1987), mean while the formation of sulphide of certain metals increase the concentration of such metals in solution. Although Cd, Cu and Zn can directly precipitate as sulphides; it is mainly Fe that generates sulphide precipitate in the estuarine environment. Fe sulphides are rapidly formed as Fe is often available in high concentrations. The dissolved sulphides produced by bacterial sulphate reduction in sediments have several possible fates; reaction with Fe oxides or Fe^{2+} to form FeS, incorporation in sediment organic matter to form organic sulfur compounds, reaction with FeS to pyrite and oxidation leading to the formation of elemental sulphur (Du Laing, 2011).

3.2 Phosphorous as a Geochemical Index

Phosphorous in the estuarine environment usually exists in various forms, mainly; dissolved inorganic, dissolved organic, particulate organic, particulate inorganic, crystalline, occluded and adsorbed P (Gikuma-Njuru et al., 2010). In water column, P may exchange between particulate and dissolved phases through physical, chemical and biological processes (Suzumura et al., 2004). The particulate form can get settled from water column or can be dissolved into water column through microbial activity and through physical or chemical dissolution.

Among the soluble P forms, only ortho-phosphate can be directly utilised by phytoplankton (Gikuma-Njuru et al., 2010) and the amount of phosphate (PO_4^{3-}) present in a water body at any given time depends on external P loading and various influence mechanisms that regulate its release and retention in sediments (Garcia and Iorio, 2003). The mechanisms involved can be of chemical (adsorption/de-sorption and precipitation/dissolution) or biological in nature (uptake or release by bacteria and algae, excretion by macrofauna), or a combination of both (e.g. anoxic conditions mediated by bacteria resulting in the release of sorbed P from iron oxides) (Slomp et al., 1996; Garcia and de Iorio, 2003).

Under aerobic conditions, PO_4^{3-} interacts with many cations such as Fe^{3+} and Ca^{2+} to form relatively insoluble compounds that precipitate out of the water column to the sediments (Spivakov et al., 1999; Wetzel, 2001). The P bound to iron hydroxides can be released into overlying water when FeOOH get reduced in the anoxic layers of the sediment (Jordan et al. 2008). When this happens under aerobic fresh water, the resulting Fe (II) can diffuse upward into aerobic sediments, and get oxidised to FeOOH that can bind phosphate, thus preventing it from diffusing to the overlying water. In anoxic layers of salt water rich sediments, sulphides formed by sulphate reduction can bind with Fe (II) leading to pyrite formation thus preventing it from diffusing to aerobic layers where it might otherwise re-oxidise and bind to phosphate (Caraco et al., 1990; Gachter and Muller, 2003; Jordan et al., 2008; Gikuma-Njuru et al., 2010). It is well established that sulphides can promote FeOOH reduction in anoxic sediments (Jensen et al., 1995). PO_4^{3-} can also co-precipitate with calcium carbonate in alkaline waters (Golterman, 1995). Furthermore, in saline water the reduction of FeOOH below the oxic layer of the sediment may release phosphate and fluoride into the water, leading to the formation of carbonate fluroapatite, a P mineral that accumulates in marine sediments (Ruttenberg, 1992; Ruttenberg and Berner, 1993). P is not redox sensitive by itself, but is strongly coupled with redox dependant cycle of Fe and organic matter as well as precipitation and dissolution of calcium carbonate.

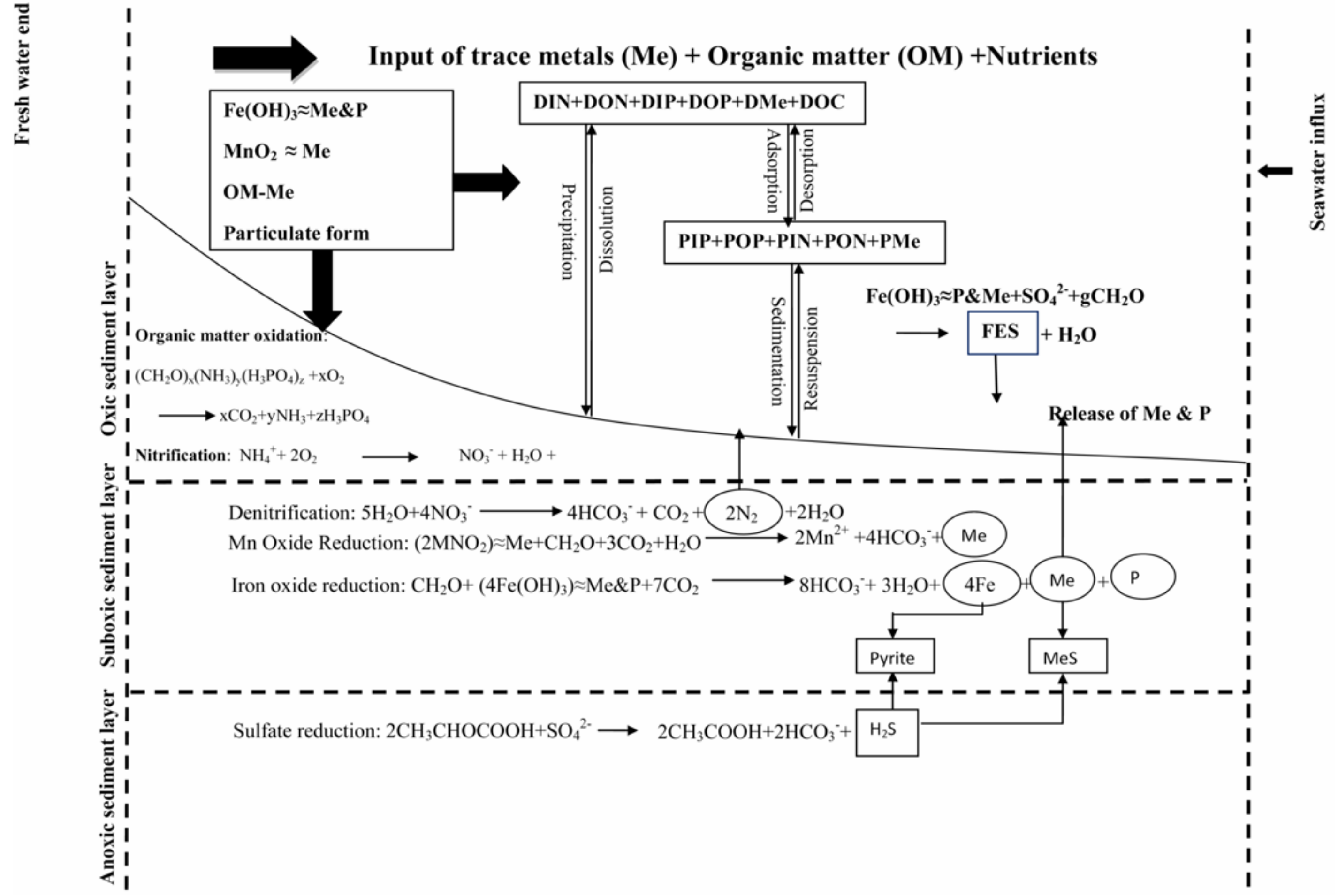

Fig.3.1 Schematic representation of major biogeochemical processes in estuaries [DIN-dissolved inorganic N, DON-dissolved organic N, DIP- dissolved inorganic P, DOP- dissolved organic P, DMe- dissloved metals, DOC-dissolved organic carbon, PIN-particulate inorganic N, PON-particulate organic N, PIP- particulate inorganic P, POP- particulate organic P, DMe- particulate metals, DOC-dissolved organic carbon]

Therefore the fractionation of P can be used as an effective tool for unravelling the redox processes acting along the salinity gradients of the Cochin estuary. The schematic representation of aforementioned geochemical processes are furnished in Fig. 3.1

3.3 Results

3.3.1 General Water and Sediment Characteristics

Water column pH (Fig. 3.2) showed slightly alkaline character in pre monsoon season with an average of 7.91 and 7.98 in surface and bottom water respectively. It displayed slightly acidic character in monsoon season except in the bottom waters of barmouth to S7 which ranged from 6.08 to 6.89 (average ± standard deviation, 6.46±0.28) in surface water and 6.00 to 7.44 (6.66 ± 0.63) in bottom water. Salinity exhibited (Fig. 3.2) large fluctuations in the study region with a bimodal pattern, which ranged from 0.02 to 32.23. Fresh water condition was prevailing during monsoon when the river run off is very high and after that salinity started to increase. An increase in salinity was again noticed during pre monsoon period and it ranged from 17 to 31.5 during pre monsoon. PO_4^{3-} showed (Fig. 3.2) a decreasing trend from bar mouth to Thanneermukkom bund in all seasons. PO_4^{3-} ranged from 0.38 to 3.55 µM (1.74± 1.14) in surface water and 0.17 to 7.19 µM (2.41 ± 1.76) in bottom water during pre monsoon. In monsoon, it ranged from below detectable limit (n.d) to 2.22 µM (0.75 ± 0.80) in surface water and n.d to 5.88 µM (1.90 ± 2.11) in bottom water. In post monsoon the surface range was between 0.23 and 1.63 µM (0.69 ± 0.40) and at the bottom, the estimated range was between 0.09 and 1.96 µM (0. 78± 0.52).

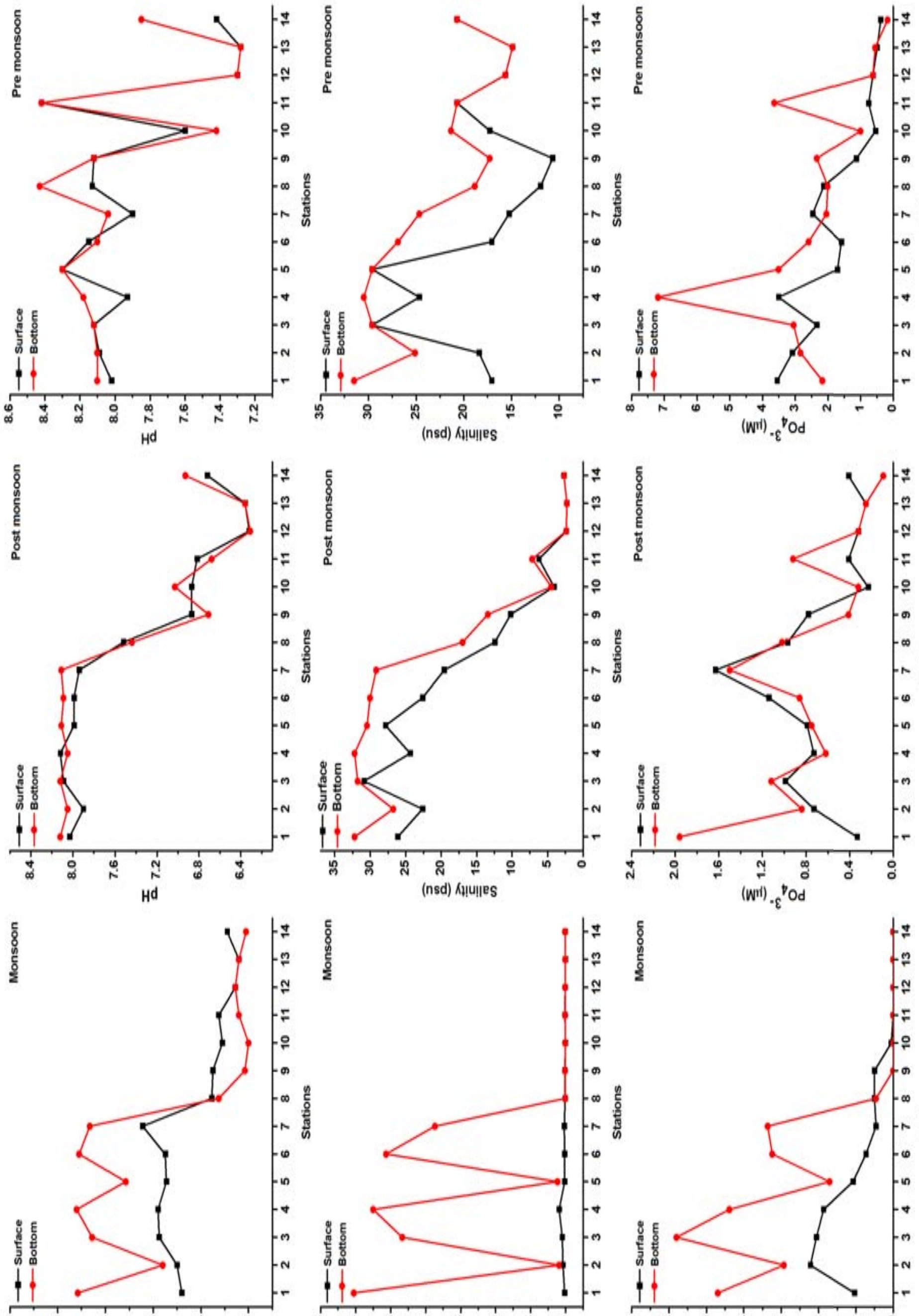

Fig. 3.2 Spatial distributions of pH, salinity and dissolve inorganic phosphate in the Cochin estuary

Surface sediments collected from Cochin estuary showed spatial variations in textural characteristics (Fig. 3.3). Sand and silt content dominated in the first three stations (barmouth region) as well as in the last four stations (southern end).

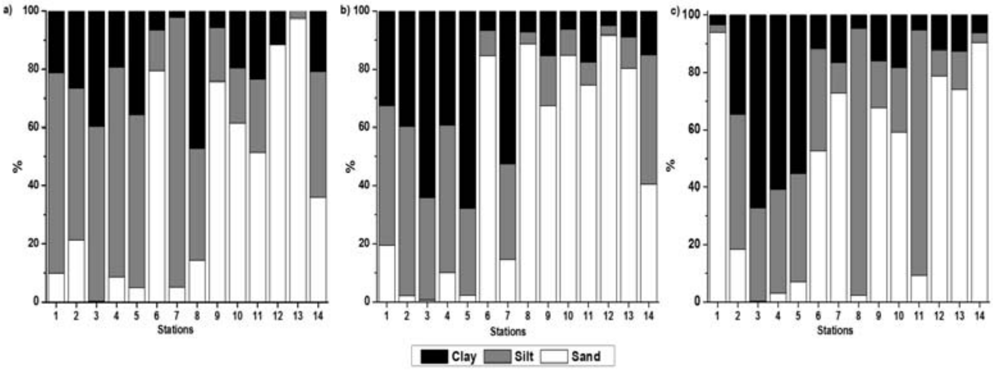

Fig. 3.3 Distribution of sand, silt and clay in surface sediments (a-monsoon, b-post monsoon and c-pre monsoon)

Table 3.1 pH and Eh in surface sediments of the Cochin estuary

Stations	pH			Eh (mV)		
	Mon	Post	Pre	Mon	Post	Pre
1	7.18±0.06	7.16±0.04	7.8±0.04	-194±5	-182±9	-185±5
2	7.24±0.07	6.93±0.03	8.23±0.06	-151±7	-190±10	-242±7
3	6.75±0.04	6.88±0.04	7.19±0.05	-170±10	-210±10	-225±5
4	6.43±0.05	7.03±0.07	7.78±0.04	-52±5	-220±7	-189±6
5	7.14±0.0	6.95±0.05	8.64±0.07	-175±10	-187±8	-160±10
6	7.13±0.08	7.41±0.06	8.34±0.07	-130±5	-164±5	-214±10
7	6.73±0.08	7.14±0.10	8.37±0.02	-102±7	-192±7	-176±9
8	6.63±0.07	6.98±0.04	7.65±0.04	-209±6	-160±8	-189±5
9	6.85±0.06	7.02±0.06	7.2±0.05	-152±4	-160±8	-212±12
10	6.59±0.05	6.9±0.13	8.02±0.11	-204±5	-175±3	-200±10
11	6.72±0.07	7.03±0.06	7.19±0.05	-200±5	-182±4	-207±10
12	6.7±0.07	7.00±0.08	7.05±0.06	-202±6	-186±5	-190±10
13	6.72±0.06	7.04±0.09	7.1±0.06	-196±6	-188±5	-188±10
14	6.83±0.04	7.09±0.10	8.11±0.06	-211±9	-215±7	-202±7

Other stations were characterised by high content of fine fractions. Sediment pH (Table 3.1) in the entire study area was slightly alkaline in pre monsoon and post monsoon while slightly acidic pH recorded in monsoon. The pH values ranged from 6.43 to 7.24 in monsoon, 6.88 to 7.41in post monsoon and 7.05 to 8.64 in pre monsoon. The sediment is characterised by reducing conditions (the average Eh is below the redoxcline, i.e. 230 mV) and Eh values (Table 3.1) were in the range of -242 to -160mV (pre monsoon), -211 to -52mV (monsoon) and -220 to -160mV (post monsoon).

3.3.2 Phosphorous Fractionation in Sediments

The variation of TP and different forms of P in the surface sediments are depicted in Fig.3.4. The concentration of TP in surface sediments ranged from 395 to 2383μgg^{-1} (1375 ± 763) in monsoon, 612 to 2190 μgg^{-1} (1435 ± 616) in post monsoon and 313 to 2239μgg^{-1} (1173 ± 558) in pre monsoon. TP in surface sediments was dominated by TIP for stations S1-S7, while TOP dominated in the rest of the stations. Fe(OOH)≈P in surface sediment was highest in monsoon (147 to 963μgg^{-1}, 510 ± 301) followed by post monsoon (103 to 503μgg^{-1}, 257 ± 127) and pre monsoon (33 to 597μgg^{-1}, 294 ± 191). Fe(OOH)≈P accounted for 4 to 61% of the total P pool in pre monsoon, 15 to 75% in monsoon and 6 to 60% in post monsoon.

$CaCO_3$≈P in the surface sediments varied from 25 to 809μgg^{-1} (292 ± 274), 44 to 945μgg^{-1} (381 ± 347) and 17 to 1355μgg^{-1} (387 ± 396) in monsoon, post monsoon and pre monsoon respectively. It constituted to about 6 to 61% of the total P pool in pre monsoon. In monsoon, its contribution was from 3 to 43% and in post monsoon 3 to 49%. Acid-OP ranged from 16 to 194μgg^{-1} (94 ± 70) in monsoon, 65 to 207μgg^{-1} (136 ± 56) in post monsoon and 32 to 194μgg^{-1} (106 ± 50) in pre monsoon.

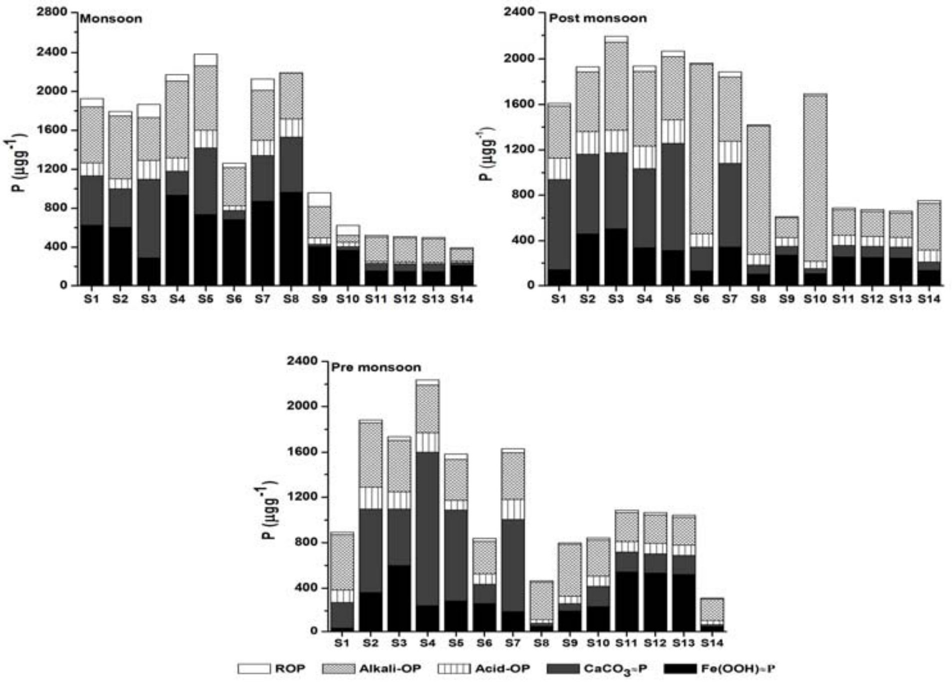

Fig. 3.4 Fractions of P in surface sediments

Alkali-OP varied from 72 to 787µgg^{-1} (412 ± 212) and 176 to 1492µgg^{-1} (734 ± 440) and 198 to 568µgg^{-1}(362 ± 109) in pre monsoon, monsoon and post monsoon respectively. ROP was found to be comparatively smaller fraction and ranged from 2 to 142µgg^{-1}(66 ± 51) in monsoon, 8 to 47µgg^{-1} (26 ± 15) in post monsoon and 7 to 48µgg^{-1}(24 ± 12) in pre monsoon.

3.4 Discussion

3.4.1 Ratios of Carbon, Nitrogen, Sulphur, Phosphorous and Iron as Redox Indicators

The OC: TP ratios (Fig. 3.5) showed large variations in the study region which ranged from 3 to 62 in pre monsoon, 9 to 47 in monsoon and 2 to 32 in post monsoon. The TN: TP ratios were very low and did not display much variation in the study region. Their values ranged from 0.4 to

6.6, 0.5 to 3.9 and 0.2 to 2.2 in pre monsoon, monsoon and post monsoon respectively. The OC: TP and TN: TP ratios were lower than the Redfield ratio (Hecky et al., 1993), indicating that the organic matter is enriched with P and tends to accumulate in surface sediments.

Chemistry of P in sediments is largely influenced by redox conditions, and the redox cycle of Fe greatly affects P geochemistry after burial (Cha et al., 2005). OC: TS ratio (Fig. 3.5) gives a qualitative indication of the redox status of the environment of deposition, when TS concentrations are high (Raiswell et al., 1987). OC: TS > 5 are considered as oxic sediment with oxygenated bottom water, OC: TS = 1.5–5 indicates deposition under periodic anoxia and OC: TS < 1.5 represents anoxic sediment with overlying anoxic water. The OC: TS values (Fig. 3.5) in the study region lie in the second category suggesting that these sediments undergo sulphate reduction below an oxygenated water column (Hedges and Keil, 1995; Renjith et al., 2011).

The involvement of Fe in the dynamic equilibrium between the sediment and water PO_4^{3-} levels has led to suggestions that the Fe dependant threshold limit exists for the sediment ability to bind phosphate (Sondergaard et al., 2003). The Fe: TP ratios were considered as a measure of free sorption sites for PO_4^{3-} ion on iron hydroxides (Jensen et al., 1992; Jensen and Thamdrup, 1993; Coelho et al., 2004). Caraco et al., (1990) suggested that the Fe: TP ratio should exceed 10 if it was to regulate P release. Fe: TP ratios lacked seasonal variations in the study region, but displayed significant spatial variation. The fresh water areas showed higher Fe: TP indicating the presence of enough Fe in surface sediments to bind with P, while lower ratios obtained towards the seaward sites suggest the saturation of sorption sites or less capacity to bind with PO_4^{3-} may result in P efflux from the sediment to the overlying water column.

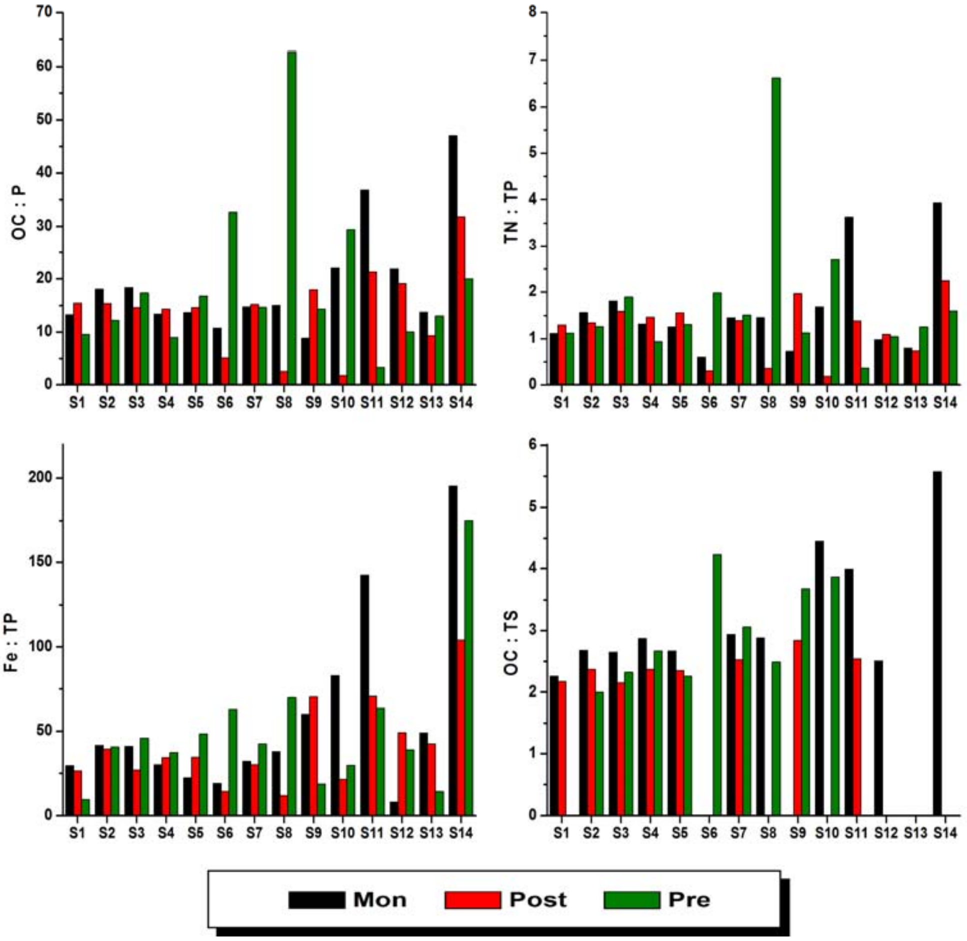

Fig. 3.5 Elemental ratios in surface sediments

3.4.2 Forms of Phosphorous in the Estuary

Phosphorous fractions undergo series of chemical and biological transformations along the salinity gradients of estuaries. Previous studies demonstrated a shift from importance of Fe(OOH)≈P to $CaCO_3$≈P with increasing salinity (Coelho et al., 2004; Hartzell et al., 2010). Oxidative decomposition of organic matter and reductive dissolution of Fe(OOH)≈P releases the P to the sediment interstitial water and a portion of released P may return to the water column. A substantial portion of released P contributes to the formation of authigenic P minerals and thereby immobilised in the sediments (Ruttenburg and Berner, 1993; Schuffert et al., 1994; Kim et al., 1999; Cha et al., 2005). ANOVA and correlation analysis were carried out to measure the statistical significance of each processes along the salinity gradients of the estuary.

TP displayed spatial variation in the study area ($p<0.01$) but did not exhibit any significant seasonal variation. In general, the distribution of P fractions and other geochemical parameters in the surface sediments of Cochin estuary displayed relationship with sediment texture. Correlation analysis (Table 3.2) has revealed that silt and clay fractions have high positive correlations with most of the sedimentary parameters. Sand fraction showed similar negative correlations. These indicate that the main factor influencing the geochemistry of surface sediments in the study region could be sediment texture. These high correlations may have resulted from the greater surface area of fine particles resulting in more adsorption sites for phosphate ions (Andrieux-Loyer and Aminot, 2001; Liu et al., 2002; Zhou et al., 2005). It is well established that organic carbon, nutrients and metals are enriched in silt and clay fractions than in sand (Krishna Prasad and Ramanathan, 2008; Wen et al., 2008; Renjith et al., 2011).

Table 3.2 Correlation between different phosphorus fractions and other geochemical parameters (n=42)

	pH	Eh	Sand	Silt	Clay	OC	TN	TC	TS	Fe	Ca	Fe (OOH) ≈P	$CaCO_3$ ≈P	Acid-OP	Alkali-OP	ROP	TP	Salinity
pH	1																	
Eh	-0.34^b	1																
Sand	-0.07	0.17	1															
Silt	-0.05	0.05	-0.86^a	1														
Clay	0.19	-0.37^b	-0.78^a	0.34^b	1													
OC	0.06	-0.11	-0.96^a	0.78^a	0.79^a	1												
TN	-0.14	-0.04	-0.89^a	0.75^a	0.70^a	0.91^a	1											
TC	-0.06	-0.07	-0.90^a	0.77^a	0.69^a	0.92^a	0.96^a	1										
TS	-0.02	-0.09	-0.87^a	0.71^a	0.72^a	0.88^a	0.87^a	0.86^a	1									
Fe	-0.03	-0.32^b	-0.68^a	0.57^a	0.54^a	0.65^a	0.59^a	0.62^a	0.43^a	1								
Fe(OOH)≈P	-0.26	0.02	-0.31^b	0.40^a	0.09	0.28	0.35^b	0.33^b	0.36^b	0.11	0.14	1						
$CaCO_3$≈P	0.17	-0.06	-0.71^a	0.58^a	0.58^a	0.72^a	0.60^a	0.60^a	0.73^a	0.25	0.19	0.13	1					
Acid-OP	-0.08	0.04	-0.59^a	0.47^a	0.49^a	0.61^a	0.54^a	0.53^a	0.67^a	0.13	0.20	0.33^b	0.80^a	1				
Alkali-OP	-0.15	0.10	-0.01	0.03	-0.02	0.04	0.07	0.02	0.01	-0.18	0.35^b	-0.12	0.13	0.22	1			
ROP	-0.29	0.23	-0.34^b	0.49^a	0.03	0.31^b	0.35^b	0.36^b	0.27	0.21	0.22	0.30	0.29	0.25	0.02	1		
TP	-0.13	0.04	-0.60^a	0.58^a	0.38^b	0.60^a	0.59^a	0.54^a	0.64^a	0.12	0.38^b	0.51	0.75^a	0.79^a	0.55^a	0.38^b	1	
Salinity	0.45^a	-0.14	-0.35^b	0.18	0.42^a	0.39^b	0.30	0.29	0.33^b	0.01	-0.02	-0.08	0.44^a	0.33^b	0.15	-0.09	0.30	1

a Correlation is significant at the 0.01 level (2-tailed). b Correlation is significant at the 0.05 level (2-tailed)

One way ANOVA of Fe(OOH)≈P has revealed significant seasonal variation ($p<0.01$) but lacked significant spatial variations. During the monsoon season, the estuary is virtually converted into a freshwater basin even in areas around the barmouth where salt water penetration occurs at depths below 5 m depth only (Menon et al., 2000; Renjith et al., 2011). In post monsoon, river discharge gradually diminishes and tidal influence gains momentum as the estuarine conditions changes to a partially mixed type, weakening stratification. This is mainly a transitional period. In pre-monsoon, the river discharge is in minimum and seawater influence is maximum upstream; the estuary is well-mixed and homogeneity exists in the water column (Menon et al., 2000). Fe(OOH)≈P showed higher concentrations in the monsoon season followed by post-monsoon and pre-monsoon and the percentage contribution of Fe(OOH)≈P to TP displayed an increasing trend from barmouth to Thannermukkom bund. $CaCO_3$≈P exhibited significant spatial difference in the study region recording higher concentrations at the barmouth region and lower concentrations towards the bund ($p<0.01$) but did not display any seasonal variation. $CaCO_3$≈P showed strong positive correlation with salinity and its concentration increased towards the barmouth region. TS concentrations were higher towards the seaward sites and it exhibited strong positive correlation with Fe.

These trends may have resulted from several processes (Coelho et al., 2004; Hou et al., 2009). The increased pH and salinity in pre monsoon and post monsoon season may inhibit phosphate adsorption onto Fe oxides/hydroxides, by shifting speciation phosphate from $H_2PO_4^-$ to HPO_4^{2-} and by changing surface charge on the Fe oxides and hydroxides (Lebo, 1991; Zwolsman, 1994). Also, the concentration of Fe oxides and hydroxides reduced in sulphide environments by the formation of solid Fe sulphides and the sulphate reduction rate may be the highest in more saline area (Paludan and Morris, 1999; Hou et al., 2009). The increased concentration of $CaCO_3$≈P may be caused by the interactions between PO_4^{3-} and $CaCO_3$ with increasing salinity (Gunnars et al., 2004; Anshumali and Ramanathan, 2007; Hou et al., 2009). Calcite is produced at high salinities through precipitation reactions, and biological activity in estuarine environment forming an adsorption substrate for dissolved phosphate (Coelho et al., 2004). $CaCO_3$≈P also showed strong positive correlations with Acid-OP which may have resulted from the mineralisation of organic P. During microbial decomposition, organic P may have transformed into authigenic fluroapatite (Anshumali and Ramanathan, 2007; Katsaounos et al., 2007; Hou et al., 2009). The southern part of the study region is well known for black clam fishery (Lakshmilatha and Appukuttan, 2002) and their shell is thick and rich (93.3% to 95.8%) in calcium carbonate (Kripa et al., 2004). Apart from live clam beds, the estuary has extensive sub fossil deposits (Renjith et al., 2011). The present results were in good agreement with this mechanism, since $CaCO_3$≈P is the most important fraction to the seaward side and Fe(OOH)≈P towards the fresh water end of the study region. Fe(OOH)≈P dominated in the entire estuary in monsoon season. The decrease of Fe(OOH)≈P with increasing salinity has been reported in many estuaries like Pamlico River-estuary (Upchurch et al., 1974), Dalware estuary (Strom and Biggs, 1982) Scheldt estuary (Hyacinthe and Van Cappellan, 2004), Mondego estuary (Coelho et al., 2004), Patuxent River-estuary (Jordan et al., 2008) and Chesapeake Bay estuaries (Hartzell and Jordan, 2010).

Acid-OP includes apatite-bound phosphate and biochemical components such as nucleic acids, lipids, and sugars that are bound to phosphate (De Groot, 1990). It showed lower concentrations in the study area which accounted for 5 to 13%, 3 to 20%, and 4 to 14% of the TP in pre monsoon, monsoon and post monsoon respectively. Acid-OP exhibited distinct seasonal ($p<0.05$) and spatial ($p<0.01$) difference with maximum concentrations in the post monsoon, and displayed an increasing trend towards the bar mouth region. The degradation of Acid-OP compounds release phosphate which is available to phytoplankton. The main component of Alkali-OP is humic substances (Golterman, 2001). P 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). This fraction also contains phytate, an organic phosphate that is common in plants and sediments (De Groot and Golterman, 1993; Dvorakova, 1998). Alkali-OP accounted for 10 to 73% in pre monsoon, 11 to 49% in monsoon and 9 to 86% in post monsoon. Alkali-OP displayed significant seasonal difference with maximum concentrations in the post monsoon and minimum in monsoon season. Higher Alkali-OP concentrations were observed in the southern part of the study region. This may be due to the flocculation and precipitation processes involving humic acids, Fe/Al oxides and dissolved reactive P complexes from advective mixing of salt water with fresh water (Coelho et al., 2004).

PO_4^{3-} displayed significant spatial ($p<0.05$) and seasonal ($p<0.01$) variations in the study region. It was characterised with maximum concentrations in the pre monsoon and minimum in post monsoon season. Abrupt increase in PO_4^{3-} concentration with increasing salinity was observed in the study region. PO_4^{3-} also displayed highly significant positive correlations with TS, $CaCO_3\approx P$ and TP. The percentage of

Fe(OOH)≈P showed a decreasing trend towards the seaward side and the Fe and TS showed strong positive correlation in all seasons. It is well established that P-Fe-S interactions promote the release of PO_4^{3-} from sediments at higher salinities (Azzoni et al., 2005; Jordan et al., 2008). Boynton and Kemp (2008) found the lowest rate of PO_4^{3-} efflux in tidal fresh water at a salinity range of 0-0.5 ($4\mu M\ P\ m^{-2}\ h^{-1}$), higher at salinities 0.5 -5 and highest at a salinity range of 5-10 ($12\mu M\ P\ m^{-2}\ h^{-1}$ and $52\mu M\ P\ m^{-2}\ h^{-1}$ respectively) in estuarine sites outside the Chesapeake Bay. Boynton and Bailey (2008) also found a similar relationship between salinity and PO_4^{3-} efflux. This PO_4^{3-} efflux can alter the relative bioavailability of N and P (Hartzell and Jordan, 2010).

Bioavailable P (BAP) in the sediment can be defined as the sum of immediately available phosphorus and potential phosphorus that can be transformed into an available form by naturally occurring physical, chemical and biological processes (Wang et al., 2009). Fe(OOH)≈P, $CaCO_3$≈P and Acid–OP were considered as a source of BAP for phytoplankton (Diaz-Espejo et al., 1999). Knowledge of P fractions is of utmost importance in determining the upper limit of the potentially BAP in aquatic ecosystems (Hou et al., 2009). The bioavailability of Fe(OOH)≈P depends primarily on sediment redox intensity (Andrieux-Loyer and Aminot, 1997; Rozan et al., 2002; Álvarez-Rogel et al., 2007). In the areas characterised by frequent change of sediment redox potential, Fe(OOH)≈P can intermittently be reduced and released from sediments into the pelagic waters (Jensen and Thamdrup, 1993; Coelho et al., 2004). Organic P could become bioavailable by microbial remineralisation (Andrieux-Loyer and Aminot, 1997; Hou et al., 2009). The spatial and seasonal variation of BAP is depicted in Fig. 3.6. The BAP fractions ranged from 25 to 79% (107 to 1770µgg^{-1}) in pre monsoon, 48 to 78% (249 to 1716µgg^{-1}) in monsoon and 13 to 71% (217 to 1465µgg^{-1}) in post monsoon. One way ANOVA of the BAP in surface sediments displayed no significant seasonal variation ($p > 0.05$). However, the BAP concentrations varied significantly among the sampling stations ($p < 0.05$). BAP displayed a decreasing trend from barmouth to Thannermukkom bund. BAP was relatively higher in the Cochin estuary which also reveals that the sediment can act as an important internal source of P for the Cochin estuarine and coastal ecosystem.

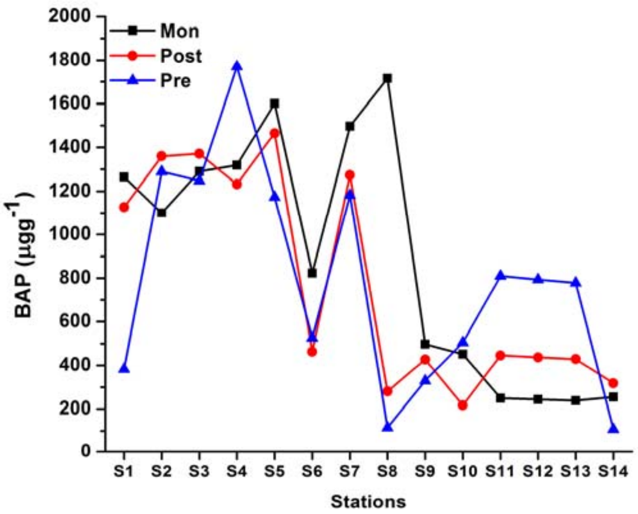

Fig. 3.6 Spatial and seasonal variations of bioavailable phosphorous (BAP) in surface sediments

The high Fe: TP ratios in the freshwater side of the estuary ensure enough sorption sites for P and saline water region is characterised with lesser sorption sites for P. The processes of reductive dissolution of iron hydroxides and biogenic or geochemical formation of calcium carbonate minerals in the saline areas is indicated by the distributional dynamics of Fe(OOH)≈P and $CaCO_3$≈P in the estuary. The input of organic matter enriched with P from rivers and from different industrial, agricultural and aquaculture activities lead to a large scale accumulation of refractory organic P in the surface sediments of the Cochin estuary.

3.5 Trace Metals Geochemistry

The sources of trace metals in estuaries include inputs from fresh water discharges, urban, domestic and industrial effluents and atmospheric deposition. Within the estuaries, trace metals are present in different physico-chemical forms or species such as dissolved, particulate and sedimentary metals. Sediments are final destination of trace metals, as a result of adsorption, desorption, precipitation, diffusion processes, chemical processes, biological activity and a combination of these phenomena. The thermodynamically stable form of metal ion in estuaries is controlled by the redox potential in the sediment water interface, which is essential in determining the fate and role of metals in biological systems. For example under oxic conditions Fe(II) get oxidised to Fe (III) which is insoluble in water and finally get deposited in sediments, lowering dissolved Fe concentrations (Du Laing et al., 2007; Du Laing, 2011). The sediment characteristics such as pH, cation exchange capacity, organic matter contents, redox conditions and salinity determine the metal sorption and precipitation processes, which is in turn related to metal mobility, bioavailability and potential toxicity (Du Laing et al., 2009).

Redox sensitive trace metal (Fe, Mn, Cu, Ni, Pb, Zn and Cd) concentrations are among the most widely used indicator of redox conditions. Fe and Mn oxides are excellent scavengers for metals and metals can exist as sulphides under anaerobic conditions and these processes affect sediment pH and Eh change (Gambrell et al., 1991;

Lery et al., 1992). Anoxic sediment conditions can exhibit significant changes in metal speciation and solubility across redox interface (Balistrieri et al., 1994). Redox sensitive elements are widely used as indicators of redox conditions. Many metals have multiple valence states and the reduced forms that exist under low oxygen conditions are more readily complexed with organic acids, taken into solid solutions by authigenic sulphides or precipitated as insoluble oxyhydroxides (Algeo and Maynard, 2004).

3.6 Distribution of Trace Metals

Fe, Mn, Mg, Co, Ni, Cu, Zn, Pb, Cr and Cd were analysed in surface sediments of the Cochin estuary and the spatial and seasonal variations of these trace metals is presented in Fig.3.7 (a, b & c). Fe showed highest concentrations in the study area which ranged from 4140 to 83000 μgg^{-1} in monsoon, 1700 to 78437 μgg^{-1} in post monsoon and 8687 to 83625 μgg^{-1} in pre monsoon, while Mn displayed large spatial and seasonal fluctuations in the study region and it ranged from 67.50 to 657.50 μgg^{-1}, 65.00 to 428.13 μgg^{-1} and 44.38 to 347.50 μgg^{-1} in monsoon, post monsoon and pre monsoon respectively.

The variations of Mg ranged from 540 to 23937μgg^{-1} in monsoon, 2856 to 23631μgg^{-1} in post monsoon and 1456 to 23372μgg^{-1} in pre monsoon, while for Co it was ranged from 5.44 to 24.09μgg^{-1}, 4.43 to 24.61μgg^{-1} and 4.89 to 19.58μgg^{-1} in monsoon, post monsoon and pre monsoon respectively. Ni ranged from 12.43 to 72.53μgg^{-1} in monsoon, 10.50 to 62.56μgg^{-1} in post monsoon and 5.91 to 60.74μgg^{-1} in pre monsoon. For Cu, the spatial variation ranged from 4.44 to 46.88μgg^{-1}, 5.25 to 32.65μgg^{-1} and 2.89 to 29.81μgg^{-1} during the three seasons. The spatial variation of Zn ranged from 16.25 to 390.63µgg^{-1} in monsoon, 23.13 to 433.13µgg^{-1} in post monsoon and 5.63 to 585µgg^{-1} in pre monsoon, while that of Pb ranged from 1.88 to 28µgg^{-1}, 6.75 to 28.75µgg^{-1} and 2.38 to 25.13µgg^{-1} respectively during three seasons. Cr varied between 13.06 and 66.04µgg^{-1} in monsoon, 11.24 and 79.16µgg^{-1} in post monsoon and 4.81 and 76.83µgg^{-1} in pre monsoon and Cd was in the range 0.31 to 5.13µgg^{-1} in monsoon, 0.16 to 4.28µgg^{-1} in post monsoon and 0.19 to 7.16µgg^{-1} in pre monsoon.

From the result of one way ANOVA, it was found that the trace metals except Mn exhibited significant spatial variations, while Mn concentrations were characterised with significant seasonal variations. Both Cd and Zn displayed peak values at S2 and other trace metals showed a gradual decrease towards the Thanneermukkom bund. The Mn concentrations showed anomalous behaviour in the study region with higher enrichment in the low saline region.

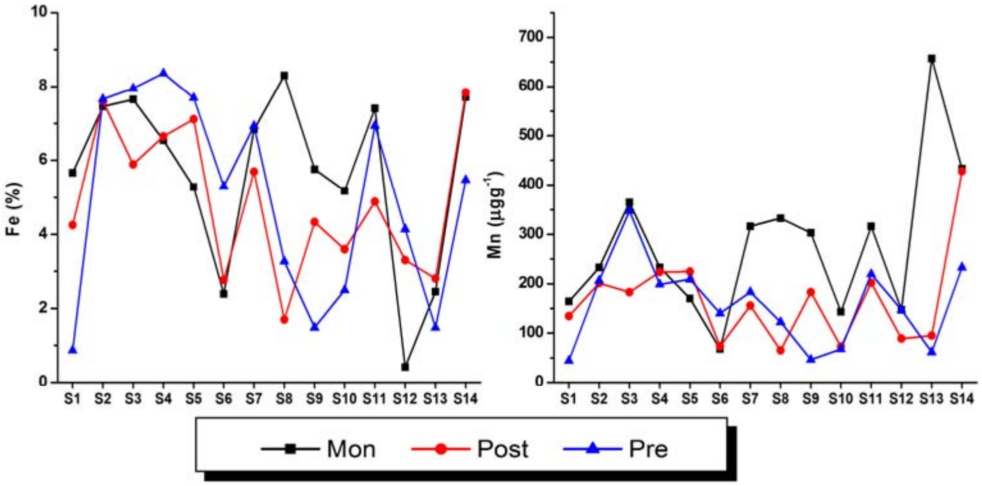

Fig. 3.7a Distributional variability of Fe and Mn in surface sediments

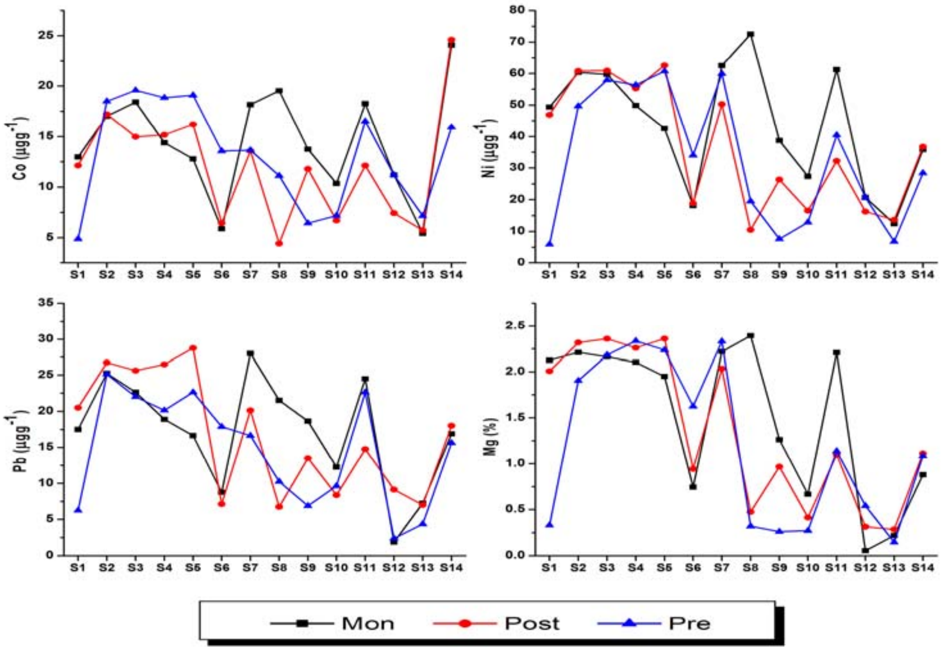

Fig. 3.7b Distributional variability of Co, Ni, Pb and Mg in surface sediments

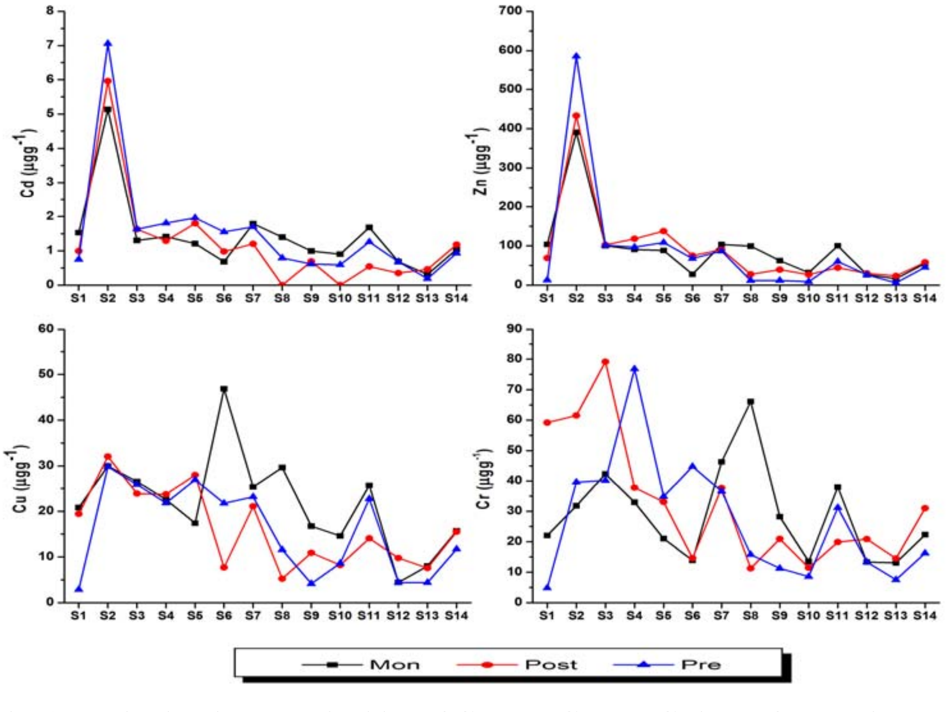

Fig. 3.7c Distributional variability of Cd, Zn, Cu and Cr in surface sediments

3.7 Discussion

There are scores of data sets available regarding the trace metal pollution and their ecological implications in surface sediments of the Cochin estuary (Balachandran et al., 2005; Nair et al., 2006; Mohan et al., 2012; Deepulal et al., 2012; Selvam et al., 2012; Ramasamy et al., 2012; Martin et al., 2012). The trace metal composition in sediments of the Cochin estuary is comparable with the aforesaid literatures. The main emphasis of the present work is to portray the redox conditions in surface sediments in the light of P fractions and trace metal composition. In the absence of a sequential extraction of metals in sediments, statistical procedures can be used for making inferences on the important pathways of elemental deposition (Isaac et al., 2005). Multivariate statistical tools like Principal Component Analysis can be effectively used to get signatures of major redox sensitive geochemical and biochemical processes acting along the salinity gradients of the estuary.

3.7.1 Principal Component Analysis

Principal Component Analysis (PCA) of geochemical parameters was done seasonally to discover and interpret various geochemical variables and thereby identifying the major geochemical processes acting in the estuary (Table 3.3). Varimax rotation was applied in order to identify the variables that are more significant to each factor based on the significance of their correlations that are expressed as factor loading (Buckley et al., 1995; Davis, 2002). The various geochemical and biochemical processes acting along the salinity gradients strongly depends on the hydrodynamic conditions prevailing in the water column. Therefore, the hydrodynamic conditions were taken into account while interpreting the PCA result. Transformations during early diagenesis and changing redox states characterise the forms of metals in estuarine environment. The sedimentary distribution of certain elements like Fe, Mn, Cu and S provide information about the occurrence of oxidised forms of metals such as oxides and hydroxides of Fe and Mn and the conversion of these species to more reduced form such as sulphides (Canfield et al., 1993; Kuzyk et al., 2011). Therefore, these elements have potential to be used as qualitative indicators of various biogeochemical processes.

The PCA generated four components for the monsoon season with a total cumulative variance of 85%. The PC1 accounted for a total variance of 27% and exhibited positive correlations with OC, TN, TS, P fractions and water column variables (salinity and phosphate). The complete freshening of the estuary takes place during the peak monsoon period (Revichandran et al., 2012). This factor indicates that the major factor which strongly influences the geochemistry of surface sediment is the hydrodynamic forcing during the monsoon season. PC2 accounted for a total variance of 25% with high positive loading for clay particles, OC, TN, TS and some of the trace metals (Cd, Ni, Co, Zn and Cr). This factor attributes to different physical and chemical processes acting on the sediment surface. Close association of clay particles with organic matter and trace metals indicate the granulometric component and also the flocculation and sedimentation of organic matter. The physical adsorption of organic matter and trace metals and subsequent sinking to the surface sediments is a significant geochemical process during the monsoon season. This component also gives an indication about the diagenetic processes acting in the surface sediments. Diagenesis, a redox process, largely mediated by microorganisms and the suitable indicators of this process is OC, TN and TS. Strong positive loading of Fe with silt particles and some of the trace metals (Pb, Ni, Mg and Cu) were observed in PC3 and accounted for a total variance of 24%. This component revealed the formation of stable Fe (oxy hydr) oxides and co- precipitation or adsorption of other trace metals (Pb, Ni and Cu) onto Fe (oxy hydr) oxides and subsequent sinking to the surface sediments.

For post monsoon season, PCA resolved three components accounting for a total cumulative variance of 89%. The close association of grain size, organic matter, trace metals (Cd, Pb, Ni, Zn, Mg, Cu, Cr), P fractions except Alk-OP and salinity in PC1 (46% of total variance) indicated the several process taking place in the salinity gradient. The adsorption of trace metals and P fractions onto clay minerals, organic matter complexation of trace metals and salinity induced flocculation of organic matter and trace metals are the major geochemical processes taking place during post monsoon season. Organic matter content and grain size are important controlling factors influencing the abundance of trace metals in estuarine sediments (Rubio et al., 2000). Adsorption of metal increases in the order: sand<silt<clay, due to an increase in their superficial area and mineral/organic matter contents (Haque and Subramanian, 1982). The presence of fine grained sediments leads to the accumulation of trace metals in contrast to the coarse grains. The larger surface area provided by finer sediments preferably adsorb metal scavenging phases such as Fe/Mn hydroxylates (Thuy et al., 2000; Bradl, 2004; Magesh et al., 2011). The grouping of OC, TN and TS pointed towards the diagenetic processes taking place in the surface sediments.

The PC2 exhibited a total variance of 31% and characterized the grouping of trace metals with organic matter and silt particles. This gave an indication about the heavy siltation process in the estuary and adsorption or co-precipitation of trace metals (Pb, Ni and Cu) onto Fe/Mn hydroxides and subsequent sinking with silt particles. Trace metal enrichment in sediments is linked to the scavenging mechanisms involving hydrous oxides. Fe and Mn being the abundant element in the study region exert a major control on the distribution of other metals in the sediments. The hydrous oxides of Fe and Mn constitute significant sinks to trace metals in estuarine environment through adsorption/co-precipitation of other trace metals and formation of complexes with various organic ligands. The grouping of salinity and phosphate and moderate negative loading of Fe(OOH)≈P in the PC3 (total variance of 11%) indicated the desorption of reductive dissolution of Fe(OOH) in the high saline area and release of Fe(OOH)≈P to the water column.

PCA resolved four components accounting for total cumulative variance of 87% during the pre monsoon season. The PC1, PC2, PC3 and PC4 accounted for a total variance of 39%, 19%, 18% and 11% respectively. PC1 was characterised by grouping of trace metals and phosphate, while significant correlations of clay particles with P fractions were observed in PC2. However, PC3 exhibited significant positive loading on silt particles, OC, TN and TS and this factor reflected the diagenetic processes operating in the sedimentary environment. The strong association of salinity, phosphate and Acid-OP is observed in PC4. The loading of trace metals in the first component indicate precipitation and settling as the major processes during pre monsoon season. Reducing condition prevails in the surface sediments during pre monsoon season and the P fractionation indicated lowest concentrations of Fe(OOH)≈P in surface sediments during this period. The high temperature induces greater bacterial activity in sediments together with the high salinity initiate sulphate reduction, denitrification and iron redox reactions (iron cycling) in sediments. These processes results in the formation of sulphide minerals such as greigite (Fe_3S_4) and mackinawite (FeS) in sediments, minerals that grows as a precursor to pyrite during early diagenetic sedimentary sulphate reduction (Sobrinho et al., 2011). PC2 is characterised with high positive loading for the P fractions. Mean while, PC3 indicates the granulometric component as well as the diagenetic pathway operating in sediments. Significant positive loading of salinity and phosphate and significant negative loading of Fe(OOH)≈P give indications about the reductive dissolution of iron oxides and subsequent release of P to the water column. Under anaerobic conditions, active sulphide formation and co-precipitation with other metal sulphides rapidly remove many trace metals from soluble form (water column and pore water) to insoluble forms (Balistrieri et al., 1994; Clark et al., 1998; Schlieker et al., 2001).

3.8 Conclusions

Sequential chemical extraction of P and distribution of trace metals were used for a better understanding of the sediment redox conditions of the Cochin estuary. The water column remains oxic throughout the year. Results of sedimentary P fractions show a shift in abundance of Fe(OOH)≈P fraction to $CaCO_3$≈P fraction with increasing salinity. An increase in TS was also observed with increase in salinity. An abrupt increase in the concentration of dissolved inorganic P with increase in salinity was observed in the study region. Fe(OOH)≈P exhibited a distinct seasonal pattern with maximum values during the monsoon season, when estuary behaves as more or less a fresh water system.

Table 3.3 Result of Principal Component Analysis

Parameters	Monsoon				Post monsoon			Pre monsoon			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC1	PC2	PC3	PC4
Sand	-0.756	-0.405	-0.268	-0.248	-0.850	-0.379	-0.147	-0.481	-0.008	-0.643	-0.098
Silt	0.404	0.361	0.801	0.033	0.532	0.743	0.138	0.389	-0.026	0.663	-0.063
Clay	0.031	0.833	0.134	-0.284	0.807	0.494	0.140	0.316	0.660	0.490	-0.064
OC	0.595	0.701	0.321	0.056	0.721	0.638	0.036	0.050	0.255	0.871	0.201
TN	0.548	0.634	0.489	0.151	0.768	0.546	0.187	0.139	0.203	0.908	0.038
TS	0.626	0.733	0.093	0.126	0.879	0.345	0.123	0.325	0.200	0.844	0.336
Cd	0.188	0.675	0.395	-0.074	0.696	0.487	0.018	0.653	0.398	0.156	0.370
Pb	0.189	0.348	0.883	0.231	0.699	0.685	0.026	0.819	0.079	0.239	0.333
Ni	0.261	0.749	0.522	0.184	0.717	0.677	0.064	0.904	0.260	0.268	-0.008
Co	-0.056	0.765	0.356	0.335	0.379	0.910	-0.112	0.942	0.134	0.239	-0.126
Zn	0.303	0.722	0.476	0.042	0.727	0.468	0.226	0.828	0.408	0.061	0.215
Fe	0.078	0.301	0.893	0.236	0.466	0.841	-0.201	0.918	0.191	0.257	-0.165
Mg	0.327	0.388	0.841	0.047	0.674	0.614	0.328	0.886	0.347	0.077	0.181
Mn	-0.162	0.019	0.140	0.961	0.243	0.904	-0.216	0.919	0.080	0.185	-0.178
Cu	0.299	0.197	0.759	-0.285	0.764	0.624	-0.022	0.868	0.194	0.375	0.097
Cr	0.254	0.586	0.485	0.421	0.763	0.510	0.053	0.846	0.310	0.300	0.051
Fe(OOH)≈P	0.585	0.207	0.448	-0.341	0.838	0.145	-0.432	0.188	0.607	0.211	-0.621
$CaCO_3$≈P	0.836	0.452	0.042	0.099	0.890	0.228	0.305	0.257	0.905	0.155	0.175
Acid-OP	0.744	0.342	0.392	-0.058	0.826	0.350	0.384	0.210	0.928	-0.042	0.149
Alk-OP	0.787	0.248	0.101	0.010	-0.028	-0.096	0.821	-0.064	0.501	0.285	0.732
ROP	0.456	-0.276	0.355	-0.254	0.743	0.521	0.009	0.298	0.794	0.253	0.150
Salinity	0.816	-0.177	0.248	-0.211	0.541	0.377	0.723	0.230	0.209	0.292	0.844
Phosphate	0.907	0.075	0.211	-0.189	0.138	-0.193	0.771	0.763	0.048	0.036	0.514
% of Variance	27	25	24	8	46	31	11	39	19	18	11

Fractionation of P in sediments of the Cochin estuary gives a mixed or metastable diagenetic character with strong seasonal signatures. The surface sediments in the entire estuarine region remain oxic during the monsoon season and gradually become reducing during the post monsoon season which in turn shifts to strong reducing conditions during the pre monsoon season. The PCA results generated by considering various P fractions and trace metals supports the presence of dynamic interchange of oxidising and reducing character of the surface sediments. It also gives an indication about the formation of minerals such as greigite (Fe_3S_4) and mackinawite (FeS) in sediments which grow as a precursor to pyrite during the early diagenetic sedimentary sulphate reduction in the pre monsoon season.

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Chapter 4

QUALITY AND SOURCES OF SEDIMENTARY ORGANIC MATTER

Contents

4.1 Introduction

The overall geochemical processes prevailing in estuaries cannot be explained by mere application of geochemical parameters such as total content of organic matter, chemo-dynamics of P and total concentrations of sedimentary trace metals. The redox status of sedimentary environment in estuaries is directly linked to the processes of organic matter remineralisation. Organic matter remineralisation regenerate nutrients in the sediment and the regenerated nutrients diffuse or transport back to the overlying water and support water column production (Dunn et al., 2008). The biogeochemical processes associated with organic matter remineralisation are not a simple function of the total quantity of organic matter present, but also depends on the quality of organic matter. Therefore, the assessment of nature and quality of organic matter is of prime importance for explaining organic matter remineralisation. The biochemical composition of sedimentary organic matter has been employed to gather information on the nature and parameters controlling the diagenetic fate of organic matter. The concentrations of labile organic biopolymers (proteins, carbohydrates, lipids and chlorophyll pigments) are considered as best tool for the evaluation of nature and quality of organic matter which can provide clear insights into the biogeochemical characterisation of sedimentary environment in estuaries (Colombo, 1996; Dell' Anno et al., 2002; Pusceddu et al., 2009, 2011; Venturni et al., 2012).

4.2 Biochemical Composition of Organic Matter

Sedimentary organic matter in estuaries is composed of complex mixture of organic molecules characterised by large variability in composition and reactivity (Middelburg and Herman, 2007). Nature and quality of organic matter largely depend on several factors including origin, composition and biochemical transformations that occur on organic particles during their descent through the water column (Cowie and Hedges, 1992). Sedimentary organic matter comprises labile and refractory organic compounds, whose abundance changes as a function of complex array of processes including degradation, heterotrophic utilisation, transformation and export (Viollier et al., 2003; Venturini et al., 2012). Refractory organic compounds include fulvic acids, humic acids and structural carbohydrates which are characterised by lower degradation and high preservation in sediments (Middleburg et al., 1999; Danovaro et al., 1999). The labile fraction of sedimentary organic matter consists of simple and/or combined organic biopolymer molecules such as proteins, carbohydrates and lipids, which are available for benthic organisms and rapidly mineralised (Venturini et al., 2012). The production or inputs of rapidly sinking particles allows the accumulation of organic materials, which have suffered a continuous process of degradation of labile compounds in the water column. Even after sedimentation, organic particles are equally subjected to continuous degradation and mixing process (Colombo, 1996; Cotano and Villate, 2006).

The labile organic compounds in sediments represent the fraction of organic matter available to the benthic community (Mayer et al. 1995). The biopolymeric carbon (BPC) fraction of sedimentary organic matter, calculated by sum of protein, carbohydrate and lipid carbon has been reported as the fraction of total organic carbon potentially available to the benthic consumers (Pusceddu et al., 2009). Organic biochemical components vary faster than the more conservative total organic carbon in response to spatial and temporal changes in the benthic trophic status associated to both natural and human-induced environmental alterations (Dell'Anno et al., 2002; Pusceddu et al., 2003, 2009; Joseph et al., 2008).

4.3 Chlorophyll Pigments in Sedimentary Organic matter

Chlorophyll pigments are abundant in estuarine environment and are recognised as markers of different processes taking place in the sea water and sediments (Szymczak-Zyła and Kowalewska, 2007). Pigments are useful as molecular biomarkers due to their taxonomic specificity and hold the potential to represent the entire phototrophic community and overall primary production. The comparative labiality and source specificity of pigments makes them a powerful tool to investigate many aspects of biogeochemical processes as well as organic matter source and history (Sun and Dai, 2005; Reuss, 2005). Sedimentary pigments can provide information on various aspects of benthic processes and biogeochemistry (Woulds and Cowie, 2009). Only a fraction of photosynthetic production from water column end up in sediment surface and the most extensive degradation of pigments take place during the deposition through the water column in the surface sediments (Cuddington and Leavitt, 1999). Oxygen concentration in sediment water interface greatly influences the pigment preservation in sediment (Sun et al., 1993).

4.4 Bulk Organic Matter Techniques

Determining the relative contribution of organic matter fuelling the biogeochemical processes in estuarine sediments remains a challenge due to the wide range of organic matter sources in estuaries as well as the spatio-temporal variations in the delivery of organic matter to the sediments. In recent years, the source characterisation of sedimentary organic matter is achieved by using combination of bulk indices (C/N ratios and $\delta^{13}C$ and $\delta^{15}N$) and molecular biomarkers. The bulk elemental and isotopic approach is based on the following two assumptions: i) the bulk elemental and isotopic ratios of sedimentary organic matter are conservative and ii) there exist a linear response to physical mixing among the end members (Liu et al., 2006; Rumolo et al., 2011).

Most of the photosynthetic organisms including trees, shrubs, phytoplankton etc. incorporate carbon into their biomass using the Calvin (C_3) pathway which discriminates against ^{13}C to produce a shift in $\delta^{13}C$ values of about -20‰ from the isotopic ratios of inorganic carbon source. Some plants (many subtropical savannah grasses and sedges) use the Hatch-Slack pathway (C_4) which leads to an isotopic shift of -7‰ from the inorganic carbon source. Other plants mostly succulents, utilise the Crassulacean Acid Metabolism (CAM) which more or less switches between the C_3 and C_4 pathway and causes the $\delta^{13}C$ values to depend on growth dynamics (Schulz and Zabel, 2006). Terrestrial organic matter produced by C_3 pathway have $\delta^{13}C$ values ranging from -30 to -26‰, while for C_4 pathway it is from -16 to -9‰ (Pancost and Boot, 2004). Simultaneous utilisation of bulk elemental and isotopic parameters helps to obtain an improved identification of source as well as fate of organic matter in estuarine sediments. The usefulness of OC/N, $\delta^{13}C$ and $\delta^{15}N$ relies on the fact that there exist characteristic source specific bulk elemental and isotopic signatures for terrigenous, anthropogenic, marine and in situ organic matter.

Organic detritus in sedimentary organic matter pool has long been recognised for its influence on the biogeochemical cycles of both water column and sediments and its importance as a benthic food resource (Mann and Lazier, 1991; Bianchi and Bauer, 2011). The organic detritus comprises planktonic materials, animal faecal pellets and vascular materials which exhibit different reactivity to leaching and remineralisation processes. The relative contribution of different sources of detritus will affect the biogeochemical cycling of organic matter. Hence, a vivid knowledge about the nature, quality and the relative contribution of different sources (e.g. planktonic *vs*. terrestrial plant materials) is of prime importance for understanding organic matter dynamics in estuaries. This chapter investigates the nature and quality of organic matter in surface sediments of Cochin estuary and evaluates the relative contribution of different sources of sedimentary organic matter by the application of bulk organic matter techniques and chlorophyll pigment distributions.

4.5 Results

4.5.1 Biochemical Composition of Sedimentary Organic Matter

TOM concentrations in the sediments varied from 1.24 to 6.15% (3.98±1.82), 0.54 to 5.77% (3.33±1.93) and 0.65 to 5.39% (3.33±1.64) during the monsoon, post-monsoon and pre-monsoon seasons respectively. TOM showed a decreasing trend from seaward to Thanneermukkom bund and remarkably high concentrations were recorded at stations S1 to S7. The spatial and seasonal distributions of biochemical components are presented in Fig. 4.1. PRT concentrations in the sediments ranged from 1.40 to 7.3mgg^{-1} (4.79±1.77), 1.09 to 12.33mgg^{-1} (5.30±3.03) and 0.51 and 9.67mgg^{-1} (4.27±2.57) during the monsoon, post monsoon and pre monsoon seasons respectively. Higher PRT concentrations were found during the post monsoon when compared with the monsoon and pre monsoon seasons. CHO concentrations in the sediments ranged from 4.17 to 14.00mgg^{-1} (8.42±3.19), 2.21 to 5.66mgg^{-1} (8.34±4.30) and 0.87 to 9.02mgg^{-1} (5.70±2.53) during the monsoon, post monsoon and pre monsoon seasons respectively. LIP concentrations in the sediments ranged from 0.01to 1.50mgg^{-1} (0.81±0.46), 0.13 to 0.93mgg^{-1} (0.51±0.28) and 0.05 to 1.99mgg^{-1} (0.93±0.64) during the monsoon, post monsoon and pre monsoon seasons respectively. BPC concentrations in the sediments ranged from 0.25 to 0.96% (0.6±0.23), 0.16 to 1.25% (0.63±32) and 0.06 to 0.79% (0.50±0.24) during the monsoon, post monsoon and pre monsoon seasons respectively.

Carbohydrates in the sediments represent a major biochemical class (BPC pools) of organic compounds during the monsoon (53.45 %), post monsoon (53.15 %) and pre monsoon (46.95 %) respectively. This is followed by proteins monsoon (37.41 %), post monsoon (40.53 %) and pre monsoon (40.23 %) and lipids monsoon (8.74 %), post monsoon (6.31 %) and pre-monsoon (12.83 %).

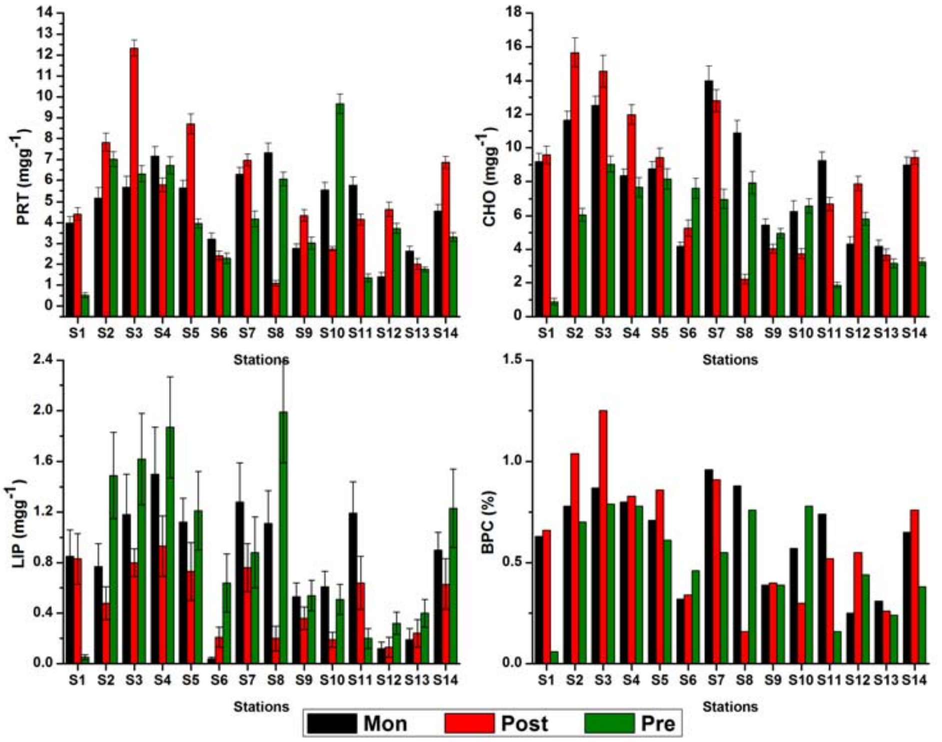

Fig.4.1 Distribution of PRT, CHO, LIP and BPC in surface sediments

The spatial and seasonal variations in Chl-*a* and Pheo are presented in Fig.4.2. Chl-*a* concentrations in the surface sediments ranged from 0.37 to 7.20µgg^{-1} (2.29±1.89), 5.72 to 18.62µgg^{-1}(10.02±4.97) and 0.02 to 17.91µgg^{-1}(6.26±3.88) in monsoon, post monsoon and pre monsoon seasons respectively. Maximum Chl-*a* concentration was recorded at station 5 during the post monsoon season, while the lowest concentration was observed at S1 during the pre monsoon season. Chl-*b* concentrations ranged from 0.05 to 6.13µgg^{-1} in monsoon, 0.09 to 5.73µgg^{-1} in post monsoon and 0.08 to 3.85µgg^{-1} in pre monsoon. Chl-*c* displayed comparatively lower concentrations in the study region which ranged from n.d to 7.20µgg^{-1} in monsoon, n.d to 4.85µgg^{-1} in post monsoon and n.d to 3.44µgg^{-1} in pre monsoon season. Pheo concentrations in the sediments ranged from 0.52 to 52.71µgg^{-1}(16.81±14.01), 7.59 to 46.65µgg^{-1}(23.46±12.76) and 2.14

to 26.90μgg^{-1}(15.86±8.22) in monsoon, post monsoon and pre monsoon seasons respectively. Highest Pheo concentration was recorded at S5 in monsoon season, while the lowest recorded at S12 at monsoon season.

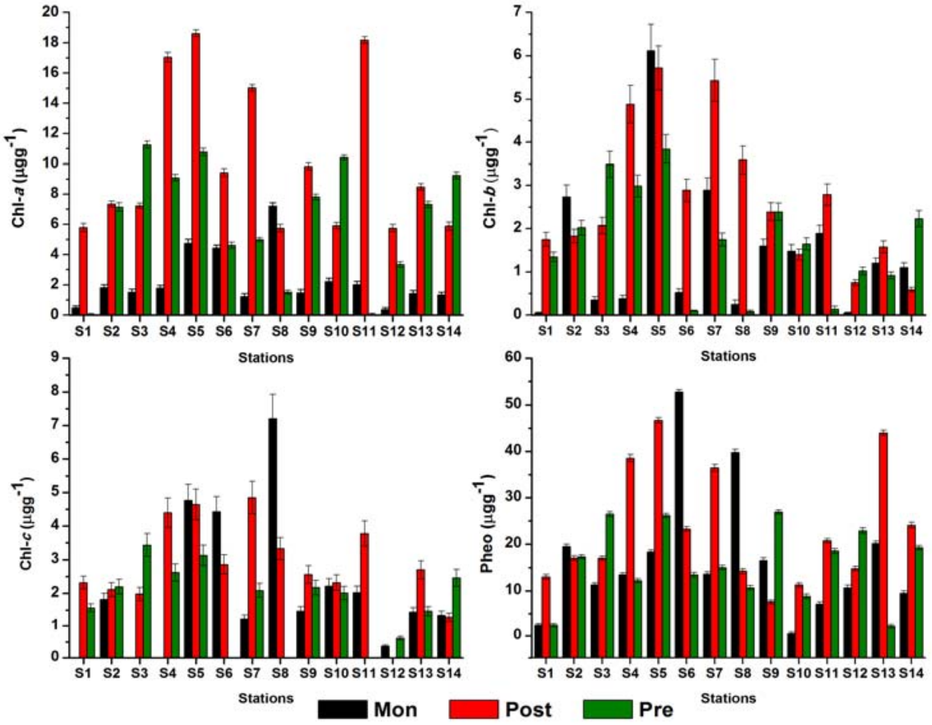

Fig.4.2 Spatial and seasonal variations of Chl-*a*, Chl-*b*, Chl-*c* and Pheo in surface sediments of the Cochin estuary (Mon-monsoon, Post-post monsoon and Pre-pre monsoon)

From the results it was found that Chl-*a* concentrations displayed highly significant seasonal variations ($p<0.001$) in the study region but lack significant differences along the sampling sites. Comparatively higher Chl-*a* concentrations were observed in post monsoon, while lower values were observed in monsoon season. However, Chl-*b* concentrations did not display any seasonal variations but it showed differences among the sampling sites ($p<0.01$). Pheo did not display any spatial and seasonal variations in the study region.

The results of correlation analysis of sedimentary biogeochemical parameters are presented in Table 4.1. All biogeochemical parameters (except Chl-*a* and Pheo) showed highly significant positive correlation with clay and silt content and negative correlation with sand, which indicated that the sediment texture is one of the main factor influencing organic matter accumulation in the study region. This phenomenon is ascribed to the close hydraulic equivalence of both organic and mud particles and/or to the relatively high absorptive capacity of fine particles for organic molecules (Cotano and Villate, 2006). OC, TN and TS also displayed significant positive correlation with the biochemical components in the surface sediments.

All the sedimentary parameters, both geochemical and biochemical showed a significant spatial variation (ANOVA $p<0.05$) except Chl-*a* and pheopigments (Table 4.2). Sediment texture displayed more significant spatial variations than seasonal variations. BPC and TN also showed significant spatial variation. Chl-*a* concentrations exhibited significant seasonal variation, but have not displayed any significant spatial variations. CHO was characterized with significant spatial and seasonal variations with the highest concentrations during the monsoon and lowest concentrations in the pre-monsoon seasons. Seasonal variations of LPD and PRT concentrations showed opposite trends. While pre-monsoon was characterised with a higher LIP and lower PRT concentrations, pre monsoon was characterized with a lower LIP and higher PRT concentrations.

Table 4.1 Pearson correlation between sedimentary parameters in the Cochin Estuarine System (n = 42).

	Sand	Silt	Clay	OC	TN	TS	Chl-*a*	Pheo	PRT	TCHO	LPD	BPC
Sand	1											
Silt	-0.84**	1										
Clay	-0.71**	0.22	1									
TOM	-0.86**	0.63**	0.74**	1								
TN	-0.83**	0.62**	0.69**	0.91**	1							
TS	-0.85**	0.61**	0.74**	0.90**	0.89**	1						
Chl-*a*	-0.12	-0.23	0.51**	0.19	0.23	0.23	1					
Pheo	-0.12	-0.14	0.40**	0.24	0.31*	0.23	0.75**	1				
PRT	-0.65**	0.39*	0.66**	0.78**	0.77**	0.69**	0.32*	0.33*	1			
CHO	-0.73**	0.55**	0.60**	0.89**	0.81**	0.79**	0.16	0.21	0.75**	1		
LPD	-0.69**	0.58**	0.50**	0.59**	0.71**	0.66**	0.08	0.06	0.51*	0.46**	1	
BPC	-0.78**	0.55**	0.69**	0.90**	0.88**	0.82**	0.24	0.27	0.92**	0.93**	0.62**	1

** Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed)

Table 4.2 The mean values of different biogeochemical parameters measured in the surface sediments of Cochin estuary and the result of ANOVA of different biogeochemical variables.

Parameters	Mean			Source of variation	*df*	F	p value
	Mon	Post	Pre				
OC (%)	2.21	1.85	1.85	Stations	13	4.72	0.000**
				Seasons	2	2.11	ns
TN (%)	0.2	0.17	0.18	Stations	13	4.58	0.000**
				Seasons	2	0.49	ns
TS (%)	0.69	0.6	0.57	Stations	13	6.19	0.000**
				Seasons	2	0.52	ns
Chl-*a* ($\mu g\ g^{-1}$)	2.29	10.02	6.26	Stations	13	1.11	ns
				Seasons	2	15.01	0.000**
Pheo ($\mu g\ g^{-1}$)	16.81	23.46	15.86	Stations	13	1.01	ns
				Seasons	2	1.69	ns
PRT ($mg\ g^{-1}$)	7.31	12.33	9.67	Stations	13	2.12	0.050*
				Seasons	2	0.8	ns
CHO ($mg\ g^{-1}$)	8.42	8.34	5.7	Stations	13	2.74	0.014*
				Seasons	2	4.55	0.020*
LPD ($mg\ g^{-1}$)	0.81	0.51	0.93	Stations	13	3.42	0.004**
				Seasons	2	5.03	0.014*
BPC (%)	0.63	0.63	0.51	Stations	13	3.04	0.008**
				Seasons	2	1.7	ns
PRT/CHO	0.59	0.64	0.75	Stations	13	1.2	ns
				Seasons	2	11.08	0.000**

***significant at the 0.01 level, *significant at the 0.05 level, ns- not significant.*

4.5.2 Bulk elemental and isotopic ratios

Fig. 4.3 shows the spatial and seasonal variability of OC and TN in surface sediments of Cochin estuary. The OC concentrations ranged from 0.85 to 3.26% (2.28±0.94) in monsoon, 0.30 to 3.03% (2.11±1.02) in post monsoon and 0.62 to 2.74% (1.91±0.81) in pre monsoon season. The spatial distribution of OC indicated that OC content is comparatively high at stations S1, S2, S3, S4 and S6 than the other stations. However, it was observed that there is no significant seasonal difference in OC content of surface sediments. Concentrations of TN were relatively lower in surface sediments of Cochin estuary, which varied from 0.07 to 0.31% (0.20±0.10), 0.03 to 0.32% (0.19±0.10) and 0.09 to 0.25% (0.18±0.06) in monsoon, post monsoon and pre monsoon respectively. TN varied significantly among the sampling sites, but it did not display any seasonal difference. The spatial and seasonal variations of TN are similar to OC.

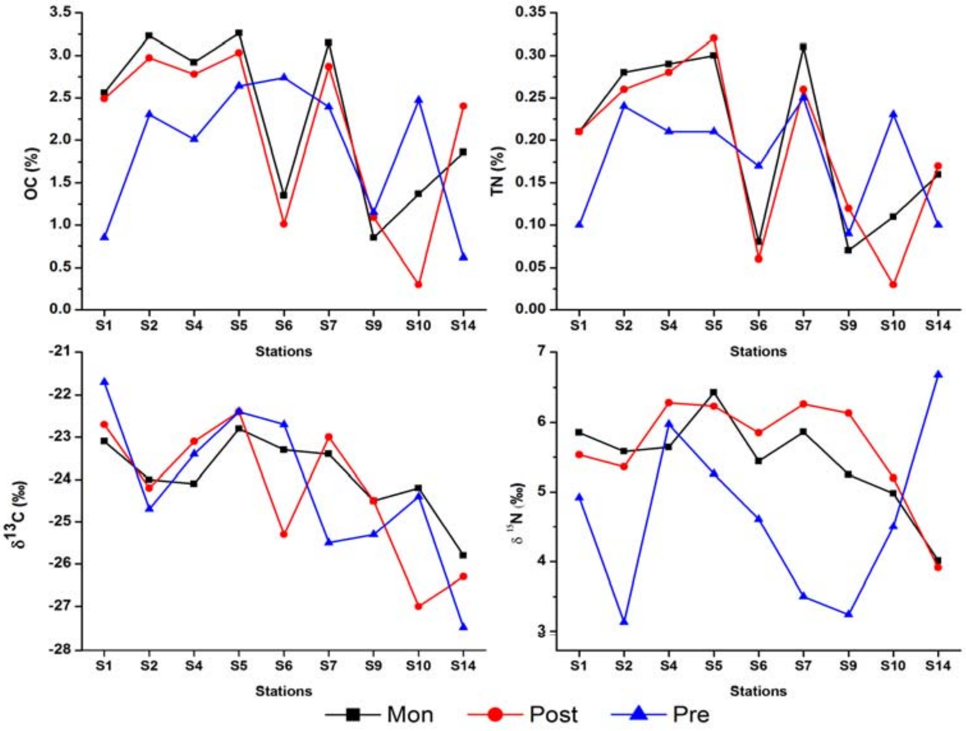

Fig.4.3 Spatial and seasonal variations of OC, TN, $\delta^{13}C$ and $\delta^{15}N$ in surface sediments

The spatiotemporal variation in $\delta^{13}C$ and $\delta^{15}N$ values in surface sediments is presented in Fig.4.3. The $\delta^{13}C$ in surface sediment of Cochin estuary ranged from -25.8 to -22.8‰ in monsoon, -27.0 to -22.4‰ in post monsoon and -27.5 to -21.7‰ in pre monsoon. $\delta^{13}C$ showed a clear spatial gradient with higher values towards the seaward side, while it lacks seasonal variation. There was an increasing heavier trend in $\delta^{13}C$ values at the gradient of increasing salinity in the overlying waters. The $\delta^{15}N$ values in the surface sediment varied from 4 to 6.4‰, 3.9 to 6.3‰ and 3.1 to 6.7‰ in monsoon, post monsoon and pre monsoon respectively. $\delta^{15}N$ did not display any spatial or seasonal variations.

4.6 Discussion

There is scarce information in literature about the biochemical compounds in sediments of the Cochin estuary. Biochemical composition of sedimentary organic matter in the mangrove ecosystems of the northern part of the Cochin estuary were studied by Joseph et al., 2008 and the observations in the present study are well within the ranges reported therein. The contribution of labile organic matter to total organic matter pool was very high in most stations.

CHO dominated among the organic biopolymers in the study region and the observed concentrations are compared with those reported in other eutrophic systems. The rampant propagation of water hyacinth during the monsoon and post monsoon season is a major ecological problem in the Cochin estuary. Comparatively higher concentration of CHO may be attributed to decay and decomposition of these floating plants in the estuary. High levels of CHO concentrations in surface sediments have also been attributed to the accumulation of aged organic detritus due to the faster utilisation of proteins than carbohydrates by micro organisms (Joseph et al., 2008; Venturini et al., 2012).

The PRT concentrations found in the present study were comparable to those reported in other eutrophic systems (Dell'Anno et al., 2002; Cotano and

Villate, 2006; Venturini et al., 2012). A number of fish peeling and processing units are situated in the banks of the Cochin estuary. The wastes from these units are dumped into the estuary where tissues undergo decomposition to liberate protein, which are finally adsorbed/settled into the surface sediments (Vasudevan, 2000; Balasubramaniam et al., 2012). PRT/CHO ratio > 1 has been attributed to the presence of fresh materials of recent origin, while PRT/CHO ratio <1 indicated to the predominance of aged organic matter in surface sediments (Danovaro et al., 1993). The PRT/CHO ratio remained <1 (Fig.4.4) in almost all stations during the study which implies the accumulation of more degraded organic matter in the estuarine sediments.

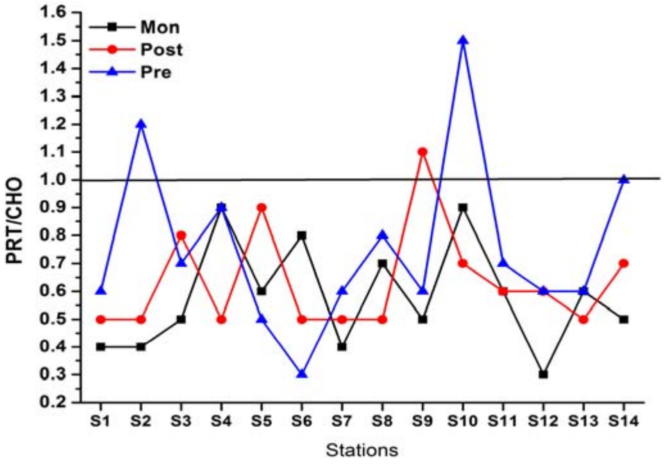

Fig.4.4 PRT/CHO ratios in surface sediments

The LIP values obtained in the present study were in good agreement with those reported for Bilbao estuary, Spain (0.97-2.5mgg^{-1}), Ligurian sea, Italy (1.0-1.4mgg^{-1}) (Cotano and Villate, 2006) and lower than those reported Rio de la Plata estuary, Uruguay (1.12-6.63 mg g^{-1}) (Venturini et al., 2012). Cochin estuary houses the 4th largest port in India and this facility currently handles export and import of container cargos (1225 vessels, 13.9 × 10^6 tons during 2005-06) at its terminal at the Willingdon

Island (Martin et al., 2012). Comparatively high LIP concentrations at stations around Cochin harbour region could be ascribed to anthropogenic inputs such as domestic sewage inputs from Cochin City and petroleum inputs. Usually high LIP values have been reported from many industrialised and urbanised estuaries because the method used to extract lipids may also recover significant quantities of organic contaminants such as hydrocarbons reflecting high degree of pollution (Galois et al., 2000; Venturini et al., 2012).

4.6.1 Benthic Trophic Status

Basic studies on the assessment of trophic status have been based on the measurement of physical, chemical and biological measurement of water column variables such as water column turbidity, inorganic nutrients (predictive variables) and Chl-*a* (responsive variables) concentrations (Zurlini, 1996; Cloern, 2001; Coelho et al., 2007). Many authors reported that the measurements of these predictive and responsive variables are insufficient to provide better insights into the trophic status of aquatic ecosystems (Izzo et al., 1997; Cognetti, 2001; Dell'Anno et al., 2002; Pusceddu et al., 2011). In shallow ecosystems like estuaries, ponds and lagoons, there is a significant contribution of Chl-*a* from microphytobenthose and macroalgae to benthic primary production (Dell'Anno et al. 2002). The re-suspension events in these shallow ecosystems might lead to an increased concentration of Chl-*a* in water column, thereby creating discrepancies in the assessment of trophic status (Conde et al. 1999; Pusceddu et al. 1999). Since these predictive and responsive variables are ineffective in shallow coastal systems, Nixon (1995) proposed a new approach for the assessment of trophic status based on the supply of total organic carbon in terms of $gCm^{-2}y^{-1}$. Even though this approach moves focus on the potential consequence of eutrophication in the benthic realm, it showed poor sensitivity because it does not consider the differential reactivity of wide range of organic compounds within the sediments (Pusceddu et al., 2009).

The benthic trophic status of sampling stations were established based on PRT and CHO threshold levels proposed by Dell'Anno et al., 2002 (Hypertophic (H): PRT>4 and CHO > 7mgg^{-1}, eutrophic (E): PRT 1.5-4mgg^{-1} and CHO = 5.7mgg^{-1} Meso-oligotrophic (MO): PRT<1.5mgg^{-1} and CHO <5mgg^{-1}). BPC and algal contribution to BPC (CChl-*a*) levels as proposed by Pusceddu et al., 2011 (eutrophic- BPC >3 mg g^{-1} and algal contribution to BPC <12%) are also used in this study. The benthic trophic classifications of the stations are presented in Table 4.3. Most of the stations are classified as hypertrophic using PRT threshold levels, while CHO concentrations reflect hypertrophic conditions around Cochin harbour region and meso-oligotrophic conditions in the inner part of the estuary. BPC and algal contribution to BPC are the reflection of the eutrophic conditions prevailing in the estuary.

The trophic classifications of the stations in the Cochin estuary based on the threshold values proposed by Dell'Anno et al., 2002 are presented in Table 4.3. Based on the CHO concentrations stations S3, S4, S5, S6 and S8 were classified as hypertrophic, whereas the stations S2, S7, S10 and S12 presented CHO concentrations close to eutrophic conditions. The bar mouth region and southern end of the study area (Thanneermukkom bund region) showed lower CHO concentrations reflecting meso-oligotrophic conditions. Almost the entire study region showed PRT concentrations higher than threshold values for hypertrophic conditions, while the BPC results suggested eutrophic conditions. An algal contribution to BPC <12%

indicate eutrophic conditions and lower values between 3 and 6 indicate hypertrophic conditions (Pusceddu et al., 2011). From the results it was clear that Cochin harbour region has lower algal contribution to BPC reflecting hypertrophic condition in that region, while eutrophic conditions were obtained towards the inner part of the estuary. From the above discussion it is obvious that different biochemical descriptors inferred different trophic conditions for the study area, hence a detailed examination is needed for assessing the applicability of these biochemical descriptors in the tropical estuarine and coastal systems. However, high BPC and PRT values and lower algal contribution to BPC indicate high degree of benthic eutrophication in the Cochin estuary.

Table 4.3 Benthic trophic classifications of sampling sites

Stations	PRT (mgg^{-1})	Trophic Status	CHO (mgg^{-1})	Trophic Status	BPC (mgg^{-1})	Trophic Status	CChl-*a* (%)	Trophic Status
S1	2.97	E	0.87	MO	5.90	E	1.38	H
S2	6.67	H	6.05	E	6.01	E	2.62	H
S3	8.10	H	9.02	H	5.81	E	2.90	H
S4	6.55	H	7.66	H	5.46	E	4.60	H
S5	6.10	H	8.14	H	5.21	E	6.15	E
S6	2.64	H	7.60	H	4.98	E	6.81	E
S7	5.81	H	6.97	E	5.13	E	3.59	H
S8	4.82	H	7.91	H	4.71	E	6.23	E
S9	3.37	H	4.94	MO	4.49	E	6.46	E
S10	5.99	H	6.58	E	4.60	E	4.95	E
S11	3.75	H	1.86	MO	4.39	E	5.07	E
S12	3.25	H	5.81	E	4.28	E	2.61	H
S13	2.13	H	3.14	MO	4.35	E	8.96	E
S14	4.90	H	3.23	MO	5.97	E	4.51	E

The Chl-*a*/Pheo (<1) and CChl-*a* (<12) indicated the enrichment of degraded phytodetritus as well as refractory organic materials in the sediments. Many previous studies demonstrated that eutrophic systems have a tendency to accumulate refractory organic matter (Pusceddu et al., 2009; Pusceddu et al., 2011; Venturini et al., 2012) and the present study agrees well with these concepts. Management strategies should be developed to reduce the organic matter load reaching the Cochin estuary.

The influx of large amount of allochthonous organic matter into the Cochin estuary was previously reported by many authors (Saraladevi et al., 1983; Thomson, 2002; Balachandran et al., 2003; Babu et al., 2006; Thottathil et al., 2008; Martin et al., 2010). The estuary receives heavy freshwater influx during summer monsoon (June-September) when 71% of annual rainfall occurs (Srinivas et al., 2003) transporting large amount of organic matter to this estuary (Balachandran et al., 2003). Very high concentrations of dissolved organic carbon and particulate organic carbon were reported from central estuary (Martin et al., 2010) which clearly indicates that a significant portion of organic matter is derived from sewage generated from Cochin metropolitan city. The southernmost part of the estuary receives organic wastes from aquaculture fields ($62km^2$), agricultural fields ($80km^2$) and coconut retting yards which increase the organic pollution in an alarming rate (Thomson, 2002; Babu et al., 2006; Martin et al., 2010). All these activities lead to changes in trophic structure and have affected the functioning of this vital ecosystem. Eutrophication induced changes in benthic community structure was recently reported by Martin et al., 2011. Out of 62 macrobenthic species reported (Saraladevi, 1986), 11species were disappeared from the estuarine system during the last two decades (Martin et al., 2011).

Sedimentary Chl-*a* and Pheo concentrations have not been reported previously from the Cochin estuary. Chl-*a* concentrations obtained were comparable with those reported in the other estuaries like Orda, Palmones and Baltic sea (Kowalewska et al., 2004; Moreno and Niell, 2004; Szymczak-

Żyła et al., 2011) and lower Chl-*a* concentrations were observed as compared with Tagus estuary (Cartaxana et al., 2006). Different biotic and abiotic factors affect the spatial and seasonal variations of chlorophyll pigments in the surface sediments (Moreno and Niell, 2004). Chl-*a* concentrations showed distinct seasonal variations with maximum concentrations in the post monsoon season, minimum in the monsoon season and also displayed a highly significant positive correlation with clay. This is in good agreement with the results of Coljin and Dijkema,1981 and Moreno and Niell, 2004, who also reported that Chl-*a* is positively correlated with the clay content and negatively correlated with hydrodynamic energy. Sedimentary pigment concentrations are dependent on the light availability and oxygen content in the water column (Kowalewska and Szymczak, 200; Kowalewska et al., 2004). The light availability at the sediment surface is affected by the variability in the hydrodynamic conditions (Moreno and Niell, 2004). During the monsoon season daily total solar radiation is less than 350 ly day^{-1} due to the formation of heavy cloud cover and high rain fall (average 400 mm day^{-1}) (Qasim, 2003). Furthermore, during the monsoon season an increase in the concentration of the suspended particulate matter due to the terrestrial and river run off enhances the water column turbidity leading to a reduction in sufficient light penetration. According to earlier reports (Madhu et al., 2007, 2010), the light attenuation coefficient in the Cochin estuary is higher during the monsoon season when compared to the pre monsoon season (average 2.4±0.4 m^{-1} and 1.2±0.4 m^{-1} respectively).

Local water column input of Chl-*a* is clearly a determinant factor of sedimentary Chl-*a* concentrations (Szymczak-Zyła and Kowalewska, 2007). The increased water column turbidity and reduction in sufficient light penetration, limit phytoplankton production in water column as well as in the benthic compartments. A major portion of the primary carbon either settles down or gets transported to the coastal regions during monsoon. High flushing of Cochin backwaters also facilitates faster removal of primary producers to the coastal regions during monsoon (Jyothibabu et al., 2006). Chl-*a*/Pheo (Table 4.4) ratios remained below 1 throughout the study period indicating the preponderance of detritus material in the surface sediments. The algal contribution to BPC (Table 4.4) displayed very low values in the study area which ranged from 0.31 to 5.46% in monsoon, 2.32 to 14.61% in post monsoon and 1.38 to 8.98% in pre monsoon season.

Table 4.4 Chl-*a*/ Pheo ratios and the percentage of algal contribution to BPC pool in the surface sediments

Stations	Chl-*a*/Pheo			%C Chl-*a*		
	Mon	Post	Pre	Mon	Post	Pre
S1	0.2	0.4	0.0	0.3	3.5	0.3
S2	0.1	0.4	0.4	0.9	2.8	4.1
S3	0.1	0.4	0.4	0.7	2.3	5.7
S4	0.1	0.4	0.7	0.9	8.2	4.7
S5	0.3	0.4	0.4	2.7	8.7	7.1
S6	0.1	0.4	0.3	5.5	11.0	4.0
S7	0.1	0.4	0.3	0.5	6.6	3.6
S8	0.2	0.4	0.1	3.3	14.6	0.8
S9	0.2	0.4	0.4	1.5	9.8	8.1
S10	0.1	0.8	0.4	1.6	7.9	5.4
S11	0.1	0.4	0.0	1.1	14.0	0.1
S12	0.7	0.5	0.4	0.6	4.2	3.1
S13	0.2	0.4	0.4	1.8	12.9	12.1
S14	0.1	0.4	0.4	0.8	3.1	9.6

4.6.2 Principal Component Analysis

The quantity and quality of sedimentary organic matter in aquatic systems are mainly controlled by the biogeochemical processes and Principal

Component Analysis (PCA) was employed to deduce it. The parameters for the PCA were selected in such a way that the component of the analysis can give an indication to the significance of biogeochemical processes. The concentrations of individual chemical species will be the net result of these processes and thereby fixing with suitable indicators, it will be possible to identify the relative significance of each process to each species.

In estuarine environment, both geochemical and biochemical processes operate in different magnitude altering the concentrations of nutrients, trace metals and organic matter. The schematic representations of the obtained outputs of PCA are depicted in Fig. 4.5 (a, b & c). PCA analysis for monsoon and post monsoon season derived similar outputs and accounted for a total variance of 83% and 89% respectively. PC1 accounted for a total variance of 61%, exhibited significant loading for texture, TOM, TN and biochemical components which revealed the grain size as the main contribution factor influencing the enrichment of organic matter in surface sediments. This factor revealed the influence of both biochemical and geochemical processes in the surface sediments. It is interesting to note that Chl-*a* and Pheo concentrations were insignificantly loaded in the first component which implies the substantial contribution of terrestrial derived organic matter in BPC pool. The aforementioned parameters were loaded as a separate component, PC2 which accounts for a total variance of 23%. The fresh algal material is more susceptible to degradation and rapidly utilised by consumers than terrestrial derived organic detritus.

In pre monsoon, the results of PCA generated two PCs which accounts for a total cumulative variance of 79%. The PC1 accounted for a total variance of 50% with the grouping of clay particles with other biochemical parameters while the PC2 which account for a total variance of 19% revealed the grouping of silt particles with LIP. This clearly indicates the dominance of biochemical processes in pre monsoon season compared to various geochemical processes. PC2 represents geochemical processes such as adsorption and flocculation. LIP being hydrophobic in character adsorbs onto silt and sand particles and ultimately sinks through the water column into the surface sediments. From PCA results it is evident that different geochemical and biochemical processes alter the organic matter concentrations in surface sediments to a greater extent. Both processes alter the nature and quantity of organic matter in estuarine systems. Therefore, a better knowledge on sources of organic matter is essential to identify major biochemical and geochemical processes in estuaries.

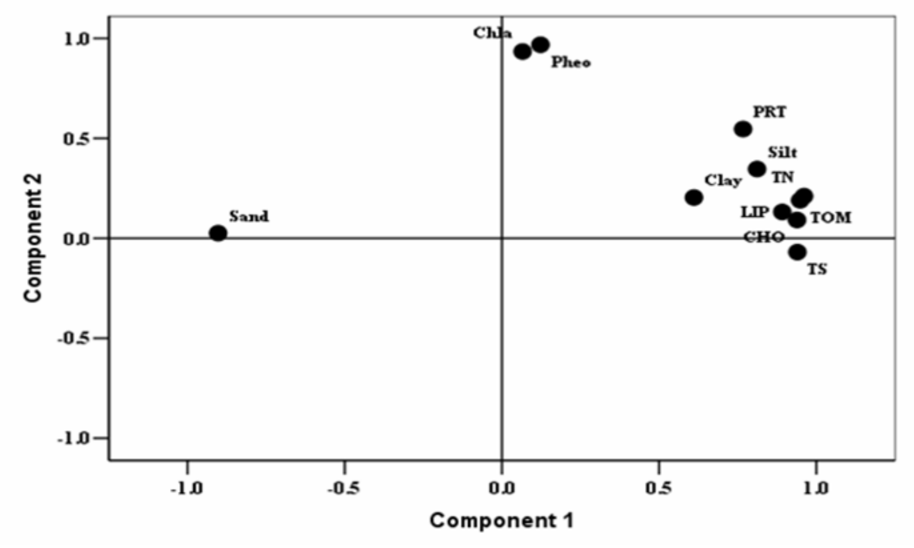

Fig.4.5a Output of PCA for monsoon

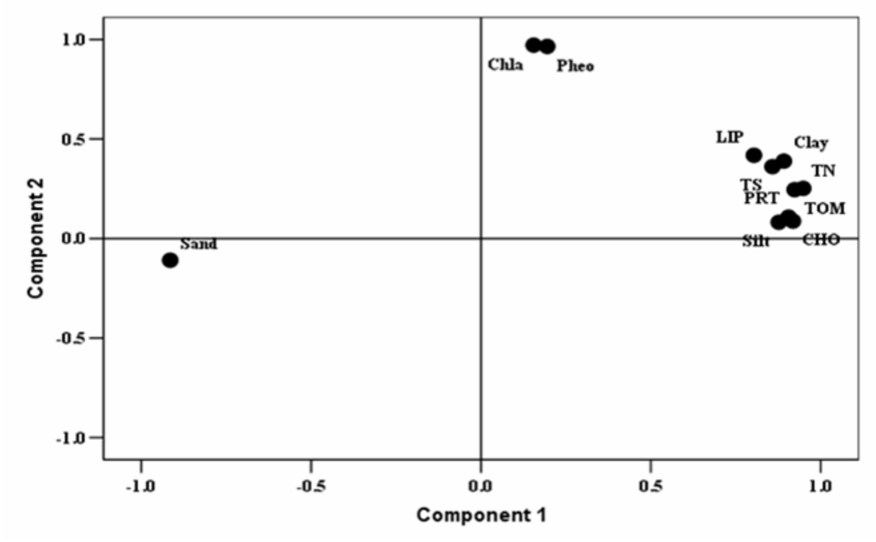

Fig. 4.5b Output of PCA for post monsoon

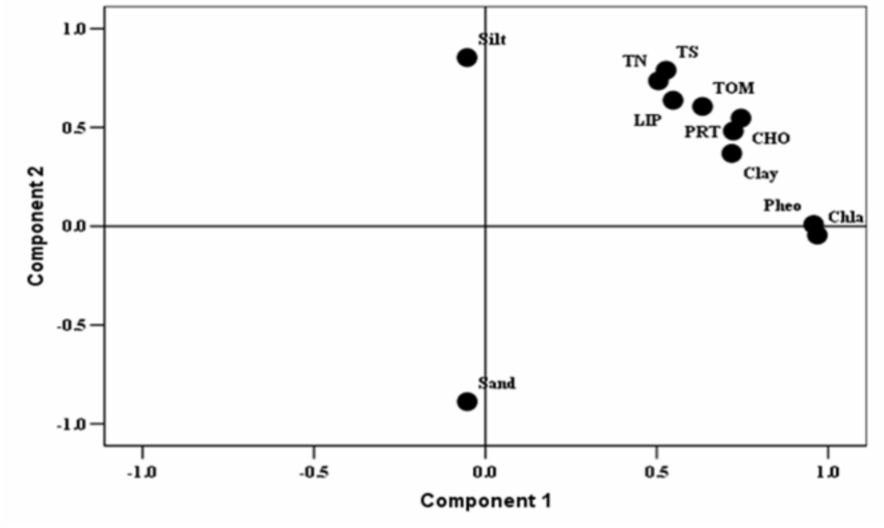

Fig. 4.5c Output of PCA for pre monsoon

4.7 Provenance of Sedimentary Organic Matter - Inference from Bulk Organic Matter Techniques

The OC concentrations measured in the sediments of the Cochin estuary during the present study agree well with the ranges reported in previous studies (Balachandran et al., 2005; Martin et al., 2010). OC and

TN concentrations showed highly significant positive correlation with fine grained (silt+clay) sediment and negative correlation with sand content (Table 4.5). Sediment grain size was found to be the main factor influencing the organic matter accumulation in surface sediments of the study region. This is attributed to the close hydraulic equivalence of both organic and mud particles and/or to the relatively high adsorptive capacity of fine particles for organic matter (Cotano and Villate 2006; Ramaswamy et al., 2008).

Table 4.5 Result of correlation analysis of bulk elemental and isotopic ratios with general sediment characteristics.

	Sand	**Silt**	**Clay**	**OC**	**TN**	**OC/TN**	**$\delta^{13}C$**	**$\delta\ ^{15}N$**
Sand	1							
Silt	-0.88[a]	1						
Clay	-0.86[a]	0.52	1					
OC	-0.83[a]	0.77[a]	0.67[a]	1				
TN	-0.88[a]	0.77[a]	0.77[a]	0.94[a]	1			
OC/TN	0.29	-0.18	-0.32	0.01	-0.31	1		
$\delta^{13}C$	-0.35	0.21	0.41[b]	0.51[b]	0.46[b]	0.12	1	
$\delta\ ^{15}N$	-0.23	0.16	0.25	0.11	0.20	-0.21	0.31	1

[a] Correlation is significant at the 0.01 level (2-tailed), [b] Correlation is significant at the 0.05 level (2-tailed).

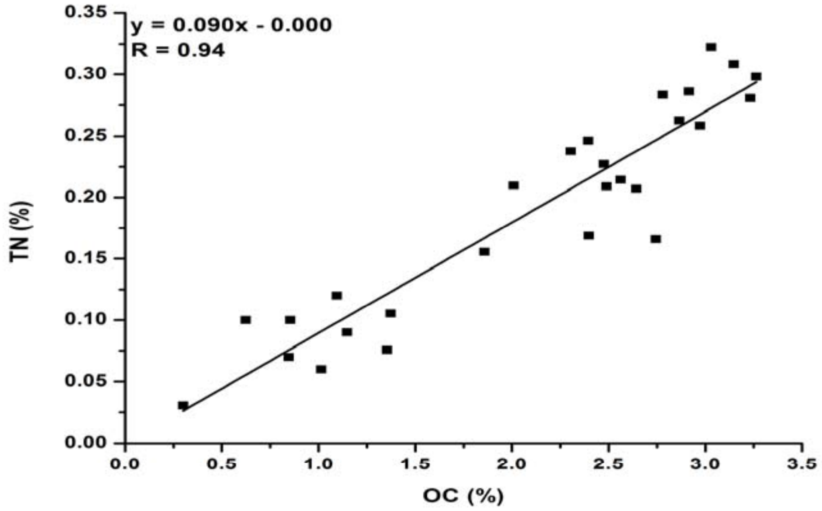

Fig. 4.6 Inter-relationship between OC and TN in surface sediments

The OC and TN exhibited a good linear relationship (Fig. 4.6) in surface sediments of Cochin estuary and the intercept of regression line passes through the origin which indicates very less adsorbed NH_4^+ ions in surface sediments. Hence, it is assumed that the measured TN concentrations can be used as a reasonable estimate of organic nitrogen (Liu et al., 2006; Rumolo et al., 2011) and OC/TN used as a better proxy to track the sources of organic matter. The total organic carbon to nitrogen (C/N) ratio has been widely used to differentiate the sources of organic matter in estuaries based on the generalisation that fresh marine autogenic organic matter has C/N ratios between 4 and 9, whereas organic matter derived from terrestrial vascular plants has C/N ratios more than 12 (Meyers 1994; Rumolo et al., 2011). The basic reason for such differences in C/N ratios between vascular plants (>12) and micro algae (5–7) are simply due to the carbohydrate-rich (e.g., cellulose)/ protein-poor and protein-rich/carbohydrate poor nature of each source respectively (Hedges et al., 1986; Orem et al., 1991; Meyers, 1997). The inorganic nitrogen species adsorbed in clay minerals can cause serious errors when applying OC/TN ratios for source characterisation of organic matter in aquatic systems (Meyers, 1994; Schubert and Calvert, 2001; Rumolo et al., 2011). In the present study it was found that the OC/TN ratios displayed high variability in the study region and devoid of any correlation with grain size data.

The OC/TN ratios (Fig. 4.7a) in the surface sediments of Cochin estuary ranged from 12 to 21 (14±3) in monsoon, 11 to 20 (13±3) in post monsoon and 7 to 19 (13±3) in pre monsoon which indicate a mixture of continental derived as well as marine materials in surface sediments. Wu et al., 2002 reported the existence of a negative correlation between the

OC/TN ratios to that of $\delta^{13}C$ values. However, the regression analysis revealed that there is no such correlation in the present study (Fig.4.7b). This result is consistent with the previous studies (Liu et al., 2006; Gao et al., 2012) which demonstrated that the decomposition processes such as autolysis, leaching and microbial remineralisation considerably alters the sedimentary OC/TN ratios in surface sediments. The OC/TN was nitrogen ratio of sedimentary organic matter is expected to increase or decrease during sediment diagenesis by the selective degradation of organic matter components (Meyers et al., 1996; Liu et al., 2006). The OC/TN ratios tend to be decrease due to the release of CO_2 and CH_4 as the degradation products, while it tend to be increase due to the rapid degradation of proteinaceous organic matter than non-nitrogen organic components (Gao et al., 2012). Hence, the organic matter degradation processes may influence the distribution of OC/TN ratios to a great extent.

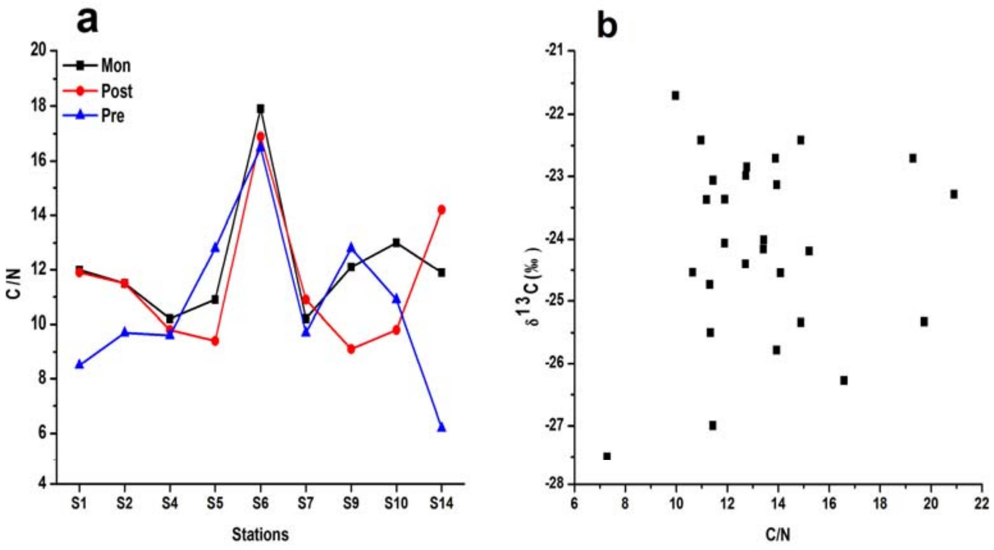

Fig. 4.7 a) C/N ratios and b) $\delta^{13}C$ Vs C/N ratio in surface sediments

Stable carbon isotopic ratios have been widely used for the source characterisation of organic matter in estuaries (Andrews et al., 1998; Liu et al., 2006; Zhang et al., 2009). Terrestrial organic matter produced by C_3 pathway have $\delta^{13}C$ values ranging from -30 to -26‰, while for C_4 pathway, it is from -16 to -9‰ (Pancost and Boot, 2004). Organic matter produced by marine phytoplankton has $\delta^{13}C$ values between -22 and -18‰ (Cifuentes et al., 1998). The $\delta^{13}C$ values of sedimentary organic matter from Cochin estuary were found to be comparable with those obtained from estuaries such as Hunts Bay, Forth estuary, Yangtze estuary and Pearl River estuary (Andrews et al., 1998; Graham et al., 2001; Liu et al., 2006; Zhang et al., 2009). The distributional characteristics of $\delta^{13}C$ values indicate that the organic matter in surface sediments of Cochin estuary is a mixture of terrestrial derived material and marine derived material. The $\delta^{13}C$ values displayed a clear seaward enrichment of heavy carbon isotope which indicates the supply of large amount of marine derived organic matter into the surface sediments towards the seaward side. Several earlier studies demonstrated the seaward increase of $\delta^{13}C$ values (Liu et al., 2006; Hu et al., 2009; Gao et al., 2012) and it is explained as the seaward increase of marine autogenous organic matter relative to the terrigenous organic matter. However, the seaward decrease of $\delta^{15}N$ values and OC/TN ratios were not observed in the present study and the $\delta^{13}C$ values were positively correlated with clay content, OC and TN concentrations, which indicate that the hydrodynamic conditions in the estuary as well as the input of anthropogenic organic matter from the nearby urban areas which influence the $\delta^{13}C$ values to a considerable degree.

Marine organic matter has mean $\delta^{15}N$ values of 5-7‰ derived from phytoplankton which normally use dissolved nitrate (Lamb et al., 2006; Gao et al., 2012). The $\delta^{15}N$ of organic matter derived from nitrogen fixing land plants is around zero, where as plants using soil nitrogen compounds have usually positive $\delta^{15}N$ values (Gaye–Haake et al., 2005). The anthropogenic organic matter derived from sewage is isotopically rich in heavy nitrogen compounds (Cole et al., 2006). The spatiotemporal distributional features of $\delta^{15}N$ values in surface sediments of Cochin estuary is rather complex. Even though the $\delta^{15}N$ values are in a narrow range of 3.1 to 6.7‰, it did not show any correlation with other physical and chemical parameters in the study region. This may be due to the modification of nitrogen isotopic composition by a wide array of biogeochemical processes. Previous studies have demonstrated that the transformation and recycling of dissolved and particulate nitrogen compounds can cause kinetic isotopic fractionation of nitrogen (Wu et al., 2003; Gao et al., 2012). The organic matter diagenesis tends to increase the $\delta^{15}N$ values in surface sediments (Liu et al., 2006). It is also reported that the nitrogen isotopic enrichment is relative to heterotrophic microorganisms and the $\delta^{15}N$ values of microbial reworked terrestrial organic matter increased from -4 to 9‰ (Caraco et al., 1998). The observed trends in the $\delta^{15}N$ values indicate that the sediment decomposition processes have considerable effects on nitrogen isotopic composition in the Cochin estuary also.

Some marine phytoplankton like *Trichodesmium* fix dissolved molecular nitrogen and $\delta^{15}N$ values of organic matter derived from them is close to zero (Altabet, 1996; Ramaswamy et al., 2008). During the present study, comparatively low $\delta^{15}N$ values were observed in pre monsoon season. This may be due to the occurrence of *Trichodesmium* in the estuary. Many authors reported the occurrence of *Trichodesmium* in the lower reaches of Cochin estuary in pre monsoon period (Gopinathan et al., 1974; Verma and Agarwal, 2000). Joseph, 2005 identified a total number of 75 cyanobacterial species in the Cochin estuary and reported a high density of these species during the pre monsoon months. The incidence of *Trichodesmium* bloom had frequently been reported form the adjacent coastal waters (Krishnan et al., 2007; Ashadevi et al., 2010).

The potential sources of organic matter in the estuarine sediments are terrestrial detritus, in situ primary production, marine materials and sewage. It was found that the $\delta^{13}C$ values were insignificantly correlated with sedimentary Chl-*a* concentrations (Fig. 4.8). It has been reported in the literature that sewage has no significant impact on the carbon and nitrogen isotopes in estuaries having strong self purification capacities (Liu et al., 2006). Cochin estuary is a dynamic positive estuary having a sporadic introduction of terrestrial organic matter; a large amount of terrestrial organic matter is transported from the catchment of six rivers to the estuary (Thottathil et al., 2008).

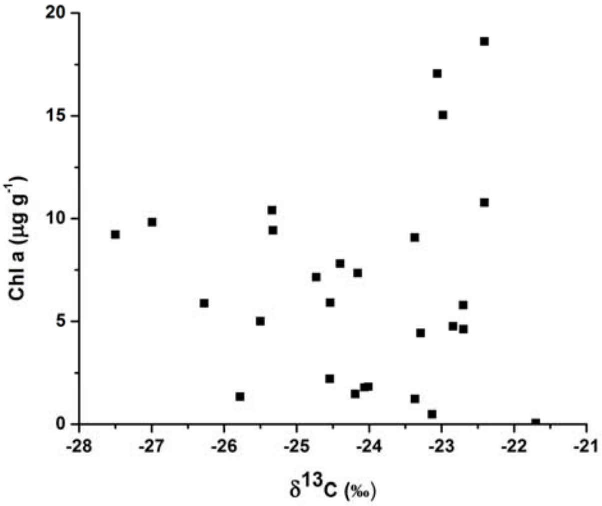

Fig.4.8 Inter-relationship between δ^{13}C and Chl-a content in surface sediments

Hence we disregarded the influence of sewage while calculating the relative proportion of terrigenous input. Furthermore, the $\delta^{15}N$ values reflected an insignificant input of sewage to the sediments only. All these converge to the assumption that organic matter derived mainly from the terrigenous and marine inputs in the surface sediments of the Cochin estuary.

The fraction of terrestrial derived organic matter in surface sediment could be quantitatively estimated using $\delta^{13}C$ based two end member mixing model proposed by Schultze and Calder, 1976. Although the bulk indices such as elemental stoichiometry and bulk isotopic composition have been widely adopted to characterise the sources of organic matter, there have been only limited studies in Indian estuaries. Therefore we are unable to compare our results with any other Indian estuaries and hence we assigned the marine and terrestrial end member values as -30‰ and -20.5‰ respectively considering those reported in previous literatures especially in Chinese estuaries (Wu et al., 2002; Jia and Peng, 2003; Liu et al., 2006; Zhang et al., 2009).

The relative contribution of terrestrial organic matter (F) was calculated using the following equation:

$$F\ (\%) = \{(\delta^{13}C_{marine} - \delta^{13}C_{measured}) / (\delta^{13}C_{marine} - \delta^{13}C_{terrestrial})\} \times 100$$

The contribution of terrestrial derived organic matter in surface sediments of Cochin estuary ranged from 13 to 74% (Fig. 4.9) in surface sediments. The terrestrial derived organic matter did not display any seasonal difference, but possess significant spatial variations and showed a decreasing trend from the inner part of the estuary to the seaward side.

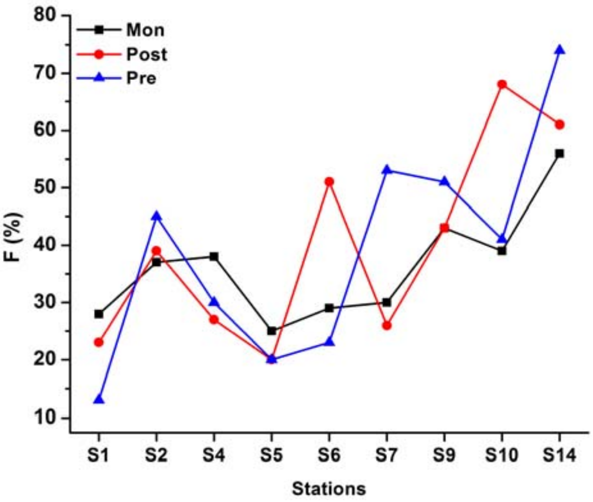

Fig.4.9 Fraction of terrestrial derived organic matter in surface sediments of the Cochin estuary.

4.8 Conclusions

The results of organic carbon content and biochemical components in the surface sediments of Cochin estuary showed comparable concentrations to those reported in highly industrialised and urbanised eutrophic estuaries. Biopolymeric carbon chains were dominated by carbohydrates, followed by proteins and lipids, pointing a nitrogen limitation for heterotrophic metabolism. The input of organic matter from rivers coupled with the discharge of organic wastes from different industrial, agricultural and aquaculture sectors leads to a large scale accumulation of organic matter in the estuarine sediments. The predominance of carbohydrates over sedimentary protein indicates faster mineralisation of proteinaceous organic matter in surface sediments and the estuary behaves as a detrital trap for the accumulation of aged organic matter. Low Chl-*a* concentrations were displayed during the monsoon when compared to other seasons were due to higher water column turbidity, heavy cloud cover and high rainfall events that limit the light availability for primary production. The threshold levels of PRT, BPC and algal contribution to BPC suggests the hypertrophic conditions prevailing in and around Cochin harbour region and eutrophic conditions towards the inner part of the estuary.

OC, TN, OC/TN ratios and $\delta^{13}C$ in surface sediments of Cochin estuary showed distinct spatial variations, while $\delta^{15}N$ did not display any spatial variations in the study region. OC and TN concentrations strongly depend on the granulometric composition of surface sediments in the study region. The $\delta^{13}C$ values ranged from -27.5 to -21.7‰ in surface sediments and a gradient was observed towards the seaward side. The $\delta^{15}N$ values were found to be more complex in surface sediments and ranged from 3.1 to 6.7‰. The fraction of terrestrial organic matter in total organic matter pool is estimated by $\delta^{13}C$ based two end member mixing model and it ranged from 13 to 74% in surface sediments. The distribution features of terrestrial organic matter exhibited significant spatial differences with higher contribution in the inner part of the estuary.

The application of biochemical descriptors and bulk indices like elemental composition and isotopic ratios are useful techniques for the assessment of total quality, benthic trophic status and relative contribution of marine and terrestrial derived organic matter in surface sediments; but the source, fate and degradation pathway of organic matter in the Cochin estuary can be deduced only by a molecular biomarker approach.

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Chapter 5

HYDROCARBONS AS BIOMARKERS

Contents

5.1 Introduction

Estuarine systems are complex environments with a broad spectrum of sub environments such as terrestrial, littoral and benthic compartments that contribute to organic matter and which have different reactivities in biogeochemical processes (Bianchi and Caunel, 2011). A significant portion of organic matter sinks through the water column and ultimately get stored in sediments (Hu et al., 2006) by the interaction of a series of physical, chemical and biological processes (Liu et al., 2006). Due to the complex nature of source and fate of organic matter, sole application of bulk indices such as elemental stoichiometry and bulk isotopic composition to characterise the sources of organic matter has certain potential limitations.

The bulk analysis of elemental signatures of total organic carbon to total nitrogen (OC/TN) and their stable isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) could provide general identification of the relative contribution of terrigenous and marine derived organic matter in estuaries (Meyers, 1997; Hu et al., 2012).

However, OC/TN ratios can be very misleading in determining the organic carbon sources in the absence of additional source proxies. Faster remineralisation of nitrogenous organic compounds can result in high OC/TN ratios leading to misidentification of source materials. The colonisation of bacterial and fungal populations on ageing vascular plant detritus can decrease OC/TN ratios since the bacterial and fungal organic matter represents a significant fraction of N pool (Tenore et al., 1982; Rice and Hanson, 1984). In sediments having low concentration of organic matter, the relative importance of inorganic N could be significant which underestimates the OC/TN ratios (Mayers, 2003).

Hydrodynamic sorting of sediment particles has been shown to be an important mechanism for the distribution of OC/TN ratios in estuarine sediments (Prahl et al., 1994; Keil et al., 1998; Bianchi et al., 2002). Large fragments of woody plant materials are entrained in coarse sediments because of higher settling rate of these large materials resulting in high OC/TN ratios in coarse grained sediments. Fine grained sediments typically have OC/TN ratios lower than those of coarse grained sediments because of higher surface area that allows greater sequestration and sorption of organic matter (Mayer, 1994; Mayer et al., 2004). Fine grained sediments have a proportion of clay minerals greater than the coarse grained sediments. Due to their negative charge, they can adsorb NH_4^+ ions from pore water resulting in decrease of C/N ratios (Meyers, 1997; Bianchi, 2007).

Stable isotopic ratios provide complementary information to OC/TN ratios and are used to identify the organic matter sources in estuarine systems (Andrews et al., 1998; Goni and Thomas, 2000; Goni et al., 2003; Wu et al., 2003; Liu et al., 2006; Rumolo et al., 2011; Gao et al., 2012;

Gireeshkumar et al., 2013). The interpretation of bulk isotopic ratios is complicated by the fact that bulk materials represent mixtures of carbon from several sources, and thus isotopic signatures reflect integration of different sources. Moreover, estuarine systems have variable input of organic matter with respect to space and time. Therefore, it is difficult to separate multiple end members mixing from the effects of matter alteration and transportation (Dittmar et al., 2001; Barros et al., 2010). To overcome the aforementioned discrepancies of bulk indices, molecular biomarkers such as hydrocarbons, fatty acids and sterols can be effectively used to explore the source and fate of organic matter in estuarine systems.

The organic matter pools entering the estuary are potentially modified by several pathways such as photolytic reactions (Amon and Benner, 1996; Miller and Moran, 1997), heterotrophic degradation (McCallister et al., 2004), respiration (Findlay et al., 1998; Moran et al., 1999), flocculation (Sholkovitz et al., 1978; Fox, 1983) and sorptive fractionation (Aufdenkampe et al., 2001). Consequently, estuaries are among the most challenging aquatic systems in which the origins, transformations and fates of organic matter can be traced (Hedges and Keil, 1999). The differential reactivity of individual organic compounds to various modification processes produce diverse range of end products causing further complexities to organic matter sources. The multi-proxy approach has been shown to be an effective way of evaluating the source, fate and degradation pathway of organic matter in estuarine systems.

5.2 Hydrocarbons as Biomarkers

The straight chain alkanes (n-alkanes) are generally regarded as molecular biomarkers. Biogenic n-alkanes are known to be biosynthesised by wide variety of both marine and terrestrial plants. In higher plants, n-alkanes are derived from epicuticular waxes, have long chain length (C_{25}-C_{35}) and an odd over even chain length predominance (Sikes et al., 2009). The shorter chain n-alkanes (C_{14} to C_{24}) are mainly from algal or phytoplankton origin (Meyers and Ishiwatari, 1993). Recent studies shown that freshwater aquatic and marine mactophytes have dominant mid chain length n-alkanes (C_{23} to C_{27}) and mangroves have slightly longer mid chain n-alkanes (Mead et al., 2005). The difference in characteristic chain lengths of marine and terrestrial plants made the distribution of n-alkanes an effective biomarker tool for assessing sources organic matter in terrestrial and marine sediments.

Branched hydrocarbons derived from isoprene units are common constituents in many bacteria or may be formed from algal organic matter during grazing and senescence. These isoprenoid hydrocarbons occur in both cyclic and acyclic forms. Pristane and phytane are two acyclic isoprenoid hydrocarbons formed by the decomposition of phytol, the side chain of Chl-*a*. Under oxidizing conditions, phytol is preferably converted to pristene which is subsequently reduced to pristane. Under anoxic conditions, phytol is subjected to reduction and dehydration resulting the formation of phytane (Killops and Killops, 2005; Bianchi and Canuel, 2011). The major hydrocarbon biomarkers are listed in Table 5.1.

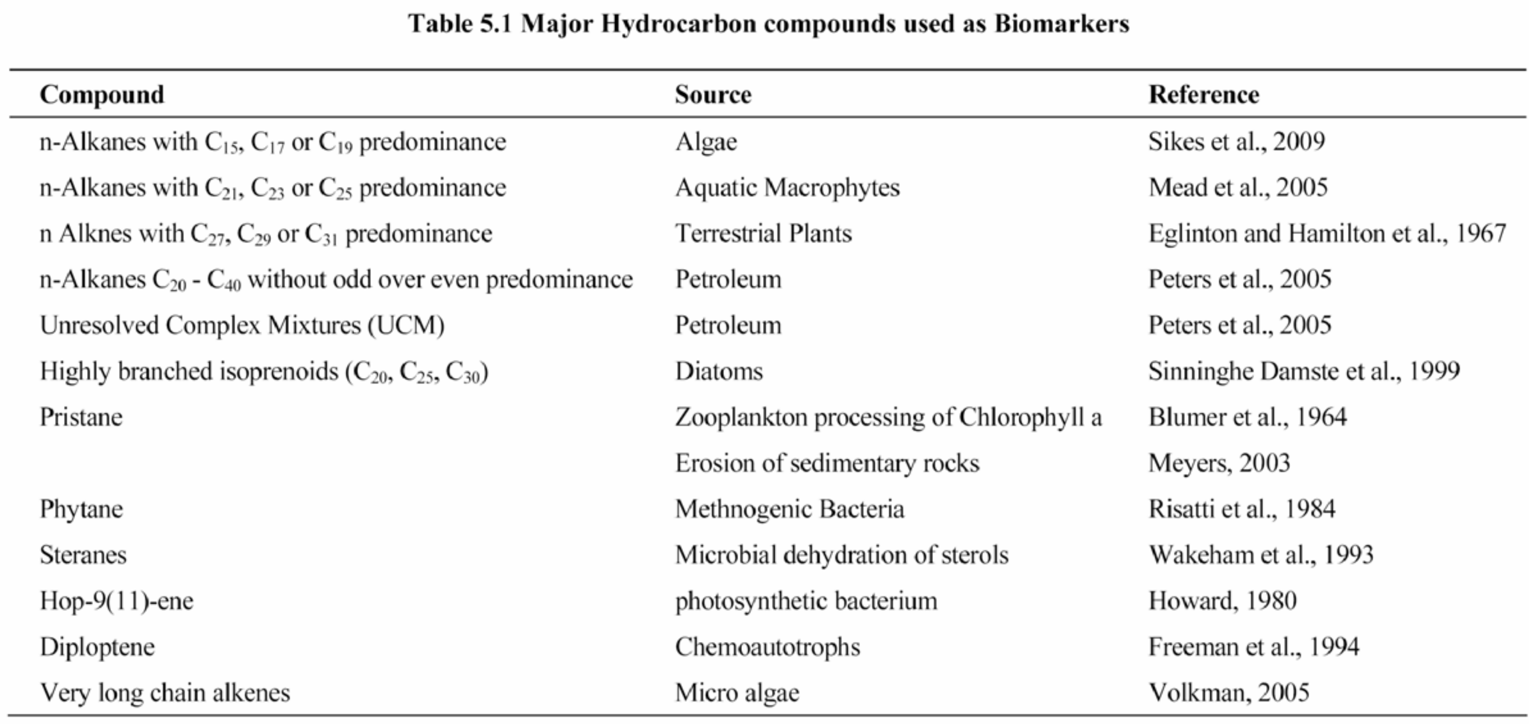

Table 5.1 Major Hydrocarbon compounds used as Biomarkers

Compound	Source	Reference
n-Alkanes with C_{15}, C_{17} or C_{19} predominance	Algae	Sikes et al., 2009
n-Alkanes with C_{21}, C_{23} or C_{25} predominance	Aquatic Macrophytes	Mead et al., 2005
n Alknes with C_{27}, C_{29} or C_{31} predominance	Terrestrial Plants	Eglinton and Hamilton et al., 1967
n-Alkanes C_{20} - C_{40} without odd over even predominance	Petroleum	Peters et al., 2005
Unresolved Complex Mixtures (UCM)	Petroleum	Peters et al., 2005
Highly branched isoprenoids (C_{20}, C_{25}, C_{30})	Diatoms	Sinninghe Damste et al., 1999
Pristane	Zooplankton processing of Chlorophyll a	Blumer et al., 1964
	Erosion of sedimentary rocks	Meyers, 2003
Phytane	Methnogenic Bacteria	Risatti et al., 1984
Steranes	Microbial dehydration of sterols	Wakeham et al., 1993
Hop-9(11)-ene	photosynthetic bacterium	Howard, 1980
Diploptene	Chemoautotrophs	Freeman et al., 1994
Very long chain alkenes	Micro algae	Volkman, 2005

The hopanes are cyclic isopreniod hydrocarbons composed of three sterioisomeric forms, namely 17α(H),21β-, 17β(H),21β(H)-and 17β(H),21α(H)-(moretanes). The major precursors for hopanes include bacteriohopanetetrol and related bacteriohopanes. These compounds upon diagenesis and catagenesis result in the formation of 17α(H), 21β(H)-hopanes or 17 β (H), 21 α (H)-moretanes (Peters et al., 2005).

5.3 Results

5.3.1 Aliphatic Hydrocarbons

Surface sediments from the Cochin estuary showed the presence of n-alkanes ranging from C_{12} to C_{33}. The total concentration of n-alkanes (Σalkanes) ranged from 6033 to 43233ngg^{-1} with average of 16797ngg^{-1} (Table 5.2). Maximum n-alkane concentrations were recorded at S3, while minimum values were observed at S5. S1, barmouth region exhibited the predominance of C_{17}, C_{19} and C_{21} n-alkane and showed a gradual decrease in higher chain n-alkane concentrations. S2 (Bolgatty) being the main mixing zone of fresh water from the river Periyar with Arabian sea water, it clearly showed the dominance of C_{25}, C_{27}, C_{29}, C_{31} and C_{33} n-alkanes with maximum concentrations of C_{31} n-alkane. S4 is characterised by higher concentrations of C_{29}, C_{31} and C_{33} n-alkanes. At the same time comparable concentrations of short chain alkanes especially C_{17}, C_{19} and C_{21} n-alkanes were also obtained. The C_{13} and C_{14} n-alkanes predominated at S5. Comparatively higher concentrations of C_{25}, C_{27}, C_{29}, C_{31} and C_{33} n-alkanes were observed at this station whereas minimum concentrations were observed for C_{15}- C_{20} alkanes. The sum of n-alkanes of terrestrial origin (C_{27}, C_{29} and C_{31}) and phytoplanktonic origin were calculated to identify the dominant sources and the order of abundance. Pristane and Phytane in surface sediments of the Cochin estuary ranged from n.d to 835ngg^{-1} and 27 to 846ngg^{-1} respectively. Both pritane and phytane showed higher concentrations at S1, S3 and S7 (Fig. 5.1). Detection of hopanes was carried out by monitoring ions at m/z=191. The major hopanes identified are presented in Table.5.3.

Table. 5.2 Distribution of n-alkanes (ngg^{-1}) in surface sediments of the Cochin estuary

Stations	S1	S2	S4	S5	S6	S7	S9	S10	S14
C_{12}	147	1	1647	656	87	266	661	11	69
C_{13}	390	1	2051	1603	227	358	1063	7	393
C_{14}	740	12	2174	1475	291	269	1575	12	439
C_{15}	678	33	1150	220	46	41	866	13	70
C_{16}	1189	187	2057	165	111	195	1561	44	104
C_{17}	1600	119	2356	143	48	35	1373	336	293
C_{18}	1081	213	1926	129	257	38	1381	44	56
C_{19}	1325	136	2208	95	54	47	1534	45	77
C_{20}	1278	282	2089	237	130	289	1448	83	227
C_{21}	1657	339	2347	886	124	155	1502	221	112
C_{22}	1059	457	1760	361	200	300	1271	175	242
C_{23}	929	560	1734	749	194	229	1237	407	231
C_{24}	948	680	1485	844	250	405	1089	429	267
C_{25}	851	1052	1577	1202	306	448	1087	961	313
C_{26}	676	927	1237	565	291	542	863	765	288
C_{27}	776	1559	1889	1267	433	638	1095	1375	448
C_{28}	527	936	999	592	270	506	580	846	224
C_{29}	501	2181	3920	1009	639	734	1351	1917	542
C_{30}	329	852	1083	382	278	424	643	857	323
C_{31}	378	2922	3709	894	921	858	1886	2810	728
C_{32}	185	673	1514	447	320	341	582	772	305
C_{33}	211	1907	2374	559	556	587	1275	1933	461

Table 5.3 Hopanes and hopenes identified from the surface sediments

Sl No	Name
1	18α-22,29,30-trisnorhopane (Ts)
2	17β (H) –22,29,30-trinorhopene (Te)
3	17α-22,29,30-trisnorhopane (Tm)
4	17α(H),21β(H)-30-norhopane (C29αβ)
5	Hop-13(18)ene (H13(18))
6	17β(H),21α(H)-30-norhopane (C29 βα)
7	17α(H),21β(H)-hopane (C30αβ)
8	17β(H),21α(H)-hopane (C30βα)
9	17α(H),21β(H)-homohopane (C31αβ 22S)
10	17α(H),21β(H)-homohopane (C31αβ 22R)
11	Gammacerane (G)
12	A'-Neogammacer-22(30)-ene or Hop-22(29)-ene or Diploptene (NG)
13	17α(H),21β(H)-bishomohopane (C32αβ 22S)
14	17α(H),21β(H)-bishomohopane (C32αβ 22R)
15	17α(H),21β(H)-trishomohopane (C33αβ 22S)
16	17α(H),21β(H)-trishomohopane (C33αβ 22S)
17	17α(H),21β(H)-tetrakishomohopane (C34αβ 22S)

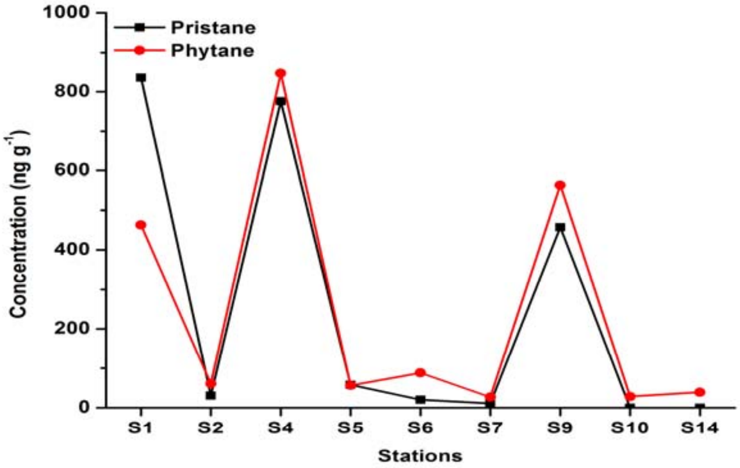

Fig. 5.1 Distribution of pristane and phytane in surface sediments

5.4 Discussion

5.4.1 Aliphatic hydrocarbons

The strong odd to even carbon preference of the high molecular weight (HMW) n-alkanes ($>C_{23}$) in surface sediments with C_{max} at C_{31} revealed prominent terrigenous contribution derived from higher vascular plant wax (Aboul-Kassim and Simoneit, 1996; Hu et al., 2013) in addition to the preferential degradation of the marine derived components (Meyers et al., 1984); while the bar mouth region displayed increasing proportion of LMW ($<C_{23}$) homologues with C_{max} at C_{17} and C_{21}, indicating a smaller terrigenous contribution with an increased fraction of marine derived OM. The n-alkanes in the sediments from the Cochin estuary showed an even over odd predominance in the short chain homologues ($\leq C_{22}$) (except at S1 and S10), which is different from those commonly reported features of n-alkane compositional patterns in the marine and freshwater sediments. This feature has also been observed in marine and freshwater sediments with various ages depositional conditions and biological sources, including plankton, microbial reworking of algal detritus and recent biogenesis of fatty acids or other lipid materials (Hu et al., 2013). The direct microbial inputs have been suggested to account for such uncommon distribution (Nishimura and Baker, 1986; Grimalt and Albaiges, 1987; Elias et al.,1997; Volkman et al., 1998). Generally, the even carbon preference of the C_{12}–C_{22} n-alkanes in marine sediments can be mainly attributed to the direct biogenic contribution from algae, bacteria, fungi and yeast species (Nishimura and Baker, 1986; Grimalt and Albaiges, 1987) as well as the potential direct petroleum-derived inputs (Ekpo et al., 2005; Harji et al., 2008). Additionally, the even predominance is also suggested to be formed

by the reduction of fatty acids under anoxic environments (Dastillung and Corbet, 1978).

The quantification of homologues of n-alkanes enables the determination of Carbon Preference Indices (CPI) providing supportive evidence for the incorporation of different hydrocarbon sources. In this study, the CPIs were calculated according to Harji et al., 2008 (CPI^a, full range n-alkanes) and (CPI^b higher chain n-alkanes).

$$\mathbf{CPI^a} = \frac{\sum_{13}^{33} \textbf{Odd Chain n} - \textbf{Alkanes}}{\sum_{12}^{32} \textbf{Even Chain n} - \textbf{Alkanes}}$$

$$\mathbf{CPI^b} = \frac{\sum_{23}^{33} \textbf{Odd Chain n} - \textbf{Alkanes}}{\sum_{24}^{32} \textbf{Even Chain n} - \textbf{Alkanes}}$$

The CPI^a and CPI^b were in the range 1.14 to 2.48 and 0.73 to 2.39 respectively. These values strongly support the above conclusion that the n-alkanes in the estuarine sediments were mostly derived from terrestrial sources with substantial contribution from biogenic and/or petroleum sources.

The total content of $C_{27}+C_{29}+C_{31}$ n-alkanes have been used as terrestrial organic matter indicator, while the total content of $C_{15}+C_{17}+C_{19}$ n-alkanes is normally considered as marine organic matter indicator (Xing et al., 2011). The total contribution of $C_{27}+C_{29}+C_{31}$ n-alkanes ranged from 9 to 43%, accounting for an average of 29% and exhibited increased inputs in the inner part of the estuary which reflected a dominant fluvial input (Fig. 5.2). This revealed the enhanced input of marine autogenous hydrocarbons towards the seaward side. The total contribution of planktonic n-alkanes ($C_{17}+C_{19}+C_{21}$) accounted for 3 to 26% with maximum contributions towards the seaward side and lower contributions towards the inner part of the estuary.

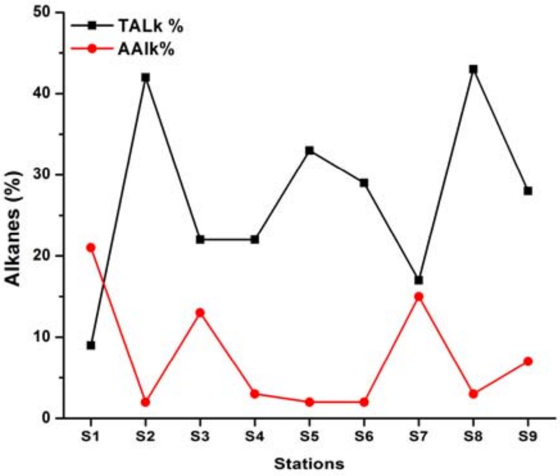

Fig. 5.2 Distribution of terrestrial derived (TAlk) and autogenous organic matter (AAlk) in surface sediments

An index based on the aforementioned n-alkanes has been proposed to estimate the relative contribution of terrestrial and marine derived organic matter *viz.* Terrigenous to Aquatic Ratio (TAR) (Meyers, 1997) even if this method may overestimate the terrigenous input due to the preferential preservation of terrestrial hydrocarbons compared to planktonic counterparts (Volkman et al., 1987). The TAR values were calculated using the equation

$$\mathbf{TAR} = \frac{C_{27} + C_{29} + C_{31}}{C_{15} + C_{17} + C_{19}}$$

The TAR values ranged from 0.5 to 23 in the surface sediments with minimum values at S1, S3 and S7, while all other estuarine sites exhibited TAR values >6. This confirmed the accumulation of terrigenous biogenic n-alkanes in the estuarine sediments.

Submerged and floating macrophytes have n-alkane biomarker compositions intermediate between algae and terrestrial vascular plants and are characterised by the dominance of odd mid chain length n-alkanes (C_{21},C_{23} or C_{25}) (Viso et al., 1993; Ficken et al., 2000). The proxy P_{aq} is often used for expressing the relative contributions of submerged macrophytes/floating plants. P_{aq} values ranged from 0.22 to 0.61. The lower values observed towards the Thanneermukkom bund may be ascribed to the input of n-alkanes from water hyacinth which proliferates in the entire estuary during the monsoon and post monsoon seasons.

$$\mathbf{P_{aq}} = \frac{\mathbf{C_{23} + C_{25}}}{\mathbf{C_{23}+C_{25} + C_{29} + C_{31}}}$$

To differentiate the probable sources of organic matter in these sediments, proxies like Σn-alkane/n-C_{16}, n-C_{17}/Pristane and n-C_{18}/Phytane, and (Pristane+Phytane)/ n-C_{17} ratios were often used. The Σn-alkanes/n-C_{16} ratio is usually <15.0 for petroleum contaminated samples (Gogou et al., 2000; Ou et al., 2004; Gao et al., 2007). The Σn-alkane/n-C_{16} is >15 in all the samples suggesting minor contribution of petroleum residues in the surface sediments. The even LMW n-alkanes showed a positive correlation with clay particles indicating the formation of n-alkanes from anoxic degradation of fatty acids. The even carbon preference for mid chain length (C_{20}-C_{26}) observed in most of the stations suggests the potential contribution of microorganism derived organic matter. The average chain length (ACL) calculated for surface sediments ranged from 21 to 28 and exhibited a positive significant correlation with TAR values. The high TAR value and elevated ACL in the Bolghatty region and inner part of the estuary indicated the preferential preservation of terrestrial derived organic matter over planktonic organic matter in surface sediments (Meyers et al., 1984; Volkman et al., 1987).

Pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethyl-hexadecane) are products of geological alteration of phytol and other isoprenoidyl natural products, and are not primary constituents of most terrestrial biota (Li et al.,1995; Gao et al., 2007; Harji et al., 2008). However, in the marine environment zooplankton and other higher marine animals can be the contributors of pristane while phytane is not only a normal component of oil but also can be synthesised by the methanogenic and photosynthetic bacteria (Steinhauer and Boehm, 1992; Sakata et al., 1997). In immature sediments, pristane and phytane are commonly produced from the phytyl side chain of chlorophyll a and b (Ragan and Chapman, 1978) and therefore can indicate algal source in marine sediments. Pristane/Phytane ratio was in the range 0.23 to 1.80 in surface sediments. The geological formation of phytane and pristane strongly depends on the depositional condition. Reducing or anoxic conditions in sediments promote the cleavage of phytyl side chain of chlorophyll a to yield phytol, which undergo reduction to form dihydrophytol and finally to phytane. Oxic environment promote the conversion of phytol to pristane by oxidation of pytol to phytenic acid followed by decarboxylation and reduction to form pristane. Stations S1 and S4 displayed pristane/phytane ratio higher than 1 and for all other stations the ratio is less than one.

5.4.2 Hopanes

The hopanes comprised a series of 17α (H), 21β (H)-compounds (C_{27}–C_{34}), maximising at the C_{30} homologue with less prominent 17β (H), 21α (H)-hopanes. The Ts/(Ts+Tm) ratios (Ts=18α-22,29,30-trisnorhopane, Tm=17α-22,29,30-trisnorhopane) and the 22S/(22S+22R) epimers of the homohopanes have been used to characterise the origin and the degree of maturation of crude oil (Hostettler et al., 1999). Gammacerene is distinguished as a biomarker for crude oil, derived from lacustrine sources also present in most of the samples indicating a minor input of petroleum hydrocarbons into the surface sediments.

The pentacyclic triterpene 17β(H), 21β(H)-hop-22(29)-ene (diploptene) has been reported from riverine, estuarine and coastal environments (Prahl, 1985; Prahl, 1992). There are several sources of diploptene including methylotrophic bacteria, cyanobacteria and certain species of ferns (Rohmer et al., 1984; Prahl, 1985). Diploptene has been identified in the present study and the relative abundance of this compound exhibited increase in concentrations towards the inner parts of the estuary. The erosion of soil derived bacterial organic matter can be considered as the major source of diploptene into the surface sediments while the presence of these compounds towards the seaward side may be attributed to some additional sources like methane oxidising bacteria.

5.5 Principal Component Analysis

PCA was employed on data sets including n-alkanes, OC, TN and sediment grain size components. PCA results are focussed on the first two principal components, which explained 83% of total cumulative variance. PC1 and PC2 generated have variance of 45% and 35% respectively (Fig. 5.3). PC1 is distinguished by highly significant loading for n-alkanes in the range C_{12} to C_{26}, indicating the biogenic contribution of marine derived organic matter and microbial derived organic matter as the main component. The highly significant correlation of LMW n-alkanes and even mid chain n-alkanes reveals the biochemical degradation pathway operating in the surface sediments. PC2 shows a high positive loading on the variables of long chain n-alkanes, OC, TN and fine grained sediments, indicating the terrigenous signature and geochemical accumulation of organic matter with respect to the grain size. The negative loading of sand content in PC1 is attributed to the lack of accumulation of organic matter in this portion. It can be found that barmouth region is distinguished by the presence of algal derived organic matter, while Bolgatty region, Fisheries harbour and Poothotta areas (S2, S5 and S10) of the estuary (Fig. 5.4) are distinguished by the accumulation of terrestrial derived organic matter. The terrestrial molecular components of the sedimentary organic matter characteristically degrade less rapidly than their marine counterparts (Zonneveld et al., 2010; Blair and Aller, 2012), and this could be partially responsible for the wide occurrence of HMW n-alkanes, especially in the central mud deposits. Stations S6, S7 and S14 are distinguished by the accumulation of terrestrial as well as even carbon n-alkanes.

Previous literatures demonstrated that the fluvial organic matter in fine grained sediment is enriched in non-woody cutin markers, and these non-woody biogenic markers are more easily associated with fine silt and clay particles which are preferentially and selectively transported towards the regions of low energy conditions, whereas the lignin rich coarser materials are deposited in the high energy regions (Prahl et al., 1994; Goni et al., 1998; Bianchi et al., 2002; Hu et al., 2013). The fine particles of the suspended sediment play a major role in fluvial organic matter transfer from land to the estuary and finally to the coastal areas (Zhang et al., 2009). Therefore, the hydrodynamic sorting and long distance transport of fine particles could possibly lead to a preferential dispersion and accumulation of terrigenous organic matter enriched with refractory matrix of land based constituents which can be distinguished by the alteration in elemental and isotopic ratios of sedimentary organic matter (Hu et al., 2013). The above mentioned factors alter the geochemical and biochemical processes to a considerable extent.

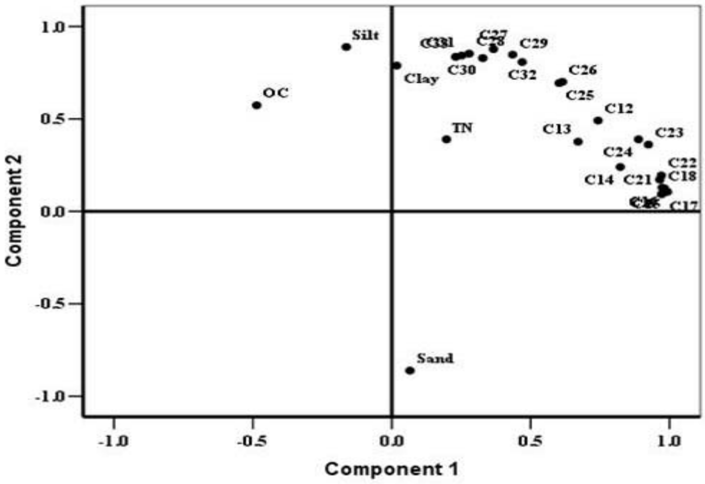

Fig. 5.3 Result of PCA

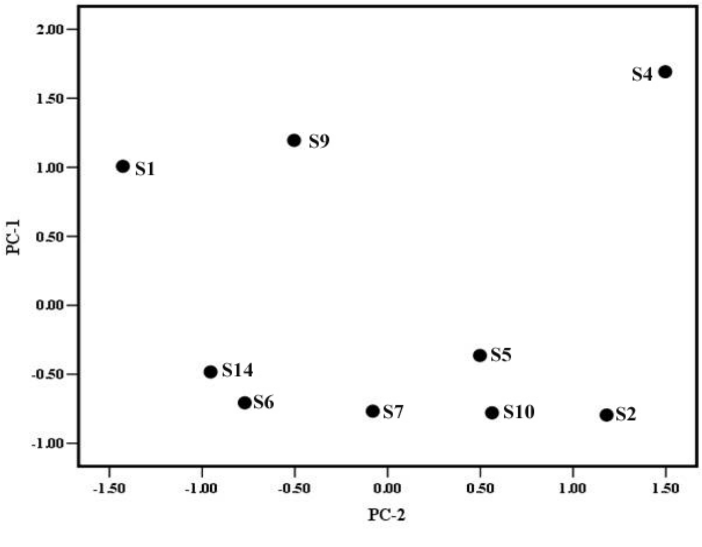

Fig. 5.4 Grouping of stations according to the PC scores

5.6 Conclusions

The higher abundance of HMW n-alkanes with higher TAR and ACL values and PCA results reveals the preferential utilisation of marine derived organic matter in biochemical degradation pathway. Also, various geochemical processes contribute to the accumulation of terrigenous sedimentary n-alkanes in the estuary. Biochemical degradation processes could be responsible for the unusual presence of even carbon predominance in the LMW homologues. The wide occurrence and higher proportion of the terrigenous n-alkanes in stations S2, S5 and S10 suggest an effective preferential dispersal and accumulation of the terrigenous organic matter with refractory matrix of land-based constituents. The biochemical degradation pathway operating in surface sediments causes substantial alterations to the planktonic organic matter in surface sediments. The hydrodynamic depositional forcing plays a dominant role not only on the selective delivery of fluvial terrigenous organic constitutes, but also on the preferential preservation of terrigenous organic matter versus the marine derived organic matter in estuarine sediments.

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Chapter 6

FATTY ACIDS AS BIOMARKERS

Contents

6.1 Introduction

Biogeochemical studies of estuarine sediments are fundamental for understanding carbon cycling and assessing the sources and fate of organic matter (Middelberg and Herman, 2007). Estuarine sediments consist of a complex mixture of organic molecules derived from a multitude of autochthonous and allochthonous sources. The effective source characterisation of organic matter cannot be achieved by the application of single biomarker compounds like n-alkanes, but need multi-proxy approaches. The fact that many n-alkanes can originate from more than one source (e.g. petroleum and bacterial inputs) emphasises the need for caution in assigning biological origins depending only on n-alkanes data. In order to get more precise and vivid knowledge regarding the sources and diagenetic processes of organic matter in estuarine sediments, lipid biomarkers such as fatty acids and sterols can be effectively used.

6.2 Fatty acids as Biomarkers

Fatty acids, the principal components of most classes of natural lipids having a great structural diversity, are present in a variety of cellular membranes and energy storage (Gurr and James, 1971) which have been extensively utilised for the characterisation of source and diagenetic fate of organic matter in estuarine systems (Niggemann and Schunert, 2006; Hu et al, 2006). Their unique distributions in various organisms (bacteria, microalgae and higher plants), distinct structural features indicative of their sources and the spectrum of reactivity exhibited by the fatty acid classes makes them an effective molecular biomarker tool (Bianchi and Canuel, 2011).

The fatty acids are typically of C_{12} to C_{36} chain length and in animals they are predominantly saturated (alkanoic acids), whereas in plants more unsaturated (alkenoic acids) and polyunsaturated acids are present. Fatty acids have predominantly even numbers of carbon atoms because they are effectively formed from acetyl (C_2) units, which are derived from glucose in the presence of various enzymes, coenzymes and carrier proteins. The important attributes of a fatty acid are its carbon chain length, the number of double bonds present and their positions, that can be represented by a simple notation scheme. For example, oleic acid can be represented by *cis*-18 :1 ω 9, where *cis* refers to the stereochemistry about the C=C bond, 18 is the number of C atoms, the number of double bonds (1) is given after the colon, and the number following ω is the position of the double bond from the opposite end to the acid group. As double bonds in polyunsaturated acids are usually conjugated, it is only necessary to give the position of the first double bond because all others follow on alternate carbon atoms. Hence arachidonic acid is 20 :4 ω 6, in which the first C=C bond occurs between C-6 and C-7, numbering from the opposite end to the acid group, and the other three C=C bonds are between C-8 and C-9, C-10 and C-11, and C-12 and C-13 (Killops and Killops, 2005).

The fatty acids show patterns related mainly to species sequence of phytoplankton populations and growth conditions (Mayzaud et al., 1990; Napolitano et al., 1997), and have been used as chemotaxonomic indicators

(Minnekin and Good Fellow, 1980; Saliot et al., 1991). Fatty acids of sinking particles have proven to be useful indicators of photosynthetic activity in surface water (Hayakawa et al., 1996; Tolosa et al., 2004). They have also been applied to elucidate particulate dynamics and the physiological state of phytoplankton (Wakeham et al., 1984; Shin et al., 2000). The relative abundances of individual fatty acids are useful in evaluating the respective importance of inputs from bacteria, microalgae, marine fauna and continental higher plants (Sargent et al., 1981; Tolosa et al., 2004). Major fatty acid biomarkers employed in this study are furnished in Table 6.1.

The saturated fatty acids (SFA) $C_{16:0}$ and $C_{18:0}$ are ubiquitous in the marine environment and can be used as a measure of total community biomass (Parkes, 1987). $C_{14:0}$ fatty acids are present in phytoplankton especially in diatoms (Reitan et al, 1994) and to a lesser extend in dinoflagellates (Napolitano et al., 1997). C_{18} - C_{22} polyunsaturated fatty acids (PUFAs) are associated with marine organisms. Their primary source is phytoplankton, while marine animals obtain PUFAs from the food web and further modify the distributions by chain elongation and desaturation of the ingested fatty acids (Napolitano et al., 1997; Volkman, 2005). $C_{20:5\ \omega3}$, $C_{16:2\omega7}$, $C_{12:2\omega7}$ and $C_{20:4\omega6}$ are used as diatom markers in marine environment (Volkman et al., 1989; Canuel et al., 1995; Pond et al., 1998). The presence of elevated amounts of $C_{22:6\omega3}$ in sediments usually indicate dinoflagellate origin (Viso and Marty, 1993; Parrish et al., 2000). C_{18} PUFAs especially with positional isomers ω-3 and ω-6 are usually found in chlorophyta, while $C_{18:2\omega6}$, $C_{18:3\omega3}$ and $C_{18:3\omega6}$ is used as markers of green algae (Napolitano et al., 1997; Meziane and Tsuchiya, 2000; Joseph et al., 2012). $C_{20:5\omega3}$ and $C_{16:4\omega3}$ are typical diatom markers (Colombo et al., 1996); 18:2ω6, 18:3ω3 and 18:3ω6 have been used as markers of green algae (Meziane and Tsuchiya, 2000); $C_{22:6\omega3}$ usually indicate dinoflagellate origin (Budge and Parrish, 1998; Carrie et al., 1998, Hu et al., 2005). Some monounsaturated fatty acids (MUFAs) such as $C_{16:1\omega5}$, $C_{16:1\omega7}$, $C_{16:1\omega9}$ are also used as signals of diatom derived organic matter (Carrie et al., 1998; Joseph et al., 2012). *Iso*-, *anteiso*-, cyclopropyl and branched fatty acids are often the most abundant fatty acids in bacteria (Parkes, 1987; Ding and Sun, 2005; Hu et al., 2006). Terrestrial plants are considered as the main source of long chain fatty acids ($C_{22} – C_{30}$) showing a strong even order predominance (Shi et al., 2001; Zimmerman and Cannel, 2001; Hu et al., 2005).

Table 6.1 Specific fatty acid molecular biomarkers used for the source characterisation of organic matter in aquatic environment

Fatty acid	Source	References
$C_{14:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$, $C_{16:1\omega9}$, $C_{16:4\omega1}$, $C_{20:5\omega3}$	Diatoms	Reitan et al., 1994; Berge et al., 1995; Carrie et al., 1998
$C_{14:1}$	Cyanobacteria	Caudales and Wells, 1992; Caudales et al., 1993.
Σ odd carbon + branched, iso and anteiso C_{15} and C_{17}, $C_{18:1\omega7}$	Bacteria	Parkes and Taylor,1983; Kaneda, 1991; Budge and Parrish, 1998; Viso and Marty, 1993
C_{16}, C_{18}	Ubiquitous biological markers of plankton in the marine environment	Parkes, 1987
$C_{16:1}$	Marine algal species	Reitan et al., 1994
$C_{18:1\omega 9}$	Brown algae	Jamieson and Reid, 1972; Johns et al., 1979
$C_{18:2\omega6}$, $C_{18:3\omega6}$, $C_{18:3\omega3}$	Green algae	Volkman et al., 1989; Dunstan et al., 1992; Zhukova and Aizdaicher,1995; Napolitano et al., 1997; Kharlamenko et al., 1995
$C_{20:1}$, $C_{24:1}$	Zooplankton	Albers et al., 1996
$C_{22:6\omega3}$	Dinoflagellates	Sargent et al., 1987; Harvey et al., 1988; Viso and Marty, 1993
Long Chain Fatty acids ($\geq$22)	Terrestrial Plants	Tulloch, 1976; Shi et al., 2001; Volkman et al., 1998
$C_{16:1}/C_{16}>1.6$, $\Sigma C_{18}/\Sigma C_{16}>2$	Diatoms	Parrish et al., 2000; Hu et al., 2006

6.3 Results

A total of 28 fatty acids were identified from the surface sediments including PUFA, MUFA, SFA and branched chain fatty acids (Br-FA) (Table 6.2). Total Fatty acid concentrations ranged from 22.56 to 440.69µgg^{-1} in surface sediments with maximum concentrations at the Fisheries harbour region and minimum concentration in the barmouth region of the estuary. 19 individual fatty acids were detected at bar mouth region (S_1) with a total fatty acid concentration of 22.50µgg^{-1}. SFA contributed 69% of total fatty acid pool, while Br-FA constituted 10% and MUFA and PUFA contributed 18% and 2.84% respectively. $C_{16:0}$ was the most abundant individual fatty acid which contributed 32% of total fatty acids. The $C_{14:0}$ and $C_{18:0}$ SFA contributed 12.7% and 9.42% respectively.

A total of 26 fatty acids were detected in the Bolgatty region with a total concentration of 194µgg^{-1}. The $C_{16:0}$, $C_{14:0}$ and $C_{18:0}$ fatty acids were dominant in the study region contributing 51.72%, 14.44% and 12.76% respectively. SFAs dominated in total fatty acid content (62%) followed by Br-FA (25%) and MUFA (15%) (Fig.6.1). Very low concentrations of PUFAs were recorded in the Bolgatty region. Comparatively high fatty acid concentrations were detected in stations S4 and S5. Among the 25 individual fatty acids detected in Sulphur Jetty region and Fisheries harbour region, $C_{16:0}$, $C_{14:0}$ and $C_{18:0}$ individual fatty acids were the dominant class. Comparatively higher concentrations of long chain fatty acids and iC_{15} were obtained in Sulphur Jetty area of the estuary.

Table 6.2 Distribution of fatty acids (µgg^{-1}) in the surface sediments

Compound	S1	S2	S4	S5	S6	S7	S9	S10	S14
$C_{12:0}$	1.15	5.70	9.03	9.41	0.59	5.89	9.21	10.57	5.17
$iC_{14:0}$	n.d	n.d	6.01	6.85	n.d	n.d	1.81	4.51	2.30
$C_{14:0}$	2.87	14.44	27.96	26.97	1.05	15.21	8.16	22.96	12.72
5,9,13-C_{14}	0.45	6.55	13.38	25.24	0.40	6.27	2.39	7.24	3.42
$iC_{15:0}$	0.93	11.41	20.48	22.31	0.63	12.94	5.25	13.55	12.27
$aC_{15:0}$	0.64	7.37	12.30	14.60	0.35	6.44	3.28	7.94	6.72
$C_{15:0}$	0.72	4.92	16.79	10.53	0.40	5.66	2.65	14.03	10.12
$iC_{16:0}$	0.31	5.26	9.84	12.19	0.27	3.83	0.59	7.57	3.30
$C_{16:1}$	1.62	7.40	16.06	14.32	0.49	11.50	2.94	11.35	9.35
$C_{16:0}$	7.21	51.72	102.40	103.98	3.89	54.50	35.51	77.12	57.83
$iC_{17:0}$	n.d	3.93	0.00	8.55	n.d	3.33	1.90	5.94	3.80
$aC_{17:0}$	n.d	4.04	8.30	11.21	n.d	n.d	2.05	5.69	n.d
$C_{17:0}$	0.40	3.38	9.53	7.22	0.26	3.59	1.93	6.67	4.85
Cyclo-C_{17C}	n.d	1.22	2.41	n.d	n.d	n.d	0.78	1.30	47.87
$C_{18:0}$	2.12	12.76	26.84	25.27	0.99	12.66	16.71	19.85	4.26
$C_{18:1\omega7}$	1.17	10.84	11.82	16.87	0.47	6.00	0.48	15.93	6.00
$C_{18:1\omega9}$	1.27	9.74	14.27	21.89	0.39	6.81	2.81	7.71	6.79
$C_{18:2\omega6}$	n.d	n.d	6.28	0.00	n.d	n.d	1.68	6.34	3.57
$C_{19:1}$	n.d	0.86	n.d	2.83	2.62	0.83	n.d	1.22	0.38
$C_{20:0}$	n.d	5.92	16.99	12.28	0.43	1.49	3.09	10.08	2.73
$C_{20:4}$	0.30	1.10	n.d	0.15	1.65	1.08	0.75	1.64	1.01
$C_{20:5}$	0.34	1.09	n.d	0.17	1.21	0.73	0.70	2.20	0.49
$C_{22:0}$	0.27	6.46	18.58	15.72	0.88	1.25	4.54	12.60	2.80
$C_{23:0}$	n.d	1.76	4.33	n.d	0.41	n.d	1.59	3.94	n.d
$C_{24:0}$	0.34	7.52	23.42	22.20	1.54	1.50	6.61	18.91	1.60
$iC_{26:0}$	n.d	2.70	18.32	17.90	1.69	n.d	4.59	6.11	4.19
$C_{26:0}$	0.26	3.70	1.71	19.61	1.74	0.44	6.21	13.74	n.d
$C_{28:0}$	0.21	2.25	9.95	12.44	1.65	n.d	5.39	7.18	n.d

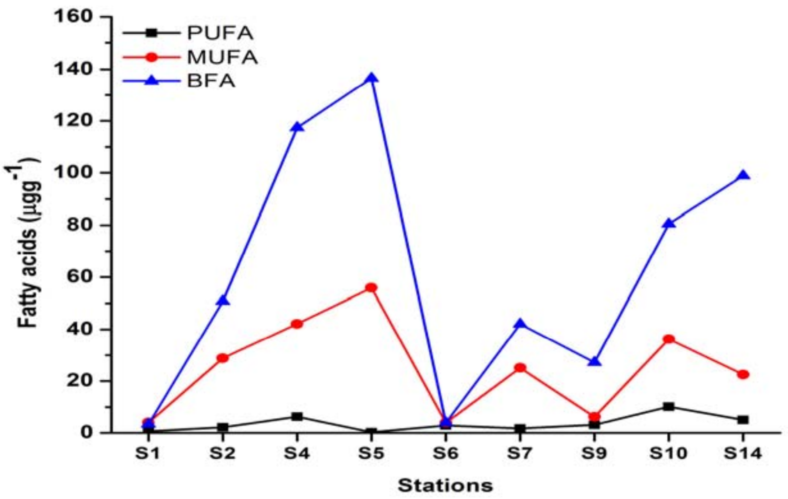

Fig. 6.1 Distribution of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), bacterial derived fatty acids (BFA) in the surface sediments

Shipyard region showed comparatively low total fatty acid concentrations (24.01μgg^{-1}). Here C_{16} fatty acids dominated, while high concentrations of $C_{19:1}$ was also recorded in this region. 21 individual Fatty acids were detected in Thevara region and $C_{16:0}$, $C_{14:0}$ and $C_{12:0}$ dominated among individual fatty acids. High concentrations of $iC_{15:0}$ and $C_{16:1}$ was also obtained in this area, while very low concentrations of long chain fatty acids were recorded. Panavalli, Poothotta and Thanneermukkom bund region of the estuary was characterised by high concentrations of Fatty acids. $C_{16:0}$, $C_{14:0}$ and $C_{18:0}$ fatty acids dominated among the individual Fatty acids. 27 fatty acids were detected in the Panavalli region with high concentration of $C_{16:0}$, $C_{14:0}$ and $C_{18:0}$.

SFAs were the major fraction contributing 47 to 76% of total fatty acid and ranged from 13.83 to 267.53μgg^{-1}. SFAs $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$, $C_{24:0}$, $C_{26:0}$ and $C_{28:0}$ were detected in all the stations. $C_{16:0}$ dominated among the SFAs followed by $C_{14:0}$ and $C_{18:0}$. Panavalli region of the estuary showed maximum SFA concentrations, while Bund region displayed minimum SFA concentrations. Br-FA constituted about 10.32 to 39.27% in the study region with maximum concentrations in the Bund region and minimum in the barmouth region. The total Br-FA concentrations were in the range 2.33 to 118.83µgg^{-1}. iC$_{14:0}$, 5, 9, 13Me-C$_{14}$, *i*C$_{15:0}$, *a*C$_{15:0}$, *i*C$_{16:0}$, *i*C$_{17:0}$ and iC$_{26:0}$ were detected in surface sediments and among the Br-FAs *i*C$_{15:0}$, *a*C$_{15}$ and *i*C$_{17}$ were present in high concentrations. PUFA constituted only a meager portion of total FAs (only 0.07 to 11.93%), which ranged from 0.31 to 10.18µgg^{-1}. MUFA constituted about 4.66 to 17.97% of total fatty acids and it ranged from 3.98 to 55.91µgg^{-1} in surface sediments. The sum of Br-FA and odd number carbon fatty acids were regarded as bacterial fatty acids (BFA) and it ranged from 3.44 to 136.58µgg^{-1}.

6.4 Discussion

The distributional dynamics of n-alkanes revealed the preferential preservation of terrestrial derived n-alkanes in surface sediments and the hydrodynamic sorting of organic matter through the estuarine salinity gradient. The obtained signatures about the biochemical degradation pathway operating in surface sediments lack clarity due to the overlapping of n-alkanes sources from bacterial degradation and petroleum input. Both microbial activity and fossil fuel inputs contribute short chain n-alkanes, but they do not have a strong odd-to-even carbon preference (Bouloubassi et al., 2001). This recurring theme of overlapping n-alkanes markers in different organic matter sources indicates that caution should be advised when using only n-alkanes to distinguish between bacterial derived organic matter and petroleum input. Combination of different class of lipid biomarkers (multi-proxy approach) can be effectively used for the better understanding of organic carbon cycle.

The total fatty acid distributions in surface sediments descended in the higher side when compared to the earlier reports from estuaries around the world (Table 6.3). The contribution of various specific, coupled and grouped fatty acids characteristics of known organic matter sources were used further to evaluate the relative importance of organic matter inputs within deposited surface sediments of Cochin estuary. Fatty acids with odd carbon number and branched fatty acids can be used as bacterial markers (Haddad et al., 1992; Rajendran et al., 1993; Bouillon and Boschker, 2006; Joseph et al., 2012). The long chain fatty acids from terrigenous source are more refractory than saturated chain fatty acids from planktonic sources and the overall order of relative reactivity between the sources is planktonic > bacterial > terrestrial (Camacho-Ibar et al., 2003). High total fatty acid concentrations were obtained in the present study. The major studies regarding the distribution of fatty acids in the estuarine sediments around the world are presented in Table 6.3.

Table 6.3 Distribution of total fatty acid concentrations reported in various estuaries

Study Area	Total Fatty acids (μgg^{-1})	Reference
Mangrove sediments of Cochin	37-95	Joseph et al., 2012
Matapuri Estuary	295-325	Alfaro et al., 2006
Pearl River Estuary	1.28-42.25	Hu et al., 2006
Oura Bay Estuary	222.6	Mfilinge et al., 2005
Yalujiang Estuary North China	4.8-32.9	Wu et al., 2004
Okinawan Estuary Japan	89.2-613.9	Meziane and Tsuchiya, 2002
Santa Monica Basin	6-40	Pearson et al., 2001
Estuaries of south Florida	7-222.8	Jaffe et al., 1996
Cochin Estuary	22.6- 440.7	Present Study

The fatty acid composition of the surface sediments of the Cochin estuary showed the predominance of saturated straight chain fatty acids including $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$. The $C_{14:0}$, $C_{16:0}$ and $C_{16:1}$ are ubiquitous biological markers of phytoplankton in the estuarine environment. The ratio of 16:1/16:0 is usually used to distinguish between diatoms and dinoflagellates and values > 1.6 have been used as an evidence for diatom derived fatty acids in different aquatic systems (Budge and Parrish, 1998; Hu et al., 2005; Joseph et al., 2012). The ratios in the study region were in the range 0.08 to 0.22. Due to the higher susceptibility of unsaturated fatty acids to the biological and chemical degradation during sedimentation, the ratio of $C_{16:1}$/ $C_{16:0}$ in surface sediments is mostly well below 1 (Birgel et al., 2004; Hu et al., 2005; Joseph et al., 2012).

Phytoplanktons are characterised by high concentrations of C_{16} fatty acids, while zooplanktons exhibit elevated amounts of C_{18} fatty acids. The ratio of the sum of all fatty acids having 18 carbon numbers to sum of fatty acids having 16 carbon numbers was proposed as diatom marker (Parrish et al, 2000). Relatively high concentrations of these fatty acids were obtained in sediments of the study area and the ratios ranged from 1.80 to 3.42. Unsaturated fatty acids 16:4ω1 can be used as diatom marker, since these fatty acids are very rarely encountered in other phytoplankton species.

The PUFA 20:5ω3 and 16:4ω1, 18:2ω6, 18:3ω3 and 18:3ω6 have been used as markers of green macro algae (Meziane and Tsuchiyu, 2000; Hu et al, 2005) where as 22:6ω5 usually used as dinoflagellate marker (Budge and Parrish, 1998; Carrie et al, 1998; Hu et al, 2005). However very low concentrations of $C_{18:2\omega6}$, $C_{20:4}$ and $C_{20:5}$ PUFAs were detected in surface sediments of the Cochin estuary. In addition to the elevated amount of $C_{16:4\omega1}$ fatty acids, diatoms produce large amount of 20:5ω3, whereas dinoflagellates generally contain higher proportions of $C_{22:6\omega5.}$

Odd carbon numbered fatty acids and branched chain fatty acids are commonly produced by bacteria, and can be used as bacterial biomarkers (Volkman et al., 1980; Haddad et al., 1992; Harvey, 1994; Rajendran et al., 1997). Several odd chain fatty acids, specifically 15:0 and 17:0 and all branched chain fatty acids are produced by both aerobic and anaerobic bacteria (Parkes and Taylor, 1983; Parrish et al., 2000; Joseph et al., 2012) and the sum of these fatty acids can be used as bacterial biomarker (Volkman et al., 1980; Haddad et al., 1992; Harey, 1994; Rajendran et al., 1997). The sum of C_{15}, C_{17} and branched chain fatty acids contributed to 15% to 46% of total fatty acid composition in the surface sediments. The high abundance of odd straight chain and branched chain fatty acids and lower concentrations of PUFA indicated that bacteria mediated biochemical degradation processes occurring in the surface sediments of Cochin estuary.

The nitrogen isotopic ratios in the surface sediments provided indication about the microbial reworking of sedimentary organic matter (Chapter 4). Nitrogen isotopic enrichment is relative to heterotrophic microorganisms and the $\delta^{15}N$ values of microbial reworked terrestrial organic matter increased from -4 to 9‰ (Caraco et al., 1998). The BFA and $\delta^{15}N$ values showed a positive correlation in the study area. Thus, the microbial mediated degradation processes appear to have significant effect on the composition of sedimentary organic matter in Cochin estuary (Fig. 6.2). Degradation of algal fatty acids represents a loss in algal materials through biochemical processes while increase of bacterial abundance indicates a new production of organic matter by bacterial biosynthesis (Sun et al., 2002).

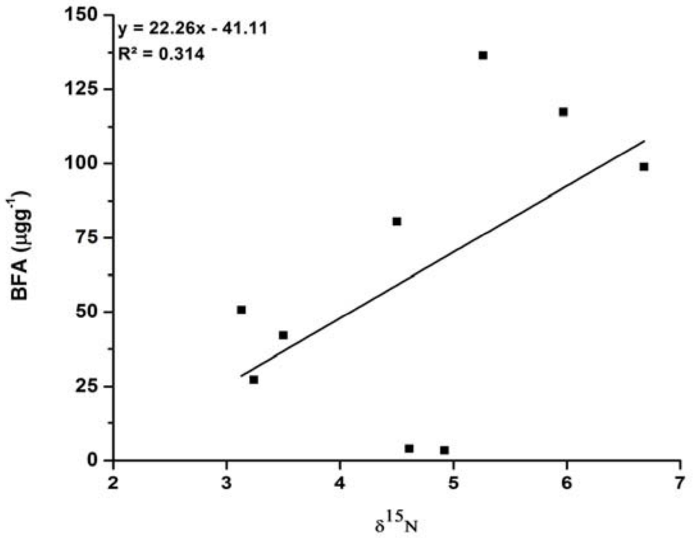

Fig. 6.2 Inter-relationship between bacterial fatty acids (BFA) and $\delta^{15}N$ in surface sediments

Bacteria can specifically biosynthesise a series of odd number C_{15} and C_{17} odd chain fatty acids, branched chain FAs and also produce $C_{18:1\omega7}$ FAs (Kanede, 1991), which have been widely used as bacterial source indicators (Wakeham and Beier, 1991; Ding and Sun, 2005). The decreased abundance of PUFA in surface sediments of the Cochin estuary is not a direct indication of the absence of phytoplankton derived organic matter, but rather the result of labiality of PUFA. The PUFAs are subjected to rapid loss by bacterial degradation and/or by zooplankton grazing (Hu et al., 2006; Dunn et al., 2008). The fatty acid biomarkers *iso*-C15:0, *anteiso*-$C_{15:0}$ and $C_{15:0}$ are abundant in sulphate reducing bacteria (Parkes and Taylor, 1983) and gram positive bacteria (Kanede, 1991). The fatty acid composition in aquatic environment is closely linked with bacterial respiratory types (aerobic, facultative anaerobic or anaerobic) and the concentrations of these biomarkers are drastically increased below the oxic/anoxic interface (Wakeham and Beier, 1991; Ding and Sun, 2005). Algal derived organic matter is rapidly assimilated and degraded as evident from the lack of polyunsaturated fatty acids, even in samples where algal contributions would be expected. This reflects not only microbial processes, but also the effects of grazing fauna such as crabs (Guest and Connolly, 2004; Guest et al., 2006). Previous studies in various estuaries and lakes already demonstrated the rapid assimilation of microalgal derived fatty acids in surface sediments (Hu et al., 2006; Volkman et al., 2008; Dunn et al., 2008).

6.5 Conclusions

The fatty acid composition in surface sediments of the Cochin estuary showed that fatty acids were mainly derived from phytoplankton, bacteria and terrestrial organic matter. The distribution of total fatty acids and bacterial fatty acids were similar, suggesting that bacteria may be a significant component of fatty acids of these sediments. The highest bacterial fatty acids concentrations were occurred in Sulphur Jetty and Shipyard (S4 and S5) region of the estuary may be due to the influence of sewage. The PUFA levels were comparatively low in the surface sediments, suggesting that most of the labile fatty acids were effectively recycled during the settling and deposition processes in the estuary.

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Chapter 7

STEROLS AS BIOMARKERS

Contents

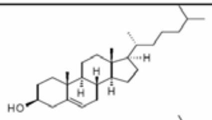
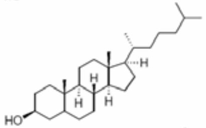
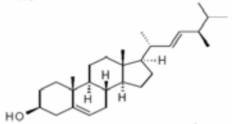
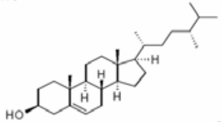
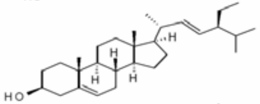
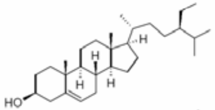
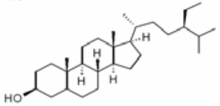
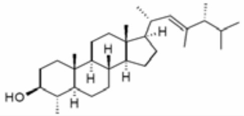
7.1 Introduction

Several neutral lipid classes are used as biomarkers (alcohols, sterols, terpenoids etc) because of their relatively high specificity in source assignments and better resistance to bacterial degradation than other classes of organic compounds (Volkman, 2006). Fatty alcohols can be efficient biomarkers for distinguishing marine and terrestrial organic matter. Short-chain ($<C_{20}$) n-alcohols are produced by marine and freshwater organisms, while long-chain ($\geq C_{22}$) n-alcohols with a strong even-over-odd carbon number preference are derived from waxes of terrestrial higher plants (Jeng et al., 2003; Treignier et al., 2006; Xu and Jaffe, 2007; Costa et al., 2011). Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), a diterpenoid alcohol present as an esterified side-chain of the chlorophyll-a molecule, is a general marker for primary producers and a suitable indicator of ''fresh'' organic matter derived from autotrophs due to its high reactivity (Jeng and Huh, 2004; Rontani and Volkman, 2003; Volkman et al., 2007; Costa et al., 2011).

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 of phospholipids bi-layers, while prokaryotic organism do not generally biosynthesise these compounds (Volkman, 1986). They represent a class of molecular biomarkers that can be used to differentiate between allochthonous, autochthonous and anthropogenic lipid carbon sources in estuarine environment. The unique structural features such as position of double bond, alkylation in the ring system and side chain, and stereochemistry makes them suitable tracers of organic matter sources. The source specificity, broad diversity and their distinguishable diagenetic products makes them excellent indicators to delineate provenance as well as the diagenetic pathway of sedimentary organic matter (Volkman, 2006; Rontani et al., 2009).

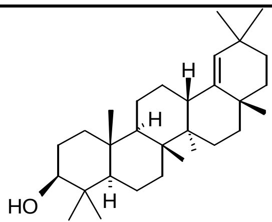
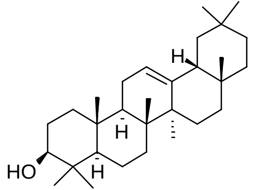
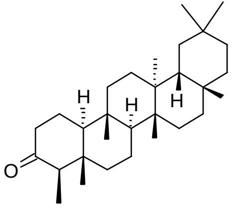
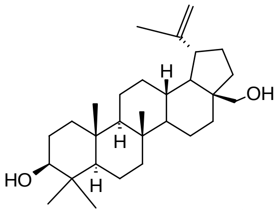
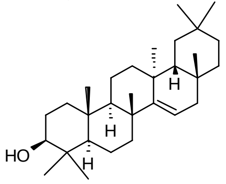
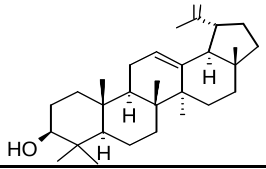
Prevalence of 4-methyl sterols such as 4α, 23, 24-trimethyl-5α-cholest-22(E)-en-3β-ol (dinosterol) indicate a significant dianoflagellate contribution, although certain diatoms have been found to synthesise these sterols (Volkman et al., 1993; Hudson et al., 2001). Even though certain algae produce C_{29} sterols, proportionately high abundance of C_{29} sterols are indicative of terrestrial inputs (Laureillard and Saliot, 1993; Li et al., 1995; Hudson et al., 2001). Terrestrial plants have been shown to have a high abundance of 24-ethylchloest-5-en-3β-ol (sitosterol), 24-methylcholest-5-en-3β-ol (campesterol) and stigmasterol (Volkman, 1986; Jaffe et al., 1995; Bianchi, 2007). The major sterol biomarkers used in the study are furnished in Table 7.1.

Table 7.1 Name and structure of major sterol biomarkers used in the study

Sl No	IUPAC Name	Common Name	Structure
1	Cholest-5-en-3β-ol	Cholesterol	
2	5α-Cholestan-3β-ol	Cholestanol	
3	24-Methylcholesta-5,22E-dien-3β-ol	Brassicasterol	
4	24-Methylcholest-5-en-3β-ol	Campesterol	
5	24-Ethylcholesta-5,22E-dien-3β-ol	Stigmasterol	
6	24-Ethylcholest-5-en-3β-ol	Sitosterol	
7	24-Ethyl-5α-cholestan-3β-ol	Stigmastanol	
8	4α, 23,24-Trimethyl-5a-cholest-22-en-3β-ol	Dinosterol	

Cholesterol is the most abundant and ubiquitous sterol in the environment due to its multiple sources (Pratt et al., 2008). Even though cholesterol is mainly animal sterol, it is also produced by other organisms including diatoms, 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 and often been used in the ratio form with other sterols. The sterol coprostanol, which is produced by bacterial reduction of cholesterol in the digestive systems of higher animals (McCalley et al., 198; Brown and Wade 1984), is largely used to characterise sewage inputs to aquatic ecosystems (Isobe et al., 2004; Cordeiro et al., 2008). Pentacyclic triterpenoids have been used to characterise the contribution of vascular plant sources in sedimentary environment (Koch et al., 2003; Boot et al., 2006). Pentacyclic triterpeniods with oleanane, ursane, taraxerane, lupane and friedoleanane skeleton is identified in mangroves and terrestrial plants but not in marine organisms. The identification of fatty alcohols, sterols and pentacyclic terpenoids can give important source information and signal of organic matter remineralisation pathway. The major pentacyclic triterpenoids employed in the study are presented in Table 7.2

Table 7.2 Major pentacyclic triterpeniod biomarkers used in the present study.

Sl No	Common Name	Structure
1	Germanicol	
2	β-Amyrin	
3	Friedelin	
4	Betulin	
5	Taraxerol	
6	Lupeol	

7.2 Results

7.2.1 Fatty alcohols in the surface sediments

A total of 18 fatty alcohols (C_{12}-C_{28}), including short chain, long chain and branched were identified from surface sediments of the Cochin estuary and the relative abundance of each fatty alcohol is furnished in Table 7.3. Six branched chain fatty alcohols were identified including 6, 10, 14-trimethylpenta-decan-2-ol, 3,7,11,15-tetramethyl-hexadecanol and phytol. Barmouth region is characterised by high abundance of phytol, short chain fatty alcohols including hexadecanol, tetradecanol and octadecanol. The long chain fatty alcohols were not identified in the barmouth region. Bolghatty region exhibited high abundance of hexadecanol, octacosanol, 13-methyl pentadecanol and tetracosanol. Unlike bar mouth region, this station showed presence of long chain fatty alcohols such as octacosanol and docosanol.

The inner part of the estuary exhibited the predominance of long chain fatty alcohols. This area is characterised by higher abundance of docosanol, hexacosanol and octacosanol. The abundance of short chain fatty alcohols decreased towards the inner part of the estuary. In all samples, there was an even over odd predominance in the n-alcohol chain length. However, in Fisheries harbour region, pentadecanol displayed high abundance. The distribution of n-alcohols is bimodal and characterised by even carbon predominance. In all the samples, n-C_{16} was the most dominant compound among short chain n-alcohols, while C_{22} and C_{28} was the most dominant in long chain alcohols.

Table 7.3 Percentage distribution of major fatty alcohols identified from the surface sediments of the Cochin estuary

Fatty Alcohol	S1	S2	S4	S5	S6	S7	S9	S10	S14
Dodecanol (C_{12})	2.61	3.75	0.43	3.54	1.78	0.90	0.68	-	0.54
Tetradecanol (C_{14})	5.91	7.63	0.44	2.49	3.33	2.56	3.03	-	1.75
Methyl Tetradecanol (Me-$C_{15:0}$)	4.12	11.57	1.10	4.52	3.67	2.86	2.09	-	3.15
Pentadecanol (C_{15})	2.02	5.02	0.00	25.23	1.11	0.79	0.73	-	0.70
6,10,14-trimethylpentadecan-2-ol	2.09	3.47	0.48	1.38	1.49	1.01	0.74	-	0.89
Hexadecanol (C_{16})	13.76	23.18	4.42	8.58	13.85	10.82	6.09	2.84	15.52
Heptadecanol (C_{17})	2.55	4.15	1.35	1.65	2.72	2.87	1.13	0.96	3.30
3,7,11,15-tetramethyl hexadecanol	-	-	1.89	-	1.70	1.93	0.99	1.29	2.35
Octadecanol (C_{18})	11.28	9.91	3.57	4.91	10.93	10.10	4.78	3.15	6.33
Phytol	38.23	-	6.30	1.15	8.93	15.18	3.15	27.30	2.59
18-methyl nonadecanol	5.57	3.57	4.35	3.47	6.53	5.60	3.75	2.84	2.24
Docosanol (C_{22})	8.86	5.50	21.28	9.48	23.01	15.36	22.85	9.77	13.85
Tricosanol (C_{23})	0.85	-	2.17	-	1.24	1.67	1.90	0.21	1.08
22-methyltricosanol	2.16	3.03	14.53	5.31	6.60	9.54	18.17	15.35	22.84
Pentacosanol (C_{25})	-	-	2.64	-	-	0.87	2.21	2.46	4.30
Hexacosanol (C_{26})	-	1.20	13.03	1.75	4.32	7.29	9.54	16.88	10.12
Heptacosanol (C_{27})	-	-	2.29	0.00	-	0.56	1.95	-	2.67
Octacosanol (C_{28})	-	18.03	19.71	26.52	8.79	10.11	16.23	16.94	5.76

7.2.2 Sterols and pentacyclic triterpenoids in the surface sediments

The identification of individual sterols is confirmed by co-injection with authentic standards and by the comparison of individual mass spectra with published MS data (Volkman, 1986; Duan, 2000). The presence and abundance of various sterols showed large variations in the study region. The major sterols identified in the surface sediments of Cochin estuary are furnished in Table 7.4.

Table 7.4 List of sterols identified in surface sediments

Name	S1	S2	S4	S5	S6	S7	S9	S10	S14
Cholesterol	+	+	-	+	+	+	-	+	+
Chloestanol	-	-	-	-	-	-	-	-	-
Brassicasterol	+	-	-	-	-	-	-	-	-
Stigmasterol	+	+	-	-	+	-	-	-	+
Stigmastanol	+	+	-	+	+	-	-	-	+
Sitosterol	+	+	-	+	-	-	+	-	+
Dinosterol	+	+	+	+	+	+	+	+	+

+ detected, -not detected

Cholesterol, cholestanol, brassicasterol, campesterol, stigmasterol, β-sitosterol, stigmastanol and dinosterol were identified in the bar mouth region. The dominance of C_{27} sterols is indicative of a zooplankton source, even though they are common in algae (Volkman, 1986; Lu and Zhai, 2006). The cholesterol contributed 29% of total sterols detected in the barmouth region. Brassicasterol, campesterol and dinosterol were the significant sterols in the barmouth region. Brassicasterol, dinostanol, cholestanol contributed 13%, 8% and 8% of total abundance respectively, while sitosterol contributed 13% of total abundance.

All estuarine stations except S4 and S10 showed the presence of cholesterol and cholestanol. Cholesterol/cholestanol ratio ranged from 0 to 4.58. Brassicasterol was detected in stations S1, S5, S6, S7 and S14. The major penatacyclic triterpenoids identified include: gernamicol, amyrin, betulin and friedelin. The entire estuarine area showed the presence of germanicol. The relative abundance of germanicol displayed an increasing pattern from barmouth region to the inner parts of the estuary. The chromatogram of pentacyclic triterpenoids are given in Appendix.

7.3 Discussion

Many previous studies pointed out the predominance of even number carbon chain between C_{12} and C_{28} as a characteristic feature of fatty alcohols in marine sediments (Sever and Parker, 1969; Volkman et al., 1981; Mudge and Norris, 1997). Short chain fatty alcohols predominantly originate from marine organisms including plankton and microalgae, but it is also reported that these compounds are derived from unspecified terrigenous sources (Mudge and Seguel, 1999; Seguel et al., 2001). Short chain fatty alcohols are also produced by freshwater organisms (Triegnier et al., 2006) and Volkman et al., 1999 identified zooplankton as major source of fatty alcohols in marine sediments.

Comparatively high abundance of short chain fatty alcohols in the barmouth region is attributed to the increased input of autogenous organic matter to the surface sediments. C_{12}, C_{14}, C_{16} and C_{18} fatty alcohols contributed 33.56% of total fatty alcohols in this region, while the long chain fatty alcohols remained below the detection level. The long chain fatty alcohols showed an increasing pattern from barmouth region to the inner part of the estuary and short chain fatty alcohols exhibited a decreasing pattern. Either C_{22} or C_{28} n-alcohols predominated in the estuarine sediments. The abundance of C_{22} and C_{24} n-alcohols may be attributed to aquatic macrophytes (Ficken et al., 1998). The predominance of long chain fatty alcohols indicates the input of terrestrial higher plants (Fukushima and Ishiwatari, 1984; Mudge and Norris, 1997; Gao et al., 2008). The relatively high abundance of allochthonous material in the inner part of the estuary is not only due the input of terrestrial organic matter but also due to the difference in reactivity of marine derived and terrestrial derived organic materials.

Odd chain and branched chain alcohols were detected in the surface sediments. Branched fatty alcohols are usually resulting from bacterial metabolism (Parkes, 1987; Mudge and Norris, 1997) of even chain length precursors. Therefore, the degree of bacterial metabolism in sediment samples can be assessed by using the ratio between the even chain precursor and odd carbon numbered methyl derivative of fatty alcohols. The presence of branched and odd chain fatty alcohols are signals of bacterial degradation processes acting in the surface sediments.

Phytol (3, 7, 11, 15-tetramethyl-2-hexadeca-1-ol) was identified in all the stations except at Bolgatty region. The relative abundance ranged from 0 to 38.23% in total fatty alcohols. The barmouth region (S1) and Poothotta region (S10) of the estuary showed high abundance of phytol. It derives from the side chain of Chl-*a* and is a typical marker of phytoplankton (Shi et al., 2001; Pearson et al., 2007). It can also arise from the hydrolysis of bacterio-chlorophyll *a* of purple sulphur bacteria (Marchand and Rontani, 2003). Previous studies reported that sedimentary bound phytol is derived from both terrestrial plants and phytoplankton (Chikaraishi and Naraoka, 2005), while free phytol in sediments is predominantly from phytoplankton inputs (Pearson et al., 2007).

3, 5, 17, 15 – hexadeca-1-ol (dihydroxyphytol) was also identified in surface sediments. Dihydroxyphytol is previously reported in faecal pellets of copipods (Prahl et al., 1984a,b) and relative percentage of dihydroxyphytol to phytol can be used to estimate the input of faecal zooplanktonic material in aquatic systems (Christodoulou et al., 2009). The dihydroxyphytol was identified only in the inner part of the estuary suggests the zooplankton grazing in the inner estuary. The isoprenoid 6, 10, 14 – trimethyl pentadecan-2-one (phytone) is an important component in many sites of the estuary and showed maximum abundance in the Bolgatty region. Phytone is the early oxidation product of phytol and the distribution of phytone can be used as an indicator of bacterial activity (Brooks and Maxwell, 1974; Rontani and Acquaviva, 1993; Marchand and Rontani, 2003; Pearson et al., 2007). Isoprenoid ketones may be produced in several processes including bacterial degradation and photosensitised oxidation of free phytol, photodegradation of chlorophyll phytyl side chains and photosensitised oxidation of some isoprenoid hydrocarbons such as pristine and phytane (Rontani et al., 1992).

Cholesterol was identified in most of the estuarine region during the present study. Cholesterol has multiple sources in aquatic systems. Even though cholesterol is the main animal sterol (Puglisi et al., 2003) it is also produced by diatoms, microbial community, macrophytes, phytoplankton and zooplankton (Reeves and Patton, 2001; Azevedo, 2003). The barmouth region showed maximum cholesterol concentration. C_{27} sterols, cholesterol and dehydrocholesterol have been used to indicate inputs of zooplankton grazing (Gagosian et al., 1981) and they have been also reported as algal/phytoplankton marker (Volkman et al., 1998). The relative high abundance of cholesterol in the barmouth region suggests an important contribution of zooplankton or other marine fauna which are well known to contain high abundances of this compound, although phytoplankton contribution cannot be excluded. The C_{28} sterol, 24-methyl cholesta-5,2-dien-3β-ol (brassicasterol) is considered as diatom marker (Brassell et al, 1982). Even though brassicasterol was detected in the inner part of the estuary (Fisheries harbour region and Shipyard region) the maximum abundance was observed in the barmouth region which indicated significant input of diatom derived organic matter in the barmouth region of the estuary. This observation is consistent with the reported result of chemotaxonomic analysis of sediments using pigment markers which identified the abundance of fucoxanthin in the barmouth region (Aneeshkumar and Sujatha, 2012).

Another C_{28} sterol detected in sediments was campesterol, which is used as a terrestrial biomarker, but they are also biosynthesised by diatoms (Xu et al., 2006). Campesterol has already been reported in mangrove sediments of Cochin (Narayanan, 2006; Kumar, 2011) and northern part of Cochin estuary (Kumar, 2011). Campesterol was detected around the Cochin harbour region and remained below detectable limit towards the Thaneermukkom bund. Previous studies showed that mangroves contain significant amount of campesterol. Maximum campesterol content was reported in mangrove species *Acanthus ilicifolius* (15.5%), while minimum concentration in *Avicennia marina* (0.9%) (Sunilkumar and Antony, 1994; Subramanian, 2000; Kumar, 2011). The abundance of campesterol in the harbour area is attributed to the close proximity of two mangrove ecosystems Murikumpadam and Mangalavanam with this area. One of the dominant mangrove species in these ecosystems includes *Acanthis ilicifolius*. Hence, the abundance of campesterol may represent a signal of mangrove derived organic matter in surface sediments of Cochin estuary.

Terrestrial organic matter can be recognised by the presence of C_{29} sterols, which are the predominant sterols produced by vascular land plants. The presence of stigmasterol detected in most of the sites. Sitosterol was detected in the barmouth region as well as in the inner part of the estuary. Sitosterol has been used as a marker of allochthonous materials in estuarine environment (Laureillard and Saliot, 1993; Mudge and Norris, 1997) due to its high abundance in land plants. In the present study, β-sitosterol did not follow the expected trend to trace the terrestrial materials with decreasing relative abundance from Thaneermukkam bund to the barmouth region. Sitosterol was found to be more concentrated in the bund region as well as in the barmouth region. A comparison of sitosterol with other lipid biomarker compounds such as n-alkanes and fatty acids, strongly suggests that the sitosterol might be a mixture of terrestrial as well as marine origin (Sangiorgi et al., 2005).

The predominance of sitosterol is reported in many microalgae including some diatom species (Volkman, 1986; Barrett et al., 1995). Hence, the high abundance of sitosterol in the barmouth region indicated the autochthonous and allochthonous additions of sitosterol into the sediment, making complexities while using it as a molecular biomarker.

As compared to the above mentioned sterols, dinosterol is considered more taxonomically specific molecular biomarker because of its predominance in dinoflagellates (Volkman et al., 1993; Mansour et al, 1999; Leblond and Chapman, 2004). The wide spread abundance of dinosterol in surface sediments of the Cochin estuary, underlines the importance of dinoflagellates of the organic matter pool in the surface sediments.

The dinosterol has been reported as an abundant sterol component in dinoflagellate species *Prorocentrum micans*, *Lingneodinium polydrum*, *Gymnodinium* and *Alexandrium tamarense* (Sangiorgi et al, 2005). The abundance of *Prorocentrium* and *Gymnodinium* was previously reported in Cochin estuary by many authors in Cochin estuary (Gopinathan et al., 1974; Martin et al., 2012). Even though dinoflagellates have been reported as the major source of dinosterol, this sterol has also been identified in certain diatom species (Budge and Parrish, 1998; Hudson et al., 2001). A recent study identified 43 species of diatoms and 40 species of dinoflagellates in the Cochin estuary (Aneeshkumar and Sujatha, 2012). Analysis of chlorophyll pigment markers identified the presence of peridinin which indicated the abundance of dinoflagellates in the Cochin estuary (Aneeshkumar and Sujatha, 2012).

The pentacyclic triterpenoids is dominated by germanicol and this biomarker compound reported in organic matter derived from mangrove *Rizhophora mangle* (Koch et al., 2003). The presence of organic matter derived from angiosperm to the total organic matter was identified by the presence of lupeol. Betulin was detected in most of the stations which indicated the presence of mangrove derived organic matter in the sediments of the study area. The triterpenoid biomarker friedoleanane was identified in estuarine sediments and this compound was reported in fluvial and lacustrine sediments whose origin is attributed to the bark and leaves of numerous angiosperms (Jaffe et al., 1996; Otto and Simoneit, 2001; Kumar, 2011).

7.4 Biomarker proxies for the Cochin Estuary

Every estuary is unique in terms of their morphological features, climatic settings, tidal incursion and chemical processes. The development of a set of biomarker proxies which suits better for a particular estuary is important in order to acquire better knowledge regarding the organic matter cycling in that estuary.

Several biomolecules are frequently used as biomarkers which are indicators of the origin of organic matter in sediments in order to derive information about the carbon cycling in estuaries. The underlying goal driving quantitative biomarker analysis is the determination of the quantity of a given compound originating from one source compared to another, with the aim of deriving information on past or present environmental conditions and/or processes (Panetta and Gelinas, 2009). When using molecular biomarkers in complex estuaries like Cochin estuary, the different transport processes, which are dominated by river discharge, vertical and lateral transport through the water column, the transport from adjacent mangrove ecosystems, urban and industrial inputs have to be considered. The study focuses on three major inputs which include: algal, terrestrial and bacterial derived organic matter.

The bulk parameter approach revealed the dominance of terrestrial derived organic matter in surface sediments. The fraction of terrestrial organic matter in the total organic matter pool ranges from 13 to 74% in the surface sediments as estimated by $\delta^{13}C$ based two end member mixing model. The $\delta^{15}N$ exhibited complex spatial and seasonal distributions in the study area. It was found that the dynamic cycling of nitrogen through various biogeochemical and organic matter degradation processes modifies the OC/TN ratios and $\delta^{15}N$ to a considerable degree. The relative contribution of terrestrial derived organic matter in surface sediments displayed a gradual increase from inner part of the estuary to the seaward side which suggest an increase in contribution of marine autogenous organic matter towards the seaward side. Biomarker analysis provides an added dimension to the understanding of the carbon cycle in estuarine systems, in many cases highlighting important details not revealed by bulk analyses alone.

The short chain homologous of n-alkanes, n-alcohols and alkanoic acids are associated with the aquatic flora. These compounds can be used to infer dynamics of algal derived organic matter in estuarine systems. It was observed that the results of n-alkanes and n-alcohols in the present study are consistent with the aforementioned general feature. Conversely, the C_{16} n-alkanoic acid showed very high concentrations than higher chain n-alkanoic acids which may be due to the influx of sewage derived organic matter in estuarine sediments. Hence the use of short chain fatty acids is inadequate to track the algal derived organic matter in this estuary. The very low abundance of PUFA makes more complexities in the source characterisation process by alkanoic acid biomarker tool. The distribution of short chain n-alkanes and the use of terrestrial to aquatic ratio is found to be the better biomarker proxy in this estuary. The distribution of phytol also can be a relevant tool for the same.

PUFAs are less resistant to diagenetic degradation than monounsaturated and saturated fatty acids (Farrington et al., 1988; Wakeham and Canuel, 1990). PUFAs were present in very low concentrations in the samples analysed which was indicative of a strongly degraded material in surface sediments which in turn provide primary indication about the bacterial mineralisation process in sediments. Further for delineating the distributional dynamics of bacterial derived organic matter, branched and odd carbon numbered fatty acids were found to be a reliable proxy in the Cochin estuary. Comparatively high concentrations of branched and odd chain concentration in sediments can be used as an effective tool for the assessment of bacterial derived organic matter. The inference about bacterial input, which can be extracted from short chain n-alkanes, is more or less masked by the overlapping of source input through petroleum spillage.

Homologues series of long-chain n-alkanes, n-alkanols and fatty acids are typical terrigenous lipids found in marine sediments (Poynter et al., 1989; Bird et al., 1995; Kuypers et al., 1999; Huang et al., 2000). These compounds are abundant constituents of terrestrial higher plant epicuticular waxes (Eglinton and Hamilton, 1963), occurring as protective coating on leaves and stems. Plant waxes can be washed off the leaf surface by rain and transported into the ocean by freshwater runoff (Bird et al., 1995). From the result of the present study, it was found that all the aforementioned biomarker compounds can provide effective source information in the study area. The triterpenoid biomarker germanicol displayed widespread abundance in the study region and this biomarker can be employed to get an account of the terrestrial derived organic matter in the estuarine sediments.

7.5 Conclusions

The significant abundance of the straight chain and branched chain fatty alcohols emphasises their potential applicability as molecular markers. The sets of sterols identified in the present study include cholesterol, cholestanol, brassicasterol, campesterol, stigmasterol, stigmastanol, sitosterol and dinosetrol. The abundance of terrestrial derived sterols underlines the prevailing importance of terrestrial end member in the sediment organic pool and their preferential preservation in the estuarine salinity gradient. The relatively high abundance of dinosterol suggested that this compound can be effectively employed to assess the dianflagellate derived organic matter in surface sediments.

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Chapter 8

SUMMARY AND CONCLUSIONS

Estuaries are connected to both terrestrial and marine environments, receiving substantial subsidies of nonliving and living material from both sources. Sedimentary organic matter in estuaries is a mixture of terrestrial and marine components, consisting of phytoplanktonic cells, organic debris, and eroded material from soils and sediments. Estuarine sediments represent a ‘‘recorder’’ of water column processes and are the final storage sites for the accumulation of autochthonous and allochthonous organic matter inputs. Chemical tracers (stable isotopes and molecular biomarkers) have been applied to identify the source, transport and fate of organic matter in estuarine systems.

The Cochin estuary is the second largest wetland system in India and is unique in terms of physiography, geology, climate, hydrology, land use and flora and fauna. There is a conspicuous lack of information about the concentrations, source and fate organic compounds in this estuary. In order to understand the relative importance of biogeochemical processes, it is necessary to characterise and quantify organic matter and to identify the relative contribution of different end members in the sedimentary organic matter pool.

The main objective of the study was to identify the major biogeochemical pathways operating in the estuary and the sources of organic matter was used as a tool to identify various biogeochemical processes. Bulk elemental and isotopic ratios were used as initial proxies, while molecular biomarker approach was used for the detailed investigation of sedimentary organic matter. The major labile biochemical organic compound classes were estimated to assess the quality and diagenetic state of organic matter in the Cochin estuary. The geochemical characteristics of the estuarine sediments were investigated using P fractionation and trace metal analysis.

P fractionation in sediments of the Cochin estuary gives a mixed or metastable diagenetic character with strong seasonal signatures. The surface sediments in the entire estuarine region remain oxic during the monsoon season and gradually become reducing during the post monsoon season which in turn shifts to strong reducing conditions during the pre monsoon season. The PCA results generated by considering various P fractions and trace metals supports the presence of dynamic interchange of oxidising and reducing character of the surface sediments.

The labile portion within the total organic matter pool was quantified in terms of biochemical components and benthic trophic status was identified using various biochemical descriptors. The results of organic carbon content and biochemical components in the surface sediments of Cochin estuary showed comparable concentrations to those reported in highly industrialised and urbanised eutrophic estuaries. Biopolymeric carbon chains were dominated by carbohydrates, followed by proteins and lipids, pointing a nitrogen limitation for heterotrophic metabolism. The input of organic matter from rivers coupled with the discharge of organic wastes from different industrial, agricultural and aquaculture sectors leads to a large scale accumulation of organic matter in the estuarine sediments.

The predominance of carbohydrates over sedimentary protein indicates faster mineralisation of proteinaceous organic matter in surface sediments and estuary behaves as a detrital trap for the accumulation of aged organic matter. Chl-*a* displayed significant seasonal pattern. Low Chl-*a* concentrations recorded during the monsoon, when compared to other seasons, were due to higher water column turbidity, heavy cloud cover and high rainfall events that limit the light availability for primary production. The threshold levels of PRT, BPC and algal contribution to BPC suggests a hypertrophic condition in and around Cochin harbour region and a eutrophic condition towards the inner part of the estuary.

OC, TN, OC/TN ratios and $\delta^{13}C$ in surface sediments of Cochin estuary showed distinct spatial variations, while $\delta^{15}N$ did not display any spatial variations in the study region. OC and TN concentrations strongly depend on the granulometric composition of surface sediments in the study region. The $\delta^{13}C$ values ranged from -27.5 to -21.7‰ in surface sediments and a gradient was observed towards the seaward side. The $\delta^{15}N$ values were found to be more complex in surface sediments and ranged from 3.1 to 6.7‰. The fraction of terrestrial organic matter in total organic matter pool is estimated by $\delta^{13}C$ based two end member mixing model and the relative contribution of terrestrial derived organic matter ranged from 13 to 74% in surface sediments. The distribution features of terrestrial organic matter exhibited significant spatial differences with higher contribution in the inner part of the estuary.

The application of biochemical descriptors and bulk indices like elemental composition and isotopic ratios are useful techniques for the assessment of total quality, benthic trophic status and relative contribution of marine and terrestrial derived organic matter in surface sediments. Due to the complex nature and differential reactivity of organic molecules bulk indices such as element stoichiometry and stable isotopic ratios are not completely successful in revealing the sources of organic matter. The molecular biomarker approach can be effectively used as a tool to characterise the sources of organic matter in estuarine sediments. Furthermore, the application of multiple molecular biomarkers (multi-proxy approach) can be a handy tool to obtain a holistic picture of different organic matter sources in estuarine systems.

The strong odd to even carbon preference of the high HMW n-alkanes in surface sediments with C_{max} at C_{31} and high abundance of even chain fatty alcohols revealed higher vascular plant derived organic matter in surface sediments. The higher abundance of HMW n-alkanes with higher TAR and ACL values as well as the PCA results reveals the preferential utilisation of marine derived organic matter in biochemical degradation pathway and various geochemical processes contribute to the accumulation of terrigenous sedimentary n-alkanes in the estuary. Biochemical degradation processes could be responsible for the unusual presence of even carbon predominance in the LMW homologues. The biochemical degradation pathway operating in surface sediments cause substantial alterations to the planktonic organic matter in surface sediments and the hydrodynamic depositional forcing plays a dominant role not only on the selective delivery of fluvial terrigenous organic constitutes, but also on the preferential preservation of terrigenous organic matter versus the marine derived organic matter in estuarine sediments.

The fatty acid composition in surface sediments of the Cochin estuary show that fatty acids mainly derived from phytoplankton, bacteria and terrestrial organic matter. The distribution of total fatty acids and bacterial fatty acids are similar, suggesting that bacteria may be a significant source of fatty acids of these sediments. The highest bacterial fatty acids concentrations were observed in Sulphur Jetty and Shipyard (S4 and S5) region of the estuary may due to the sewage discharge. The PUFA levels are comparatively low in the surface sediments, suggesting that most of the labile fatty acids were effectively recycled during the settling and deposition processes in the estuary.

The significant abundance of the straight chain and branched chain fatty alcohols emphasises their potential applicability as molecular markers. The sets of sterols identified in the present study include cholesterol, cholestanol, brassicasterol, campesterol, stigmasterol, stigmastanol, sitosterol and dinosetrol. The abundance of terrestrial derived sterols underlines the prevailing importance of terrestrial end member in the sediment organic pool and their preferential preservation in the estuarine salinity gradient. The relatively high abundance of dinosterol suggested the important contribution of dinoflagellates to the sediment organic matter pool.

The bulk geochemical and lipid biomarkers proxies suggest the overwhelming terrestrial contribution of organic matter in inner parts of the estuary with gradual decrease towards the barmouth region. Even though the autochthonous derived organic matter is a minor contributor, it plays an important role in regulating the biochemical processes in estuary. The n-alkanes can be effective for the determination algal and terrestrial derived organic matter, while fatty acid biomarkers stand effective for the determination of bacterial derived organic matter. The higher proportion of saturated fatty acids and branched fatty acids compared to poly and mono unsaturated counterparts reflect the preferential degradation of the unsaturated components and active microbial reworking in the sediment-water interface.

Thus the application of multiple molecular biomarkers could be used effectively to characterise multiple organic matter sources in estuaries and their biogeochemical transformations. The information extracted from of molecular biomarkers can be applied to identify the environmental changes occurred in the estuary and these information has valid significance while formulating management decisions. The understanding of geochemical response to the changes in environmental conditions in recently deposited sediments is also essential for the realistic interpretation of geochemical proxies derived from core sediments in paleoenvironmental studies.

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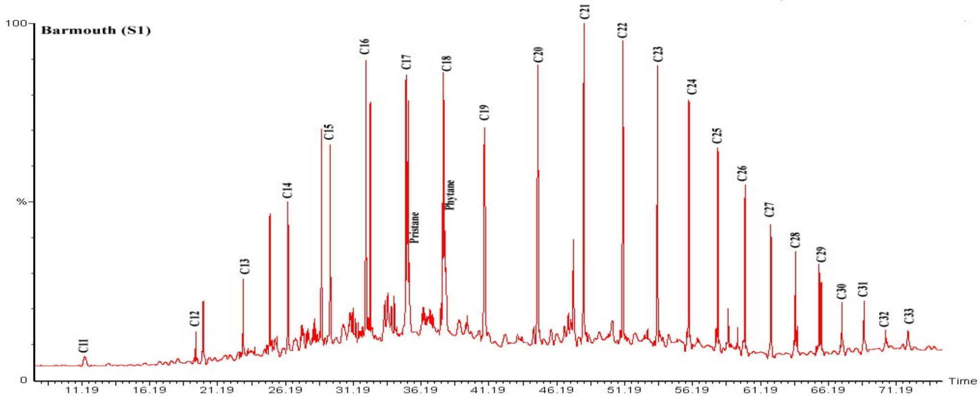
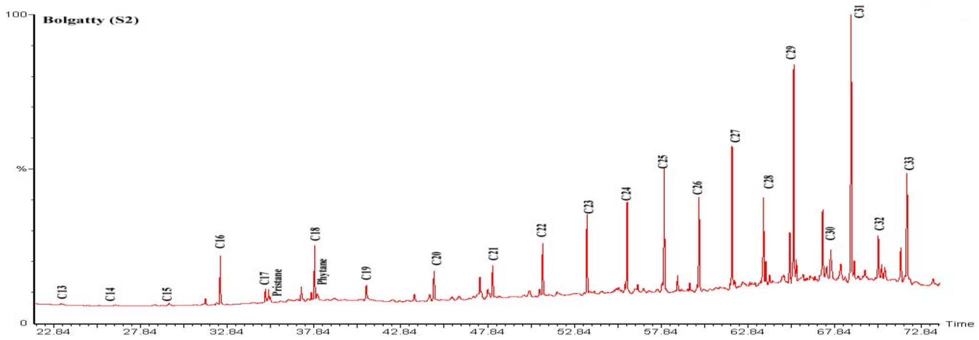
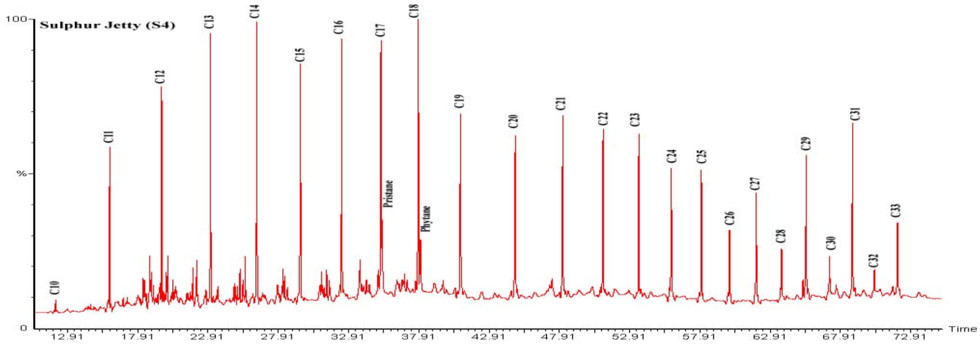
APPENDIX

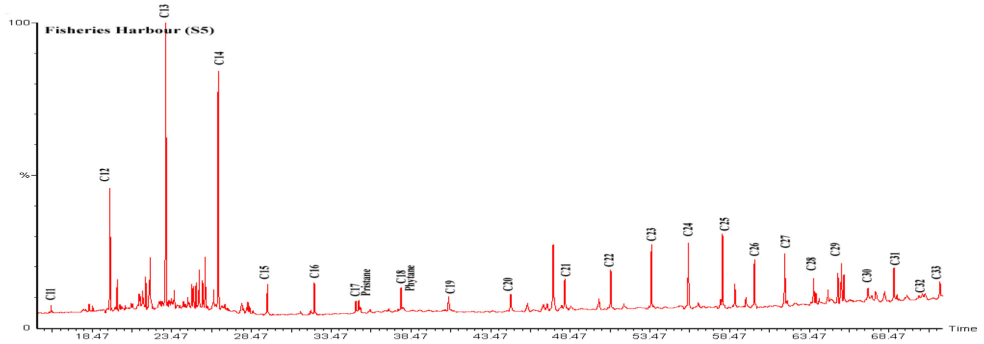
Appendix -1

Total Ion Chromatogram of Hydrocarbon fraction in surface sediments of the Cochin estuary








Fisheries Harbour (S5)
100
%
0
C11
C12
C13
C14
C15
C16
C17
Pristane
C18
Phytane
C19
C20
C21
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C23
C24
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C26
C27
C28
C29
C30
C31
C32
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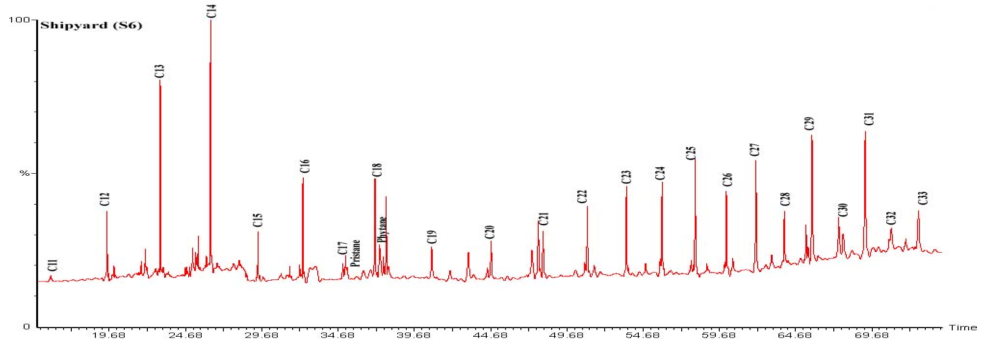
58.47
63.47
68.47
Time


Shipyard (S6)
100
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C11
C12
C13
C14
C15
C16
C17
Pristane
C18
Phytane
C19
C20
C21
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C23
C24
C25
C26
C27
C28
C29
C30
C31
C32
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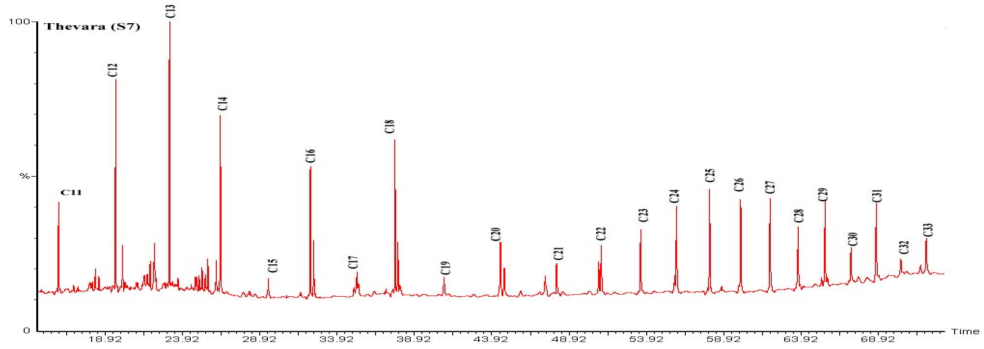
64.68
69.68
Time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C29
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C31
C32
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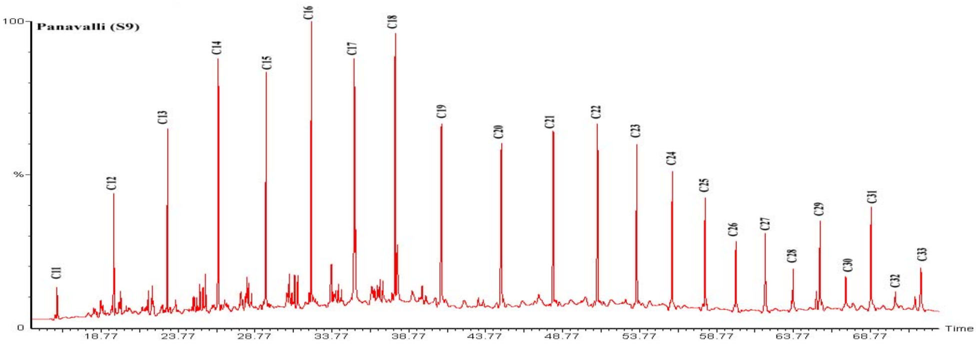
Time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C22
C23
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C26
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C29
C30
C31
C32
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63.77
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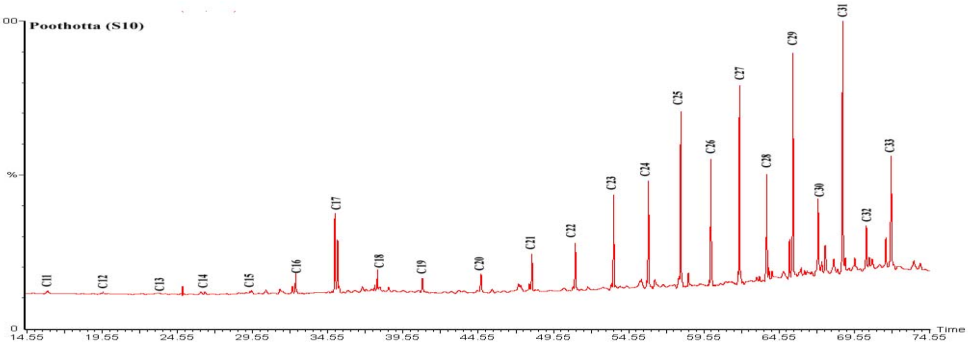
Time


Poothotta (S10)
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C30
C31
C32
C33
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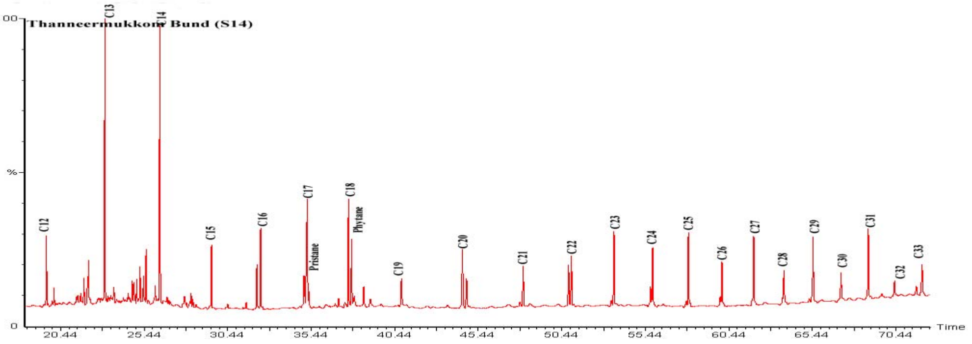
69.55
74.55
Time


Thanneermukkom Bund (S14)
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C32
C33
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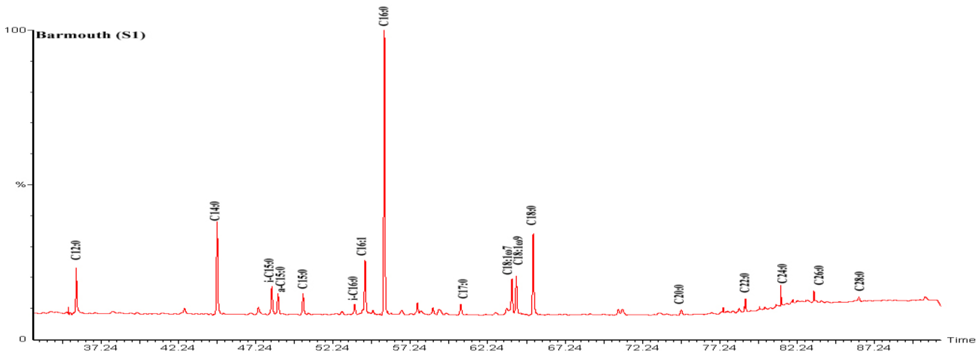
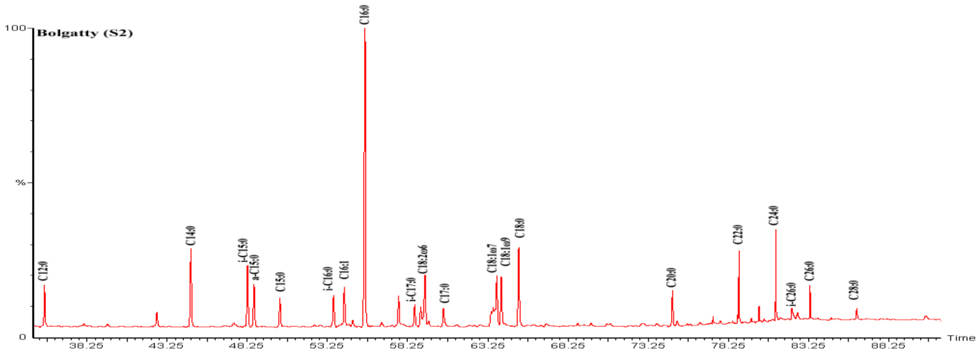
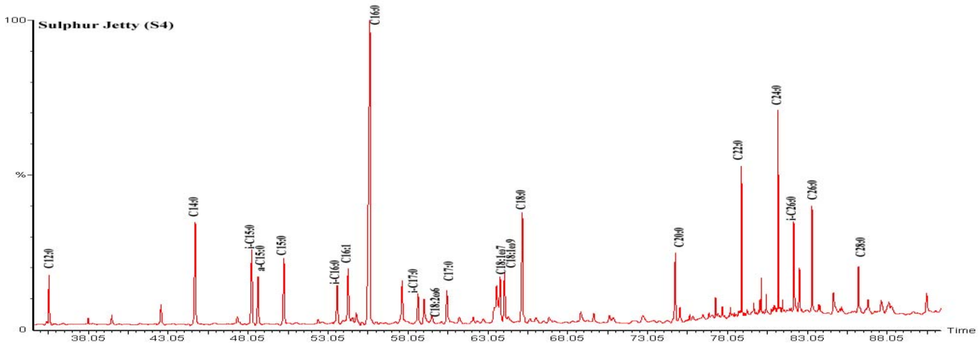
45.44
50.44
55.44
60.44
65.44
70.44
Time

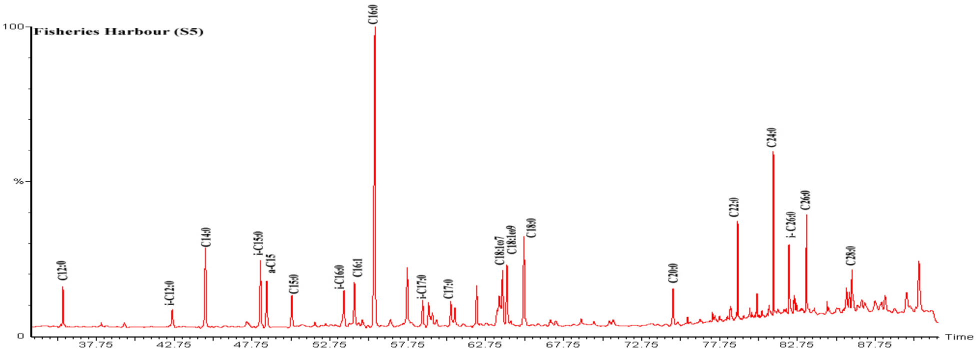
Appendix -2

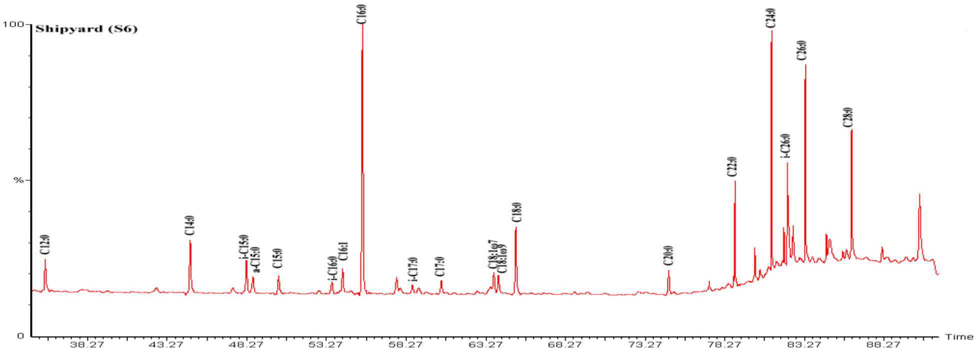
Total Ion Chromatogram of fatty acid fraction in surface sediments of the Cochin estuary

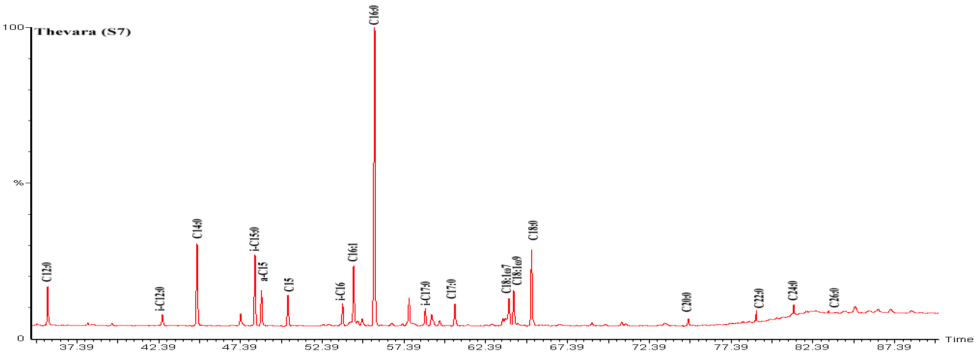


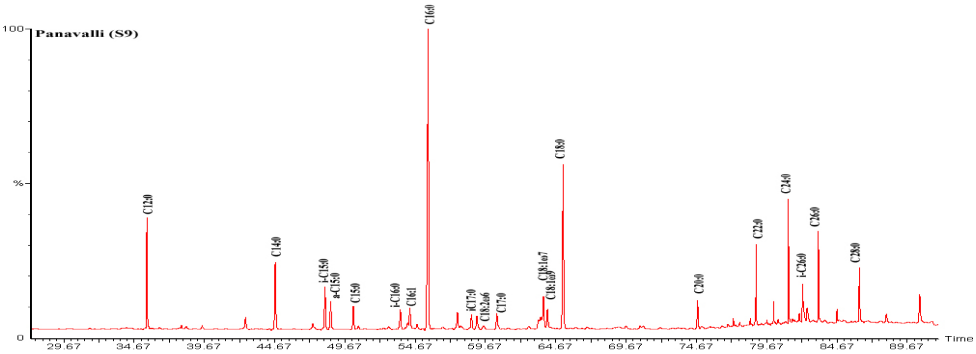


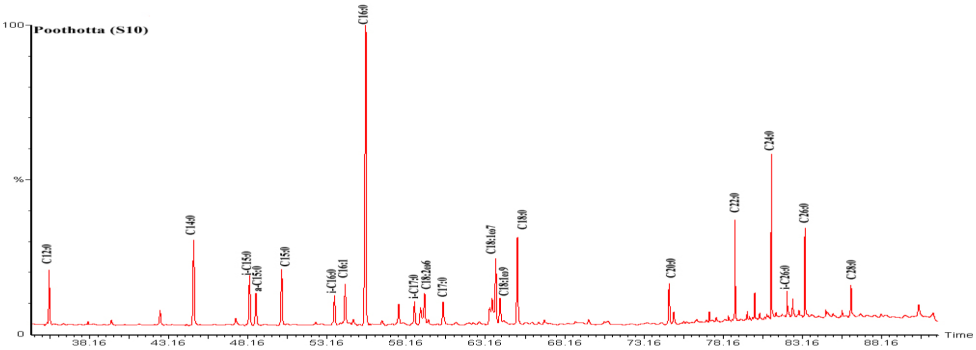


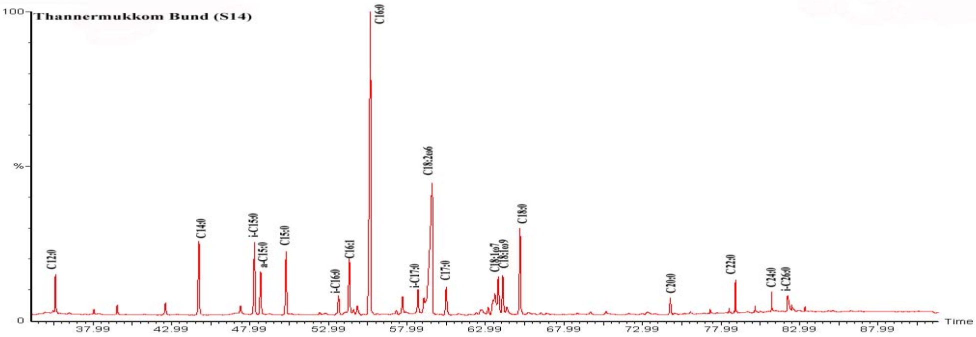

100
%
0
Fisheries Harbour (S5)
C12:0
i-C12:0
C14:0
i-C15:0
a-C15
C15:0
i-C16:0
C16:1
C16:0
i-C17:0
C17:0
C18:1ω7
C18:1ω9
C18:0
C20:0
C22:0
C24:0
i-C26:0
C26:0
C28:0
37.75
42.75
47.75
52.75
57.75
62.75
67.75
72.75
77.75
82.75
87.75
Time


100
%
0
Shipyard (S6)
C12:0
C14:0
i-C15:0
a-C15:0
C15:0
i-C16:0
C16:1
C16:0
i-C17:0
C17:0
C18:1ω7
C18:1ω9
C18:0
C20:0
C22:0
C24:0
i-C26:0
C26:0
C28:0
38.27
43.27
48.27
53.27
58.27
63.27
68.27
73.27
78.27
83.27
88.27
Time


100
%
0
Thevara (S7)
C12:0
i-C12:0
C14:0
i-C15:0
a-C15
C15
i-C16
C16:1
C16:0
i-C17:0
C17:0
C18:1ω7
C18:1ω9
C18:0
C20:0
C22:0
C24:0
C26:0
37.39
42.39
47.39
52.39
57.39
62.39
67.39
72.39
77.39
82.39
87.39
Time

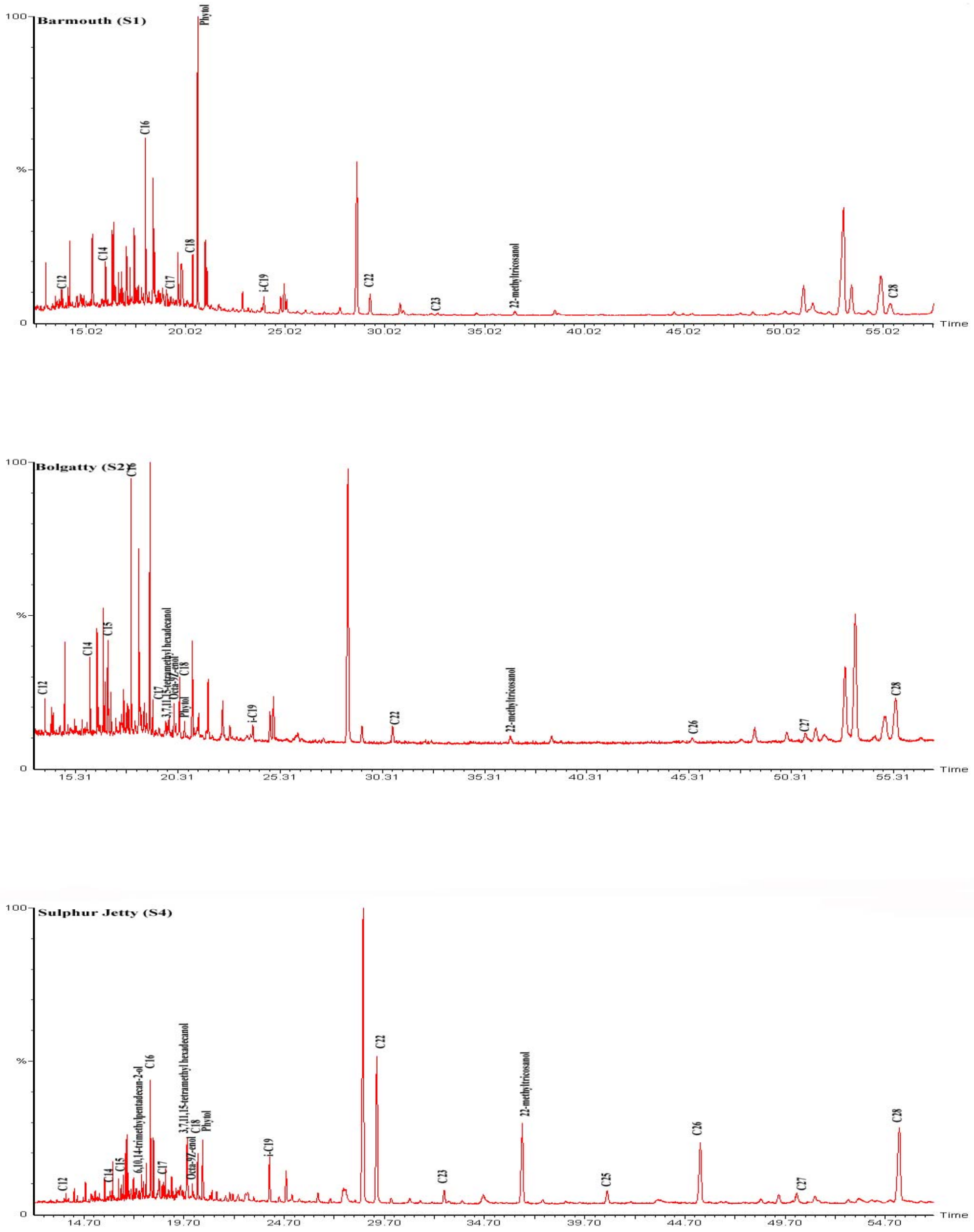

Panavalli (S9)
100
%
0
C12:0
C14:0
i-C15:0
a-C15:0
C15:0
i-C16:0
C16:1
C16:0
iC17:0
C18:2ω6
C17:0
C18:1ω7
C18:1ω9
C18:0
C20:0
C22:0
C24:0
i-C26:0
C26:0
C28:0
29.67
34.67
39.67
44.67
49.67
54.67
59.67
64.67
69.67
74.67
79.67
84.67
89.67
Time

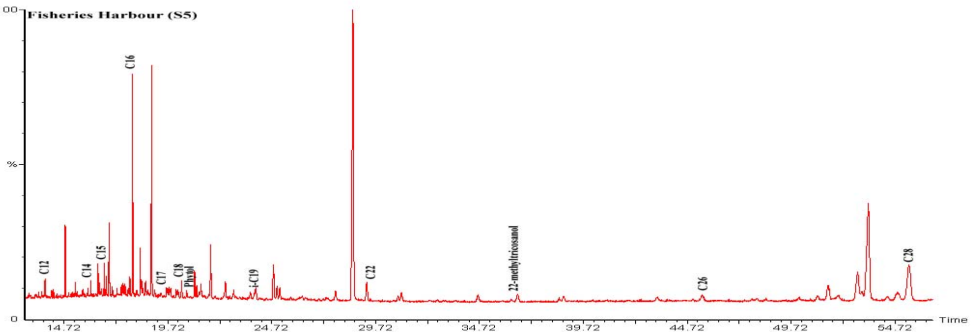

Poothotta (S10)
100
%
0
C12:0
C14:0
i-C15:0
a-C15:0
C15:0
i-C16:0
C16:1
C16:0
i-C17:0
C18:2ω6
C17:0
C18:1ω7
C18:1ω9
C18:0
C20:0
C22:0
C24:0
i-C26:0
C26:0
C28:0
38.16
43.16
48.16
53.16
58.16
63.16
68.16
73.16
78.16
83.16
88.16
Time

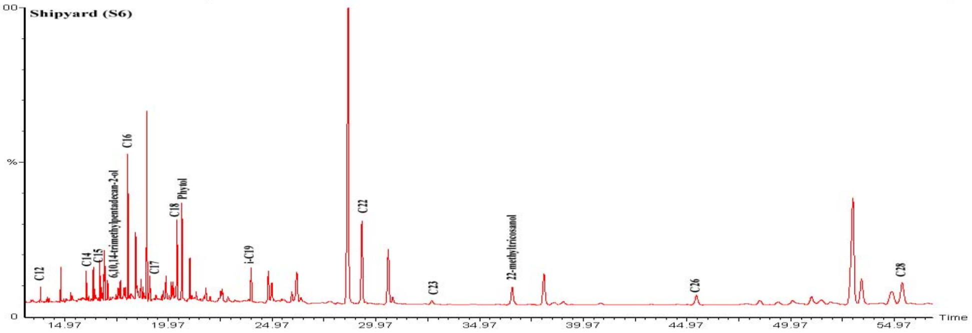

Thannermukkom Bund (S14)
100
%
0
C12:0
C14:0
i-C15:0
a-C15:0
C15:0
i-C16:0
C16:1
C16:0
i-C17:0
C18:2ω6
C17:0
C18:1ω7
C18:1ω9
C18:0
C20:0
C22:0
C24:0
i-C26:0
37.99
42.99
47.99
52.99
57.99
62.99
67.99
72.99
77.99
82.99
87.99
Time

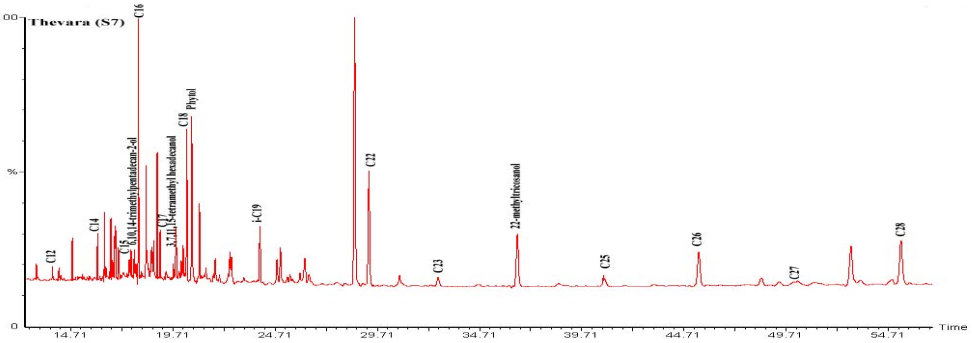
Appendix -3a

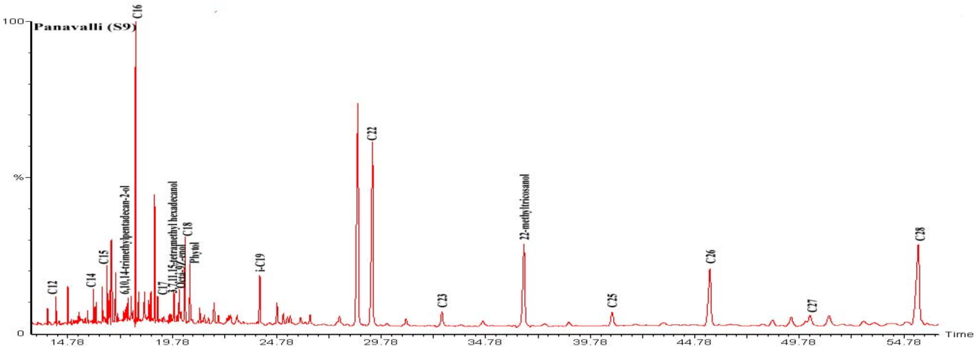
Total Ion Chromatogram of fatty alcohols in surface sediments of the Cochin estuary

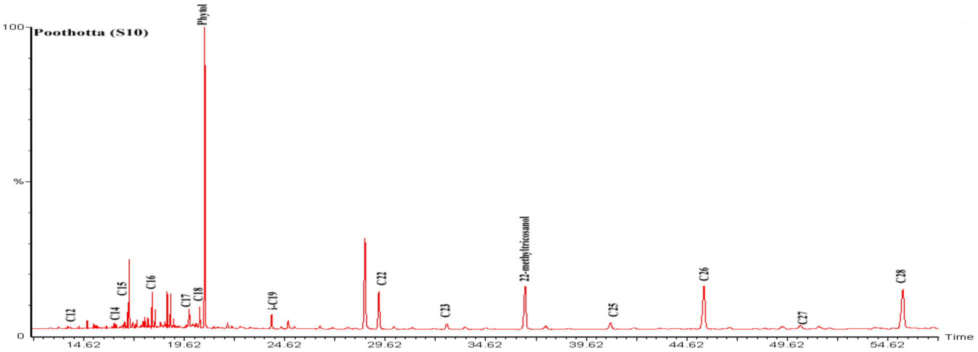


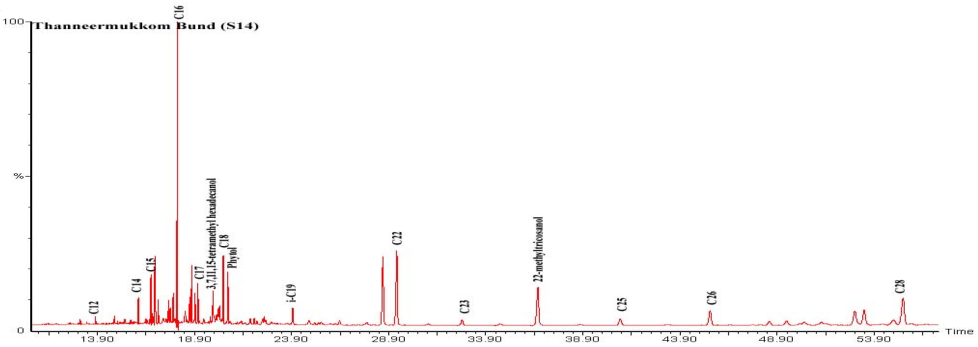

Fisheries Harbour (S5)
100
%
0
C12
C14
C15
C16
C17
C18
Phytol
i-C19
C22
22-methyltricosanol
C26
C28
14.72
19.72
24.72
29.72
34.72
39.72
44.72
49.72
54.72
Time


Shipyard (S6)
100
%
0
C12
C14
C15
6,10,14-trimethylpentadecan-2-ol
C16
C17
C18
Phytol
i-C19
C22
C23
22-methyltricosanol
C26
C28
14.97
19.97
24.97
29.97
34.97
39.97
44.97
49.97
54.97
Time


Thevara (S7)
100
%
0
C12
C14
C15
6,10,14-trimethylpentadecan-2-ol
C16
C17
3,7,11,15-tetramethyl hexadecanol
C18
Phytol
i-C19
C22
C23
22-methyltricosanol
C25
C26
C27
C28
14.71
19.71
24.71
29.71
34.71
39.71
44.71
49.71
54.71
Time

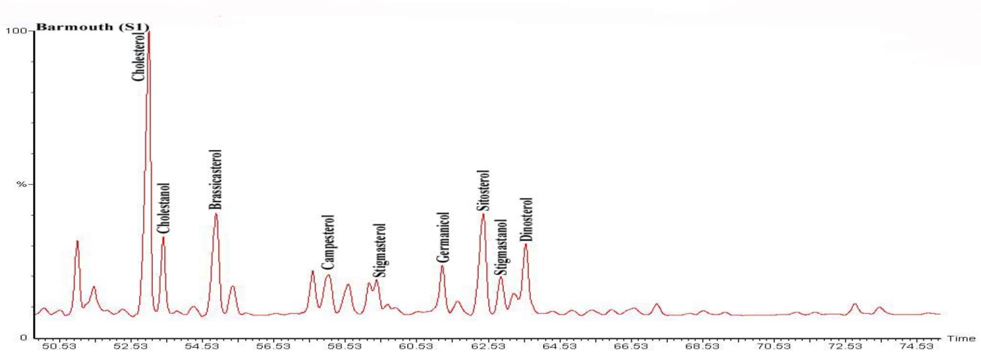

Panavalli (S9)
100
%
0
C12
C14
C15
6,10,14-trimethylpentadecan-2-ol
C16
C17
3,7,11,15-tetramethyl hexadecanol
Octa-9Z-enol
C18
Phytol
i-C19
C22
C23
22-methyltricosanol
C25
C26
C27
C28
14.78
19.78
24.78
29.78
34.78
39.78
44.78
49.78
54.78
Time

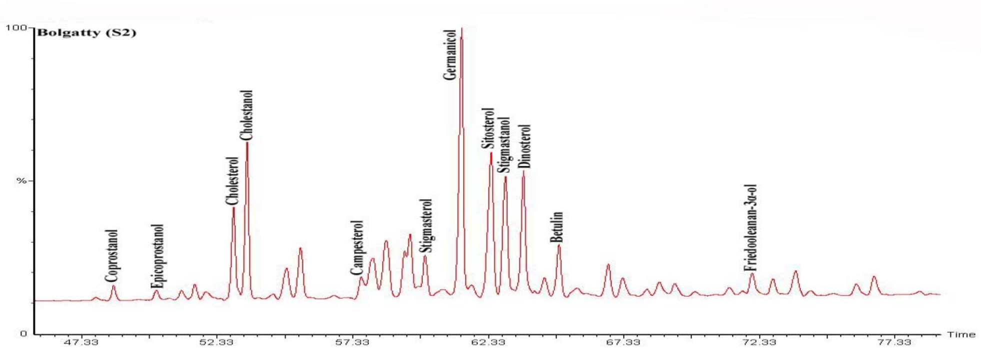

Poothotta (S10)
100
%
0
C12
C14
C15
C16
C17
C18
Phytol
i-C19
C22
C23
22-methyltricosanol
C25
C26
C27
C28
14.62
19.62
24.62
29.62
34.62
39.62
44.62
49.62
54.62
Time

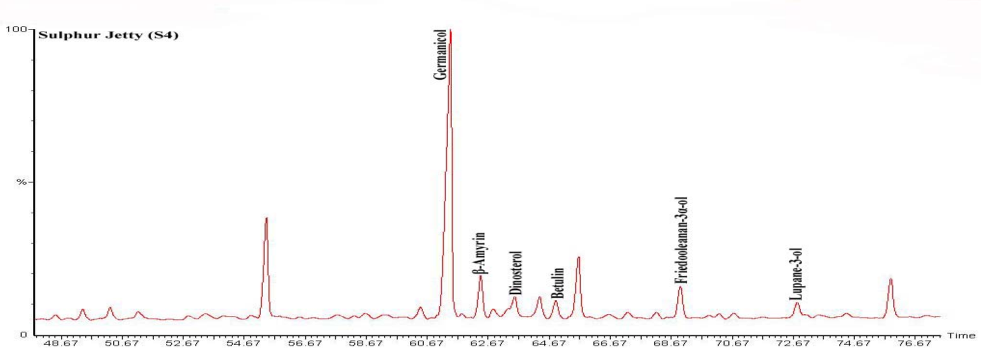

Thanneermukkom Bund (S14)
100
%
0
C12
C14
C15
C16
C17
3,7,11,15-tetramethyl hexadecanol
C18
Phytol
i-C19
C22
C23
22-methyltricosanol
C25
C26
C28
13.90
18.90
23.90
28.90
33.90
38.90
43.90
48.90
53.90
Time

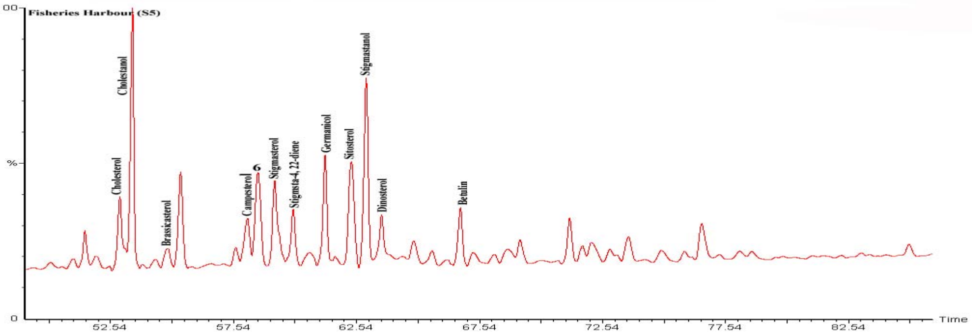
Appendix -3b

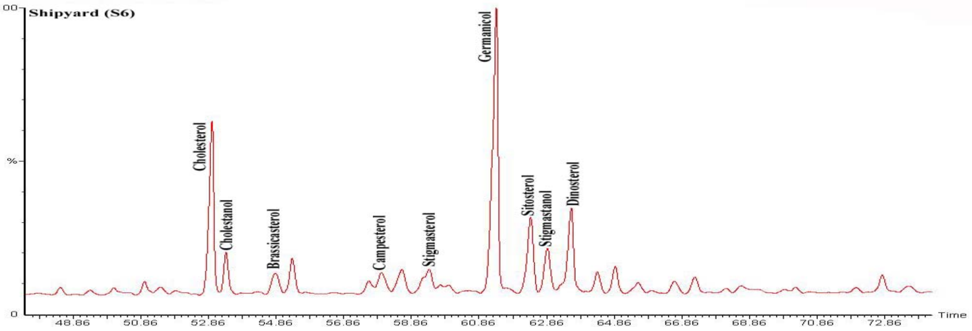
Total Ion Chromatogram of Sterols and pentacyclic triterpenoids in surface sediments

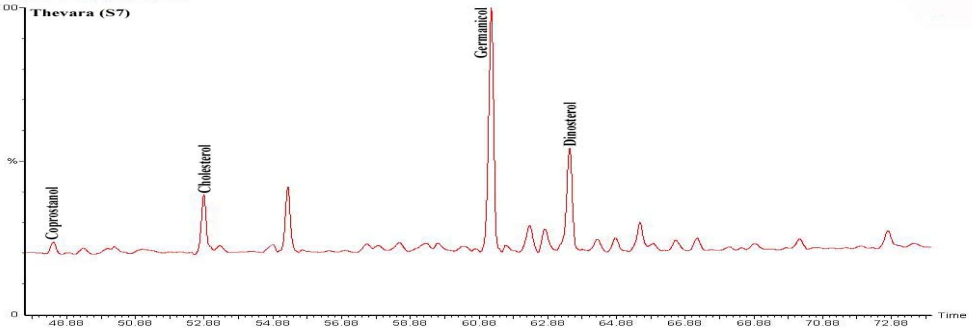


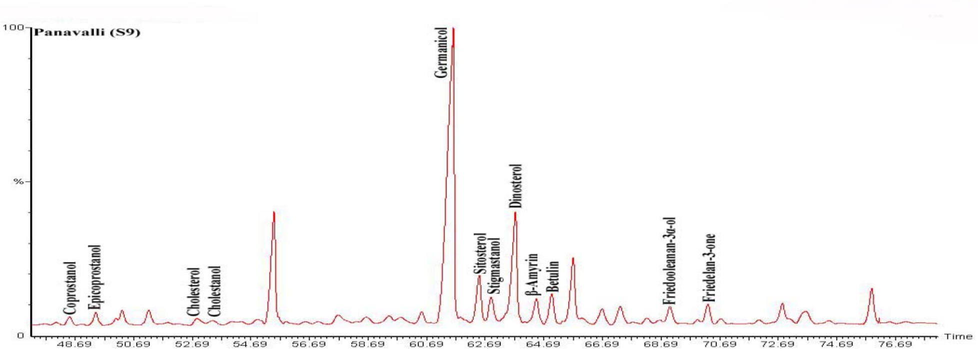


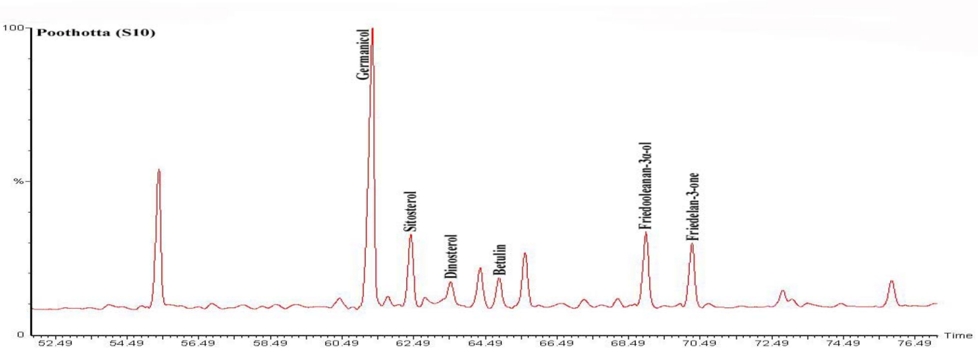


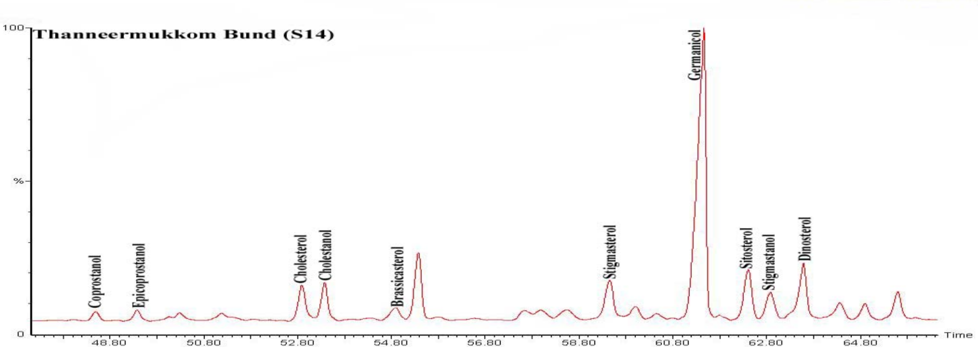

100
Fisheries Harbour (S5)
%
0
Cholesterol
Cholestanol
Brassicasterol
Campesterol
6
Stigmasterol
Stignsta-4, 22-diene
Germanicol
Sitosterol
Stigmastanol
Dinosterol
Betulin
52.54
57.54
62.54
67.54
72.54
77.54
82.54
Time


100
Shipyard (S6)
%
0
Cholesterol
Cholestanol
Brassicasterol
Campesterol
Stigmasterol
Germanicol
Sitosterol
Stigmastanol
Dinosterol
48.86
50.86
52.86
54.86
56.86
58.86
60.86
62.86
64.86
66.86
68.86
70.86
72.86
Time


100
Thevara (S7)
%
0
Coprostanol
Cholesterol
Germanicol
Dinosterol
48.88
50.88
52.88
54.88
56.88
58.88
60.88
62.88
64.88
66.88
68.88
70.88
72.88
Time


100
%
0
Panavalli (S9)
Coprostanol
Epicoprostanol
Cholesterol
Cholestanol
Germanicol
Sitosterol
Stigmastanol
Dinosterol
β-Amyrin
Betulin
Friedooleanan-3α-ol
Friedelan-3-one
48.69
50.69
52.69
54.69
56.69
58.69
60.69
62.69
64.69
66.69
68.69
70.69
72.69
74.69
76.69
Time


100
%
0
Poothotta (S10)
Germanicol
Sitosterol
Dinosterol
Betulin
Friedooleanan-3α-ol
Friedelan-3-one
52.49
54.49
56.49
58.49
60.49
62.49
64.49
66.49
68.49
70.49
72.49
74.49
76.49
Time


100
%
0
Thanneermukkom Bund (S14)
Coprostanol
Epicoprostanol
Cholesterol
Cholestanol
Brassicasterol
Stigmasterol
Germanicol
Sitosterol
Stigmastanol
Dinosterol
48.80
50.80
52.80
54.80
56.80
58.80
60.80
62.80
64.80
Time

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List of Publications

[1] Gireeshkumar, T.R., Deepulal, P.M., Chandramohanakumar, N., 2013. Distribution and sources of sedimentary organic matter in a tropical estuary, south west coast of India (Cochin estuary): A baseline study. *Marine pollution bulletin*, 66(1-2), 239–45.

[2] Gireeshkumar, T.R., Deepulal, P.M., Chandramohanakumar, N., 2013. Phosphorous Speciation in Surface Sediments of the Cochin Estuary, *Environmental Monitoring and Assessment.* DOI 10.1007/s10661-012-2729-3.

[3] Gireeshkumar, T.R., Deepulal, P.M., Chandramohanakumar, N., Ratheeshkumar, C.S., Renjith,K.R., Rejomon George., Organic Matter Enrichment and Benthic Trophic Status of the Cochin Estuary, Tropical Estuary South West Coast of India (Under Review, *Environmental Earth Science*).

[4] Deepulal, P. M., Gireesh kumar, T. R., Sujatha, C. H., Rejomon George., 2012. Chemometric study on the trace metal accumulation in the sediments of the Cochin Estuary—Southwest coast of India. *Environmental Monitoring and Assessment*, 184, 6261–6279

[5] Deepulal, P.M., Gireeshkumar, T.R., Sujatha, C.H., 2012. Behaviour of REE's in a tropical estuary and adjacent continental shelf Southwest coast of India: Evidence from anomalies. *Journal of Earth System Science,* 121(5), 1215-1227.

[6] Manju, M.N., Resmi, P, Gireeshkumar, T.R., Ratheesh Kumar C.S., Rahul, R., Joseph, M.M., Chandramohanakumar, N., 2012. Assessment of Water Quality Parameters in Mangrove Ecosystems along Kerala Coast: A Statistical Approach. *International Journal of Environmental Research*, 6(4), 893-902.

[7] Joseph, M. M., Ratheesh Kumar, C. S., Renjith, K. R., Gireesh Kumar, T. R., Chandramohanakumar, N., 2010. Phosphorus fractions in the surface sediments of three mangrove systems of southwest coast of India. *Environmental Earth Sciences*, 62(6), 1209-1218.

[8] Ratheesh Kumar, C. S., Joseph, M. M., Gireesh Kumar, T. R., Renjith, K.R., Manju, M. N., Chandramohanakumar, N., 2010. Spatial Variability and Contamination of Heavy Metals in the Inter-tidal Systems of a Tropical Environment. *International Journal Environmental Research*, 4(4), 691-700.

[9] Joseph, M. M., Ratheeshkumar, C.S., Gireeshkumar, T.R., Renjith, K.R and Chandramohanakumar, N., 2008). Biogeochemistry of surficial sediments in the intertidal systems of a tropical environment. *Chemistry and Ecology*, 24, (4), 247-258.

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