Fingerprinting of Mangrove Ecosystems along Northern Kerala Coast using Biomarker Approach

Thesis submitted to
Cochin University of Science and Technology
in partial fulfilment of the requirements
for the degree of
Doctor of Philosophy
in
Marine Chemistry
Under the Faculty of Marine Sciences

by

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April 2015

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Ph.D. Thesis under the Faculty of Marine Sciences

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Certificate

This is to certify that the thesis entitled **"Fingerprinting of Mangrove Ecosystems along Northern Kerala Coast using Biomarker Approach"** is an authentic record of the research work carried out by Ms. Resmi. P, 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.

I further certify that the corrections and modifications suggested by the audience during the pre-synopsis seminar and recommended by the Doctoral Committee of Ms. Resmi. P are incorporated in the thesis.

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Declaration

I hereby declare that the thesis entitled “**Fingerprinting of Mangrove Ecosystems along Northern Kerala Coast using 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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March, 2015

Acknowledgement

Over the past four years it has taken to complete my Ph. D thesis, many people have helped me along the way, and I am eternally thankful for their assistance. I would like to take this opportunity to thank all the persons who have made it possible for me to commence and complete this enormous task. I would like to acknowledge them for their efforts, cooperation and collaboration that have worked towards the successes of this study.

I am deeply grateful to my supervising guide, Professor (Dr.) N. Chandramohanakumar, for patiently taking me through this difficult task of environmental research. I express my deep and sincere gratitude to my guide for conceptualisation and implementation of this research topic, in addition to his peerless guidance and motivation all the way through my doctoral research. He is not only my academic supervisor, but also a real mentor and friend who let me know how to treat life with patience and fair-mindedness.

I am thankful to Dr. Sujatha C.H., Head, Department of Chemical Oceanography for her valuable suggestions and encouragement during the tenure of my work. I do not have words to acknowledge Professor (Dr.) Jacob Chackko and Professor (Dr.) S. Muraleedharan Nair, for their help and encouragement through the course of my research.

I am grateful to Professor (Dr.) Sajan. K Dean, School of Marine Sciences and Professor (Dr.), K. Mohan Kumar, Director, Faculty of Marine Science for providing facilities for the research work.

I am grateful to Mr (Dr) . Shaiju P Technical Assistant for his support and encouragement in the course of my research work. I owe special thanks to my Senior and fellow research scholar, Ms. Manju. M. N for her unstinted support all through the course of this research work. My special thanks to Dr.

Gireeshkumar. T. R and Dr. Ratheesh Kumar. C. S for their support to carry out my field as well as laboratory work, regarding extraction, derivatisation and GC-MS analysis of organic compounds. I am also grateful to Udayakrishnan. P. B for rendering his help in the final stages of the Research work. I extend my deep gratitude to Saritha. S, Salas. P. M, Dr. Proshob Peter K.J, Nebula Murukesh, Movitha Mohandas, Dr. Manju Mary Joseph, and Sanil Kumar for their companionship, inspired advice, continuous encouragement and support.

I extend my special gratitude towards Dr. Anindya Sarkar, Professor, Geology and Geophysics, IIT Khagpur for stable carbon isotope analysis. I thank staff of Sophisticated Instrumentation Facility, CUSAT for CHNS analysis.

I acknowledge the help and encouragement rendered by my colleagues, Dr. Deepulal. P. M, Ragi A.S., Leena P.P., Dr. Renjitha Raveendran., Dr. Martin G.D., Akhil P Soman., Shameem, K., Manu Mohan, Bindu, K. R., Kala K. Jacob, Sudheesh, V., Ramzi Habeeb., Libin Baby, Mridul Rag, Eldhose Cheriyan, Dr. Renjith K R and Dr. Radika Dileep.

I thank office staff of Department of Chemical Oceanography and Cochin University of Science and Technology for helping the administrative work of my thesis.

I am also thankful to my in laws for their patience and supports to complete my research work. I would like to thank my parents and sister for their support and inspiration over the years. I have no words to thank my husband Sumesh. K, for his patience, advice and mental support in the entire course of my work. My Daughter Dheerasmera, she suffered a lot in the course of my Ph.D work, thanks to her also…

Resmi. P

Preface

Mangrove forests are the most productive and bio-diverse wetlands on earth. It generate a large amount of litter in the form of leaves, branches, twigs, inflorescence and other debris and provides habitat for diverse flora and fauna of marine and terrestrial origin such as bacteria, fungi, algae, lichens, zooplankton, benthos, birds, reptiles and mammals. These systems act as nursery for many fishes and shellfishes. The other sources may also provide important organic carbon inputs; including allochthonous riverine or marine material, autochthonous production by benthic or epiphytic micro- or macroalgae, and local water column production by phytoplankton. Since mangrove sediments are very complex which receives autochthonous and allochthonous organic matter inputs, the information extracted from the analysis of mangrove sediments is the fingerprint of both natural and human-induced changes.

Bulk organic matter characterisation (stable carbon analysis and elemental composition) along with analysis of biochemical components can be used as a tool to characterize sources of organic matter. Due to the complexity of organic matter sources, biogeochemistry of mangrove sediments could not be explained effectively by using bulk organic matter characterisation or biochemical composition and hence the application of chemical biomarkers has become widespread. This molecular information is more specific and sensitive for characterising sources of organic matter, and further allows for the identification of multiple sources. Lipids can be used as effective biomarkers of organic matter in coastal systems, which represent an important component of organic matter with a diversity of compound classes (e.g., hydrocarbons, fatty acids, n-alkanols and sterols).

The mangrove forest in North Malabar extend from Calicut to Kasaragod districts represents about 83 % of mangrove cover in the State, but the scientific investigations on these mangrove ecosystems are scanty. So mangrove ecosystems of northern Kerala Coast were selected for the present study which includes: Kunjimangalam, Pazhayangadi, Pappinissery, Thalassery and Kadalundi.

The thesis is divided into 8 chapters. Chapter 1 provide the introduction of the research work and review the biomarker compounds used for source characterization. The chapter also comprises aim and scope of the present study. Chapter 2 deals with description of the study region and also includes the general methods adopted for the present study. Chapter 3 Described general water quality parameters and sedimentary characteristics such as grain size and elemental characteristics. This chapter also devoted to phosphorus fractionation using sequential extraction methods. In Chapter 4 stable carbon isotope and biochemical composition is used to recognize origin and quality of organic matter. Stable carbon analysis and biochemical composition of the sediment was unsuccessful in explaining organic matter sources in the selected mangrove ecosystems, so biomarker concept was introduced. Chapter 5 discussed the use of hydrocarbon as biomarkers to identify sources of organic matter. The difference in characteristic chain lengths of marine and terrestrial plants made the distribution of n-alkanes an effective biomarker tool. But significant mangrove, marine macrophyte, and/ or significant riparian–aquatic inputs introduce sources with non- unique n-alkane compositions, making difficult the sources determination by n-alkane chain length alone. In Chapter 6 fatty acids are employed as biomarkers for assessing the sources and fates of organic matter in these complex systems. Fatty acids used to trace origin of organic matter in different environments structural diversity, source specificity and relative stability. But poly unsaturated fatty acids can be degraded by heterotrophs that affect concentrations as well as their relative proportions. Therefore Chapter 7, pentacyclic triterpenoids and sterols, a common chemical constituents of marine and terrigenous plants and have been employed as biomarkers since they are structurally diverse and have long term stability. Finally Chapter 8 includes the overall summary of the present study. This chapter also try to extract the use of muti-marker approach for assessing source characteriation.

Dedicated to my family…

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List of Abbreviations

Max	Maximum
Min	Minimum
avg	Average
Stdv	Standard Deviation
TAR	Terrestrial/Aquatic Ratio
CPI	Carbon Preference Index
ACL	Average Chain Length
UCM	Unresolved Complex Matrix
MOM	Marine Organic Matter
TOM	Terrestrial Organic Matter
LMW	Low Molecular Weight alkane
HMW	High Molecular Weight alkane
Pr	Pristane
Py	Phytane
Mon	Monsoon
Post-mon	Post Monsoon
Pre-mon	Pre Monsoon
ND	Not Detected
TOC	Total Organic Carbon
TN	Total Nitrogen
TS	Total Sulphur
TP	Total Phosphorus
TOM	Total Organic Matter
Fe(OOH) ≈P	Iron Bound Phosphorus
$CaCO_3$ ≈P	Calcium bound Phosphorus
Acid-OP	Acid Soluble Organic Phosphorus
Alkali-OP	Alkali Soluble Organic Phosphorus
ROP	Residual Organic Phosphorus
Chl *a*	Chlorophyll *a*
Chl b	Chlorophyll b
Chl c	Chlorophyll c
Pheo	Pheopigments
PRT	Proteins
CHO	Carbohydrates
LIP	Lipid

T & L	Tannin and Lignin
BSA	Bovine Serum Albumin
BPC	Biopolymeric Carbon
FAME	Fatty Acid Methyl Ester
GC-MS	Gas Chromatography-Mass Spectrometer
GC-FID	Gas Chromatography-Flame Ionisation Detector
BSTFA	N,O-Bis(trimethylsilyl) trifluroacetamide
BAME	Bacterial Acid Methyl Esters
ANOVA	Analysis of Variance
SPSS	Statistical Package for Social Sciences
PCA	Principal Component Analysis
PC	Principal Component
DO	Dissolved Oxygen
DIP	Dissolved Inorganic Phosphate
TDN	Total Dissolved Nitrogen
TDP	Total Dissolved Phosphorus
DIN	Dissolved Inorganic Nitrate
F%	Relative Contribution of terrestrially derived organic matter
Al-OC	Algal derived organic carbon
PAH	Polyaromatic hydrocarbon
FA	Fatty Acid
TFA	Total fatty acids
CAM	Crassulacean Acid Metabolism
L/S ratio	Long chain to Short chain ratio of n-fatty alcohols
OM	Organic Matter
i	iso
a	anteiso
SCFA	Short Chain Fatty Acids
LCFA	Long Chain Fatty Acids
SFA	Saturated Fatty Acids
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
BrFA	Branched Fatty Acids

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Chapter 1

INTRODUCTION

Contents
1.1 General Introduction
1.2 Molecular Biomarkers
1.3 Aim and Scope of the study

1.1 General Introduction

Mangrove forests, the coastal intertidal habitats in the tropical and subtropical region, generate litter in large amounts in the form of leaves, branches, twigs, inflorescence and other debris. They possess precious natural resources and fulfil important ecological functions (Rönnbäck, 1999; Wang et al., 2011; Weng et al., 2013) such as shoreline protection, entrapment of heavy metals (Harbison, 1986; Clark et al., 1998), litterfall subsidy (Twilley et al., 1986), nursery grounds (Robertson and Duke, 1987; Mumby et al., 2004; Siple and Donahue, 2013) and sediment stabilisation (Posey, 1987). Sedimentation in mangrove ecosystems may develop the new land and drive back the sea coast, e.g., in Palembang of Indonesia (north coast of Sumatra- Wolters, 1979). Mangroves show high adaptability to extreme environmental conditions, such as broad range of salinity, water-logged environments, and contrasting redox conditions (Ranjan et al., 2011).

Mangroves, acting as net sinks or sources of dissolved and suspended organic matter, supplies nutrients to estuaries and adjacent coastal systems (Boto and Bunt, 1981; Alongi, 1996; Alongi et al., 1998; Dittmar and Lara, 2001). A fraction of mangrove detritus escapes degradation and retains within their sediments, and the other fraction lose to adjacent coastal waters by tidal forcing. Crab burrows, which shields organic carbon to wash away by tides (Kristensen, 2008) enhance organic carbon retention by storing leaf litter, mangrove propagules and other organic material from different sources (Dye and Lasiak, 1987; Micheli et al., 1991; Micheli, 1993; Dahdouh-Guebas et al., 1997). Mangrove forests may be similar to tropical rainforests (Alongi et al., 2001), but contain stocks of organic carbon larger than upland forests (Donato et al., 2011). So mangrove forests have also importance in the global carbon budget (Chmura et al., 2003; Bouillon et al., 2008; Breithaupt et al., 2012) with net primary production of 218 Tg yr^{-1}, ≈21 Tg for particulate organic carbon (OC) export, 24 Tg to dissolved OC export, 42 Tg to CO_2 efflux (Bouillon et al., 2008) and between 26 and 34 Tg to OC burial (McLeod et al., 2011; Breithaupt et al., 2012).

A range of other sources may also provide important organic carbon inputs; including allochthonous riverine or marine material (e.g., sea grasses), autochthonous production by benthic or epiphytic micro- or macro-algae, and local water column production by phytoplankton (Twilley et al., 1992; Bouillon et al., 2004; Kristensen, et al., 2008). These processes generate debris of phytoplankton, and also support organisms that feed directly or indirectly on phytoplankton. Though mangrove detritus also act as a food resource (Tenore, 1977; Rice, 1982; Tenore et al., 1982; Mann and Lazier, 1991), nutritive value of mangrove litter is low. Microbial processing, which makes detritus nutrient-rich (Odum, 1971; Lee, 1990; Ashton et al., 1999; Kathiresan and Bingham, 2001; Rajendran and Kathiresan, 2000, 2004, 2007), is accompanied by the flow of carbon and nitrogen from primary producers to consumers, but the mechanism is not clearly understood. Denitrifying bacteria (Flores-Mireles et al., 2007), N_2 fixing bacteria (Toledo et al., 1995; Rojas et al., 2001; Flores-Mireles et al., 2007), anaerobic ammonia oxidising (ANAMMOX) bacteria (Meyer et al., 2005), sulphur reducing and oxidising bacteria have been detected in mangrove ecosystems. Since mangrove sediments receive autochthonous and allochthonous organic matter inputs, the information extracted from the analysis of mangrove sediments is the fingerprint of both natural and human-induced changes.

The redox status of mangrove soil is controlled by a number of factors; the composition and concentration of the inorganic and organic components, tidal elevation, water content, microbial activity, and extent of anthropogenic input (Alongi, 2009). The stability and chemical form of phosphorus is a function of the redox status and other environmental conditions, which regulate its retainment and release from the sediment phase (Pardo et al., 2003; House, 2003; Jarvie et al., 2005; Katsaounos et al., 2007). Under oxic conditions phosphate adsorbs onto positively charged clay particles, iron and aluminium oxides, but release at the sediment–water interface in the event of anoxia. Therefore, different forms of sedimentary phosphorus have been reported as a key to understanding the redox status of the system, which can be achieved by sequential extraction of sedimentary phosphorus.

Bulk organic matter characterisation (stable carbon analysis and elemental composition) along with analysis of biochemical components can be used as a tool to characterize the sources of organic matter. C_{12} and C_{13}

have natural abundance of 98.9% and 1.1% respectively, but during biologically mediated kinetic fractionation the products get enriched with lighter isotope due to differential partitioning from higher energy properties (Emerson and Hedges, 2008). It will result in the depletion of heavy isotope in the product and these processes are controlled by: (1) carbon sources, (2) isotopic effects during assimilation processes in producer organisms, (3) isotopic effects during metabolism and biosynthesis, and (4) cellular carbon budgets (Hayes, 1993; Bianchi, 2007). Thus, carbon isotopic ratios are useful in distinguishing the marine and terrestrial organic matter sources in sediments. But, these general trends get more complicated when factors such as different food sources (e.g., terrestrial versus aquatic), size and physiology of the organisms involved (Incze et al., 1982; Hughes and Sherr, 1983; Goering et al., 1990; Megens et al., 2002).

Elemental ratios of sediments, may be altered to different extents by one of the following ways; selective decomposition of organic matter during (Meyers, 1997) or the colonization of bacterial and fungal populations on “ageing” of vascular plant detritus (due to the typically low (e.g., 3 to 4) C:N ratios found in bacteria) (Tenore et al., 1982). Due to the complexity of organic matter sources, biogeochemistry of mangrove sediments cannot be explained effectively by using bulk organic matter characterisation or biochemical composition and the application of chemical biomarkers has become widespread. The molecular information extracted from biomarkers, which is more specific and sensitive, helps for source characterisation of organic matter with multiple or complex origins. Lipids can be used as an effective biomarker of organic matter in coastal systems, which represent an important component of organic matter with a diverse compound class (e.g., hydrocarbons, fatty acids, n-alkanols, and sterols).

1.2 Molecular Biomarkers

The term “biomarker” originated in 1960s from early product precursor relationships (Streibl and Herout, 1969) and then Eglinton and Calvin (1967) introduces the term as “chemical fossils”. Then evolved the term “biological markers” (Hunt, 1979), and was then contracted to the popular term “biomarker”. . Molecular organic biomarkers, whose extensive use as proxies for both sources and process of organic matter in natural systems (Meyers, 1997; Volkman et al., 1998; Volkman, 2006) can be related to geological and environmental transformations. These compounds undergo chemical transformations while retaining enough structural characteristics. Many of these compounds, which can be related to particular groups of organisms (e.g., terrestrial plants, bacteria, microalgae, heterotrophs, etc.) sometimes used to distinguish specific sub-groups of organisms (e.g., mangroves, sulphate reducing bacteria, diatoms and dinoflagellates). Biomarkers can be identified (if present) in macro- and microfossils (Otto and Simoneit, 2001), and also in volatile, liquid, and solid carbonaceous matter (Summons, 1993). Cyclic and branched hydrocarbons, acids, and hetero compounds (e.g., terpenoids) are generally stable over long periods, and thus are useful indicators of biosynthesis in the older geological record. The use of biomarkers as indicators of biogenic, paleoenvironmental, and geochemical processes on Earth has been widely accepted (Mackenzie et al., 1982; Johns, 1986; Simoneit et al., 1986; Brassell, 1992; Imbus and McKirdy, 1993; Mitterer, 1993; Simoneit, 1998).

Lipids, among biomarkers, are particularly useful because they can reveal valuable information on OM sources at the molecular level (Meyers, 2003). Aliphatic lipids such as alkanes, alkenes, fatty acids, fatty alcohols, alkenones, esters, wax esters, amides, etc carry a strong carbon number predominance inherited from their biosynthesis, and their homolog distribution can reflect biogenic origin (e.g., marine vs. terrestrial vegetation; Simoneit, 1977a, b). Mass spectrometry is an important method to identify these homologous lipids (Simoneit and Mazurek, 1982; Evershed, 1992; Murphy, 1993; White et al., 1997; Simoneit et al, 1999; Simoneit, 2005). The same precursor-product relationship has also been documented for steroids (Simoneit, 1995) and numerous other natural product precursors (Johns, 1986; Simoneit, 1998).

Higher-plant biomarkers were discovered initially in brown coals, peat, and resins (amber) (Johns, 1986). The homologous aliphatic compounds such as n-alkanes (C27, C29, C31, C33), n-alkanols, and n-alkanoic acids (both homologous series have dominant C24, C26, C28, or C30), with lesser amounts of other oxygenated homologous species have strong carbon number pre-dominances and are derived from the epicuticular waxes and related lipids of higher plants. The terpenoid biomarkers and its derivative products, originating from higher plants, are formed by the reductive and oxidative alteration during their transport by diagenesis in sedimentary environments, or by thermal transformation processes (Currie and John, 1989; Poinsot et al., 1995; Versteegh et al., 2004). Reductive alteration generally yields the parent compound skeleton with various isomerisations of chiral centers and, in some cases, loss of carbon due to decarboxylation and other reactions. Oxidative alteration, mainly occurs, by successive ring aromatizations, usually commence from a ring that has a functional group (e.g., OH, C=C, C=O; typically on ring-A) by direct dehydrogenation, dehydration, ring rearrangement, or ring opening and subsequent loss. Thus, one would expect to find oxidized and reduced products, as well as unknown and novel substances during organic geochemical analysis.

The tetracyclic triterpenoids, such as lanostane, euphane, onocerane, dammarane, limonin, and cucurbitacin, are found in vascular plants, and the first four have been reported in sedimentary rocks also (Kimble et al., 1974; Johns, 1986). The phytosterols (mainly C29, minor C28 skeletons) have been used as indicators for higher plants, though many marine algae also produce these sterols with same or different side chain alkyl substitutions (Johns, 1986; Simoneit, 2005). Many pentacyclic triterpenoids (e.g., the oleanane, ursane, taraxerane, lupane, friedelane, serratane, or bauerane skeletons) are higher plant biomarkers and the most frequently encountered in sedimentary environments are ursanes and oleananes (Johns, 1986; Wang and Simoneit, 1990). Higher plant biomarkers, a geochemical tool to identify terrestrial organic detritus in global compartments (e.g., atmosphere, sediments, etc.), helps to study the export of carbon by rivers to the ocean (Prahl et al., 1994) and the source identification of petroleum and the correlation of oils with their source rocks (Ekweozor and Telnaes, 1990; ten Haven, et al., 1992; Peters and Moldowan, 1993). Higher plant biomarkers are also useful in paleoenvironmental (Conte and Weber, 2002a, b) and environmental studies (e.g., source characterisation of atmospheric organic matter) (Simoneit, 1977a, 1989; Peltzer and Gagosian, 1989; Schauer et al., 1996). Along with naturally-derived saturated and aromatic hydrocarbons (and sometimes oxygenated analogs), polynuclear aromatic hydrocarbons (PAHs) from anthropogenic origins were also found in contemporary studies (Simoneit, 1977a; LaFlamme and Hites, 1979; Wakeham et al., 1980; Tan and Heit, 1981).

Mid chain length aliphatic lipids such as alkanes, n-alkanoic acids, n-alkenoic acid and n-alkanols <C19 in sedimentary mixtures have been attributed predominantly to microorganism inputs (Simoneit, 1977b; Cranwell et al., 1987; Medeiros and Simoneit, 2008) such as algae and bacteria.

Planktonic inputs such as dinoflagellates and diatoms can also be predicted by the identification of related sterols like dinosterol (Volkman et al., 1993) and brassicasterol (Volkman, 1986).

The hopanoids are widely used as cyclic biomarkers for bacteria (Ensminger et al., 1974; Ourisson et al., 1979, 1982; Kajukova et al., 1981). The 17α(H),21β(H)-hopanes that range from C27 to C35 (no C28) were encountered in numerous ancient sediments and petroleum, and diagenesis and maturation of the microbial precursors were elucidated (Ourisson et al., 1982; Rohmer, 1993). The extended hopanes or homohopanes (C31-C35) is related to the bacteriohopanepolyols in bacteria and lower hopane pseudo-homologs related to C30 precursors such as diploptene and diplopterol (Peters and Moldowan, 1991). Diploptene (17α(H),21β(H)-hop-22(29)-ene), a wide spread hopane, whose occurrence is attributed to multiple bacterial sources (Rohmer et al., 1992) such as cyanobacteria, purple non-sulfur bacteria, acetic acid bacteria, nitrifying bacteria, methylotrophic and methanotrophic bacteria, is originated either direct or from its diagenetic precursor diplopterol (Summons et al., 1994; Elvert et al., 2001). Hopanes, which undergo reduction during diagenesis, whose 17 β(H),21β(H)-22R configuration is thermally unstable, undergo epimeric transformations to βα-hopanes (moretanes) and αβ-hopanes (Peters and Moldowan, 1991).

1.3 Aim and Scope of the Study

Mangroves, which dominate coastal vegetation in tropical ecosystems, cover about 161,000 km^2 (Spalding et al., 1997) and produce high amounts of organic carbon especially in the form of leaf litter (Dittmar and Lara, 2001). Total area of mangroves in India is about 4461 km^2, constituting 0.41% its geographical area (Jagtap et al., 2002) and Kerala, southernmost state on the coast of Arabian Sea, have a mangrove cover of 17 km^2 (Chand Basha, 1992). Wetlands of southern Kerala have been lost over years due to reclamation, conversion for industrial use, dumping of solid waste, discharge of untreated municipal sewage and industrial effluents (Manju et al., 2012). A recent study by Radhakrishnan et al., 2006 pointed out 83% of the mangrove cover in the state is spread over four northern districts - Kasargod, Kannur, Kozhikode and Malappuram, and Kannur District has the major share. The focus of the study is restricted to mangroves occurring in the northern Kerala coast since reported works on these systems are scanty. Quantitative information on true mangroves of Thalassery, Pappinissery and Kunjimangalam (Khaleel, 2005) and Frequency and abundance of Marine fungi of Kadalundi and Kunjimangalam mangroves were reported (Gayatri and Raveendran, 2009). Other earlier reported works were Environmental challenges to the mangrove wetlands of North Malabar (Khaleel and Jaleel, 2009) and studies on water quality parameters of five mangrove ecosystems of northern Kerala coast (Manju et al., 2012).

Litter production, a significant process in carbon dynamics of mangrove ecosystems, was evident from the literature. A litter production of 1350 g of dry weight (DW) m^2 in northern Brazil (Mehlig, 2001) and net primary production of 3000 g C m^2 yr^{-1} in Australia (Clough, 1998) were reported. Bacterial abundance in mangrove sediments of tropical Australia was reported as 1011 cells per gram in dry surface sediment (Gillan and Hogg, 1984; Koch et al., 2005), hence Microbial processes are vital components in mangrove sediments. Along with litter production and bacterial abundance, organisms such as fungi, algae, lichens, zooplankton, benthos, birds, reptiles and mammals were also found to be associated with the mangrove systems. Apart from the autochthonous production marine and riverine organic matter can also be brought into the system. The different sources of the organic pool and its burial in mangrove systems, which is a critical issue when constructing the C budget, have been rarely studied in Indian scenario.

Stable isotopes have long been used to distinguish among different OM sources (Bouillon et al., 2001, 2004; Dittmar et al., 2001; Cook et al., 2004) but bulk organic carbon pools entering these environments, make the understanding of controls on the isotopic composition complex. Therefore, instead of stable isotopes, identification biomarkers (lipids, e.g., n-alkanes, n-alkanoic acids, terpenoids, steroids) augment valuable information such as sources, transport and diagenetic state of sedimentary, particulate and dissolved organic carbon in coastal systems (Cunha et al., 2002; McCallister et al., 2006a,b; Medeiros and Simoneit, 2008).

The objectives of the present study are as follows

- To find the redox characteristics of the mangrove ecosystems using P fractionation
- To assess the nature and quality of bulk sedimentary organic matter using biochemical composition
- To explore the spatio-temporal distributional dynamics of stable carbon isotopes and elemental compositions in surface sediments from the mangrove ecosystems and to calculate the 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 evaluate seasonal variation of biomarkers in the mangrove ecosystems.

References

Alongi, D. M., 1996. The dynamics of benthic nutrient pools and fluxes in tropical mangrove forests. Journal of Marine Research, 54, 123–148.

Alongi, D. M., Ayukai, T., Brunskill, G. J., Clough, B. F., Wolanski, E., 1998. Sources, sinks, and export of organic carbon through a tropical, semi-enclosed delta (Hinchinbrook Channel, Australia). Mangroves and Salt Marshes, 2, 237–242.

Alongi, D. M., Wattayakorn, G., Pfitzner, J., Tirendi, F., Zagorskis, I., Brunskill, G.J., Davidson, A., Clough, B.F., 2001. Organic carbon accumulation and metabolic pathways in sediments of mangrove forests in southern Thailand. Marine Geology, 179, 85-103.

Alongi, D. M., 2009. The Energetics of Mangrove Forests. Springer Science 216.

Ashton, E. C., Hogarth, P. J., Ormond, R., 1999. Breakdown of mangrove litter in a managed mangrove forest in Peninsular Malaysia. In: Dodd, R.S. (Ed.), Diversity and Function in Mangrove Ecosystems. Hydrobiologia, 413,77–88.

Bianchi, T. S., 2007. Biogeochemistry of Estuaries. Oxford University Press, New York.

Boto, K. G., Bunt, J. S., 1981. Tidal export of particulate organic matter from a northern Australian mangrove system. Estuarine, Coastal and Shelf Science, 13, 247–255.

Bouillon, S., Mohan, P. C., Sreenivas, N., Dehairs, F., 2001. Sources of suspended organic matter and selective feeding by zooplankton in an estuarine mangrove ecosystem as traced by stable isotopes. Marine Ecology Progress Series, 208, 79–92.

Bouillon, S., Koedam, N., Baeyens, W., Satyanarayana, B., Dehairs, F., 2004. Selectivity of subtidal benthic invertebrate communities for local microalgal production in an estuarine mangrove ecosystem during the post-monsoon period, Journal of Sea Research, 51, 133-144.

Bouillon, S., Connolly, R. M., Lee, S. Y., 2008. Organic matter exchange and cycling in mangrove ecosystems: Recent insights from stable isotope studies. Journal of Sea Research, 59, 44-58.

Brassell, S. C., 1992. Biomarkers in recent and ancient sediments: The importance of the diagenetic continuum. In: Whelan JK, Farrington JW, editors. Organic matter—Productivity, accumulation, and preservation in recent and ancient sediments. Columbia University Press, New York.

Breithaupt, J. L., Smoak, J. M., Smith, T. J., Sanders, C. J., Hoare, A., 2012. Organic carbon burial rates in mangrove sediments: Strengthening the global budget. Global Biogeochemical Cycles, 26, 1-11.

Chand Basha, S., 1992. Mangroves of Kerala-A fast disappearing asset. Indian Forester, 118, 175-190.

Chmura, G. L., Anisfeld, S. C., Cahoon, D. R., Lynch, J. C., 2003. Global carbon sequestration in tidal, saline wetland soils. Global Biogeochemical Cycles, 17, 1111.

Clark, M. W., Mc Conchie, D., Lewis, D. W., Saenger, P., 1998. Redox stratification and heavy metal partitioning in *Avicennia*-dominated mangrove sediments: a geo- chemical model. Chemical Geology, 149, 147–171.

Clough, B., 1998. Mangrove forest productivity and biomass accumulation in Hinchinbrook Channel, Australia. Mangroves and Salt Marshes, 2, 191–198

Conte, M. H., Weber, J. C., 2002a. Plant biomarkers in aerosols record isotopic discrimination of terrestrial photosynthesis. Nature, 417, 639–641.

Conte, M. H., Weber, J. C., 2002b. Long-range atmospheric transport of terrestrial biomarkers to the Western North Atlantic. Global Biogeochemical Cycles, 16, 1–17.

Cook, P. L. M., Revill, A. T., Clementson, L. A., Volkman, J. K., 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. Marine Ecology Progress Series, 280, 39-54.

Cranwell, P. A., House, T. F., Organic, U. K., Unit, G., Close, C., 1987. Lipids of aquatic organisms as potential contributors to lacustrine sediments—ll. Organic Geochemistry, 11, 513-527.

Cunha, L. C., Serve, L., Blazi, J. L., 2002. Neutral sugars as biomarkers in the particulate organic matter of a French Mediterranean river. Organic Geochemistry, 33, 953–964.

Currie, B. R., Johns, R. B., 1989. An organic geochemical analysis of terrestrial biomarkers in a transect of the Great Barrier Reef Lagoon. Australian Journal of Marine & Freshwater Research, 40, 275–284.

Dahdouh-Guebas, F., Verneirt, M., Tack, J. F., Koedam, N., 1997. Food preferences of Neosarmatium meinerti de Man (Decapoda: Sesarminae) and its possible effect on the regeneration of mangroves. Hydrobiologia, 347, 83–89.

Dittmar, T., Lara, R. J., 2001. Driving Forces behind Nutrient and Organic Matter Dynamics in a Mangrove Tidal Creek in North Brazil. Estuarine, Coastal and Shelf Science, 52, 249-259.

Dittmar, T., Fitznar, H. P., Kattner, G., 2001. Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D-and L-amino acids. Geochimica et Cosmochimica Acta, 65, 4103-4114.

Donato, D. C., Kauffman, J. B., Murdiyarso, D., Kurnianto, S., Stidham, M., Kanninen, M., 2011. Mangroves among the most carbon-rich forests in the tropics. Nature Geoscience, 4, 293–297.

Dye, A. H., Lasiak, T. A., 1987. Assimilation efficiencies of fiddler crabs and deposit feeding gastropods from tropical mangrove sediments. Comparative Biochemistry and Physiology, 87, 341–344.

Eglinton, G., Calvin, M., 1967. Chemical fossils. Scientific American, 216, 32-43.

Ekweozor, C. M., Telnaes, N., 1990. Oleanane parameter: Verification by quantitative study of the biomarker occurrence in sediments of the Niger delta. Organic Geochemistry, 16, 401–413.

Elvert, M., Whiticar, M. J., Suess, E., 2001. Diploptene in varved sediments of Saanich Inlet : indicator of increasing bacterial activity under anaerobic conditions during the Holocene. Marine Geology, 174, 371-383.

Emerson, S. R., Hedges, J. I., 2008. Chemical oceanography and the marine carbon cycle. Cambridge University Press Cambridge, New York.

Ensminger, A, van Dorsselaer, A, Spyckerelle, C, Albrecht, P, Ourisson, G., 1974. Pentacyclic triterpenes of the hopane type as ubiquitous geochemical markers: Origin and significance. In: Tissot B, Bienner F, editors. Advances in organic geochemistry, 1973. Paris: Editions Technip, 245–260.

Evershed, R. P., 1992. Mass spectrometry of lipids. In Lipid Analysis. A Practical Approach. Hamilton R, Hamilton S. (eds). IRL Press, Oxford.

Flores-Mireles, A. L., Winans, S. C., Holguin, G., 2007. Molecular characterization of diazotrophic and denitrifying bacteria associated with mangrove roots. Applied and environmental microbiology, 73, 7308-7321.

Gayatri, R. N., Raveendran, K., 2009. Frequency and Abundance of Marine Mycoflora in Mangrove Ecosystem of North Malabar, Kerala (India). Academic Journal of Plant Sciences, 2, 65-68.

Gillan, F. T., Hogg, R. W., 1984. A method for the estimation of bacterial biomass and community structure in mangrove- associated sediments. Journal of Microbiological Methods, 2, 275–293.

Goering, J., Alexander, V., Haubenstock, N., 1990. Seasonal variability of stable isotope ratios of organisms in a north Pacific Bay. Estuarine, Coastal and Shelf Science, 30, 239-260.

Harbison, P., 1986. Mangrove muds - a sink and a source for trace-metals. Marine Pollution Bulletin, 17, 246–250.

Hayes, J. M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence, Marine Geology, 113, 111–125.

House, W., 2003. Geochemical cycling of phosphorus in rivers. Applied Geochemistry, 18, 739–748.

Hughes, E. H., Sherr, E. B., 1983. Subtidal food webs in a Georgia estuary: $\delta^{13}C$ analysis. Journal of experimental marine biology and ecology, 67, 227-242.

Hunt, J. M., 1979. Petroleum Geochemistry and Geology, W. H. Freeman, San Francisco.

Imbus, S. W., McKirdy, D. M., 1993. Organic geochemistry of pre-cambrian sedimentary rocks. In: Engel MH, Macko SA, editors. Organic geochemistry. Pergamon Press, New York.

Incze, L. S., Mayer, L. M., Sherr, E. B., Macko, S. A., 1982. Carbon inputs to bivalve mollusks: a comparison of two estuaries. Canadian Journal of Fisheries and Aquatic Sciences, 39, 1348-1352.

Jagtap, T. G., Murthy, P., Komerpant, D., 2002. Mangrove ecosystems of India: major biotic constituents conservation and management, In: Wetland conservation and management, Hosetti. B. B (Ed.), Pointer publishers, Jaipur, India.

Jarvie, H. P., Jurgens, M. D., Williams, R. J., Neal, C., Davies, J. J. L., Barrett, C., White, J., 2005. Role of river bed sediments as sources and sinks of phosphorus across two major eutrophic UK river basins: the Hampshire Avon and Herefordshire Wye. Journal of Hydrology, 304, 51–74.

Johns, R. B., 1986. Biological markers in the sedimentary record. Amsterdam: Elsevier.

Kajukova, G. I., Pustulnikova, C. D., Abruitina, N. N., Golovkina, L. S., Meshtueva, V. L., Petrov, A. A., 1981. Structural variations in hopane type hydrocarbons. Neftechimiya, 21, 803–811.

Kathiresan, K., Bingham, B. L., 2001. Biology of mangroves and mangrove ecosystems. Advances in Marine Biology, 40, 81-251.

Katsaounos, C. Z., Giokas, D. L., Leonardos, I. D., Karayannis, M. I., 2007. Speciation of phosphorus fractionation in river sediments by explanatory data analysis, Water Research, 41, 406-418.

Khaleel, K. M., 2005. Study of the quantitative structure of true mangroves present in mangal forest of Thallichery, Pappinissery and Kunjimangalam of Kannur district. Indian forestor, 131, 81-89.

Khaleel, K. M., Jaleel, C. A., 2009. Environmental challenges to the mangrove wetlands of North Malabar (Kerala), India: Their sustainable development and influence on local people. Knowledge and Management of Aquatic Ecosystems, 392, 03.

Kimble, B. J., Maxwell, J. R., Philp, R. P., Eglinton, G., Albrecht, P., Ensminger, A., Arpino, P., Ourisson, G., 1974. Tri- and tetra-terpenoid hydrocarbons in the Messel oil shale. Geochimica et Cosmochimica Acta, 38, 1165–1181.

Koch, B. P., Lara, J., Kattner, G., Harder, J., 2005. The effect of selective microbial degradation on the composition of mangrove derived pentacyclic triterpenols in surface sediments. Organic geochemistry, 36, 273-285.

Kristensen, E., 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. Journal of Sea Research, 59, 30-43.

Kristensen, E., Bouillon, S., Dittmar, T., Marchand, C., 2008. Organic carbon dynamics in mangrove ecosystems : A review. Aquatic botany, 89, 201-219.

LaFlamme, R. E., Hites, R. A., 1979. Tetra-and pentacyclic, naturally-occurring, aromatic hydrocarbons in recent sediments. Geochimica et Cosmochimica Acta, 43, 1687–1691.

Lee, S. Y., 1990. Primary productivity and particulate organic matter flow in an estuarine mangrove-wetland in Hong Kong. Marine Biology, 106, 453–463.

Mackenzie, A. S., Brassell, S. C., Eglinton, G., Maxwell, J. R., 1982. Chemical fossils: the geologic fate of steroids. Science, 217, 491-504.

Manju, M. N., Resmi, P., Gireesh Kumar, 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, 893-902.

Mann, K. H., Lazier, J. R. N., 1991. Dynamics of Marine Ecosystem, Blackwell Scientific Publications Inc., Boston, United States.

McCallister, S. L., Bauer, J. E., Canuel, E. A., 2006b. Bioreactivity of estuarine dissolved organic matter: a combined geochemical and microbiological approach. Limnology and Oceanography, 51, 94–100.

McCallister, S. L., Bauer, J. E., Ducklow, H. W., Canuel, E. A., 2006a. Sources of estuarine dissolved and particulate organic matter: a multi-tracer approach. Organic Geochemistry, 37, 454–468.

McLeod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., Lovelock, C. E., Schlesinger, W. H., Silliman, B. R., 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. Frontiers in Ecology and the Environment, 9, 552–560.

Medeiros, P. M., Simoneit, B. R. T., 2008. Multi-biomarker characterization of sedimentary organic carbon in small rivers draining the Northwestern United States, 39, 52-74.

Megens, L., van der Plicht, J., de Leeuw, J.W., Smedes, F., 2002. Stable carbon and radiocarbon isotope compositions of particle size fractions to determine origins of sedimentary OM in an estuary. Organic Geochemistry 33, 945–952.

Mehlig, U., 2001. Aspects of mangrove tree primary production in an equatorial mangrove forest in Brazil, Ph.D Thesis submitted to Center for Tropical Marine Ecology, University of Bremen, Germany.

Meyers, P. A., 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic and paleoclimatic processes. Organic Geochemistry, 27, 213–250.

Meyers, P. A., 2003. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. Organic Geochemistry, 34, 261–289.

Meyer, R. L., Risgaard-Petersen, N., Allen, D. E., 2005. Correlation between anammox activity and microscale distribution of nitrite in a subtropical mangrove sediment. Applied and Environmental Microbiology, 71, 6142-6149.

Micheli, F., Gherardi, F., Vannini, M., 1991. Feeding and burrowing ecology of two East African mangrove crabs. Marine Biology, 111, 247–254.

Micheli, F., 1993. Feeding ecology of mangrove crabs in North Eastern Australia: mangrove litter consumption by Sesarma messa and Sesarma smithii. Journal of Experimental Marine Biology and Ecology, 171, 165–186.

Mitterer, R. M., 1993. The diagenesis of proteins and amino acids in fossil shells. In: Engel MH, Macko SA, editors. Organic geochemistry. Plenum Press, New York.

Mumby, P. J., Edwards, A. J., Arias-Gonzalez, J. E., Lindeman, K. C., Blackwell, P. G., Gall, A., Gorczynska, M. I., Harborne, A.R., Pescod, C.L., Renken, H., Wabnitz, C. C. C., Llewellyn, G., 2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. Nature, 427, 533–536.

Murphy, R. C., 1993. Mass spectrometry of lipids. Handbook of Lipid Research Vol. 7, Plenum Press, New York.

Odum, E. P., 1971. Fundamentals of Ecology. Philadelphia: WB Saunders Company, USA.

Otto, A., Simoneit, B. R. T., 2001. Chemosystematic and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz formation. Saxony, Germany. Geochimica et Cosmochimica Acta, 65, 3505-3527.

Ourisson, G., Albrecht, P., Rohmer, M., 1979. The hopanoids. Paleochemistry and biochemistry of a group of natural products. Pure and Applied Chemistry, 51, 709–729.

Ourisson, G., Albrecht, P., Rohmer, M., 1982. Predictive microbial biochemistry—From molecular fossils to prokaryotic membranes. Trends in Biochemical Sciences, 7, 236–239.

Pardo, P., Rauret, G., Lopez-Sanchez, J. F., 2003. Analytical approaches to the determination of phosphorus partitioning patterns in sediments. Journal of Environmental Monitoring, 5, 312–318.

Peltzer, E. T., Gagosian, R. B., 1989. Organic geochemistry of aerosols over the Pacific Ocean. In: Duce RA, Riley JP, Chester R, editors. Chemical oceanography. Vol. 10, Academic Press, New York.

Peterst, K. E., Moldowan, J. M., 1991. Effects of source , thermal maturity , and biodegradation on the distribution and isomerization of homohopanes in petroleum. Organic Geochemistry, 17, 47-61.

Peters, K. E., Moldowan, J. M., 1993. The biomarker guide—Interpreting molecular fossils in petroleum and ancient sediments. Englewood Cliffs, Prentice Hall, NJ.

Poinsot, J., Adam, P., Trendel, J. M., Connan, J., Albrecht, P., 1995. Diagenesis of higher plant triterpenes in evaporitic sediments. Geochimica et Cosmochimica Acta, 59, 4653–4661.

Posey, M. H., 1987. Influence of relative mobilities on the composition of benthic communities. Marine Ecology Progression Series, 39, 99–104

Prahl, F. G., Ertel, J. R., Gon˜I, M. A., Sparrow, M. A., Eversmeyer, B.. 1994. Terrestrial organic carbon contributions to sediments on the Washington margin. Geochimica et Cosmochimica Acta, 58, 3035–3048.

Radhakrishnan, C., Gopi, K. C., Palot, M. J., 2006. Mangroves and their faunal associates in Kerala. Occasional paper No 246. Records of Zoological Survey of India. Zoological Survey of India, Calicut, Kerala, 81.

Rajendran, N., Kathiresan, K., 2000. Biochemial changes in decomposing leave of mangrove. Chemistry and Ecology, 17, 91–102.

Rajendran, N., Kathiresan K., 2004. How to increase juvenile shrimps in mangrove waters? Wetland Ecology and Management, 12, 179-188.

Rajendran, N., Kathiresan, K., 2007. Microbial flora associated with submerged mangrove leaf litter in India. Revista de Biología Tropical, 55, 393–400.

Ranjan, R. K., Routh, J., Ramanathan, A., Klump, J. V., 2011. Elemental and stable isotope records of organic matter input and its fate in the Pichavaram mangrove–estuarine sediments (Tamil Nadu, India). Marine Chemistry, 126, 163-172.

Rice, D. L., 1982. The detritus nitrogen problem: new observations and perspectives from organic geochemistry. Marine Ecology Progression Series, 9, 153–162.

Robertson, A. I., Duke, N. C., 1987. Mangroves as nursery sites — comparisons of the abundance and species composition of fish and crustaceans in mangroves and other near-shore habitats in tropical Australia. Marine Biology, 96, 193–205.

Rohmer, M., Bisseret, P., Neunlist, S., 1992. The hopanoids, prokaryotic triterpenoids and precursors of ubiquitous molecular fossils. In Biological Markers in Sediments and Petroleum (Edited by Moldowan J. M., Albrecht P. and Philp R. P.), Prentice-Hall, Englewood Cliffs, New York.

Rohmer, M., 1993. The biosynthesis of triterpenoids of the hopane series in the eubacteria: A mine of new enzyme reactions. Pure and applied chemistry, 65, 1293–1298.

Rojas, A., Holguin, G., Glick, B. R., Bashan, Y., 2001. Synergism between Phyllobacterium sp.(N2-fixer) and Bacillus licheniformis (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microbiology Ecology, 35, 181-187.

Rönnbäck, P., 1999. The ecological basis for economic value of seafood production supported by mangrove ecosystems. Ecological Economics, 29, 235–252.

Schauer, J. J., Rogge, W. F., Hildemann, L. M., Mazurek, M. A., Cass, G. R., Simoneit, B. R. T., 1996. Source apportionment for airborne particulate matter using organic compounds as tracers. Atmospheric Environment, 30, 3837–3855.

Simoneit, B. R. T., 1977a. Diterpenoid compounds and other lipids in deep-sea sediments and their geochemical significance. Geochimica et Cosmochimica Acta, 41, 463–476.

Simoneit, B. R. T., 1977b. The Black Sea, a sink for terrigenous lipids. Deep-Sea Research, 24, 813–830.

Simoneit, B. R. T., Mazurek, M. A., 1982. Organic matter of the troposphere—II. Natural background of biogenic lipid matter in aerosols over the rural Western United States. Atmospheric Environment, 16, 2139–2159.

Simoneit, B. R., Grimalt, J. O., Wang, T. G., Cox, R. E., Hatcher, P. G., Nissenbaum, A., 1986. Cyclic terpenoids of contemporary resinous plant detritus and of fossil woods, ambers and coals. Organic Geochemistry, 10, 877-889.

Simoneit, B. R. T., 1989. Organic matter of the troposphere—V: Application of molecular marker analysis to biogenic emissions into the troposphere for source reconciliations. Journal of Atmospheric Chemistry, 8, 251–275.

Simoneit, B. R. T., 1995. Evidence for organic synthesis in high temper- ature aqueous media—facts and prognosis. ISSOL 1993. Origins of Life and Evolution of Biospheres, 25, 119–140.

Simoneit, B. R. T., 1998. Biomarker PAHs in the environment. In: Hutzinger O, editor-in-chief, Neilson AH, editor. The handbook of environmental chemistry. PAH sand related compounds. Vol. 3. Part I. Springer- Verlag, Berlin

Simoneit, B. R., Schauer, J. J., Nolte, C. G., Oros, D. R., Elias, V. O., Fraser, M. P., Rogge, W. F., Cass, G. R., 1999. Levoglucosan, a tracer for cellulose in biomass burning and atmospheric particles. Atmospheric Environment, 33, 173-182.

Simoneit, B. R. T., 2005. A Review of Current Applications of Mass Spectrometry For Biomarker/ Molecular tracer elucidations. Mass Spectrometry Reviews, 24, 719-765.

Siple, M. C., Donahue, M. J., 2013. Invasive mangrove removal and recovery : Food web effects across a chronosequence. Journal of Experimental Marine Biology and Ecology, 448, 128-135.

Spalding, M. D., Blasco, F., Field, C. D., 1997. World Mangrove Atlas. International Society for Mangrove Ecosystems, Okinawa, Japan.

Streibl, M., Herout V., 1969. Terpenoids-especially mono-, sesqui-, di- and triterpanes. In Organic Geochemistry--Methods and Results (Edited by Eglinton G. and Murphy M. T. J.), Springer, Berlin.

Summons, R. E., 1993. Biogeochemical cycles: a review of fundamental aspects of organic matter formation, preservation and composition. In: Engel MH, Macko SA, editors. Organic geochemistry, principles and applications. Plenum Press, New York.

Summons, R. E., Jahnke, L. L., Roksandic, Z., 1994. Carbon isotopic fractionation in lipids from methanotrophic bacteria: relevance for interpretation of the geochemical record of biomarkers. Geochimica et Cosmochimica Acta, 58, 2853-2863.

Tan, Y. L., Heit, M., 1981. Biogenic and abiogenic polynuclear aromatic hydrocarbons in sediments from two remote Adirondack lakes. Geochimica et Cosmochimica Acta, 45, 2267–2279.

ten Haven, H. L., Rohmer, M., Rullko¨tter, J., 1992. Early diagenetic transformation of higher-plant triterpenoids in deep-sea sediments from Baffin Bay. Geochimica et Cosmochimica Acta, 56, 2001–2024.

Tenore, K. R., 1977. Growth of Capitella capitata cultures on various levels of detritus derived from different sources. Limnology and Oceanography, 22, 936–941.

Tenore, K. R., Cammen, L., Findlay, S. E. G., Phillips, N., 1982. Perspectives of research on detritus: do factors controlling the availability of detritus to macro-consumers depend on its source? Journal of Marine Research, 40, 473–490.

Toledo, G., Bashan, Y., Soeldner, A., 1995. In vitro colonization and increase in nitrogen fixation of seedling roots of black mangrove inoculated by a filamentous cyanobacteria. Canadian journal of microbiology, 41, 1012-1020.

Twilley, R. R., Lugo, A. E., Pattersonzucca, C., 1986. Litter production and turnover in basin mangrove forests in southwest Florida. Ecology, 67, 670–683.

Twilley, R. R., Chen, R. H., Hargis, T., 1992. Carbon sinks in mangroves and their implications to carbon budget of tropical coastal ecosystems. Water, Air and Soil Pollution, 64, 265-288.

Versteegh, G. J. M., Schefuß, E., Dupont, L., Marret, F., Sinninghe Damste, J.S., Jansen, J.H.F., 2004.Taraxerol and Rhizophora pollen as proxies for tracking past mangrove ecosystems. Geochimica et Cosmochimica Acta, 68, 411–422.

Volkman, J. K. 1986. A review of sterol markers for marine and terrigenous organic matter. Organic Geochemistry, 9, 83–99.

Volkman, J. K., Barrett, S. M., Dunstan, L. G A., Jeffrey, S. W., 1993. Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom, Organic Geochemistry, 20, 7-15.

Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L., Gelin, F., 1998. Microalgal biomarkers: a review of recent research developments. Organic Geochemistry, 29, 1163–1179.

Volkman, J. K., 2006. Marine OM: Biomarkers, isotopes and DNA; Springer-Verlag, Heidelberg.

Wakeham, S. G., Schaffner, C., Giger, W., 1980. Polycyclic aromatic hydrocarbons in recent lake sediments-II. Compounds derived from biogenic precursors during early diagenesis. Geochimica et Cosmochimica Acta, 44, 415– 429.

Wang, T. G., Simoneit, B. R.T., 1990. Organic geochemistry and coal petrology of Tertiary brown coal in Zhoujing Mine, Baise Basin, South China: 2. Biomarker assemblage and significance. Fuel, 69, 12–20.

Wang, Y., Huang, Y., Qiu, Q., Xin, G., Yang, Z., Shi, S., 2011. Flooding greatly affects the diversity of *arbuscular mycorrhizal* fungi communities in the roots of wetland plants. PLoS ONE, 6, 24512.

Weng, B., Xie, X., Yang, J., Liu, J., Lu, H., Yan, C., 2013. Research on the nitrogen cycle in rhizosphere of Kandelia obovata under ammonium and nitrate addition. Marine Pollution Bulletin, 76, 227-240.

White, D. C., Ringelberg, D. B., Macnaughton, S. J., Alugupalli, S., Schram, D., 1997. Signature lipid biomarker analysis for quantitative assessment in situ of environmental microbial ecology. In: Eganhouse RP, editor. Molecular markers in environmental geochemistry. American Chemical Society, Symp Ser, 671, 22–34.

Wolters, O. W., 1979. A note on Sungsang village at the estuary of the Musi river in southeastern Sumatra: A reconsideration of the historical geography of the Palembang region. Indonesia, 33-50.

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Chapter 2

MATERIALS AND METHODS

Contents

2.1 Description of Study Area
2.2 Sampling
2.3 Analytical Methodology
2.4 Statistical Analysis of Data

2.1 Description of Study Area

The mangrove ecosystems mainly restricted to the tropical and sub-tropical zones, between latitude 32^0 N and 33^0 S (Macintosh, 1984). World's dominant mangrove zones are constrained by Indo-West Pacific region, and are largely distributed in South-East Asia, North-East Australia and south East Africa (Naskar and Mandal, 1999). A "World Mangrove Atlas" has been constructed delineating the geographical distribution of existing mangroves around the world (Spalding, 2010) and it roughly covers 137,760 km^2 along the coasts of the world (Giri et al., 2011). Rodin, et al. (1975), estimated that mangrove area in Asia and Oceania is 478 X 10^5hectare, excluding those from many Oceanic Islands and in the Indian sub-continent, the Sundarbans comprises about 60% and the rest is distributed in the Bay Islands and 8 maritime States of India (Naskar and Mandal, 1999; Mukhopadhyay, 2000).

There are only a few comprehensive baseline studies which have documented the status of mangroves in Kerala. It has been reported that, in the recent past Kerala, had 70,000 ha under mangrove cover (Mohanan, 2004). Wetlands around the central region have been lost over years due to reclamation, conversion to industrial use, dumping of solid waste, discharge of untreated sewage from municipal waste, effluents from industries and encroachment for construction. A recent study by Radhakrishnan et al., 2006 pointed out mangrove vegetation in four northern districts of Kerala - Kasargod, Kannur, Kozhikode and Malappuram - is approximately 3,500 hectare, which represents about 83 % of mangrove cover in the State (Manju et al., 2012). Kannur District has the major share of it. The mangrove wetlands of Northern Kerala coast, containing 14 true mangrove species and 40 mangrove associates, has been the main focus of the present study.

A reconnaissance survey was conducted to find the true mangrove forest systems and five sampling sites were identified (Fig. 2.1 and Table 2.1) as Kunjimangalam, Pazhayangadi, Pappinissery, Thalassery and Kadalundi. Kunjimangalam is the estuarine environment formed by Pullamcode River and Kunjimangalam River (station 1) which is located 2 km away from coastline. Abundant species diversity is found in the Kunjimangalam belt, 25 species of mangrove associates are also observed in these regions. Many areas, including Kunjimangalam, still have a vast extent of mangroves that are untouched. Kunjimangalam is rich in rare species of mangroves including *Avicennia sp.*, *Excoecaria sp.*, *Rhizophora sp.* and *Lumnitzera racemosa*. Pazhayangadi (station 2) is situated about 3-4 km away from coastline, and was found to be almost free from human activities. *Avicennia marina, Avicennia officinalis, Aegiceras corniculatium* and *Rhizophora mucronata* are the major species found at this station. Pappinissery (station 3) is at a distance of 4 to 5 km from the coastline and the sources of contamination here are mainly attributed to urban and sewage runoff. Out of the 12 species of true mangrove plants (*Avicennia officinalis, Avicennia marina, Rhizophora mucronata, Rhizophora apiculata, Sonneratia caseolaris, Sonneratia alba, Aegiceras corniculatum, Excoecaria agallocha, Kandelia candel, Bruguiera cylindrica, Bruguiera sexangula* and *Lumnitzera racemosa*) observed, 5 are in common occurrence in most of the places while 3 species are very rare. Thalassery (Station 4), where mangrove area spans over 0.313 sq kilometres, which is in the vicinity of estuarine mouth, has large population of *Rhizophora, Avicennia officinalis, Acanthus illcifolius, Excoecaria agallocha, Aegiceras corniculatum* and *Thespesia populnea*. Kadalundi estuary, a notable destination for migratory birds in Kerala, the mangrove cover is estimated to be 28 ha with ten mangrove species. It is situated near the bar mouth of Kadalundi river consist of mangrove plants such as *Rhizophora mucronata, Excoecaria agallocha, Aegiceros corniculatum,* and *Acanthus ilicyfolius* etc. (CMFRI, 2002; Manju et al., 2012). Thalassery and Kadalundi are situated in the close proximity of Arabian Sea and are subjected to the semi-diurnal tidal action in the range 0.09 to 1.48 m.

Table 2.1 Details of sampling sites selected for the study

Sl No	Station Name	Latitude	Longitude
1	Kunjimangalam	12^0 03' 14"	75^0 14' 28"
2	Pazhayangadi	12^0 01' 39"	75^0 16' 17"
3	Pappinissery	11^0 56' 8"	75^0 21' 13"
4	Thalassery (Dharmadam)	11^0 45' 59"	75^0 28' 52"
5	Kadalundi	11^0 07' 51"	75^0 49' 58"

2.2 Sampling

Sediments and water samples were collected from 5 locations in Calicut, Kannur districts during October 2009, May 2010 and August 2010 representing post-monsoon pre-monsoon and monsoon, seasons respectively. Among these seasons, monsoon period accounts for the 65 - 70% of total annual rainfall, and in pre-monsoon the rainfall is minimum (Aype, 2005).

Water samples were collected using a clean plastic bucket, transferred to clean plastic bottles and transported to the laboratory on ice and stored in a deep freezer (-20^{0}C) till analysis. Sediment samples were collected using a Van Veen grab (0.042 m^2). Samples were collected in triplicate from each station and the average value for each parameter was reported. pH measurements and fixing of water samples for dissolved oxygen were done in situ and other parameters were determined in the laboratory without delay.

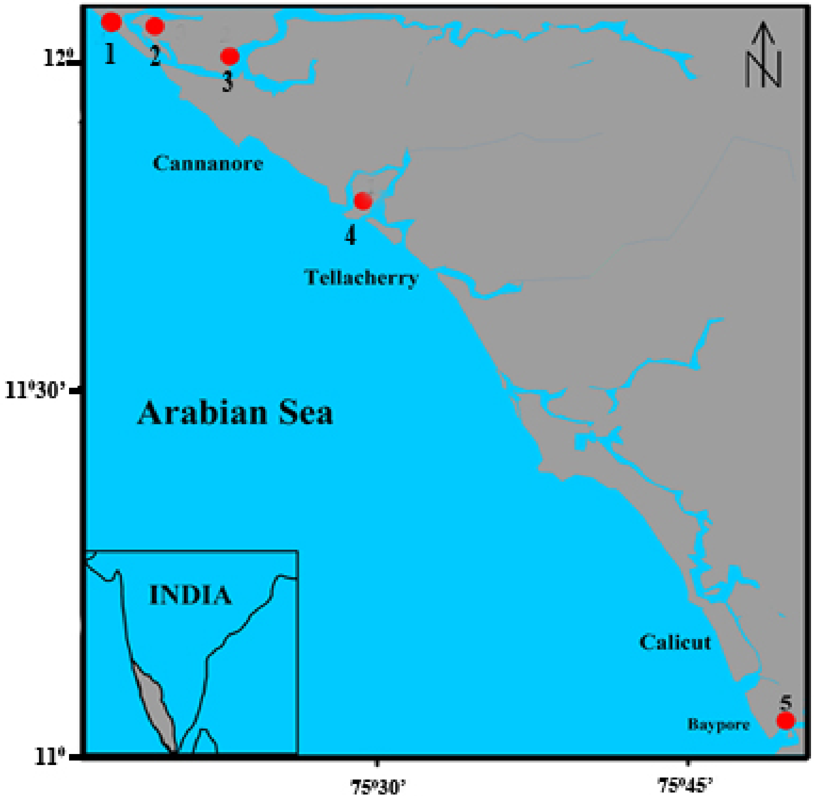

Fig. 2.1 Map showing sampling stations

2.3 Analytical Methodology

2.3.1 General Water and Sediment Characteristics

The water samples were analysed following methods suggested by Grasshoff, 1999 and APHA, 1999. All the colorimetric estimations were done using double beam spectrophotometer (Analytik jena Specord 200).

pH was measured using a portable pH meter (Eutech pH tester 10). Salinity was computed from chlorinity values, estimated by Argentometric (Mohr-Knudsen) titration. The Winkler's method was employed for estimation of dissolved oxygen. Nitrite was estimated by colorimetric method after complexation with N-naphthyl ethylene diamine and sulphanilamide. Nitrate was reduced to nitrite in cadmium column and estimated spectrophotometrically. Estimation of ammonia and phosphate were carried out using indophenol blue and molybdenum blue methods respectively. For the estimation of total nitrogen, the sample was first oxidized with alkaline persulphate and estimated as nitrite after reduction (Strickland and Parsons, 1972; Grasshoff, 1999).

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). Vario EL III CHN-S Analyser was used for the analysis of Total Nitrogen (TN) and Total Sulphur (TS). For the analysis of Total Organic Carbon (TOC), the sample was pre-treated using 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 to remove salts and finally freeze-dried and analysed using CHN-S analyser. Samples were run with blank cups in order to correct the carbon and nitrogen associated with tin/silver cups and the elemental analyser was calibrated using acetanilide standards. The detection limits for TOC and TN are 0.07 and 0.01 % respectively.

2.3.2 Sequential extraction of sedimentary phosphorus

The sequential extraction scheme by De Groot and Golterman (1993) 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.

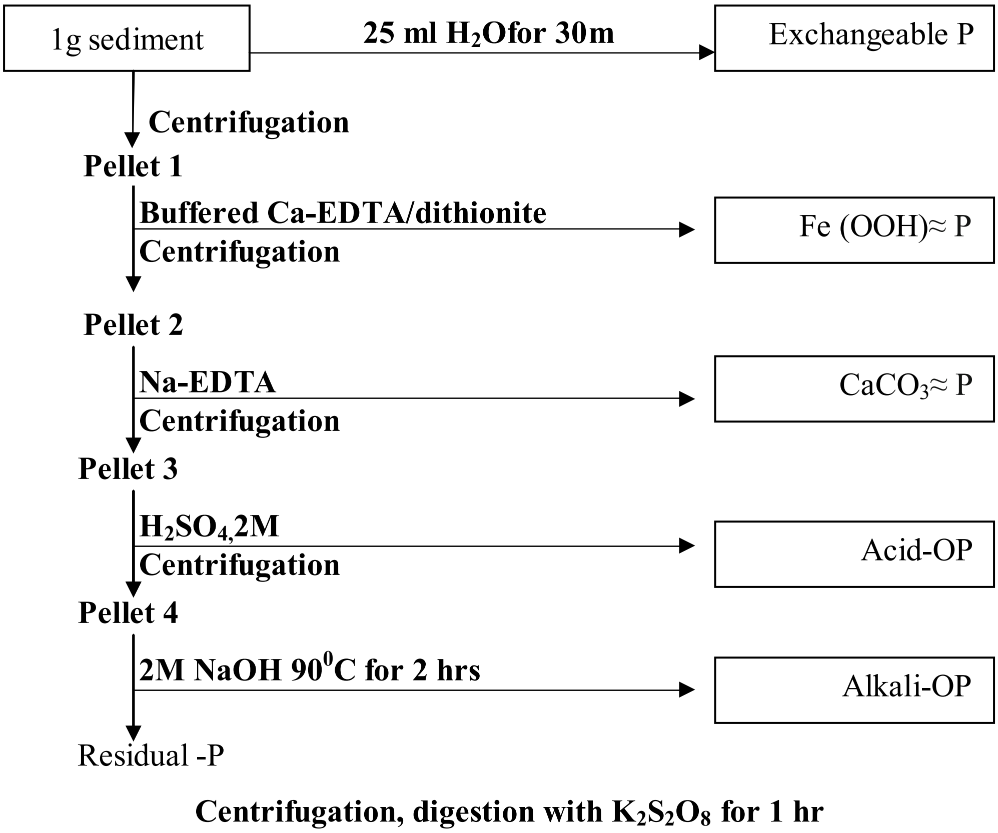

Fig. 2.2 Extraction scheme for the phosphorus fractionation in surface sediments

The extraction scheme is given in Fig. 2.2. Exchangeable phosphorus was extracted using distilled water. Iron-bound 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 $CaCO_3{\approx}P$.

In the next step, acid soluble organic P (Acid-OP) was extracted with H_2SO_4 and then the alkali soluble organic P (Alkali-OP) with 2 M NaOH at 90^0C for 2 hrs. Residual organic phosphorus (R-OP) 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 were expressed on the dry weight basis.

2.3.3 Biochemical Composition

Total protein (PRT) analysis was carried out after extraction with NaOH (1M, 2hrs at 80^0C) and determined according to Hartree (1972) modified by Rice (1982) using bovine serum albumin (BSA) as the standard. Carbohydrate was extracted using 2M H_2SO_4 (2hrs at 100^0C) and were determined as glucose equivalents, described by Gerchacov and Hatcher (1972). Total lipids (LPD) were expressed as cholesterol equivalents, determined by extracting 1g freeze-dried homogenised sediment samples at 60^0C (30 min), using 30 ml 2:1 chloroform: methanol (Bligh and Dyer, 1959) and analysed by the method of Barnes and Blackstock (1973). Tannin and lignin in sediments were extracted using 0.05M NaOH at 60^0 for 90 minutes and estimated spectrophotometrically by the sodium tungstate-phosphomolybdic acid method (Nair et al., 1989; APHA, 1995), using tannic acid as the standard. Blanks for each analysis were performed with pre-combusted sediments at $450\text{-}480^0C$ 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 biopolymeric carbon (BPC), a reliable estimate of the labile organic matter expressed as the sum of protein, lipid and carbohydrate carbon equivalents (Fabiano et al., 1995) was also determined.

Chlorophyll and Pheopigments (Pheo) were then measured according to the method of Lorenzen (1967) following the extraction with 90% acetone (24 hrs in the dark at 4^0C). After centrifugation, the supernatant was used for the determination of Chlorophyll a (Chl a), Chlorophyll b (Chl b) and Chlorophyll c (Chl c) and then acidified with 0.1 N HCl to estimate the amounts of Pheopigments (Plante-Cuny, 1974).

Also, the protein to carbohydrate (PRT: CHO) and lipid to carbohydrate (LPD:CHO) ratios were calculated and used as indicators of biochemical degradation processes (Galois et al., 2000).

2.3.4 Stable Carbon Isotope Analysis

Stable carbon isotope analysis of Total Organic Matter (δ^{13}C) was carried out using Flash EA interfaced with IRMS (FINNIGAN DELTA PLUS XP, Thermo Electron Corporation) at IIT, Kharagpur. Before the analysis, sediment sample was treated with 2N HCl to remove inorganic carbon present in the sample and was washed with distilled water until traces of chlorine removed. Stable carbon isotope abundances are presented as δ^{13}C values and are expressed relative to the PDB (Pee Dee Belemnite) standard:

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

Where δ ‰ stands for δ^{13}C, and R_{sample} and $R_{reference}$ are the isotopic ratios of the sample and reference, respectively.

2.3.4 Extraction of Molecular Biomarkers

The method described by Harvey (1994) was selected for the biomarker analysis. Freeze dried sediment samples were soxhlet extracted for 72 hrs with a mixture of 2:1 dichloromethane (DCM): methanol (MeOH). The extracts were combined and evaporated to dryness using rotary evaporation. The extracted residue was subjected to mild alkaline hydrolysis by refluxing using 6 % methanolic KOH (70 ^{o}C for 6 hours). After the sample was cooled, the neutral lipids were partitioned from the alkaline solution into hexane, which was separated and used for the analysis of neutral lipids. The neutral fraction was separated into individual compound classes using silica gel column chromatography by eluting with hexane, hexane and DCM mixture (1:1), dichloromethane and methanol, respectively viz. saturated hydrocarbons, aromatic hydrocarbons, and polar compounds (Otto and Simoneit, 2001). The fractions were dried under nitrogen. The fraction eluted with dichloromethane was converted to trimethyl silyl derivative using N, O-Bis(trimethyl silyl) trifluroacetamide (BSTFA) and pyridine for 3 hrs at 70°C.

The remaining aqueous layer containing the fatty acid salts was acidified to pH 2. Fatty acids in this polar lipid fraction were recovered separately into DCM. The polar lipid fraction containing the fatty acids was evaporated to dryness using rotary evaporation. It is then converted to fatty acid methyl esters (FAMEs) by treating with 10 ml of 12% BF_3/MeOH (Sigma Aldrich) (70^{o}C for 30 minutes). The FAMEs were subsequently extracted from the reaction solution using DCM. The DCM layer was evaporated to dryness, and the extract was then re-dissolved in 1ml acetone for chromatographic analysis.

2.3.4.1 Gas chromatography-flame ionisation detector (GC-FID) analysis of n-alkanes

The n-alkanes were separated from neutral fraction by silica column chromatography (Otto and Simoneit, 2001). The alkanes fraction was then evaporated to 1ml under ultra high purity nitrogen prior to concentration determination on gas chromatograph (GC-FID) equipped with a 30m×0.25mm i.d. DB-5 column, 0.25 µm film thickness. Oven temperature was held at 50^0C for 5 min and then increased to 300^0C at 3°C/min and kept at 300^0C for 5m. The injector temperature was held at 250^0C with detector temperature of 325 ^{0}C; N_2 was the carrier gas with flow rate 2 ml/min. Identification of individual compounds was achieved by comparison of GC retention times with those of standard compounds. Quantification was made in comparison with authentic standards (C_7-C_{40} Sigma Aldrich).

2.3.4.2 Gas chromatography-Mass spectrometry (GC-MS) analysis of hydrocarbons

The hydrocarbon fraction eluted with n-Hexane was evaporated to 1ml under ultra high purity nitrogen prior to concentration determination by GC-MS using a Perkin Elmer Clarus GC 620 GC, equipped with MS detector and 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 held at 40°C for 3min and was increased to 180°C at a rate of 4°C per min and held for 5 min. Then the temperature was again increased to 290°C at a rate of 4°C per min and held for 10 min. The detector was held at 290°C and helium was used as the carrier gas. Full data acquisition was obtained with the use of MS Turbo Mass version 5.3.2. Branched hydrocarbons, alkenes, n-alkyl cyclohexane (m/z 83) and hopanes (m/z 191) were identified from the mass spectra. Compounds are identified by comparing mass spectra obtained with literature and library data and interpretation of mass spectrometric fragmentation patterns

2.3.4.3 Gas chromatography-Mass spectrometry (GC-MS) analysis of fatty acids

FAMEs analysis was carried out by gas chromatography-mass spectrometry (GC-MS) using a Perkin Elmer Clarus GC 620 GC, equipped with MS detector and a non-polar HP ultra-double-fused silica capillary column (30 m, 0.32 mm internal diameter, 0.25 mm film thickness). Operating conditions were as follows: ion source of 200°C electron voltage 70 eV. Spectra were scanned from 50 to 600 m/z with a scan time of 1.50 s. A two-step temperature program was used: from 50°C to 200°C at 2°C per min and held for 5min. Then the temperature was again increased from 200°C to 280°C at 10°C per min. It is then again held 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, Bacterial Acid Methyl Esters BAMEs-47080-U). Sample FAMEs were also injected in the above-mentioned condition and their concentrations were determined from the calibration plot.

2.3.4.4 Gas chromatography-Mass spectrometry (GC-MS) analysis of triterpenoids and fatty alcohols

Analysis was carried out by GC-MS using a Perkin Elmer Clarus GC 620 GC, equipped with MS detector and 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).

2.4 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. The log (x+1) transformation was used to obtained normal distribution of the data set. Factor analysis was used to extract information from the most meaningful parameters (Katsaounos et al., 2007) and varimax rotation was used for deriving Principal Components (PC). Factor loadings were considered significant if the components were >0.50.

References

APHA, 1995.Standard methods for the examination of water and wastewater, American Public health association. Washington DC.

APHA, 1999. Standard methods for examination of water and waste water. American Public health association. Washington DC.

Aype Thomas P, 2005. South-West Monsoon Rainfall of Kerala and its Variability. Thesis submitted to the Cochin University of Science And Technology, India.

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animal tissues: detailed investigation of the sulphophosphovanillin method for "total" lipids. Journal of Experimental Marine Biology and Ecology, 12,103–118.

Bligh, E. G., Dyer, N. J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911–917.

CMFRI, 2002. Central Marine Fisheries Research Institute, Technical extension series, Marine Fisheries Information Services Technical Extension Series, 172.

De Groot, K. J., Golterman, H. L., 1993. On the presence of organic phosphate in some Camargue sediments: evidence for the importance of phytate. Hydrobiologia, 252, 105–116

Fabiano, M., Danovaro, R., 1994. Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. Hydrobiologia, 277, 71–84.

Fabiano, M., Danovaro, R., Fraschetti, S., 1995. A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediments of the Ligurian Sea (northwestern Mediterranean). Continental Shelf Research, 15, 1453–1469.

Folk, R. L., 1980. Petrology of sedimentary rocks. Hemphill Publishing Company, Austin, Texas

Galois, R., Blanchard, G., Seguignes, M., Huet, V., Joassard, L., 2000. Spatial distribution of sediment particulate organic matter on two estuarine mudflats: a comparison between Marennes-Oléron Bay (France) and the Humber estuary. Continental Shelf Research, 20, 1199-1217.

Gerchacov, S. M., Hachter, P. G., 1972. Improved technique for analysis of carbohydrates in sediment. Limnology and Oceanography, 17, 938–943.

Giri, C., Ochieng, E., Tieszen, L. L., Zhu, Z., Singh, A., Loveland, T., Duke, N., 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecology and Biogeography, 20, 154-159.

Grasshoff, K., Ehrhardt, M., Kremling, K.. 1999. Methods of Seawater Analysis. Verlag Chemie, Weinheim.

Hartree, E. F., 1972. Determination of proteins: a modification of the Lowry method that give a linear photometric response. Analytical Biochemistry, 48, 422-427.

Harvey, H. R., 1994. Fatty acids and sterols as source markers of organic matter in sediments of the North Carolina continental slope. Deep Sea Research II, 41, 783–796.

Katsaounos, C. Z., Giokas, D. L., Leonardos, J. D., Karayannis, M. I., 2007. Speciation of phosphorus fractionation in river sediments by explanatory data analysis. Water Research, 41, 406–418.

Logan, G. A., Eglinton, G., 1994. Biogeochemistry of the Miocene lacustrine deposit, at Clarkia, northern Idaho, U.S.A. Organic Geochemistry, 21, 857-870.

Lorenzen, C. J., 1967. Determination of chlorophyll and pheo-pigments by spectrophotometricequations. Limnology and Oceanography, 12, 343–346.

Macintosh, D. J. 1984 Ecology and productivity of Malaysian mangrove crab populations (Decapoda: Brachyura). In Proceedings of the Asian Symposium on Mangrove Environment, Research and Management (Soepadmo, E., Rao, A. N. & Macintosh, D. J., eds). University of Malaya and UNESCO.

Manju, M. N., Resmi, P., Gireesh Kumar, T. R., Kumar, R., 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, 893-902.

Mohanan, C., 2004. "Conservation of the fragile ecosystem diversity of Kerala", Centre for Earth Science Studies. Internal Report, 381-398.

Mukhopadhyay, S. K., Jana, T. K., De, T. K., Sen, S., 2000.Measurement of exchange of CO_2 in mangrove forest of Sunderbans using micrometeorological method. Tropical Ecology, 41, 57-60.

Nair, R. R., Ittekkot, V., Manganini, S. J., Ramaswamy, V., Haake, B., Degens, E. T., Desai, B. N., Honjo, S., 1989. Increased particle flux to the deep ocean related to monsoons. Nature, 338, 749-751

Naskar, K., Mandal. R., 1999. Ecology and biodiversity of Indian mangroves Part I Global status.Daya publishing House, New Delhi.

Otto, A., Simoneit, B. R. T., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz formation, Saxony, Germany. Geochimica et Cosmochimica Acta, 65, 3505–3527.

Philp, R. P., 1985. Fossil fuel biomarkers.Applications and Spectra, Elsevier, Amsterdam.

Plante-Cuny, M. R., 1974. Evaluation par spectrophotométrie des teneurs en chlorophyl-a fonctionelleer en phaeopigments des substrates meublesmarins. O.R.S.T.O.M. Nosy-Bé, 45

Radhakrishnan, C., Gopi, K. C., Palot, M. J., 2006. Mangroves and their faunal associates in Kerala.Occasional paper No 246. Records of Zoological Survey of India.Zoological Survey of India, Calicut, Kerala.

Rice, D. L., 1982. The detritus nitrogen problem: new observations and perspectives from organic geochemistry. Marine Ecology Progress Series, 9, 153–162.

Rodin, L. E., Bazilevich, N. I., and Rozov, N. N., 1975. Phytomass and annual productivity of the Earth's land area: in Reichle, D.E., Franklin, J.F., And Goodall, D.W., eds., Productivity of the World's Main Ecosystems: National Academy of Sciences, Washington, 13-26.

Spalding, M., 2010.World atlas of mangroves.Routledge.

Strickland, J. D. H., Parsons T. R., 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Bd., Canada.

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Chapter 3

WATER QUALITY PARAMETERS, GENERAL SEDIMENTARY CHARACTERISICS AND PHOSPHORUS FRACTIONATION

Contents

3.1 Water Quality Parameters
3.2 General Sedimentary Characteristics
3.3 Sequential Extraction of Sedimentary Phosphorus
3.4 Conclusion

3.1 Water Quality Parameters

3.1.1 Introduction

Mangrove ecosystems are considered to be the most complex ecosystems (Naskar and Mandal, 1999) for its ambience being rich in organic production and nutrients (Manson et al., 2005). Moreover, in mangrove water system, concentration gradient of dissolved compounds or nutrients with temporal and spatial variation become more complex, when primary production by microalgae is active (Harrison et al., 1997; Trott and Alongi, 1999). The nutrients such as inorganic phosphorus, nitrogen, potassium, and organic carbon are distributed to adjacent coastal, marine and terrestrial ecosystems through active and passive transport (Odum, 2000; Gonneea et al., 2004). Changes in proportions of dissolved nutrients in the mangrove water caused by supply from agricultural seepage, aquaculture effluents, human settlements, etc., may lead to changes in nutrient stoichiometric ratios (Si:N, N:P and

Si:P) and have its impacts on the coastal food web dynamics (Moncheva et al., 2001; Zhao et al., 2005). Hence, water quality parameters are indicative of various hydrodynamic processes in and around the system.

3.1.2 Results

Results of water quality parameters and ANOVA are shown in Table 3.1 and are depicted in Fig. 3.1. pH displayed significant seasonal variation ($P< 0.01$) and the lower values were observed in post-monsoon (avg.± stdv:7.24±0.11). Lower values of salinity were observed in monsoon except for Thalassery and Kadalundi and higher values in pre-monsoon season (avg. ± stdv:33.65±2.54 ppt). Except Kunjimangalam, (avg.±stdv: 5.64±3.20 mgL^{-1}) higher concentration of Dissolved Oxygen (DO) was observed in monsoon (avg.± stdv:5.81±1.42 mgL^{-1}) and lower concentration in pre-monsoon (avg.± stdv:4.52±3.35 mgL^{-1}). Alkalinity displayed significant seasonal variation ($P <0.01$), exhibiting much higher values in pre-monsoon (avg.± stdv:135.80 ± 18.78 mg $CaCO3L^{-1}$) and lower values in monsoon (avg.± stdv:42.49±24.51 mg $CaCO3L^{-1}$).

Nitrate, dissolved inorganic phosphate (DIP), Total Dissolved Nitrogen (TDN) and Total Dissolved Phosphorus (TDP), all of which recorded the highest concentration in monsoon, displayed a significant seasonal variation ($P<0.01$). Lower concentrations of nitrate and TDP were associated with pre-monsoon (except Thalassery), but DIP was lower in post-monsoon. Nitrite concentration ranged from 0.15 to 0.55 μmL^{-1} (avg.± stdv:0.28±0.16 μmL^{-1}) in post- monsoon, 0.29 to 0.99 μmL^{-1}(avg.± stdv:0.64±0.25 μmL^{-1}) in pre-monsoon and 0.31 to 0.99 μmL^{-1}(avg.± stdv:0.50±0.28 μmL^{-1}) in monsoon.

Table 3.1: Variations of Water Quality parameters and the results of ANOVA in the present study region

	Max	Min	Avg	Stdv	Spatial Variation		Seasonal Variation	
					F	P-value	F	P-value
pH	8.05	7.10	7.66	0.37	0.90	0.51	13.22	0.00**
salinity, ppt	35.97	0.24	16.09	14.36	1.16	0.40	27.09	0.00**
DO, mgL^{-1}	9.34	0.51	5.00	2.16	1.28	0.35	0.39	0.69
Alkalinity, mg CaCO3L^{-1}	167.33	22.31	77.91	46.39	0.30	0.87	25.72	0.00**
Nitrite, µML^{-1}	0.99	0.15	0.47	0.26	1.31	0.34	3.17	0.10
Nitrate, µML^{-1}	20.79	0.29	6.36	6.68	2.88	0.09	17.79	0.00**
Ammonia, µML^{-1}	98.09	ND	27.57	31.70	2.79	0.10	1.26	0.33
Phosphate, µML^{-1}	15.07	0.59	5.82	5.68	0.97	0.47	65.66	0.00**
Silicate, µML^{-1}	106.60	7.33	44.90	31.36	1.26	0.36	4.90	0.04*
TDN, µML^{-1}	188.38	10.26	88.35	61.71	0.61	0.67	21.13	0.00**
TDP, µML^{-1}	22.88	1.53	9.68	7.94	1.17	0.39	26.97	0.00**
Chl a, µgL^{-1}	40.86	ND	7.79	11.13	3.25	0.07	5.04	0.04*
Chl b, µgL^{-1}	6.00	ND	1.48	1.65	1.21	0.38	6.26	0.02*
Chl c, µgL^{-1}	13.80	ND	2.92	3.80	2.08	0.18	7.77	0.01**
pheopigments, µgL^{-1}	31.01	ND	10.74	11.22	3.72	0.05*	3.52	0.08
Mg, mgL^{-1}	1362.80	8.63	476.87	467.35	4.23	0.04*	39.31	0.00**
Ca, mgL^{-1}	453.13	8.02	123.19	125.03	1.06	0.43	9.53	0.01**
Na, mgL^{-1}	7400.00	50.00	2525.06	2536.57	1.97	0.19	45.03	0.00**
K,mgL^{-1}	320.00	3.30	103.85	101.77	1.47	0.30	21.67	0.00**
Li, mgL^{-1}	3.20	ND	1.08	1.11	1.04	0.44	19.54	0.00**
SO_4^{2-} mgL^{-1}	4166.67	12.00	1330.89	1499.53	2.36	0.14	39.66	0.00**
Iron mgL^{-1}	1.43	0.04	0.34	0.38	4.44	0.03*	0.34	0.72

*significant at 0.05 level, **significant at 0.01 level, Fcritical spatial variation3.84, Fcritical seasonal variation4.46

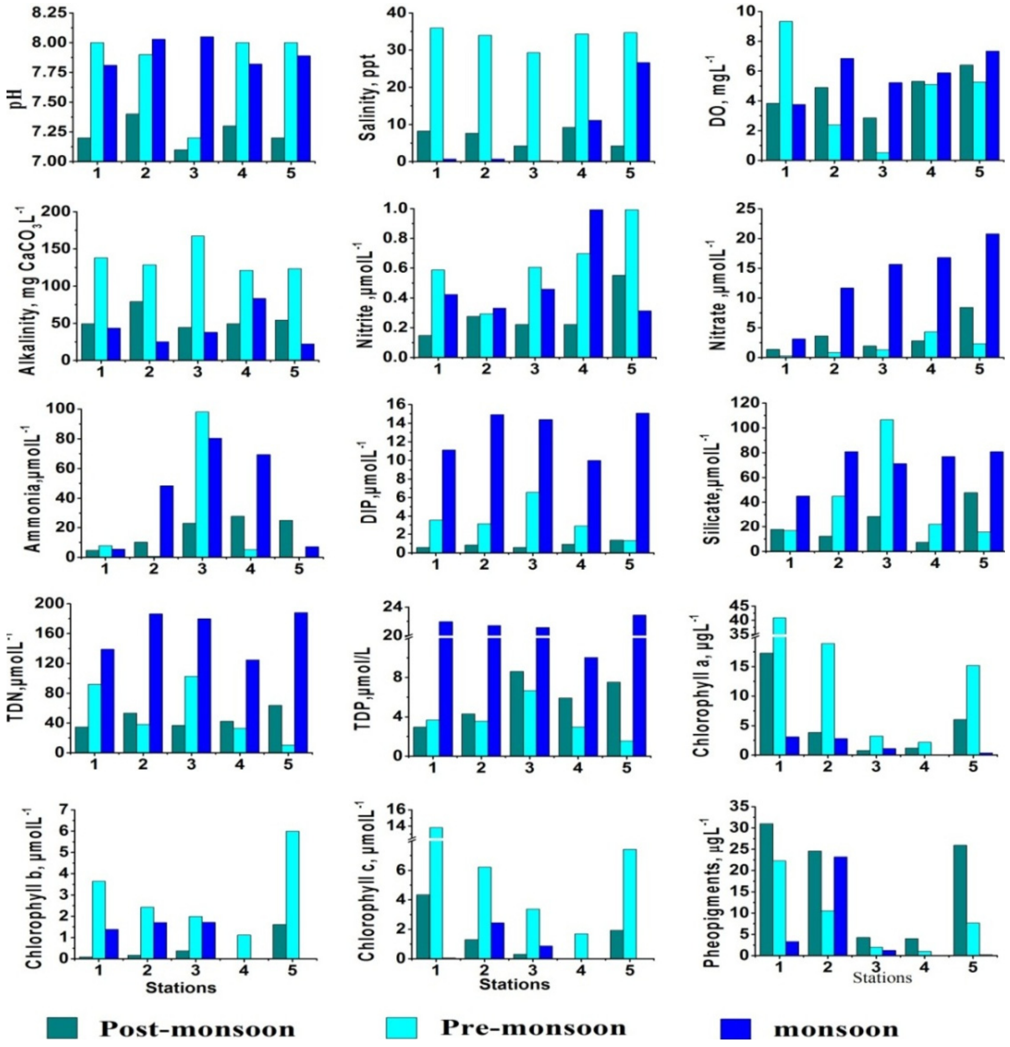

Fig. 3.1: Variation of water quality parameters in the present study region

Appreciably higher concentration of ammonia was reported from Pappinissery (avg.±stdv:67.10± 39.33 μmL^{-1}) and lower concentration from Kunjimangalam (avg.±stdv:6.04 ± 1.64 μmL^{-1}). DIP showed significant seasonal variations with much higher concentration in monsoon (avg.± stdv:13.09 ± 2.37 μmL^{-1}) and lower concentration in post-monsoon (avg.±stdv:0.86±0.32 μmL^{-1}). Silicate also followed the same trend except in Pappinissery. TDN was found to vary from 34.55 to 63.69 µmL^{-1} (avg.± stdv:46.17± 12.20 µmL^{-1}), 10.26 to 102.78 µmL^{-1} (avg.±stdv:55.19±40.10 µmL^{-1}) and 124.78 to 188.38 µmL^{-1} (avg.±stdv:163.68±29.67 µmL^{-1})in post monsoon, pre monsoon and monsoon respectively.

Significant seasonal variation for chlorophyll pigments were observed from ANOVA (P <0.05). The samples collected in pre-monsoon recorded comparatively higher chlorophyll pigments, but in monsoon lower concentration of Chl a and Chl c was reported and lower concentration of chl b was observed in post-monsoon. In post monsoon, comparatively higher Pheopigments were recorded in all stations. High value of Chl a was reported from Kunjimangalam (avg.±stdv:20.41±19.08 µmL^{-1}) followed by Pazhayangadi (avg.±stdv:8.52±9.01 µmL^{-1}). Highest concentration of pheopigments was observed at Pazhayangadi (avg.±stdv:19.44 ± 7.75µmL^{-1}) followed by Kunjimangalam (avg.±stdv:18.86 ± 14.16 µmL^{-1}).

3.1.3 Discussion

Salinity exhibited minimum values in monsoon due to freshwater runoff. The highest salinity was reported in Kunjimangalam, due to the landlocked nature of the sampling site. Compared to other stations, Thalassery and Kadalundi situated in the close proximity of Lakshadweep Sea, therefore prominent tidal activity can alter salinity of these systems. In aquatic systems, oxygenation is the result of the balance between the process of photosynthesis, degradation of organic matter, re-aeration (Granier et al., 2000), and physicochemical properties of water (Aston, 1980). The organic pollution by domestic sewage at Pappinissery resulted in the depletion of dissolved oxygen. The dissolved oxygen supersaturation

(132 %) was observed at Kunjimangalam (pre-monsoon) resulted from the phytoplankton photosynthesis and is confirmed by the higher chlorophyll content. Limited tidal rhythm and flushing also contributed to the dissolved oxygen super saturation. Dissolved gas supersaturation (DGS) can be produced in rivers and lakes which have high densities of plankton, aquatic plants, and algae (White et al., 1991).

According to Solanki et al., 2010, the proportion of different forms of nitrogen in any water body is determined by the balance between assimilation, mineralization, nitrification, denitrification and nitrogen fixation. Pappinissery received large quantity of domestic garbage and poultry waste, which resulted in the reducing environment and recorded elevated concentration of nutrients (especially NH_4^+) and low DO. In Pappinissery and Pazhayangadi, burrowing crabs produced sediment micro-pores which are reducing in nature resulting in the building-up of ammonium concentration, by the process of anaerobic ammonification (Smith et al., 1991).

In monsoon, concentration of nitrate and phosphate increases considerably due to land drainage and anthropogenic input. Faecal contamination and excreta of birds enhanced the phosphate concentration in these mangrove ecosystems. The lower content observed in post monsoon and summer seasons might be due to decreased runoff, adsorption to sediments and phytoplankton intake (Ramakrishnan et al., 1999). Inorganic phosphate (DIP) was positively correlated with pH (Table.3.2), which may be due to the release of phosphorus from iron and aluminium oxides by ligand exchange reactions (Boers, 1991) under favourable conditions. Weathering process and land run-off mainly contribute to silicate concentration in these mangrove ecosystems.

Maximum and minimum in the DIN: DIP ratio (dissolved inorganic nitrogen to dissolved inorganic phosphate ratio) was reported from Pappinissery (post monsoon) and Pazhayangadi (pre-monsoon season) respectively. Low values in pre-monsoon and monsoon are characterised by the nitrogen depletion, which might be accounted by the condition of slow regeneration of NO_3^-, when compared to PO_4^{3-}. In Pazhayangadi, major portion of TDN was in organic form because organic nitrogen from decaying plant remains are slowly converted to NH_4^+ by biological degradation (Valentina and Joseph, 2005). Sewage from excreta being richer in nutrients seemed to enhance eutrophication process in the study region. Sorption of nutrients into the sediment matrix followed by its absorption by trees and benthic microorganisms may reduce their concentrations in pore-water and make the mangrove forest an effective sink for nutrients (Alongi, 1996). From the interrelationships between nutrients (Table.3.2), it could be inferred that these nutrients originated from the same source.

Chlorophyll is considered as the most reliable index of phytoplankton biomass and the ratio of Chl a to pheopigments provides the first-hand information on the physiological status of phytoplankton. Higher pheopigments, compared to Chl a, in post-monsoon and monsoon seasons, indicated the presence of more detrital matter in these environments. This could be attributed to the decomposition of mangrove leaf litter and other organic matter, from the sediment and community structure, harbouring in the surrounding water (Tripathy et al., 2005). The pre-monsoon season showed the reverse trend due to the growth of phytoplankton in the high light intensity and low turbulent waters of mangrove ecosystems (Manju et al., 2012).

Table 3.2: Correlation matrix for the water quality parameters (n=15) in the mangrove ecosystems of northern Kerala Coast

Parameters	pH	Salinity	DO	Alkalinity	NO_2^-	NO_3^-	Ammonia	PO_4^{3-}	SiO_4^{2-}	TN	TP	Chl a	Chl b	Chl c	Pheo	DIN	Mg	Ca	Na	K	Li	So_4^{2-}	Fe
pH	1																						
Salinity	.35	1																					
DO	.36	.01	1																				
Alkalinity	.09	.79**	-.29	1																			
NO_2^-	.41	.41	.18	.51	1																		
NO_3^-	.37	-.28	.42	-.53*	.15	1																	
Ammonia	-.10	-.28	-.24	.06	.23	.36	1																
PO_4^{3-}	.56*	-.24	.17	-.4	.06	.77**	.44	1															
SiO_4^{2-}	.14	-.12	-.15	-.05	.16	.60**	.73**	.76**	1														
TDN	.42	-.33	.27	-.45	-.04	.75**	.48	.96**	.74**	1													
TDP	.34	-.5	.17	-.67**	-.2	.70**	.29	.91**	.60**	.91**	1												
Chl a	.21	.54*	.29	.48	.07	-.49	-.4	-.35	-.4	-.29	-.47	1											
Chl b	.44	.53*	.11	.55*	.53*	-.33	-.14	-.12	-.12	-.2	-.26	.56*	1										
Chl c	.28	.64**	.25	.62**	.22	-.48	-.28	-.31	-.29	-.28	-.48	.96**	.72**	1									
Pheo	-.25	-.18	.34	-.09	-.33	-.3	-.31	-.35	-.34	-.21	-.29	.49	.06	.41	1								
DIN	-.02	-.3	-.14	-.04	.25	.52*	.98**	.55*	.79**	.58*	.4	-.46	-.19	-.35	-.34	1							
Mg	.06	.79**	-.29	.92**	.42	-.48	.11	-.36	-.03	-.41	-.64**	.39	.33	.5	-.23	.01	1						
Ca	-.02	.64**	-.54*	.78**	.14	-.53*	-.03	-.41	-.03	-.49	-.58*	.35	.37	.43	-.18	-.13	.74**	1					
Na	.15	.84**	-.32	.92**	.34	-.52*	-.04	-.38	-.09	-.47	-.65**	.48	.44	.57*	-.17	-.14	.94**	.88**	1				
K	.09	.77**	-.43	.90**	.33	-.49	.03	-.37	-.01	-.46	-.63**	.37	.38	.47	-.23	-.06	.91**	.93**	.98**	1			
Li	.09	.77**	-.45	.90**	.4	-.43	.12	-.31	.08	-.41	-.58*	.32	.44	.45	-.30	.03	.91**	.91**	.96**	.98**	1		
So_4^{2-}	.35	.87**	.08	.81**	.53*	-.49	-.32	-.44	-.38	-.52*	-.68**	.68**	.69**	.78**	.00	-.38	.78**	.57*	.81**	.72**	.71**	1	
Fe	.27	-.25	.06	-.18	-.14	.04	.00	0.20	.07	.17	.19	-.09	.23	.02	.40	.01	-.33	-.07	-.21	-.18	-.21	-.16	1

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

The positive correlations obtained between salinity and cation concentrations pointed towards the same origin of investigated elements possibly by the intrusion of sea water. In Pappinissery, Pazhayangadi and Kunjimangalam seasonal patterns of measured parameters were modulated by precipitation, evaporation and temperature, but in Thalassery and Kadalundi along with these factors, tidal influx of sea water also had a profound influence. Negative correlation of calcium with DO in the present study, suggests the uptake of calcium by primary producers (Cohen et al., 1999). Sodium, potassium, lithium, calcium and sulfate contents varied seasonally while concentration of magnesium displayed both seasonal and spatial variation and iron exhibited a significant spatial variation only.

3.1.4 Principal Component Analysis

Principal component analysis (PCA), one of the best statistical techniques to identify different processes such as natural or anthropogenic flux operating in the system, is established by employing linear relationships from selected set of variables (Iyer et al., 2003). Principal components are eigenvectors formed from the linear combinations of original variables (Wunderlin et al., 2001) and Varimax rotation is applied to separate PC loadings so that the dispersion is maximum and the number of large and small coefficients are minimum (Richman, 1986). Major processes operating in the system may be identified as sea water intrusion, mangrove diagenesis, river runoff, primary production etc. Seawater intrusion and signatures of primary production may be inferred from the analysis of cations and anions and pigment analysis respectively.

The 85% of the total variance was explained by four factors. The first factor contributed 40.76% of the total variance (Table 3.3 and 3.4) and depicted strong positive loadings on all major elements, alkalinity and salinity, and negative loading on TDP. It suggested the intrusion of sea water was the important variable controlling the distribution of major elements. Negative loadings on TDP, which gave the information that higher concentration of TDP occurred in lower salinity waters, is attributed to increased precipitation and high runoff during the wet season (Valentina and Joseph, 2005).

Table 3.3: Total Variance Explained for water quality parameters using principal component analysis

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	10.66	48.46	48.46	10.66	48.46	48.46	8.97	40.76	40.76
2	3.45	15.70	64.16	3.45	15.70	64.16	3.64	16.53	57.29
3	2.91	13.24	77.40	2.91	13.24	77.40	3.57	16.21	73.50
4	1.67	7.61	85.01	1.67	7.61	85.01	2.53	11.51	85.01
5	0.89	4.03	89.03						
6	0.81	3.67	92.70						
7	0.62	2.82	95.52						
8	0.43	1.95	97.47						

The variables in the second factor were characterised by strong positive loadings on ammonia, DIP, silicate and total nitrogen and very weak negative loading on DO which accounted for 16.53 % of the total variance. Second factor can be considered as a consequence of the nutrient input from mangrove diagenesis. The third factor contributed 16.21% of the total variance exhibiting strong positive loadings on chlorophyll pigments. Chlorophyll pigments formed a separate group which gives the inference that nutrient concentrations have no control over primary production.

Table 3.4: Factor loading for Principal component analysis using Varimax

	Component			
	1	**2**	**3**	**4**
pH	-.097	.125	.212	.892
Salinity	.831	-.241	-.009	.207
DO	-.465	-.489	-.080	.600
Alkalinity	.896	-.016	.301	.062
Nitrite	.449	.205	-.003	.666
Nitrate	-.570	.283	-.492	.417
Ammonia	-.223	.612	-.361	-.263
Phosphate	-.325	.739	-.094	.512
Silicate	-.176	.886	-.050	.166
TDN	-.505	.673	-.193	.137
TDP	-.707	.588	-.302	.081
Chla	.284	-.351	.824	-.003
Chlb	.244	.158	.791	.365
Chlc	.409	-.149	.838	.103
Pheophytin	-.182	-.435	.728	-.357
Magnesium	.951	-.237	-.009	-.087
Calcium	.924	-.162	.088	-.169
Na	.951	-.250	.059	-.058
K	.959	-.199	.038	-.069
Li	.957	.098	.119	.100
Sulphate	.887	-.379	.150	.023
Fe	-.396	.004	.571	.033

Fourth factor reflected 11.51 % of the total variance that have a loading on pH, DO and nitrite. The positive influence of dissolved oxygen and pH on nitrification rates can be predicted by this factor.

The plot between PC1 and PC2 is drawn (Fig.3.2), in which the loadings of variables have been plotted. Nutrients (except nitrite) along with pH are present in the IVth quadrant. The results showed that major ions formed a group that reflected the sea water intrusion. Hence, autogenic production, mainly from mangrove litter diagenesis associated with pH variations is the major processes governing the concentration of nutrients.

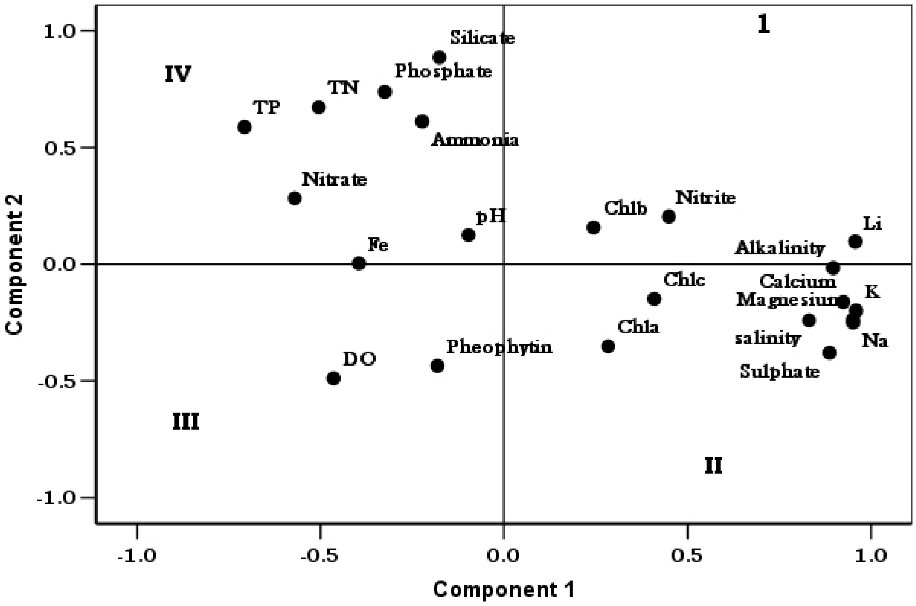

Fig. 3.2: Output of PCA analysis

3.2 General Sedimentary Characteristics

Textural characteristics along with bulk elemental analysis were carried out to evaluate the general sedimentary parameters of the system.

3.2.1 Bulk parameters-elemental composition

The abundance and ratios of important elements in biological cycles (e.g., C, H, N, O, S and P) provide a basic understanding of organic matter cycling. The Nitrogen (N) content reflects the nutritional status and the carbon (C) content reflects both energy flow and the material recycling, thus C and N are key elements for wetland biogeochemistry research (Bouillon et al., 2002; Zhou et al., 2007). Organic matter is usually quantified based on its total organic carbon (TOC) content and its profiles in a sedimentary sequence (Stein and Rack, 1992), provides direct evidence for changes in depositional patterns (Schulz and Zabel, 2009).

The bulk C information in combination with other biologically important elements (e.g., C-to-N ratio) was used for the investigation of source characterisation of sediments (Meyers, 1997). The characteristics C:N ratios of vascular plants (>20) and microalgae (5–7) are due to their carbohydrate-rich or protein-poor and protein-rich or carbohydrate- poor nature, respectively. Hence, elemental analyses were widely used for the source characterisation of mangrove ecosystems and some of the previous studies are given in Table 3.5.

3.2.2 Results

Results of variation of textural characteristics and ANOVA are shown in Table 3.6 and are depicted in Fig. 3.3. In the present study, textural characteristics showed significant spatial variation for sand and silt (P value<0.05) but no seasonal variation was observed. In pre-monsoon Kunjimagalam, Thalassery and Kadalundi were more sandy than post-monsoon, while Pappinissery and Pazhayangadi were shifted to clayey end of the tri-linear diagram. In monsoon Pappinissery again shifted to sandy end while Kadalundi shifted to clayey end.

The results of analysis of TOC (Total organic carbon), TN (Total Nitrogen) and TS (total Sulfur) are depicted in Fig. 3.4 and results of ANOVA are shown in Table 3.6. The behaviour of TOC and TN was almost similar and they displayed spatial variation (P value <0.01) in the present study. Pappinissery and Pazhayangadi showed higher concentration of TOC and TN.

Table 3.5: Previous works on elemental analysis of mangrove sediments

Parameter	Study Area	Concentration	Reference
C/N ratio	sea grass meadow in Arcachon Bay	7.1±0.4 to 15.0±2.9 %	Dubois et al., 2012
Corg	Pichavaram mangrove sediments	1.2 ± 0.52%	Ranjan et al., 2011
Ntotal		0.08±0.03%	
C/N ratio		16.6±4.1%	
C-org (%)	mangrove soils under R. mangle	2·80 %	Lacerda et al., 1995
Nitrogen (%)		0·16 %	
C/N		17·5 %	
TOC	mangrove-fringed coast in French Guiana	0.7 to 19 %	Marchand et al., 2003
Total N		around 0.1 %	
TS		0 to 1.2%	
SOC	Three stations from mangrove ecosystem in cochin	71.46 mg/g, 87.92 mg/g and 32.97 mg/g	Sebastian and Chacko, 2006

Table 3.6: Variations of general sedimentary characteristics and the results of ANOVA in the present study region

					Spatial Variation		Seasonal Variation	
	Max	**Min**	**Avg**	**Stdv**	**F**	**P-value**	**F**	**P-value**
sand, %	83.20	1.65	44.70	26.37	5.08	0.02*	0.32	0.73
Clay, %	80.06	15.99	34.74	17.86	3.28	0.07	0.53	0.61
Silt, %	42.52	0.81	20.55	15.15	3.88	0.05*	2.75	0.12
TOC, %	9.02	0.60	3.87	3.23	13.87	0.00**	1.78	0.23
TN, %	0.58	0.07	0.27	0.18	11.00	0.00**	1.09	0.38
TS, %	2.23	0.15	0.87	0.87	3.62	0.06	0.19	0.83
TOC/TS	28.73	2.60	7.63	7.30	0.94	0.49	0.97	0.42
TOC/TN	20.61	5.99	13.45	4.75	1.05	0.44	3.19	0.10
TOC/TN$_{corrected}$	27.69	9.60	17.40	5.74	1.10	0.42	0.31	0.85

*significant at 0.05 level, **significant at 0.01 level, Fcrtitical spatial variation3.84, Fcrtitical seasonal variation4.46

The concentration of TOC and TN in Pazhayangadi remains almost invariant throughout the study (avg. ± stdv:7.63 ± 0.18 % and 0.46 ± 0.05 for TOC and TN respectively). TS concentrations were below detectable limit for the sediment samples from Kunjimangalam in post-monsoon and pre-monsoon, and Kadalundi in pre-monsoon.

3.2.3 Discussion

3.2.3.1 Textural characteristics

Seven types of textural facies have been observed in the study area namely, sandy, clayey, clayey sand, sandy clay, silty clay and mixed type (more or less in equal proportion of sand, silt and clay). Among these, clayey sand followed by mixed type was the dominant one. Kunjimangalam in monsoon belongs to the sand domain while Pazhayangadi in pre-monsoon season occupies clayey domain. With the reduction of tidal flow and a decrease in turbulence, a higher amount of fine sediments would be expected to settle in Pappinissery and Pazhayangadi. Tidal rhythm modifies the sediments in Kadalundi mangrove ecosystem; hence it displayed greater variation in texture compared to other areas.

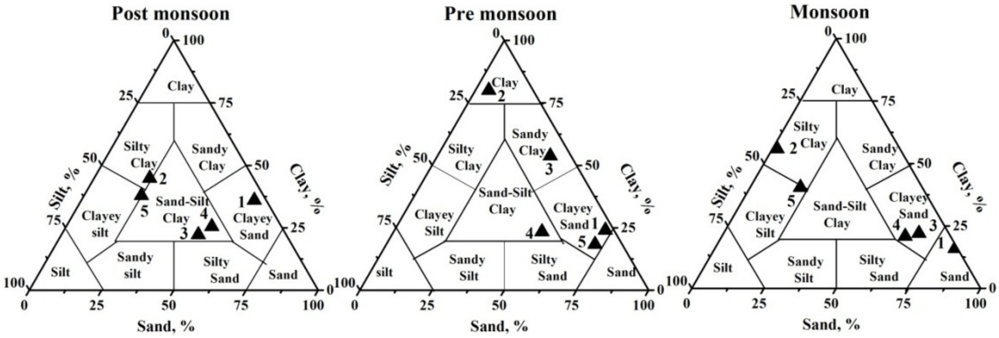

Fig. 3.3: Tri-linear diagram showing Textural facies in the present study region

3.2.3.2 Elemental composition

Elevated concentration of TOC may be related to decomposition of dead organisms and mangrove detritus, domestic sewage and anthropogenic inputs. Much higher concentration was observed in pre-monsoon at Pappinissery. Pazhayangadi also showed comparatively high concentration of TOC. The higher concentration of TOC associated with clayey nature of sediment, thick vegetation followed by large net primary production (Wang et al., 2013a). Along with these factors, restricted tidal activity may also contribute to higher TOC at Pappinissery and Pazhayangadi. Pappinissery and Kunjimagalam showed higher TOC in pre-monsoon and lower in monsoon. Manju et al., 2012 reported the presence of sewage pollution at Pappinissery, which received large quantity of domestic garbage and poultry waste, resulted in a reducing environment which recorded elevated concentration of nutrients (especially NH_4^+) in combination with low DO in water column. Hence, domestic sewage may also be a contributing factor at Pappinissery. TOC, in the present study, showed positive correlation with clay (R^2=0.69, n=15) and negative correlation with sand (R^2=-0.60, n=15). Accordingly, organic carbon in the sediment is controlled by textural attributes and largely associated with finer particles than the coarser.

On seasonal basis, Thalassery and Kadalundi displayed higher TOC in pre-monsoon and post-monsoon respectively. Thalassery and Kadalundi are situated in the close proximity of Lakshadweep Sea and are subjected to the semi-diurnal tidal action in the range 0.09 to 1.48 and caused observed variations in TOC. It creates conditions unfavourable to soil development, in particular by stripping the litter and remobilizing the organic particles and nutrients further downstream (Bailey and Guimond, 2009; Saint- Laurent et al., 2008). This was in line with lower TOC found for Thalassery and Kadalundi.

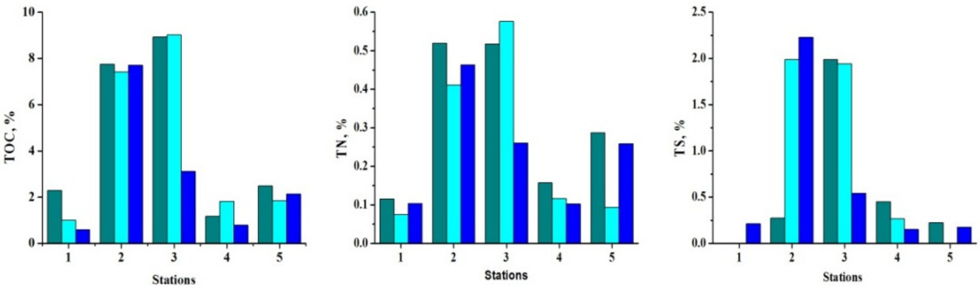

Fig. 3.4: Variation of elemental composition in the study region

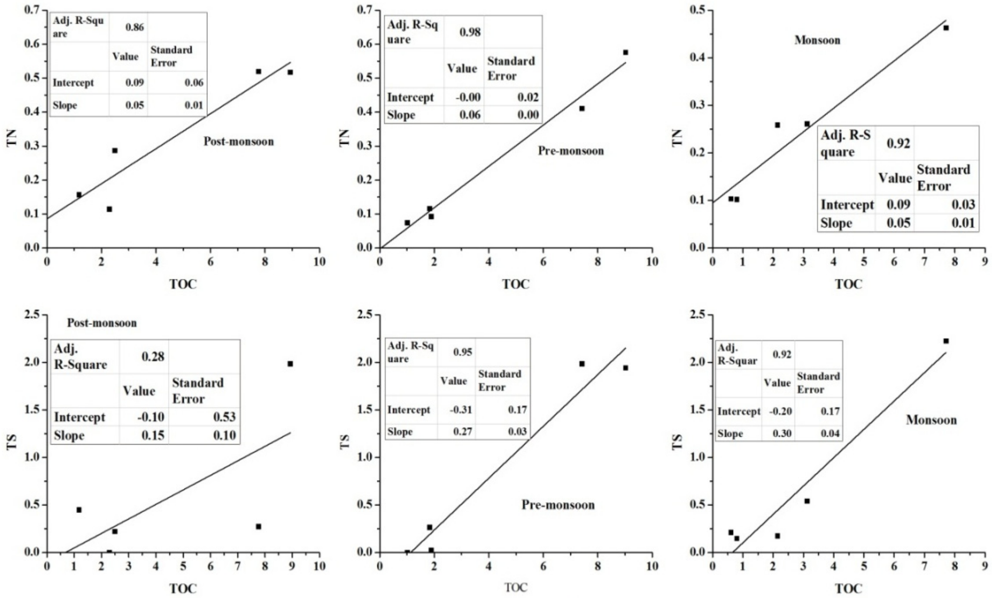

Fig. 3.5: Plot showing correlation of TOC with TN and TS

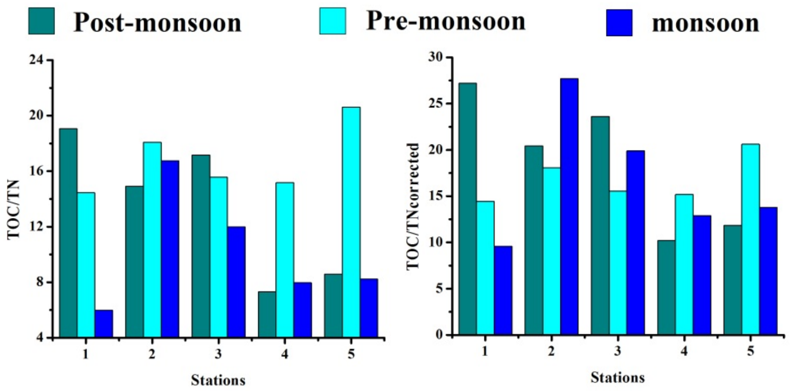

Fig. 3.6: Variation of TOC to TN ratio in the study region

TN displayed highly significant spatial variation ($p < 0.01$).Generally, soils richer in organic carbon have higher total nitrogen content also (Calvert, 2004; Brady and Weil, 2008). This might be due to the mineralization of organic matter (Cookson et al., 2005; Brady and Weil, 2008) and was found to be true for the present study also. The positive correlation between TOC and TN indicated that the quantity of organic carbon and nitrogen were similar during their removal or conversion (Ye et al., 2013). Moreover, it appears that the TOC and TN were related to clay as evident from their positive correlation. It might be thought that the large surface area of the clay can be accounted for significant adsorption of elements such as C and N (Àlvarez et al., 2003).

Pazhayangadi showed appreciably high concentration of TS in the monsoon season. TOC and TS was positively correlated in pre-monsoon ($R^2=0.95$, n=15) and monsoon ($R^2=0.92$, n=15) which might be due to the increased proliferation of sulfate-reducing bacteria with metabolizable organic matter (Leventhal, 1983; Berner and Raiswell, 1983). This was evident from the scatter plots of TOC (%) and TS (%) for pre-monsoon and monsoon as shown in the Fig.3.5. In post-monsoon, the relationship was not linear which indicate TOC and TS were independent of each other. In pre-monsoon the plot showed linear relationship (R^2=0.95). But when the scatter plot extrapolates to x- axis it did not pass through the origin representing accumulation of metal sulfides in the sediment (Rosenbauer., 2009). Under anoxic conditions, the SO_4^{2-} in top soil could be easily deoxidized (Li et al., 2009) and the reactive sulfide was produced as a by-product of microbial sulfate reduction. It may then react with iron, and gets precipitated as iron sulfide minerals which settle down on the underlying sediment (Lin and Morse, 1991; Huerta-Diaz and Morse, 1992; Morse and Rowe, 1999). Monsoon also followed the same trend (R^2=0.92).

The accumulation of organic carbon in the mangrove ecosystems is primarily controlled by two competing contributions, autochthonous (input from mangrove litter addition and primary production) and allochthonous (terrestrial input from land runoff and marine sources) input. Stoichiometric ratios of nutrients are employed to determine the origin and transformation of organic matter based on the generalization that organic matter derived from marine plankton has an atomic C/N ratio between 4 and 10 while that from mangrove plants have TOC/TN ratio is above or equal to 20 (Prahl et al., 1994; Yamamuro., 2000). The TOC/TN ratio ranged from 7.31 to 19.05 in post-monsoon, 14.44 to 20.60 in pre-monsoon and 5.99 to 16.75 in monsoon pointing towards the contributions from both marine and terrestrial input (Fig. 3.6). Thalassery in all seasons, Kunjimangalam in monsoon and Kadalundi in post-monsoon and monsoon showed the ratio, less than 10. TOC/TN in Thalassery and Kadalundi reflected the predominance of organic matter derived from the marine input. Sandy sediments of Kunjimangalam permit the penetration of oxygenated water, which helps to intensify the oxidation of organic matter. The wide range of TOC/TN from 5.99 to 20.60 indicate a highly complex ecosystem.

Though we expect mangrove litter addition as the major source of sedimentary organic matter in the system, the ratio was less than 20 in almost all the samples. Generally, lower values of C/N ratios in sediments are most likely contributed by microbial biomass which are nitrogen rich (Muzuka and Shunula, 2006). During mangrove leaf decomposition, bacteria colonized in decaying leaves may cause depletion in the dissolved organic matter (Benner et al., 1986, 1988) and carbon or nitrogen is released preferentially according to the oxic/anoxic conditions (Meyers et al., 1996; Liu et al., 2006). Selective decomposition of organic matter during humification and early diagenesis may alter the ratio, and thus providing false source signatures (Meyers, 1997; Jia and Peng, 2003; Hsieh, 2006; Liu et al., 2006).

During degradation or remineralisation of organic matter ammonia is released, and that can be re-absorbed to the soil resulting in a fall in TOC/TN ratio (Meyers, 1997; Yang et al., 2011). This adsorption may violate major assumption that sedimentary TN exclusively reflects N bound to organic matter for calculating TOC/TN ratio. Accordingly, a close covariance between TOC and TN of sediments is expected and regression of these two variables must result in a line that intersects at zero values of TOC and TN (Fig. 3.5). The plot between TOC and TN in post-monsoon showed a linear relationship (R^2=0.86) with positive slope, but the regression did not pass through the origin. It suggests that a significant fraction (26.99%) of the TN in these sediments is inorganic (presumably as NH_4^+ adsorbed on clays, Muller, 1977; Meyers, 1997). Monsoon season also showed the same trend (R^2=0.92) with 39.93 % of inorganic nitrogen. Pre-monsoon season also exhibited a good linear relationship (R^2=0.98), but the intercept of regression line passes through the origin which indicates very less adsorbed NH_4^+ ions. Consequently, measured

TN concentration as a rational estimate of organic nitrogen can only be used in pre-monsoon (Liu et al., 2006; Rumolo et al., 2011; Gireeshkumar et al., 2013). So in order to evade the inference of adsorbed NH_4^+ ions, TOC/TN was recalculated from the scatter plot. $TOC/TN_{corrected}$ was ranged from 10.21 to 27.21 in post-monsoon and 9.60 to 27.69 in monsoon. By comparing the plots of TOC/TN and $TOC/TN_{corrected}$ in figure 3.6 it is obvious that a significant improvement was obtained in majority of the cases. By applying this correction more samples showed TOC/TN ratio above 20, including Pazhayangadi (monsoon and post-monsoon) Pappinissery and Kunjimangalam (post-monsoon).

In theory, the C:S ratios in sediment should be a useful indicator of anoxic and hypoxic conditions in aquatic systems (Cornwell and Sampou, 1995). TOC/TS ratio ranged from 2.60 to 28.24 in post-monsoon, 3.73 to 6.82 in pre-monsoon and 2.85 to 11.90 in monsoon. Higher TOC/TS ratio was observed at Pazhayangadi in post-monsoon. Pappinissery and Pazhayangadi presented with crab burrows which are reductive in nature. The main product of sulfate reduction, hydrogen sulfide, is volatile and can escape from the sediment, particularly when bioturbation of the surface sediment supports this transport (Clark et al., 1998).

3.3 Sequential Extraction of Sedimentary Phosphorus

Mangrove forests can actively influence the mobility of nutrients (Nielsen and Andersen, 2003; Bosire et al., 2005), among them phosphorus and nitrogen has received much attention owing to their effects on estuarine organisms (Kennish, 1991; Ferreira et al., 1996). Sedimentary phosphorus can exist in various bioavailable forms (Reddy and D'Angelo, 1994; Kufel and Kufel 1997) and in shallow wetlands it can function either as a temporary sink or as the source for P depending on their physicochemical properties (Richardson, 1985). Phosphorus retention and release in mangrove sediments depends on several factors including pH, redox potential, tidal inundation, the nature of phosphorus compounds supplied to the sediment–water interface, sedimentation rate, bioturbation, diagenetic processes, etc. (Ingall and Van Cappellen, 1990; Ruttenberg and Berner, 1993; Silva and Sampaio, 1998; Schenau and de Lange, 2001). The sedimentary phosphorus cycle is characterized by the burial of inorganic phosphorus which is found in combination with amorphous and crystalline forms of iron, magnesium, aluminium, and calcium (Lukkari et al., 2009) via precipitation or adsorption (clay particles and iron and aluminium oxides) under oxic conditions.

Phosphorus release in shallow ecosystems are characterised by the event of anoxia on the sediment surface or diffusion of dissolved phosphate into the water column (Selig, 2003) or by re-suspension (Tessenow, 1972; Schindler et al., 1977) of bottom material erosion (Bengtsson and Hellström, 1992). Generally phosphorus release from iron oxides is in parallel with sulfide production (Caraco et al., 1989) in which reduction of iron-oxides promotes phosphorus release (Jensen et al., 1995; Howarth et al., 1995). Through the decomposition of organic matter, a large amount of phosphorus can be re-released leaving behind refractory organic matter which can store phosphate for a relatively long period (Arias et al., 2001).

Numerous methods for chemical sequential extraction procedures have been available in order to identify different phosphorus forms (Williams et al., 1971; Hieltjes and Lijklema, 1980; Psenner et al., 1988; Golterman and Booman, 1988) and the method proposed by De Groot and Golterman (1993) was used in the present study. Compared to other methods, chelating agents allow specific extraction of inorganic phosphorus leaving organic phosphorus (Golterman, 1996) with no destruction. This method allows to identify six fractions which includes: Water soluble fraction (Exchangeable-P), Iron bound phosphorus (Fe(OOH)≈P), calcium bound fraction (CaCO3≈P), acid soluble organic phosphorus (Acid-OP), alkali soluble organic phosphorus (Alkali-OP) and Residual organic phosphorus (ROP). Iron- and calcium-bound inorganic fractions and acid soluble organic fractions of phosphorus are generally considered to be bioavailable (Diaz-Espejo et al., 1999). Alkali-OP includes organically bound P attached to the humic / fulvic acids along with inorganic P compounds soluble in bases (Kozerski and Kleeberg, 1998). The R-OP, which can be considered as the immobile fraction of phosphorus, is generally constituted by organic phosphorus.

3.3.1 Results

Total phosphorus (range 272.75- 1781.85 $mgkg^{-1}$) in surface sediments of mangrove ecosystems along northern Kerala Coast displayed spatial variation (P value <0.05) and lies within the global range (100–1600 $mgkg^{-1}$; Alongi et al., 1992) except for Kadalundi during monsoon season (1781.85 $mgkg^{-1}$). The results of ANOVA are shown in Table 3.7. Thalassery, Pazhayangadi and Kadalundi followed similar trend with higher values during monsoon and lower during pre-monsoon (Fig.3.7). Comparatively lower concentration of TP was reported from Kunjimangalam and for this station a maximum and minimum value of TP was recorded in monsoon and post-monsoon. Trend in Pappinissery was opposite to Kunjimangalam, for it higher values were in post-monsoon and lower in monsoon.

Table 3.7: Variations of Phosphorus fractions and the results of ANOVA in the present study region

					Spatial Variation		Seasonal Variation	
	Max	Min	Avg	Stdv	F	P-value	F	P-value
Exchangeable-P, mgkg^{-1}	14.78	1.98	6.60	3.66	0.85	3.60	0.53	0.08
Fe(OOH)≈P, mgkg^{-1}	557.70	36.50	255.64	154.66	2.77	0.10	10.23	0.00**
CaCO3≈P, mgkg^{-1}	326.84	78.03	133.87	63.10	1.73	0.24	2.22	0.17
Acid-OP, mgkg^{-1}	194.89	35.10	92.84	42.52	1.84	0.22	3.20	0.10
Alkali-OP, mgkg^{-1}	905.22	19.76	259.68	272.55	2.80	0.10	5.49	0.03*
Res-OP, mgkg^{-1}	91.85	10.79	39.81	25.83	12.72	0.00**	0.92	0.44
TP, mgkg^{-1}	1781.85	272.75	788.45	451.17	4.06	0.04*	3.51	0.08
Bioavilable-P, mgkg^{-1}	948.47	236.39	482.36	205.21	2.29	0.15	7.76	0.01**

*significant at 0.05 level, **significant at 0.01 level,
Fcrtitical spatial variation 3.84, Fcrtitical seasonal variation4.46

Fe(OOH)≈P (Fig.3.8) which represented 9 to 44.86% of total P followed the similar seasonal variation as that of TP. Fe(OOH)≈P and Alkali-OP displayed seasonal variations (P value <0.05) and residual-P showed spatial variation(P value <0.01). Concentration of $CaCO_3$≈P was found to be almost invariant in the entire systems except Kadalundi. Acid-OP was found to be higher during pre-monsoon season in all stations except Kadalundi. In Kadalundi, the fraction of Acid-OP was found to be higher during post-monsoon season (113.21 mgkg^{-1}), but remained invariant during pre-monsoon (71.10 mgkg^{-1}) and monsoon (70.29 mgkg^{-1}). Lower concentration of Acid-OP was observed in post-monsoon except Pappinissery and Kadalundi. Except for Pappinissery, monsoon was earmarked for comparatively higher concentration of Alkali-OP (humic-P) and the maximum concentration was observed in Kadalundi (905.22 mgkg^{-1}). Comparatively lower Alkali-OP was observed during pre-monsoon at Pappinissery, Pazhayangadi and Kadalundi, but in post-monsoon at Thalassery and Kunjimangalam. Kunjimangalam during post-monsoon showed minimum concentration for this fraction (19.76 mgkg^{-1}). R-OP showed highest concentration during pre-monsoon in all stations except Kadalundi and similar patterns were observed for Acid-OP.

Exchangeable-P contribution to the TP pool was very low and was below 2.5%. ROP in post-monsoon and monsoon represent <6% of the TP pool. But ROP fraction was appreciably higher during pre-monsoon season (<12%). Exchangeable-P followed by ROP fraction represented the lowest contributor towards TP in all seasons. Fe(OOH)≈P contribution to the TP pool, was considerably high in post-monsoon and low in pre-monsoon. Thalassery in post-monsoon (30.86%) and pre-monsoon (32.84 %) and Kunjimangalam in post-monsoon season (40.54 %) showed considerably higher contribution of $CaCO_3$≈P to the TP pool and all other stations recorded <24 %. Acid-OP showed comparatively higher contribution to the TP pool in pre-monsoon and fairly low (<14 %) contribution in post-monsoon and monsoon. Alkali-OP contributed higher concentration to the TP pool in monsoon (except at Thalassery) and was fairly high at Pazhayangadi (55.18%), Kunjimangalam (47.54 %) and Kadalundi (50.80 %). Fe(OOH)≈P was the highest contributor to the TP in post-monsoon except Kunjimangalam where the $CaCO_3$≈P was the major contributor. During pre-monsoon Fe(OOH)≈P was the major fraction except Thalassery ($CaCO_3$≈P was the major contributor) and Kunjimangalam (Acid-OP was the major contributor). In monsoon, (Fe(OOH)≈P was the major contributor except for Thalassery and Pappinissery where Alkali-OP was the major fraction.

3.3.2 Discussion

The mangroves (intertidal, subtidal zones) and the coral reef platforms, have the highest P accumulation rates per unit area (Monbet et al., 2007), so the remobilization from these systems are most significant (Alongi et al., 2005) with >30% can be recycled by diagenetic processes. Total phosphorus displayed significant spatial variation and was comparatively higher at Pazhayangadi and Kadalundi which may be due to the difference in geographical and geomorphological locations. These results indicated the occurrence of different trapping mechanism and the capacity to retain phosphates by mangrove sediments. The existence of denser vegetation, low flow rate and clayey sediment nature at Pazhayangadi facilitates the trapping of nutrients. According to Wang et al., (2013b), a significantly higher phosphorus adsorption capacity was found in higher vegetated region, and this situation resulted from the oxidative environment of roots, which caused the higher ratio of active Fe/Al oxides in the vegetated than in the mudflat region.

Influences of biotic processes like bioturbation, plant uptake, transpiration and root zone decomposition are some factors which affect the mobilization of P in wetlands (Renjith, 2006). Bioturbation was active at Pappinissery and Pazhayangadi that affect the fractionation by bringing material from surface layer down into the underlying reducing sediment and vice versa.

The close proximity of Thalassery and Kadalundi to the Lakshadweep Sea results in the modification of nutrients by suspension events. In Kadalundi (except pre-monsoon season) silt and muddy sand was dominant textural facies. A shift to sandy texture in concomitance with decrease in TP concentration was noticed during pre-monsoon. Because when grain size becomes finer, the surface area of sediment increases and the various forms of phosphorus are easily adsorbed (Carman and Wulff, 1989; Frankowski and Bolałek, 1997). Sand has typically a very low P sorption potential because of its low concentration of amorphous Fe and Al (Bridgham et al., 2001).

Kunjimangalam also associated with low phosphorus concentration due to its sandy texture.

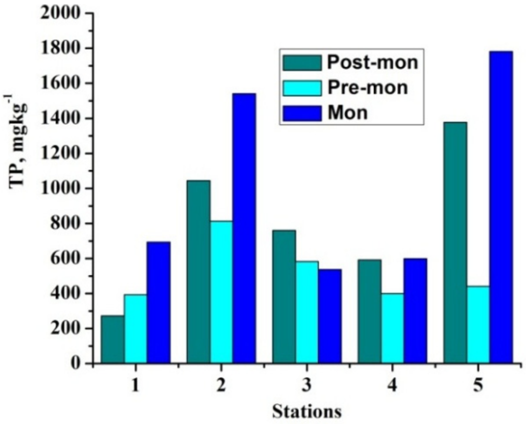

Fig. 3.7: Variation of TP in the study region

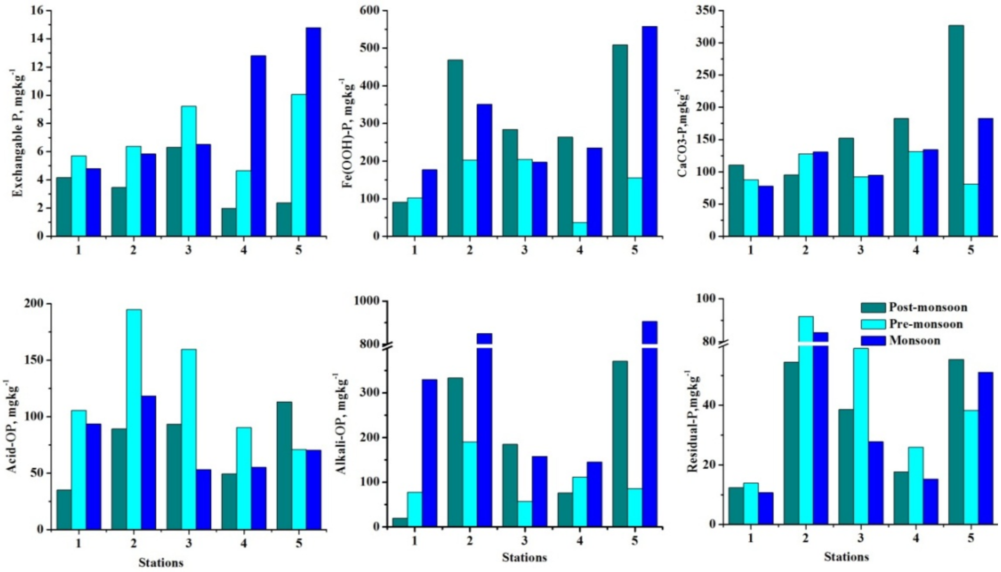

Fig. 3.8: Variation of phosphorus fractions in the study region

TP was comparatively higher in monsoon, except for Pappinissery. In water column also DIP was higher in monsoon and lower in post-monsoon. Phosphorus from land runoff and decaying leaves may enter the water column and get accumulated in the sediment. Holmboe et al., (2001) states that excess DIP was efficiently taken up by the sediment particles and primarily retrieved in the iron bound fractions; hence the sediment may act as a phosphorus sink.

TP was comparatively lower in pre-monsoon except for Pappinissery and Kunjimangalam. Increase in microbial activity (Vicente et al., 2003; Sinke and Cappenberg, 1988), the reduction of Fe oxide-bound phosphate in anoxic sediment (Fe(OOH)≈P), mineralization of organic matter and enhanced primary production in connection with improved light penetration (van Luijn et al., 1995; Woodruff et al., 1999; Søndergaard et al., 2003) are the possible reasons for lower concentration of TP during pre-monsoon season.

Exchangeable-P fraction was very low since it might be escaped into the water column as it is formed. Higher Exchangeable-P during pre-monsoon in Pappinissery, Pazhayangadi and Kunjimangalam may be linked with phosphorus release from different fractions. Froelich et al. (1979), has been observed large increase in pore water phosphate during mobilization of iron bearing oxy hydroxy coatings. Lower concentration of exchangeable-P is observed in post-monsoon which might be linked with phosphorus adsorption to the sediment.

Bioassay results revealed that Fe(OOH)≈P is a bioavailable P source for phytoplankton growth (Mesnage and Picot, 1995) and is considered to be more a redox-sensitive P fraction. Most of the sediments in the estuary or wetland may originate from the erosion of red soil in the upstream, and the red soil is rich in Fe/Al oxides. The presence of oxic layer in the surface sediment favored adsorption of P by the ferric hydroxide-bound fraction which agrees with the observations of numerous authors (Gomez et al., 1998; Detenbeck and Brezonik, 1991; Nair and Balchand, 1993).

Table 3.8 Correlation matrix for the phosphorous fractions (n=15)in the mangrove ecosystems of northern Kerala Coast

	SOC	Sand	Clay	Silt	Fe(OOH)≈ P	$CaCO_3$≈P	Acid-OP	Alkali-OP	Residual- P	TN	TS	Fe	Mn	Ca	TP	salinity	DO	DIP	TDP	Chla
SOC	1																			
Sand	-0.60*	1																		
Clay	0.69**	-0.82**	1																	
Silt	0.43	-0.57*	0.39	1																
Fe(OOH)≈P	0.33	-0.46	0.35	0.51*	1															
CaCO3≈P	0.01	-0.33	0.23	0.69**	0.44	1														
Acid-OP	0.53*	-0.54*	0.53*	0.10	0.14	0.00	1													
Alkali-OP	0.19	-0.60*	0.22	0.52*	0.66**	0.33	0.35	1												
Res-OP	0.80	-0.80**	0.75**	0.65**	0.50*	0.28	0.65**	0.51*	1											
TN	0.95**	-0.60*	0.67**	0.48	0.53*	0.13	0.56*	0.34	0.78**	1										
TS	0.80**	-0.60*	0.56*	0.29	0.24	0.07	0.61*	0.23	0.64**	0.80**	1									
Fe	0.50*	-0.67**	0.49	0.79**	0.82**	0.68**	0.42	0.77**	0.76**	0.65**	0.44	1								
Mn	0.59*	-0.71**	0.71**	0.63**	0.55*	0.55*	0.53*	0.43	0.83**	0.61*	0.50*	0.79**	1							
Ca	0.73**	-0.59*	0.85**	0.32	0.21	0.18	0.64*	-0.04	0.67**	0.69**	0.56*	0.40	0.67**	1						
TP	0.36	-0.70**	0.45	0.64**	0.84**	0.52*	0.39	0.92**	0.66**	0.54*	0.35	0.92**	0.63*	0.23	1					
Salinity	-0.06	0.09	0.19	0.02	-0.32	-0.03	0.21	-0.36	0.10	-0.15	-0.17	-0.14	0.15	0.44	-0.27	1				
DO	-0.54*	-0.01	-0.33	0.22	0.08	0.24	-0.39	0.34	-0.25	-0.53*	-0.58*	0.06	-0.20	-0.48	0.17	-0.14	1			
DIP	-0.13	-0.20	0.02	-0.12	0.14	-0.18	0.13	0.49	0.06	-0.02	0.15	0.08	-0.11	-0.34	0.33	-0.29	0.10	1		
TDP	-0.01	-0.21	-0.04	0.10	0.50*	0.15	-0.03	0.66**	0.05	0.20	0.24	0.39	-0.02	-0.38	0.59*	-0.68**	0.13	0.77**	1	
Chl a	0.70**	-0.61*	0.72**	0.43	0.47	0.34	0.79**	0.36	0.82**	0.80**	0.73**	0.70**	0.74**	0.73**	0.59*	0.14	-0.49	0.13	0.15	1

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

The Fe(III) concentration in mangrove sediments may cycle seasonally due to Fe-sink imposed by microbial sulfate reduction and the production of pyrite (FeS_2) and iron-monosulfides (FeS), alternating with a season of net oxidation. In Kadalundi and Kunjimangalam higher Fe(OOH)≈P was noticed in monsoon which might be due to the presence of an oxic layer favoring phosphorus adsorption. Metal oxides (e.g., of Fe(III) and A1) carry a net positive charge at low pH favouring the association between metal oxides and anions such as phosphate, though this trend is counteracted by increased electrolyte concentrations (Barrow et al., 1980; Hawke et al., 1989; Morris and James, 1999). In Thalassery, Pappinissery and Pazhayangadi higher concentration was associated with post-monsoon, which might be due to the presence of an oxic layer or due to increased sedimentation rate resulted from the reduced flow (Katsaounos et al., 2007). Statistical analysis reflected a good correlation between Fe and P (Table 3.8), confirming the prevalence of Fe(OOH)≈P, and conversely the thorough enrichment of P in Fe-rich phase.

Fe(OOH)≈P displayed identical seasonal variation except for Pappinissery and Kunjimangalam, with lowest values in pre-monsoon. Alkaline conditions promoted by sulfate reduction and charge shift of Fe-oxides from positive to negative by increased salinity (Stumm and Morgan, 1981) reduces phosphorus adsorption. Recent studies in mangrove sediments have shown that more than 80% of the anaerobic carbon oxidation is mediated by microbial iron reduction, when reactive Fe(III) exceeds about 35 $\mu mol/cm^3$ (Kristensen et al., 2008). The mangrove sediments, continuously receives high amount of freshly produced organic material. High organic input creates the potential for a high mineralization rate, but when the supply of oxiders are insufficient, de-sulfurication and sulfur cycling may become important (Holmer and

Storkholm, 2001). Reactive iron immobilises sulfides as highly insoluble FeS and FeS_2, which decreases the potential of phosphorus sorption and thereby the potential phosphorus release from the sediment (Kleeberg and Schubert, 2000; Perkins and Underwood, 2001; Søndergaard et al., 2003).

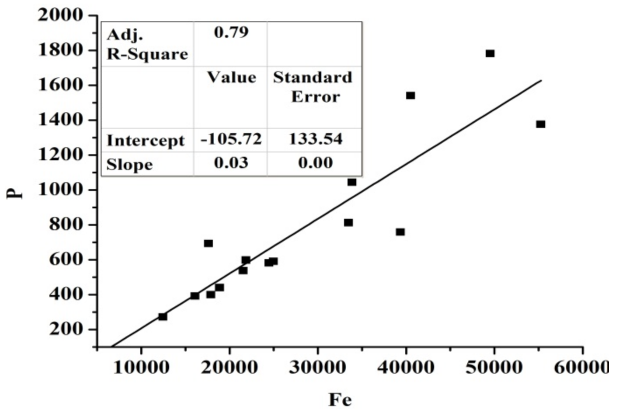

Fig. 3.9: Scatter plot between Fe and TP in the present study

From scatter plot of Iron and TP (Fig. 3.9), it could be possible to inference FeS production since the regression line does not pass through the origin.

The Fe:P ratio is considered as a measure of free sorption sites for phosphate ions on iron oxyhydroxide surfaces and the total Fe:P ratio above 15 (w:w) is enough for Fe to control the benthic flux of P from sediment to oxic water (Jensen et al., 1992). But according to Caraco et al., 1993, Fe:P ratio should exceed 10 if it was to regulate phosphorus release. Rydin and Brunberg, 1998, also described the formation of an effective oxic barrier with the above Fe:P ratio. The ratio was above 25 in all stations. Therefore, there seems to be enough Fe in surface sediments to bind P in the present study region. The presence of threshold in the present study can be supported by the strong positive relationship between the concentrations of phosphorus and iron (Søndergaard et al., 2001).

Lower values of Fe:P were observed in monsoon. Thalassery, Pazhayangadi and Kadalundi showed higher values during pre-monsoon, whereas others during post-monsoon. Pre-monsoon is characterised by Fe:P above the threshold limit, even though it may control phosphorus release. Monsoon season showed lower ratio which may due to higher Fe(OOH)≈P from mineralisation of organic matter.

Fe(OOH)≈P followed by $CaCO_3$≈P was the highest contributor to the TP during post-monsoon except Kunjimangalam ($CaCO_3$≈P was the major contributor). Generally it is found that Fe(OOH)≈P is dominant with a more acidic pH, while $CaCO_3$≈P becomes dominant in alkaline pH. However, a relatively constant profile was observed which might be necessarily due to masking of precipitation by bioturbation or other processes occurring at the sediment– water interface (Van der Zee et al., 2002). Concentration of $CaCO_3$≈P was found to be almost invariant in the entire systems except Kadalundi. In Kadalundi the fraction was modified by texture of sediments and showed higher concentration in post-monsoon and lower concentration in pre-monsoon. $CaCO_3$≈P was found to be positively correlated with silt, but the negative correlation with sand is not significant. Absence of correlation between $CaCO_3$≈P and Ca suggested that $CaCO_3$≈P was almost inert phosphorus form. This observation was also noticed by Gonsiorczyk et al., (1998) in German hard water lakes. In the opinion of Golterman, (1988) in lakes with high $CaCO_3$ content (10-30%) phosphorus will be absorbed first by iron hydroxide even if it present in small quantities and only when the sorption capability of iron hydroxide is depleted, it will bind with calcium. There is no significant correlation between TP and Ca, as P geochemistry appears to be controlled mainly by Fe. $CaCO_3$≈P was positively correlated with silt, Fe and Mn.

The phosphorus which fixes on the fresh organic material gives compounds of the acid soluble organic phosphorus while the phosphorus which associates with more stable forms of organic material gives humic acid bound phosphorus forms. Acid-OP includes apatite-bound phosphate and biochemical components such as nucleic acids, lipids and sugars which bound to phosphate (De Groot and Golterman, 1990) which could be easily mineralized by microbes, hence becomes bioavailable easily (De Groot and Golterman, 1993). This fraction usually represents only a small part of the sediment phosphorus (Mesnage and Picot, 1995) and in the present study the fraction was appreciably higher in pre-monsoon (except Kadalundi). The higher concentration of Acid-OP in the sediments in an ecosystem of France (Seine estuary) is interpreted as input of organic material from microalgae (Mesnage et al., 2002) was in good agreement with the present study.

Alkali-OP compounds or most probably organically bound P attached to the humic / fulvic acids and phytate phosphate which require strong alkaline media to solubilize, along with inorganic P compounds soluble in bases (Kozerski and Kleeberg, 1998). Phytate or phytic acid (inositol hexaphosphate) is an organic phosphate that is widely spread in plants, soils, and aquatic sediments (Joseph et al., 2010), which can form complexes that are highly resistant to bacterial action (De Groot and Golterman, 1993; Newman and Reddy, 1993). But some bacterial strains also contain phytase, which may be involved in the production or mineralization of phytate (Golterman, 2001; Garc and Iorio, 2003). Mangroves are expected to contain a high proportion of org-P due to their high organic matter content (Boto, 1988; Alongi et al., 1992), but the labile fraction was very low in these mangrove sediments signalling to the dead organic matter accumulation (Joseph et al., 2008). Similar enrichment of organic phosphorus in the mangrove zone because of high rate of organic matter production and retention were observed by Prasad and Ramanathan, (2010) in Pitchavaram mangrove sediments.

The highly coloured NaOH extracts from the surface sediments indicated the presence of large amounts of humic compounds (Golterman et al., 1998). Alkali-OP displayed significant seasonal variation with highest concentration during monsoon season, which might be due to the fact that the ability of sediment humic acids to bind P decreases in response to increasing salinity (Paludan and Morris, 1999). The land-derived materials carried by runoff also contributed towards the Alkali-OP. In monsoon Alkali-OP was the major fraction of TP in all stations except Thalassery and Pappinissery (Fe(OOH)≈P is the major contributor).

Alkali-OP displayed positive correlation (Table 3.8) with Fe(OOH)≈P (R^2=0.66) and Fe (R^2=0.77) suggesting an interaction between those two fractions. In freshwater environments oxides of Fe and A1 form complexes with dissociated and negatively charged surfaces of humic acids, and phosphate may bind to these complexes by ligand exchange on the oxide surfaces thus forming organic-metal complexes of large molecular size (Gerke and Hermann, 1992).

R-OP exhibited higher concentration in pre-monsoon for except Kadalundi and follows similar seasonal variation as Acid-OP. R-OP represents <6% during post-monsoon and monsoon season to the TP pool. But it was comparatively higher during pre-monsoon season which represent <12% to the total phosphorus pool. No seasonal variation was observed in the R-OP fraction of sediments. This fact reinforces the non- available nature of the R-OP fraction as found in other similar studies (Diaz-Espejo et al., 1999;

De Groot and Fabre, 1993). R-OP fraction found to be correlated with SOC, clay, silt, Acid-OP, TN, TS, Fe, Mn, Ca, and Chl a.

The correlation of Chl a with Acid-OP and R-OP suggested that these P forms consists of phyto- and/or zooplankton- or detritus-derived P. Either Silt (no significant correlation exist with WP, Fe(OOH)≈P, Acid-OP, TN and TS) or clay (no significant correlation with WP, Fe(OOH)≈P, $CaCO_3$≈P, Acid-OP) showed positive correlation with almost all parameters. Sand showed negative correlation with all parameters. These indicate that the main factor influencing the geochemistry of the surficial sediments in the study region could be the sediment texture (Renjith et al., 2011). TDP was found to be positively correlated with Alkali-OP, Dissolved inorganic phosphorus and TP in sediment and it showed negative correlation with salinity.

Iron- and calcium-bound inorganic fractions and acid soluble organic fractions of phosphorus are generally considered to be bioavailable (Diaz-Espejo et al., 1999).But Fe(OOH)≈P is more important than $CaCO_3$≈P in terms of potential availability of phosphorus under the redox (Eh) variations observed in the mangroves sediments (Caraco et al., 1989; Silva and Mozeto, 1997). Bioavailable fractions of phosphorus displayed significant seasonal variation and were above 60% of TP during post-monsoon and pre-monsoon seasons. During monsoon, all stations except Thalassery (71%) and Pappinissery (64%) showed lower concentrations. Since the bulk of the total phosphorus is bioavailable, these mangrove sediments have the potential to act as source of phosphorus to the overlying water column (Joseph et al., 2010). At Thalassery and Kunjimangalam during post-monsoon followed by monsoon contributed more bioavailable-P to the TP. Concentration of bioavailable-P was found to be higher in Kadalundi followed by Pazhayangadi.

Except R-OP all phosphorus fractions are considered to be refractory. In post-monsoon and monsoon refractory fraction was found to be exceed 94% of the TP pool, but in pre-monsoon it was slightly lower but still showed much higher contribution (>88 %). Kadalundi followed by Pazhayangadi have comparatively higher concentration of refractory fraction (Fig. 3.10).

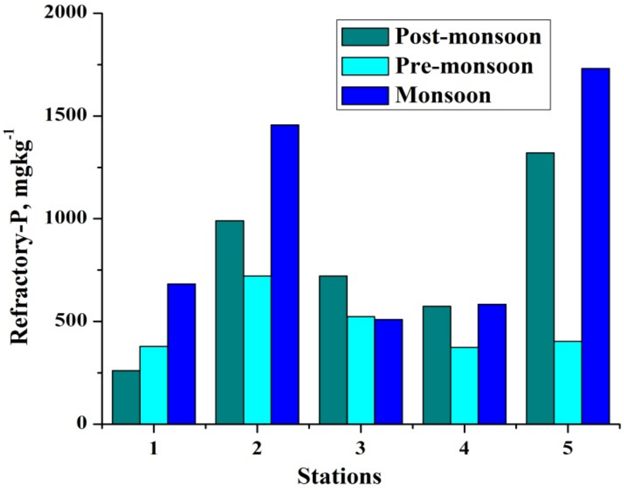

Fig. 3.10 Variation of refractory-P in the study region

Thalassery and Kadalundi are regularly flooded by the tide; the repeated re-suspension of the sediments caused by the wave action can favour exchange of orthophosphate from the solid to the liquid phases. Active recycling of organic matter is taking place at Thalassery. It does not show any build-up of Alkali–OP in any season and the organic forms may get converted to inorganic forms. Since Thalassery is in the vicinity of Lakshadweep Sea salinity played an active role in determining the formation of inorganic forms i.e., Fe(OOH)≈P or $CaCO_3$≈P. In post-monsoon and monsoon Fe(OOH)≈P was dominated whereas during pre-monsoon $CaCO_3$≈P was dominant fraction. The system may not be completely anoxic due to tidal rhythms, bioturbation activities and root nodules of mangrove micro-pores. During high tide, the system may be saline and anoxic but during low tide it may be in direct contact with the atmosphere. The highly dynamic condition exists in the system and the Fe(OOH)≈P was found to be the dominant one. In Pappinissery also such build of Alkali-OP was not found and Fe(OOH)≈P was the dominant inorganic fraction.

In Pazhayangadi, monsoon season showed build-up of Alkali-OP either from mangrove litter addition or from land runoff. Such build-up of Alkali-OP was also found in Kunjimangalam and Kadalundi, but its concentration was not too high in Kunjimangalam. In Kunjimangalam, $CaCO_3$≈P was the dominant inorganic fraction during post-monsoon, but it was Fe(OOH)≈P during pre-monsoon and monsoon. The system was sandy in nature which decreases adsorption of phosphorus onto the sediment and also resulted in the oxidation of organic matter. In Kadalundi, major inorganic fraction was found to be Fe(OOH)≈P in all seasons. Even in the most saline sites, it is possible that the supply of Fe exceeds the availability of sulfides in the sediments, allowing for the formation of ferrous phosphate minerals (Hartzell et al., 2010). The dissolved P released from organic matter is absorbed during Fe oxide precipitation in the oxidized layer, thereby fixing most of the dissolved P in solid phase and concurrently prevent it from diffusing to the overlying water column (Cha et al., 2005).

Retention and cycling of organic matter is high in the interior mangrove zone, therefore, significant amount of P is adsorbed into the organic matrix and subsequently released by the microbial degradation into the overlying water column influencing the dissolved phosphorus dynamics (Prasad and Ramanathan, 2010). Although anoxic conditions enhance release of phosphorus associated with metal oxides and oxy-hydroxides (Wetzel, 2001; Kalff, 2002), release of organic bound P gets decreased under anoxic conditions leading to its accumulation at higher rates than less stable fractions (Gale et al., 1992).

3.3.3 Principal component analysis

Principal component analysis with varimax rotation is used to identify major geochemical processes operating in the system by grouping the parameters under observation with their interrelationship. Major identifiable processes in the system are diagenetic process, redox conditions favoring precipitation and dissolution along with the adsorption to clay minerals or organic matrix. The association between clay minerals and phosphorus fractions gives the information about the control of sediment granulometry over phosphorus. TOC, TN and TS indicates the redox status of the system.

Table 3.9 Total Variance Explained for phosphorus fractions using principal component analysis

	Initial Eigen values			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Components	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	7.984	53.22	53.22	7.984	53.225	53.225	5.935	39.56	39.56
2	2.373	15.81	69.04	2.373	15.817	69.042	4.389	29.25	68.82
3	1.349	8.991	78.03	1.349	8.991	78.033	1.382	9.210	78.03
4	.800	5.335	83.36						
5	.758	5.056	88.42						
6	.537	3.583	92.00						
7	.502	3.348	95.35						
8	.323	2.151	97.50						
9	.176	1.177	98.68						
10	.088	.590	99.27						

The 78.03 % of variance was explained by three factors (Table 3.9 and 3.10). First factor explained 39.56% of the total variance which showed positive loading on TOC, clay, TN, TS, Acid-OP, ROP and calcium with negative loading on sand. This factor pointed towards the adsorption which is controlled by the fine fractions of sediments and diagenetic process (Hou et al., 2009). Second factor explained 29.25% of the total variance with positive loading on silt, Fe(OOH)≈P, CaCO3≈P, Alkali-OP, Fe and Mn along with weak negative loading on sand.This factor gives the inference of adsorption processes. Association between Fe(OOH)≈P, Alkali-OP and Fe indicated the adsorption of Fe onto the humic acid matrix to form humic acid metal complex. Third factor explained 9.21 % of total variance with loading only on exchangeable-P.

Table 3.10 Factor loading for Principal component analysis using varimax rotation for phosphorus fractions

	Component		
	1	**2**	**3**
TOC	.901	.152	.045
Sand	-.675	-.524	.018
Clay	.832	.234	-.178
Silt	.263	.783	-.251
Exchangeable-P	.026	.038	.809
Fe(OOH)≈P	.184	.801	.102
CaCO3≈P	-.029	.728	-.561
Acid-OP	.742	.096	.182
Alkali-OP	.095	.824	.361
R-OP	.784	.518	.091
TN	.837	.319	.065
TS	.816	.125	.141
Fe	.392	.901	-.027
Mn	.630	.603	-.151
Ca	.883	.034	-.291

In the plot between factor 1 and 2 (Fig. 3.11) almost all parameters under investigation lies in the Ist quadrant in two clusters. Variables with positive loadings in PC1 forms the Ist cluster and those in PC2 forms the second cluster. Sand lies in the IIIrd quadrant since sandy nature significantly reduces the phosphorus adsorption due to the reduction in surface area. Exchangeable – P lies in the centre of the plot which gives the inference that it can be formed from any process such adsorption, desorption, redox or diagenetic alterations.

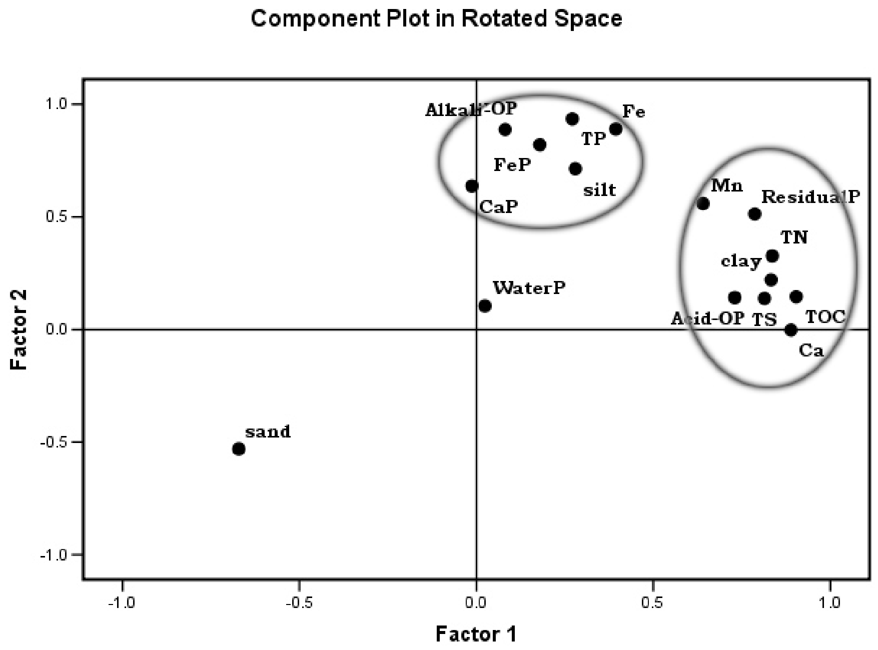

Fig. 3.11: Plot between PC1 and PC2

3.4 Conclusion

Water quality analysis, general sedimentary characteristics and phosphorus fractionation were carried out in five mangrove ecosystems along northern Kerala Coast. The dissolved oxygen supersaturation (132 %) was noticed at Kunjimangalam during pre-monsoon which might be due to higher rate of photosynthesis and limited flushing. All major elements showed positive correlation with salinity indicating saline influx from adjacent estuarine system. Higher amount of fine sediments would be expected to settle in Pappinissery and Pazhayangadi with the reduction of tidal flow and a decrease in turbulence while tidal rhythm modifies the texture in Kadalundi. Scatter plot between TOC and TN showed the inference of inorganic nitrogen during post-monsoon and monsoon while plot between TOC and TS gives accumulated metal sulfide in the sediment, but the effect was more pronounced during pre-monsoon season. Textural characteristics, vegetation, and geographical and geomorphological locations were found to modify TP in the study region. For almost all stations Fe(OOH)≈P and/or Alkali-OP was major fraction. The Alkali-OP was found to be higher in sediments from Pazhayangadi, Kunjimangalam and Kadalundi during monsoon season since fresh water condition favors its complexation. From the PCA analysis, we can infer that the adsorption and diagenetic alterations along with complex formation with metals are the major processes controlling the phosphorus dynamics in the sediment.

References

Alongi, D. M., 1996. The dynamics of benthic nutrient pools and fluxes in tropical mangrove forests. Journal of Marine Research, 54, 123-148.

Alongi, D. M., Boto, K. G., Robertson, A. I., 1992. Nitrogen and phosphorus cycles. In: Robertson, A.I., Alongi, D.M. (Eds.), Tropical Mangrove Ecosystems: American Geophysical Union, Washington D.C.

Alongi, D. M., Ramanathan, A. L., Kannan, L., Tirendi, F., Trott, L. A., Prasad, M. B. K., 2005. Influence of human-induced disturbance on benthic microbial metabolism in the Pichavaram mangroves, Vellar–Coleroon estuarine complex, India. Marine Biology, 147, 1033-1044.

Alvarez-Salgado, X. A., Figueiras, F. G., Perez, F. F., Groom, S., Nogueira, E., Borges, A., Chou, L., Castro, C. G., Moncoiffe, G., Rios, A. F., Miller, A. E. J., Frankignoulle, M., Savidge, G., Wollast, R., 2003. The Portugal coastal counter current off NW Spain: new insights on its biogeochemical variability. Progress in Oceanography, 56, 281–321.

Arias, C. A., Bubba, M. D., Brix, H., 2001. Phosphorus removal by sands for use as media in subsurface flow constructed reed beds. Water Research, 35, 1159– 1168.

Aston, S. R., 1980. Nutrients dissolved gasses and general biochemistry in estuaries. In: Chemistry and biogeochemistry of estuaries, E. Olausson and I. Cato (Eds.), Wiley, New York.

Bailey, S. E., Guimond, J. K., 2009. Above-ground biomass and nutrient limitation in relation to river connectivity in Montane Flood-plain Marshes. Wetlands, 29, 1243–1254.

Barrow, N. J., Bowden, J. W., Posner, A. M., Quick, J. P., 1980. Describing the effects of electrolyte on adsorption of phosphate by a variable charge surface. Australian Journal of Soil Research, 18, 395-404.

Bengtsson, L., Hellström, T., 1992. Wind-induced re-suspension in a small shallow lake. Hydrobiologia, 241, 163–172.

Benner, R., Hodson, R. E., Kirchman, D., 1988. Bacterial abundance and production on mangrove leaves during initial stages of leaching and biodegradation. Archiv fur Hydrobiologie, 31, 19-26.

Benner, R., Moran, M. A., Hodson, R. E., 1986. Biogeochemical cycling of lingo-cellulosic carbon in marine and freshwater ecosystems: Relative contributions of prokaryotes and eucaryotes. Limnology and Oceanography, 31, 89-100.

Berner, R. A., Raiswell, R., 1983. Burial of organic carbon and pyrite sulfur in sediments over Phanerozoic time: a new theory. Geochimica et Cosmochimica Acta, 47, 855–862.

Boers, P, C., 1991. The influence of pH on phosphate release from lake sediments. Water Research, 25, 309-311.

Bosire, J. O., Dahdouh-guebas, F., Kairo, J. G., Kazungu, J., Dehairs, F., Koedam, N., 2005. Litter degradation and CN dynamics in reforested mangrove plantations at Gazi Bay, Kenya. Biological Conservation, 126, 287-295.

Boto, K. G., 1988. The phosphorus cycle. In A. D. Agate, C. V. Subramanian and M. Vannucci (eds), Mangrove Microbiology. UNDP/UNESCO Regional Project (RAS/86/1988), New Delhi.

Bouillon, S., Koedam, N., Raman, A. V., Dehairs, F., 2002. Primary producers sustaining macro-invertebrate communities in intertidal mangrove forests. Oecologia, 130, 441–448.

Brady, N. C., Weil, R. R., 2008. The Nature and Properties of Soils, fourteenth edition. Pearson, Columbus, OH, USA.

Bridgham, S. D., Johnston, C. A., Schubauer-Berigan, J. P., Weishampel, P., 2001. Spatial controls over phosphorus sorption in soils and coupling with surface water in two riverine wetlands. Soil Science Society of America Journal, 65, 577–588.

Calvert, S. E., 2004. Beware intercepts: interpreting compositional ratios in multi-component sediments and sedimentary rocks. Organic Geochemistry, 35, 981-987.

Caraco, N. F., Cole, J. J., Likens, G. E., 1993. Sulfate control of phosphorus availability in lakes – a test and re-evaluation of Hasler and Einsele model. Hydrobiologia, 253, 275–280.

Caraco, N. F., Cole, J. J., Likens, G. E. 1989. Evidence for sulfate controlled phosphorus release from sediments of aquatic systems. Nature, 341, 316–318.

Carman, R., Wulff, E., 1989. Adsorption capacity of phosphorus in Baltic Sea sediments. Estuarine Coastal Shelf Science, 29, 447-456.

Cha, H. J., Lee, C. B., Kim, B. S., Choi, M. S., Ruttenberg, K. C., 2005. Early diagenetic re-distribution and burial of phosphorus in the sediments of the south-western East Sea (Japan Sea). Marine Geology, 216, 127-143.

Cohen, M. C. L., Lara, R. J., Ramos, J. F., da F., Dittmar T., 1999. Factors influencing the variability of Mg, Ca and K in waters of a mangrove creek in Braganca, North Brazil. Mangroves and Salt Marshes, 3, 9-15.

Clark, M. W., McConchie, D., Lewis, D. W., Saenger, P., 1998. Redox stratification and heavy metal partitioning in Avicennia-dominated mangrove sediments: a geochemical model. Chemical Geology, 149, 147–171.

Cookson, W. R., Abaye, D. A., Marschner, P., Murphy, D. V., Stockdale, E. A., Goulding, K. W. T., 2005. The contribution of soil organic-matter fractions to carbon and nitrogen mineralization and microbial community size and structure. Soil Biology and Biochemistry, 37, 1726–1737.

Cornwell, J. C., Sampou, P. A., 1995. Environmental controls on iron sulfide mineral formation in a coastal plain estuary. In ACS Symposium Series, Washington, DC: American Chemical Society, 612, 224-242.

De Groot J-C., Fabre, A., 1993. The impact of desiccation of a freshwater marsh (Garcines Nord, Camargue, France) on sediment-water-vegetation interactions. Part 3: The fractional composition and the phosphate adsorption characteristics of the sediment. Hydrobiologia, 252, 105-116.

De Groot, C. J., Golterman, H. L., 1990. Sequential fractionation of sediment phosphate. Hydrobiologia, 192, 143–149.

De Groot, C. J., Golterman, H. L., 1993. On the presence of organic phosphate in some Camargue sediments: evidence for the importance of phytate. Hydrobiologia, 252, 117–126.

Detenbeck, N. E., Brezonik, P. L., 1991. Phosphorus sorption by sediments from a soft-water seepage lake1. An evaluation of kinetic and equilibrium models. Environmental Science and Technology, 25, 395–403.

Diaz-Espejo, A., Serrano, L., Toja. J., 1999.Changes in sediment phosphate composition of seasonal ponds during filling. Hydrobiologia, 392, 21-28.

Ferreira, M. F., Chiu, W. S., Cheok, H. K., Cheang, F., Sun, W., 1996. Accumulation of nutrients and heavy metals in surface sediments near Macao. Marine Pollution Bulletin, 32, 420–426.

Frankowski, L., Bolałek, J., 1997. Phosphate desorption from the sediments in the Pomeranian Bay (Southern Baltic). Oceanological Studies, 1, 205–214.

Froelich, P. N., Klinkhammer, G. P., Bender, M. L., Luedtke, N. A., Heath, G. R., Cullen, D., Dauphin, P., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et Cosmochimica Acta, 43, 1075–1090.

Gale, P. M., Reddy, K. R., Graetz, D. A., 1992. Mineralization of sediment organic matter under anoxic conditions. Journal of Environmental Quality, 21, 394–400.

Garc, A. R., Iorio, A. F. D., 2003.Phosphorus distribution in sediments of Morales Stream (tributary of the Matanza-Riachuelo River, Argentina).The influence of organic point source contamination. Hydrobiologia, 492, 129-138.

Gerke, J., Hermann, R., 1992. Adsorption of orthophosphate to humic-Fe-complexes and to amorphous Fe-oxide. Zeitschriftfür Pflanzenernährung und Bodenkunde, 155, 233–236.

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, 239–45.

Golterman, H. L., 2001. Phosphate release from anoxic sediments or 'What did Mortimer really write?' Hydrobiologia, 450, 99–106.

Golterman, H. L., 1988. The calcium- and iron-bound phosphate phase diagram. Hydrobiologia, 159, 149–151.

Golterman, H. L., 1996. Fractionation of sediment phosphate with chelating compounds: a simplification and comparison with other methods. Hydrobiologia, 335, 87-95.

Golterman, H. L., Booman, A., 1988. Sequential extraction of iron-phosphate and calcium phosphate from sediments by Chelating agents. Internationale Vereinigungfuer Theoretische und Angewandte Limnologie. Verhandlungen, IVTLAP.

Golterman, H. L., Paing, J., Serrano, L., Gomez. E., 1998. Presence of and phosphate release from polyphosphates or phytate phosphate in lake sediments. Hydrobiologia, 364, 99- 104.

Gomez, E., Fillit, M., Ximenes, M. C., Picot, B., 1998. Phosphate mobility at the sediment–water interface of a Mediterranean lagoon (etangduM´ ejean), seasonal phosphate variation. In Oceans, Rivers and Lakes: Energy and Substance Transfers at Interfaces Springer Netherlands.

Gonneea, M. E., Payton, A., Herrera-Silveira, J. A., 2004.Tracing organic matter sources and carbon burial in mangrove sediments over the past 160 years. Estuarineand Coastal Shelf Sciences, 61, 211-217.

Gonsiorczyk, T., Casper, P., Koschel, R., 1998. Phosphorus binding forms in the sediment of an oligotrophic and an eutrophic hard-water lake of the Baltic district (Germany). Water Science and Technology, 37, 51–59.

Granier, J., Billen, G., Palfner, L., 2000. Understanding the oxygen budget and related ecological processes in the river Mosel: the RIVERSTRAHLER approach. In Man and River Systems Springer, Netherlands.

Grignon-Dubois, M., Rezzonico, B., Alcoverro, T., 2012. Regional scale patterns in seagrass defences: Phenolic acid content in Zosteranoltii. Estuarine coastal and shelf science, 114, 18-22.

Harisson, P. H., Khan, N., Yin, K., Saleem, M., Bano, N., Nisa, M., Ahmed, S. I., Rizvi, N., Azam, F., 1997.Nutrient and phytoplankton dynamics in two mangrove tidal creeks of the Indus river delta, Pakistan. Marine Ecology Progress Series, 157, 13–19.

Hartzell, J. L., Jordan, T. E., Cornwell, J. C., 2010. Phosphorus Burial in Sediments Along the Salinity Gradient of the Patuxent River, a Subestuary of the Chesapeake Bay (USA). Estuaries and Coasts, 33, 92-106.

Hawke, D., Carpenter, P. D., Hunter, K. A., 1989. Competitive adsorption of phosphate on goethite in marine electrolytes. Environmental science & technology, 23, 187-191.

Hieltjes, A. H. M., Lijklema, L., 1980. Fractionation of inorganic phosphates in calcareous sediments.Journal of Environmental Quality, 9, 405–407.

Holmboe, N., Kristensen, E., Andersen, F.Ø., 2001. Anoxic decomposition in sediments from a tropical mangrove forest and the temperate Wadden Sea: impacts of N and P addition experiments. Estuarine, Coastal and Shelf Science, 53, 125–140.

Holmer, M., Storkholm, P., 2001. Sulfate reduction and sulfur cycling in lake sediments: a review. Fresh water Biology, 46, 431– 451.

Hou, L. J., Liu, M., Yang, Y., Ou, D. N., Lin, X., Chen, H., Xu, S. Y., 2009. Phosphorus speciation and availability in intertidal sediments of the Yangtze Estuary , China. Applied Geochemistry, 24, 120-128.

Howarth, R. W., Jensen, H. S., Marino, R., Postma, H., 1995. Transport to and processing of P in near-shore and oceanic waters, In Thiessen, H. [ed.], Scope 54, Phosphorus in the global environment. Transfers, cycles and management. Wiley.

Hsieh, M., 2006. Geochemical characterization of organic matter in Victoria Harbour sediments, Hong Kong. PhD thesis submitted to the University of Oklahoma, Japan.

Huerta-Diaz, M. A., Morse, J. W., 1992. Pyritization of trace metals in anoxic marine sediments. Geochimica et Cosmochim Acta, 56, 2681– 2702.

Ingall, E. D., van Cappellen, P., 1990. Relation between sedimentation rate and burial of organic phosphorus and organic carbon in marine sediments. Geochimica et Cosmochim Acta, 54, 373–386.

Iyer, C. S., Padmanabha, Sindhu, M., Kulkarni, S. G., Tambe, S. S., Kulkarni, B. D., 2003. Statistical analysis of the physico– chemical data on the coastal waters of Cochin. Journal of Environmental Monitoring, 5, 324-327.

Jensen, H. S., Kristensen, P., Jeppesen, E., Skytthe, A., 1992. Iron:phosphorus ratio in surface sediment as an indicator of phosphate release from aerobic sediments in shallow lakes. Hydrobiologia, 235, 731–743.

Jensen, H. S., Mortensen, P. B., Andersen, F. O., Jensen. A., 1995. Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. Limnology and. Oceanography, 40, 908–917.

Jia, G., Peng, P., 2003. Temporal and spatial variations in signatures of sedimented organic matter in Lingding Bay (Pearl estuary), Southern China. Marine Chemistry, 82, 47–54.

Joseph, M. M., Kumar, C. R., Kumar, T. G., Renjith, K. R., Chandramohanakumar, N., 2008. Biogeochemistry of surficial sediments in the intertidal systems of a tropical environment. Chemistry and Ecology, 24, 247-258.

Joseph, M. M., Ratheeshkumar, C. S., Renjith, K. R., Gireeshkumar, T. R., Chandramohanakumar, N., 2010. Phosphorus fractions in the surface sediments of three mangrove systems of southwest coast of India. Environmental Earth Sciences, 62, 1209-1218.

Kalff, J., 2002. Limnology: inland water ecosystems New Jersey: Prentice Hall.

Katsaounos, C. Z., Giokas, D. L., Leonardos, J. D., Karayannis, M. I., 2007. Speciation of phosphorus fractionation in river sediments by explanatory data analysis. Water Research, 41, 406–418.

Kennish, M. J., 1991. Ecology of estuaries: anthropogenic effects, CRC press.

Kleeberg, A., Schubert, H., 2000. Vertical gradients in particle distribution and its elemental composition under oxic and anoxic conditions in a eutrophic lake, Scharmutzelsee, NE Germany. Archivfuer Hydrobiologie, 148, 187–207.

Kozerski, H. P., Kleeberg, A., 1998. The sediments and benthic- pelagic exchange in the shallow lake Muggelsee (Berlin, Germany). International Review of Hydrobiology, 83, 77–112.

Kristensen, E., Bouillon, S., Dittmar, T., Marchand, C., 2008. Organic carbon dynamics in mangrove ecosystems: a review. Aquatic Botany, 89, 201–219.

Kufel, I., Kufel, L., 1997. Eutrophication processes in a shallow, macrophyte-dominated lake–nutrient loading to and flow through Lake Łuknajno (Poland). Hydrobiologia, 342, 387-394.

Lacerda, D., Ittekkot, V., Patchineelama, S. R., 1995. Biogeochemistry of Mangrove Soil Organic Matter: a Comparison Between *Rhizophora* and *Avicennia* Soils in South-eastern Brazil. Estuarine, Coastal and Shelf Science, 40, 713–720.

Leventhal, J. S., 1983. An interpretation of carbon and sulfur relationships in Black Sea sediments as indicators of environmental deposition. Geochimica et Cosmochimica Acta, 47, 133–137.

Li, X. H., Liu, J. S., Sun, X. J., Zhu, Z. L., Zhang, X. J., 2009. Composition and vertical distribution of soil sulfur in wetland soil under Calamagrosi sangustifolia in Sanjiang Plain. Journal of Ecology and Rural Environment, 25, 34–38.

Lin, A., Morse, J. W., 1991.Sulfate reduction and iron sulfide mineral formation in Gulf of Mexico anoxic sediments. American Journal of Science, 291, 55– 89.

Liu, M., Hou, L. J., Xu, S. Y., Ou, D. N., Yang, Y., Yu, J., Wang, Q., 2006. Organic carbon and nitrogen stable isotopes in the intertidal sediments from the Yangtze Estuary, China. Marine Pollution Bulletin, 52, 1625–1633.

Lukkari, K., Leivuori, M., Kotilainen, A., 2009. The chemical character and behaviour of phosphorus in poorly oxygenated sediments from open sea to organic-rich inner bay in the Baltic Sea. Biogeochemistry, 96, 25-48.

Manju, M. N., Resmi, P., Gireesh Kumar 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, 893-902.

Manson, F. J., Loneragan, N. R., Harch, B. D., Skilleter, G. A. and Williams, L., 2005. A broad-scale analysis of links between coastal fisheries production and mangrove extent: a case-study for north-eastern Australia. Fisheries Research, 74, 69–85.

Marchand, C., Lallier-Vergès, E., Baltzer, F., 2003.The composition of sedimentary organic matter in relation to the dynamic features of a mangrove-fringed coast in French Guiana. Estuarine, Coastal and Shelf Science, 56, 119-130.

Mesnage, V., Bonneville, S., Laignel , B., Lefebvre, D., Dupont , J. P., Mikes, D. 2002. Filling of a wet land (Seine estuary, France): natural eutrophication or anthropogenic processes? A sedimentological and geochemical study of wetland organic sediments. In Nutrients and Eutrophication in Estuaries and Coastal Waters, Springer, Netherlands.

Mesnage, V., Picot, B., 1995.The distribution of phosphate and its relation with eutrophication of a Mediterranean coastal lagoon. Hydrobiologia, 297, 29–41.

Meyers, P. A., 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Organic Geochemistry, 27, 213–250.

Meyers, P. A., Silliman, J. E., Shaw, T. J., 1996. Effects of turbidity flows on organic matter accumulation, sulfate reduction, and methane generation in deep sea sediments on the Iberia Abyssal Plain. Organic Geochemistry, 25, 69–78.

Monbet, P. H., Brunskill, G. J., Zagorskis, I., Pfitzne, J., 2007. Phosphorus speciation in the sediment and mass balance for the central region of the Great Barrier Reef continental shelf (Australia). Geochimica et Cosmochimica Acta, 71, 2762–2779.

Moncheva, S., Gotsis-Skretas, O., Pagou, K., Kraster, A., 2001. Phytoplankton blooms in Black Sea and Mediterranean coastal ecosystems subjected to anthropogenic eutrophication: similarities and differences. Estuarine, Coastal and Shelf Science, 53, 281–295.

Morris, C. P., James. T., 1999. Distribution and speciation of phosphorus along a salinity gradient in intertidal marsh sediments. Biogeochemistry, 45,197-221.

Morse, J. W., Rowe, G, T., 1999. Benthic biogeochemistry beneath the Mississippi River Plume. Estuaries, 22, 206–214.

Muller, L. D., 1977. Mineral separation. In: J. Zussman (Ed.): Physical Methods in Determinative Minralogy. Academic Press, London.

Muzuka, A. N. N., Shunula, J. P., 2006. Stable isotope compositions of organic carbon and nitrogen of two mangrove stands along the Tanzanian coastal zone. Estuarine, Coastal and Shelf Science, 66, 447–458.

Nair, S. M., Balchand, A. N., 1993. Phosphate–phosphorus adsorption characteristics of sediments from a very high productive coastal zone. Toxicological& Environmental Chemistry, 39, 81–95.

Naskar, K., Mandal. R., 1999. Ecology and biodiversity of Indian mangroves Part I Global status. Daya publishing House, New Delhi.

Newman, S., Reddy, K. R., 1993. Alkaline phosphatase activity in the sediment-water column of a hypereutrophic lake. Journal of environmental quality, 22, 832-838.

Nielsen, T., Andersen, F. Ø., 2003. Phosphorus dynamics during decomposition of mangrove (*Rhizophora apiculata*) leaves in sediments. Journal of Experimental Marine Biology and Ecology, 293, 73-88.

Odum, E. P., 2000. Tidal marshes as outwelling/pulsing systems. In: Concepts and controversies in tidal marsh ecology. Weinstein, M. P, Kreeger, D. A (eds). Kluwer, Dordrecht, Netherlands.

Paludan, C., Morris. J. T., 1999. Distribution and speciation of phosphorus along a salinity gradient in intertidal marsh sediments.Biogeochemistry, 45,197-221.

Perkins, R. G., Underwood, G. J. C., 2001. The potential for phosphorus release across the sediment–water interface in a eutrophic reservoir dosed with ferric sulfate. Water Research, 35, 1399–1406.

Prahl, F. G., Ertel, J. R., Goni, M., Sparrow, M. A., Eversmeyer, B., 1994. Terrestrial organic-carbon contributions to sediments on the Washington margin. Geochimica et Cosmochimica Acta, 58, 3035–3048.

Prasad, M. B. K., Ramanathan, A. L., 2010. Characterization of phosphorus fractions in the sediments of a tropical intertidal mangrove ecosystem. Wetlands ecology and management, 18, 165-175.

Psenner, R., Bostrom, B., Dinka, M., Petterson, K., Pucsko, R., Sager, M., 1988. Fractionation of phosphorus in suspended matter and sediment. Arch. Hydrobiol. Beih.Ergebn.Limnol, 30, 98–103.

Ramakrishnan, R., Perumal, P., Santhanam P., 1999. Spatio-temporal variations of hydrographical features in the Pichavaram mangroves and Mohi aqua farm, Southeast coast of India. In: Proc. Intl. Sem. Appl. Hydrogeochem., Annamalai University, Annamalai Nagar, India, Published by Dept. of Geology, Annamalai University, Chidambaram, Tamil Nadu.

Ranjan, R. K., Routh, J., Ramanathan, A. L., Klump, J. V., 2011. Elemental and stable isotope records of organic matter input and its fate in the Pichavaram mangrove–estuarine sediments (Tamil Nadu, India). Marine Chemistry, 126, 163–172.

Reddy, K. R., D'angelo, E. M., 1994. Soil processes regulating water quality in wetlands. Global wetlands: Old world and new, 309-324.

Renjith, K. R., 2006. Chemodynamics and Ecohydrology of a Tropical Estuary. PhD thesis submitted to Cochin University of Science and Technology.

Renjith, K. R., Chandramohanakumar, N., Joseph, M. M., 2011. Fractionation and bioavailability of phosphorus in a tropical estuary, Southwest India. Environmental monitoring and assessment, 174, 299-312.

Richardson, C. J., 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. Science, 228, 1424-1427.

Richman, M. B., 1986. Rotation of principal components. Journal of climatology, 6, 293–335.

Rosenbauer, R. J., Swarzenski, P. W., Kendall, C., Orem, W. H., Hostettler, F. D., Rollog, M. E., 2009. A carbon, nitrogen, and sulfur elemental and isotopic study in dated sediment cores from the Louisiana Shelf. Geo-Marine Letters, 29, 415-429.

Rumolo, P., Barra, M., Gherardi, S., Marsella, E., Sprovieri, M., 2011. Stable isotopes and C/N ratios in marine sediments as a tool for discriminating anthropogenic impact. Journal of Environmental Monitoring, 13, 3399-3408.

Ruttenberg, K. C., Berner. R. A., 1993. Authigenic apatite formation and burial in sediments from non-upwelling continental margin environments. Geochimicaet Cosmochimica Acta, 57, 991–1007.

Rydin, E., Brunberg, A. K., 1998. Seasonal dynamics of phosphorus in Lake Erken surface sediments. Archiv fur Hydrobiologie (Arch Hydrobiol), 51, 157–167.

Saint-Laurent, D., St-Laurent, J., Lavoie, L., Ghaleb, B., 2008. Use geopedological methods for the evaluation of sedimentation rates on river floodplains. Catena, 73, 321–337.

Schenau, S. J., De Lange, G. J., 2001. Phosphorus regeneration vs. burial in sediments of the Arabian Sea. Marine Chemistry, 75, 201–217.

Schindler, D. W., 1977. Evolution of phosphorus limitation in lakes. Science, 195, 260–262.

Schulz, H. D., Zabel, M., 2009. Marine Geochemistry 2nd revised, updated and extended edition. Springer, Berlin Heidelberg, New York.

Sebastian, R., Chacko, J., 2006. Distribution of organic carbon in tropical mangrove sediments (Cochin, India). International journal of environmental studies, 63, 303-311.

Selig, U., 2003. Particle size-related phosphate binding and P-release at the sediment – water interface in a shallow German lake. Hydrobiologia, 492, 107-118.

Silva, C. A. R., Mozeto, A. A., 1997. Release and retention of phosphorus in mangrove sediments: Sepetiba Bay, Brazil. In "Mangrove Ecosystem Studies in Latin America and Africa". UNESCO, Paris.

Silva, C. A. R., Sampaio, L. S., 1998. Speciation of phosphorus in a tidal floodplain forest in the Amazon estuary. Mangroves and Salt Marshes, 2, 51–57.

Sinke, A. J. C., Cappenberg, T. E., 1988. Influence of bacterial processes on the P release from sediments in the eutrophic Loosdrecht lakes, the Netherlands. Arch. Hydrobiol. Beih.Ergebn.Limnol, 30, 5–13.

Smith, T. J., Boto, K. G., Frusher, S. D., Giddins, R. L., 1991. Keystone species and mangrove forest dynamics: The influence of burrowing by crabs on soil nutrient status and forest productivity. Estuarine, Coastal and Shelf Science, 33, 419 - 432.

Solanki, V. R., Hussain, M. M., Raja, S. S., 2010. Water quality assessment of Lake Pandu Bodhan, Andhra Pradesh State, India. Environmental monitoring and assessment, 163, 411-419.

Søndergaard, M., Jensen, J. P., Jeppesen, E., 2001. Retention and internal loading of phosphorus in shallow, eutrophic lakes. The Scientific World, 1, 427–442.

Søndergaard, M., Jensen, J. P., Jeppesen, E., 2003.Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia, 506, 135–145.

Stein, R., Rack, F. R., 1992. A 160,000-year high-resolution record of quantity and composition of organic carbon in the Santa Barbara Basin (Site 893). In proceedings-ocean drilling program scientific results. National Science Foundation.

Stumm, W., Morgan, J. J., 1981. Aquatic Chemistry An introduction emphasizing chemical equilibria in natural waters. Wiley interscience.

Tessenow, U., 1972. Lösungs, Diffusions und Sorption sprozesse in der Oberschicht von Seen-sedimenten.Ein Lang- zeitexperimentunteraeroben und anaeroben Bedingungen in Flie ßgleichgewicht. Archivfür Hydrobiologie. Supplement, 3, 353–398.

Tripathy, S. C., Ray, A. K., Patra, S., Sarma, V. V, J., 2005. Water quality assessment of Gautami-Godavari mangrove estuarine ecosystem of Andhra Pradesh, India during September 2001. Earth System Sciences, 114, 185-190.

Trott, L. A., Alongi, D. M., 1999. Variability in surface water chemistry and phyto- plankton biomass in two tropical, tidally dominated mangrove creeks. Marine and Freshwater Research, 50, 451–457.

Valentina, G. C., Joseph, N. B., 2005. Spatial patterning of water quality in Biscayne Bay, Florida as a function of land use and water management. Marine Pollution Bulletin, 50, 1416-1429.

Van der Zee, C., Slomp, C. P., van Raaphorst, W., 2002. Authigenic P formation and reactive P burial in sediments of the Nazare canyon on the Iberian margin (NE Atlantic). MarineGeology, 185, 379–393.

vanLuijn, F. V., van der Molen, D. T., Luttmer, W. J., Boers, P. C. M., 1995. Influence of benthic diatoms on the nutrient release from sediments of shallow lakes recovering from eutrophication. Water Science and Technology, 32, 89–97.

Vicente, I. D., Serrano, L., Amores, V., Clavero, V., 2003. Sediment phosphate fractionation and interstitial water phosphate concentration in two coastal lagoons (Albuferas de Adra , SE Spain). Hydrobiologia, 492, 95–105.

Wang, G., Guan, D., Peart, M. R., Chen, Y., Peng, Y., 2013a. Ecosystem carbon stocks of mangrove forest in Yingluo Bay, Guangdong Province of South China. Forest Ecology and Management, 310, 539-546.

Wang, L., Ye, M., Li, Q., Zou, H., Zhou, Y., 2013b. Phosphorus speciation in wetland sediments of Zhujiang (Pearl) River Estuary, China. Chinese Geographical Science, 23, 574-583.

Wetzel, R. G., 2001. Limnology: lake and river ecosystems, 3rd edn. Academic Press, San Diego, CA, USA.

White, P. A., Kalff, J., Rasmussen, J. B., Gasol, J.M., 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microbial Ecology, 21, 99-118.

Williams, J. D. H., Syers, J. K., Harris, R. F., Armstrong, D. E., 1971. Fractionation of inorganic phosphate in calcareous lake sediments.Soil Science Society of America, Proceedings, 15, 250–255.

Woodruff, S. L., House, W. A., Callow, M. E., Leadbeater, B. S. C., 1999. The effects of biofilms on chemical processes in surficial sediments. Freshwater Biology, 41, 73–89.

Wunderlin, D. A., Diaz, M. P., Ame, M. V., Presce, S. F., Hued, A. C., Bistoni, M. A., 2001. Pattern recognition techniques for the evaluation of spatial and temporal variations in water quality. A case study: Suquia river basin (Cordoba-Argentina). Water Research, 35, 2881–2894.

Yamamuro, M., 2000.Chemical tracers of sediment organic matter origins in two coastal lagoons. Journal of Marine Systems, 26, 127–134.

Yang, S., Tang, M., Yim, W. W. S., Zong, Y., Huang, G., Switzer, A. D., Saito, Y., 2011. Burial of organic carbon in Holocene sediments of the Zhujiang (Pearl River) and Changjiang (Yangtze River) estuaries. Marine Chemistry, 123,1-10.

Ye, W., Wang, A., Chen, J., 2013. Temporal–spatial variation and source analysis of carbon and nitrogen in a tidal wetland of Luoyuan Bay. Acta Ecologica Sinica, 33, 150–157.

Zhao, S. J., Jiao, N. Z., Shen, Z. L., Wu, Y. L., 2005. Causes and consequences of changes in nutrient structure in the Jiaozhou Bay. Journal of Integrative Plant Biology, 47, 396–410.

Zhou, J., Wu, Y., Kang, Q., Zhang, J., 2007. Spatial variations of carbon, nitrogen, phosphorus and sulfur in the salt marsh sediments of the Yangtze Estuary in China. Estuarine, Coastal and Shelf Science, 71, 47-59.

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Chapter 4

STABLE CARBON ISOTOPES AND BIOCHEMICAL COMPOSITION OF SEDIMENTS

Contents

4.1 Introduction
4.2 Results
4.3 Discussion
4.4 Principal component Analysis
4.5 Conclusions

4.1 Introduction

Two general approaches used to characterize organic matter in natural mixtures are bulk and molecular-level techniques. Bulk elemental ratios such as C/N and C/S, are the representative of the major component of organic matter that provides an overview of the system. But, these ratios, for example, TOC/TN ratios of sediments, may be altered to different extents by one of the following ways; selective decomposition of organic matter during humification and early diagenesis (Thornton and McManus, 1994; Meyers, 1997; Jia and Peng, 2003; Hsieh, 2006; Liu et al., 2006) or the colonisation of bacterial and fungal populations on “ageing” of vascular plant detritus (due to the typically low (e.g., 3 to 4) C to N ratios found in bacteria) (Tenore et al., 1982; Rice and Hanson, 1984). The elemental ratios are also affected by artifacts in the standard procedure like carbonate carbon removal (Meyers, 2003), or poor sorting in coarse-grained sediments (Schulz and Zabel, 2006). Hence, techniques like stable isotopic carbon analysis, biochemical composition and pigment analysis, were employed to investigate the diagenetic state of organic matter in surface sediments.

4.1.1 Stable Carbon Isotope

Stable isotopic carbons of organic substances are measured via isotope ratio mass spectrometry in which samples are combusted to convert organic matter into gases (e.g. CO_2, N_2) which are then ionized and the ratios of the isotopes quantified. The use of isotopes as tracers of biological, physical and geological processes helps to identify different ocean water masses, and marine versus terrestrially derived organic matter (Deegan and Garritt, 1997; Vizzini and Mazzola, 2003; Ogrinc et al., 2005; Vizzini et al., 2005; Usui et al., 2006; Tesi et al., 2007; Gao et al., 2008). They also integrate the results of on-going processes such as the passage of organic elements up the trophic levels, climate change, marine productivity, and the formation and melting of continental glaciers. Stable isotopic signatures carry over geologic time and cannot be readily altered by equilibration or degradation processes, even though severe changes occur in its chemical composition (Emerson and Hedges, 2008).

$\delta^{13}C$ values can be used as a tool for distinguishing C_3 and C_4 plants due to the non-overlapping distribution along their photosynthetic pathways (O'Leary, 1981). Studies focusing on the natural abundances of carbon and nitrogen isotopes have been successfully used in the sediments of mangrove ecosystems to trace source inputs of terrestrial and aquatic organic matter (Bouillon et al., 2002a, b; Lehman et al., 2002; Fourqurean and Schrlau, 2003; Rundegren et al., 2003) and a few of them are listed in Table 4.1.

4.1.2 Biochemical analysis

All organisms are composed of the same basic chemical classes, of these carbohydrates, proteins and lipids are geochemically more important. In addition, higher plants contain significant quantities of lignin, a major component of their supportive tissue.

Only a small fraction of the mangrove leaf litter is directly consumed by benthic organisms and the rest must be fractionated before entering the food chain (Velimirov, 1986). Labile fraction of organic matter mainly consists of carbohydrates, lipids and proteins, and is more readily available to benthic consumers (Fabiano et al., 1995; Dell' Anno et al., 2002). The carbohydrate was the major biochemical component determined since it is a reliable index of the readily assimilable organic matter in sediments (Verlencar et al., 2006). Carbohydrates are important in the production of fats, proteins etc. and proteins account for most of the nitrogen present in organisms. Lignins, tannins and related compounds are characterized by phenolic (hydroxy-aromatic) structures. They derive from monosaccharide units, which are common in plants, but animal tissues do not have.

Quantity and quality of organic matter in surface sediments, accomplished with the use of biochemical indices like protein, carbohydrate, lipids, protein to carbohydrate ratio and lipid/carbohydrate, affects benthic fauna dynamics and metabolism (Grant and Hargrave, 1987; Grémare et al., 2002). Thus, origin of particles, the factors controlling their diagenesis (Colombo et al., 1996), quality of organic materials as a food source for benthic consumers (Fabiano and Danovaro, 1994; Mayer et al., 1995; Danovaro and Fabiano, 1996; Gremare et al., 1997; Rossi et al., 2001; Rossi and Lardicci, 2002; Cividanes et al., 2002) and the trophic status of coastal marine systems

(Dell Anno et al., 2002; Pusceddu et al., 2003) can be explored using biochemical indices. For e.g., since bacterial metabolism largely involves proteins than carbohydrates (Newell and Field, 1983), the protein to carbohydrate ratio (PRT: CHO) in sediments can be assigned to determine the age of sedimentary organic matter (Danovaro et al., 1993; Cividanes et al., 2002). High PRT:CHO ratios describes a living organic matter or newly generated detritus (Danovaro et al., 1993) and low ratios suggest the presence of aged organic matter (Danovaro et al., 1993; Joseph et al., 2008). Thus the biochemical composition of sedimentary organic matter has valid significance in determining origin, and the quality and quantity of organic matter, (Joseph et al., 2008) in different marine ecosystems (Dell'Anno et al., 2002).

Table 4.1 List of previous studies on stable carbon isotope

Study Area	Concentration	Reference
Plum Island salt marsh sediments	-19.4‰ ±0:1 to -23.3‰±0.3 ‰	Wang et al., 2003
Bagamoyo mangrove forest average	-25.5‰	Muzuka and Shunula, 2006
Kisakasaka mangrove forest average	-9.4‰	
Mangroves of Celestun lagoon	-28.79‰	Gonneea et al., 2004
Mangroves of Chelem	-26.55‰	
Mangroves of Terminos	-28.93‰	
Columbia lake	-26.1‰ to -20.9‰	Dunn et al., 2008
Pichavaram mangrove sediments	-28.92 ‰ to -25.34 ‰	Bala Krishna Prasad and Ramanathan, 2009
Pichavaram mangrove sediments	-24.3‰±1.8‰	Ranjan et al., 2011

Microphytobenthos and macroalgae contribute more to the primary production in shallow coastal ecosystems than by phytoplankton (Barranguet et al., 1996; Lucas et al., 2000), hence the present study envisage sediment phytopigment concentrations as a mode for tracing the amount of organic matter produced by photosynthesis (Cahoon and Cooke, 1992; Lucas et al., 2000; Manini et al., 2001). Previous studies on chlorophyll pigments are shown in Table 4.2.

Table 4.2 List of previous studies on Chlorophyll a

Study area	Concentration	Reference
Northrern Adriatic Sea	1.5 to 21.9 μgg^{-1}	Denovaro et al., 2001
Mattarelle	1 to 31.6 μgg^{-1} 17.7 to 5.6 and 8.3 to 4.9 mgg^{-1}, 28.8 ± 7.7 μgg^{-1} and 19.9 ± 3.9 μgg^{-1}	Dell'Anno et al., 2002
intertidal sediments of Coombabah Lake	0.43 to 14.4 μgg^{-1}	Dunn et al., 2008
Alaskan Beaufort Sea	~11.5$\mu g\ m^{-2}$	Belicka et al., 2009
mangrove sediments Tanzania	13.9 ± 3.9 μgg^{-1}	Penha-Lopes et al., 2010
Arcachon Bay	8.9±3.7 μgg^{-1}	Dubois et al., 2012
New Caledonia mangrove sediments	83.4 ± 49.6 μgg^{-1}	Leopold et al., 2013

4.2 Results

Variation of stable carbon isotopes and biochemical composition are shown in Table 4.3.

4.2.1 Stable isotope Analysis and Biochemical Composition

The $\delta\ ^{13}C$ for mangrove leaf was -32.49 ‰ while that of phytoplankton it was -20.71‰. The results of analysis of $\delta\ ^{13}C$ from surface sediments are shown in annexure 3. Most enriched values of $\delta\ ^{13}C$ has reported from Kadalundi (avg.±stdv:25.15 ‰ ± 0.24 ‰) followed by Thalassery (avg.±stdv:27.47‰ ± 0.27 ‰). All stations, except Kunjimangalam, showed more depleted values during pre-monsoon season (avg.±stdv:27.14 ‰). In Pappinissery (avg.±stdv:28.23‰±0.16‰), Pazhayangadi (avg.±stdv: 28.79±0.36‰) and Kunjimangalam (avg.±stdv:27.92±0.70‰) seasonal variation follows the order post-monsoon< pre-monsoon <monsoon.

Table 4.3: Variation of Biochemical components and Stable Carbon isotopes and the results of ANOVA

					Spatial Variation		Seasonal Variation	
	Max	**Min**	**Avg**	**Stdv**	**F**	**P-value**	**F**	**P-value**
CHO, mgg^{-1}	71.61	3.34	17.23	17.39	2.68	0.11	4.22	0.06
LPD, mgg^{-1}	13.01	1.03	4.31	3.45	6.12	0.01**	0.10	0.91
PRT, mgg^{-1}	33.00	0.18	6.54	10.38	1.36	0.33	9.95	0.01**
Chl a, μgg^{-1}	26.27	3.53	10.35	6.17	1.68	0.25	0.82	0.47
Chl b, μgg^{-1}	5.02	0.96	2.72	1.33	3.64	0.06	0.71	0.52
Chl c, μgg^{-1}	6.19	0.84	2.81	1.53	1.64	0.26	0.63	0.55
Pheo , μgg^{-1}	32.94	3.71	14.62	8.65	2.49	0.13	1.14	0.37
T&L, mgg^{-1}	5.20	0.29	2.22	1.48	13.31	0.00**	2.01	0.20
PRT/CHO	2.21	0.04	0.51	0.70	1.31	0.35	17.29	0.00**
LPD/CHO	0.71	0.10	0.38	0.18	3.70	0.05*	0.03	0.97
BPC, mgg^{-1}	35.16	2.28	10.69	8.54	6.17	0.04*	4.46	0.05*
$\delta^{13}C$, ‰	-25.01	-29.19	-27.51	1.34	52.85	0.00**	2.97	0.11

*P value significant at <0.05 level ** P value significant at <0.01 level
Fcritical spatial variation 3.84 F critical spatial variation 4.46

Carbohydrates followed by Lipids were the major biochemical component in the present study region. CHO recorded higher concentration during post-monsoon (except Thalassery) but did not display significant spatial or temporal changes. All stations except Thalassery and Pappinissery showed minimum concentration during pre-monsoon. PRT displayed significant seasonal variations ($p<0.01$), while LPD displayed significant spatial in the study region. Compared to other seasons the post monsoon showed very high concentration for protein especially at Pappinissery and Pazhayangadi. T & L also showed higher concentration during post-monsoon (except Thalassery and Pappinissery) and lower concentration during the pre-monsoon season (except Pappinissery). T & L displayed significant spatial variation and showed much higher concentration in Pazhayangadi (avg.±stdv:4.06±1.19 mgg^{-1}) followed by Pappinissery (avg.±stdv:3.47±0.83 mgg^{-1}) and lowest concentration at Kunjimangalam (avg. ± stdv:-0.97±.59 mgg^{-1}).

BPC displayed significant spatial variation ($p < 0.01$) with highest concentration during monsoon. Concentration of BPC ranged from 3.75 to 35.16 mgg^{-1} (avg.±stdv:16.10±11.62 mgg^{-1}), 2.28 to 13.44 mgg^{-1} (avg.±stdv: 7.30±4.91 mgg^{-1}), 3.65 to 19.21 mgg^{-1} (avg.±stdv:8.66±6.31 mgg^{-1}) in post-monsoon, monsoon and pre-monsoon respectively.

4.2.2 Pigment Analysis

Thalassery and Kadalundi were characterised by higher chlorophyll pigment during post-monsoon, but all other stations had higher concentration of chlorophyll pigment during pre-monsoon. Pappinissery (avg. +stdv: 14.90 ± 10.02 μgg^{-1}, 3.26 ± 1.64 μgg^{-1}, 3.88 ± 2.19 μgg^{-1} respectively for Chl a, b & c) followed by Pazhayangadi (avg.±stdv: 13.88 ± 5.03 μgg^{-1}, 4.23 ± 0.50 μgg-^{1}, 3.92 ± 0.30 μgg^{-1} respectively for Chl a, b & c) showed comparatively higher concentration for pheopigments. Much lower concentration of chlorophyll pigment was reported in Kunjimangalam (avg.±stdv: 5.21 ± 1.59 μgg^{-1}, 1.24 ± 0.2 μgg^{-1}, 1.57 ± 0.71 μgg^{-1} respectively for Chl a, b & c) during monsoon season. In the present study, Chl a concentration is comparable to that of highly productive areas. But compared to the higher input of labile components from mangrove leaf litter, its contribution to the biopolymeric fraction is low.

4.3 Discussion

4.3.1 Stable isotopic carbon analysis

Carbon isotopes constitute a powerful tool for identifying carbon sources (Peterson et al., 1985; Lepoint et al., 2000; Pinnegar and Polunin, 2000; Vander Zanden and Rasmussen, 2001; Vizzini et al., 2002; Vizzini and Mazzola, 2003, 2006; Augley et al., 2007; Carlier et al., 2007), produced by various processes photosynthesis, sedimentation, degradation and transformation (Boutton, 1991). Terrestrial primary producers generally have lower $\delta^{13}C$ values than marine producers (Haines and Montague, 1979; Riera and Richard, 1996; Bouillon et al., 2000) due to the differences in the isotopic composition of inorganic and organic nutrient sources (Ostrom and Fry, 1993). Terrestrial organic matter produced by C_3 and C_4 pathway have $\delta^{13}C$ values ranging from -30 to -26‰ and -16 to -9‰ (Pancost and Boot, 2004) respectively, but for marine phytoplankton it ranged from -22 to -18‰ (Cifuentes and Eldridge, 1998).

$\delta\ ^{13}C$ values of sediments (Fig. 4.1) are found to be enriched relative to mangrove plant materials (unpublished data by Nebula), an observation that is in agreement with other reported works (Bouillon et al., 2002a; b, Muzuka and Shunula, 2006). This suggests the possibility of dilution of mangrove signature through mixing with material enriched in $\delta\ ^{13}C$. The material is probably derived from microbial biomass, oceanic nitrate, planktonic production and macrophytes that thrive well in near-shore waters (Fry and Sherr, 1984; Gearing, 1988).

Table 4.4 Correlation analysis of biochemical parameters in the mangrove ecosystems of northern Kerala coast

	TOC	TN	TS	Sand	Clay	Silt	CHO	LPD	PRT	Chl a	Pheo-pigment	T&L	BPC	$\delta^{13}C$
TOC	1													
TN	.947**	1												
TS	.803**	.800**	1											
Sand	-.605*	-.595*	-.604*	1										
Clay	.693**	.673**	.558*	-.824**	1									
Silt	.432	.482	.294	-.569*	.389	1								
CHO	.752**	.683**	.441	-.574*	.645**	.388	1							
LPD	.879**	.846**	.810**	-.656**	.682**	.303	.679**	1						
PRT	.541*	.541*	.215	-.109	.226	.368	.498	.442	1					
Chl a	.699**	.797**	.727**	-.609*	.721**	.432	.434	.522*	.125	1				
Pheo-pigments	.686**	.764**	.737**	-.715**	.728**	.475	.417	.547*	-.018	.965**	1			
T&L	.887**	.942**	.708**	-.576*	.614*	.439	.745**	.884**	.558*	.632*	.610*	1		
BPC	.885**	.837**	.670**	-.670**	.728**	.419	.932**	.894**	.538*	.538*	.533*	.890**	1	
$\delta^{13}C$	-.524*	-.510	-.487	.252	-.299	.100	-.191	-.692**	-.434	-.111	-.105	-.581*	-.439	1

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

Most depleted values were observed from Pazhayangadi followed by Pappinissery and most enriched values from Kadalundi followed by Thalassery. The distance from Lakshadweep Sea has a great influence in controlling $\delta\ ^{13}C$ value of present study region. In mangrove ecosystems, large quantities of organic material may be deposited particularly in those areas that are frequently inundated by tides (Muzuka and Shunula, 2006). Hence, enrichment of $\delta\ ^{13}C$ signature in Kadalundi and Thalassery vindicate that there is a subtle contribution of marine organic matter into these mangrove ecosystem from the adjacent coastal water. Kunjimangalam in pre-monsoon and Pappinissery and Pazhayangadi through all the season showed comparatively depleted values of $\delta\ ^{13}C$, pertaining to the input of terrestrially derived organic matter. In Kunjimangalam, during pre-monsoon season, a dissolved oxygen supersaturation due to excessive algal growth has been reported for the water column. This excessive algal growth may be accounted for the depletion of $\delta^{13}C$ during pre-monsoon season.

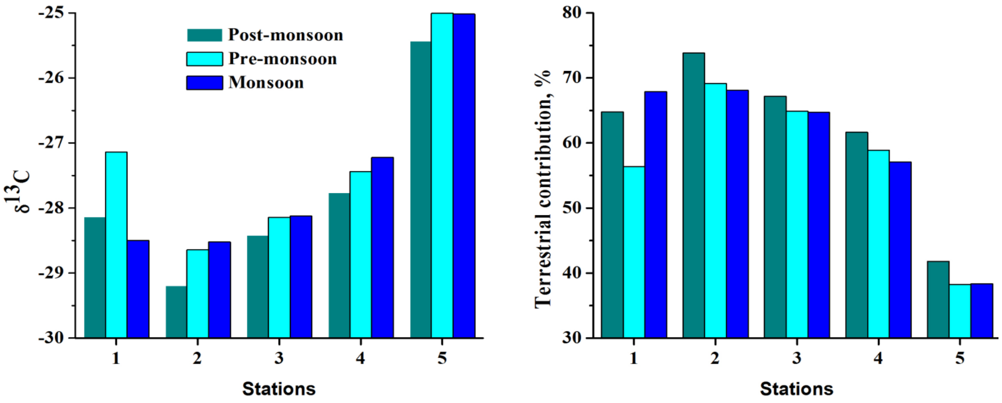

Fig. 4.1: Variation of $\delta^{13}C$ and terrestrial contribution calculated from two end member model in the mangrove ecosystems of northern Kerala coast

Sackett and Thompson, 1963 observed a gradient for the $\delta^{13}C$ value of near-shore sediment samples as a function of marine and terrestrial organic carbon due to mixing, and developed an isotopic mixing equation

$$\delta^{13}C = F_t\, \delta^{13}C_t + F_m\, \delta^{13}C_m \quad \ldots\ldots\ldots\ldots (1)$$

where, $\delta^{13}C = \delta^{13}C$ of the sediment sample, $\delta^{13}C_t = \delta^{13}C$ of terrestrial organic carbon in the region, $\delta^{13}C_m = \delta^{13}C$ of marine sediments in the region, F_t= fraction of terrestrial organic carbon in the sediment sample, F_m = fraction of marine organic carbon in the sediment sample (1 - F_t).

In order to evaluate the fraction of terrestrially derived organic matter (F%), Schultze and Calder, 1976 rearrange (1) as:

$$F\,(\%) = (\delta^{13}C_{marine} - \delta^{13}C_{measured}) / (\delta^{13}C_{marine} - \delta^{13}C_{terrestrial}) * 100 \quad \ldots (2)$$

For the calculation of terrestrial organic matter F%, in the mangrove ecosystems of the present study region terrestrial end member is taken as -32.49 ‰ ($\delta^{13}C_{terrestrial}$ -$\delta^{13}C$ of mangrove leaf from Kerala, unpublished data by Nebula) and marine end member as -20.5 ‰ (δ^{13}Cmarine) (Jia and Peng, 2003).

The terrestrial contribution in the present study region (Fig. 4.1) showed significant spatial variation (P value <0.01) and was varied from 41.83 to 73.80 % (avg.±stdv:61.85±12.05 %), 38.25 to 69.14 % (avg.±stdv: 57.51±12.87 %) and 38.35 to 68.15 % (avg.±stdv:59.22±12.50 %) during post-monsoon, pre-monsoon and monsoon season respectively. Kadalundi, which has showed clear distinction among the selected stations with comparatively lower terrestrial contribution (avg.±stdv:39.47±2.04 %), is situated near the estuarine mouth and more contribution from marine organic matter in this station substantiates the above observation. All other stations showed higher values (>55%) indicated predominance of terrestrially derived organic matter. Pazhayangadi (avg.±stdv:70.35±3.04 %) followed by Pappinissery (avg.±stdv:65.60± 1.38 %) showed appreciably higher F% reflect the preservation of mangrove signatures due to clayey texture and/or thick mangrove vegetation in these stations. In Kunjimangalam, lower F% was noticed in pre-monsoon, which corresponds to dissolved oxygen supersaturation produced by phytoplankton production. All stations except Kunjimangalam showed higher terrestrial contribution during post-monsoon, which may be accounted for the litter fall season of mangroves. Previous studies showed a highest leaf litter fall between December and March and higher fruit fall between December and January (Duke, 1990; Imgraben and Dittmann, 2008).

The contribution of marine algae (F′) could be estimated by the following expression (Hu et al., 2006):

$F' = 100 - F\ (\%)$ ....(3)

From this the content of algal-derived organic carbon (Al-OC) can be calculated according to the following equation,

$\text{Al-OC} = \text{TOC} \times F'$ ....(4)

Algal derived organic carbon contribution (Fig. 4.2) to the TOC was found to be higher at Pappinissery (avg.±stdv:-.2.40± 1.13 %) and Pazhayangadi (avg.±stdv:-2.26 ± 0.21 %), since these stations are organically rich. Kunjimangalam (avg.±stdv:-0.48 ± 0.31 %) contributed lowest algal organic carbon to the total pool because of its sandy nature.

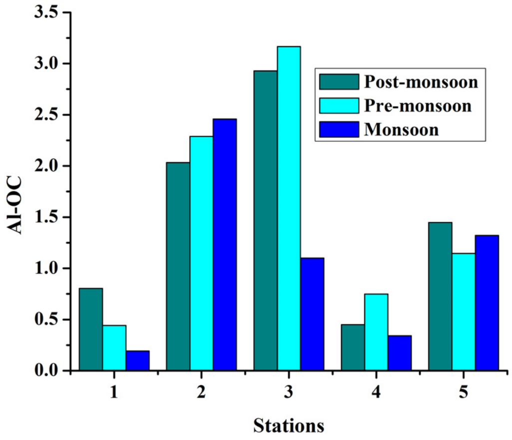

Fig. 4.2: Spatial and seasonal variation of Al-OC in the study region

4.3.2 Biochemical composition

Proteins, carbohydrates and lipids were analysed to assess the origin, quality and quantity of sedimentary organic matter present in the mangrove ecosystems. Carbohydrates represent the largest fraction of photosynthetically assimilated carbon in the biosphere (Cowie and Hedges, 1984). Carbohydrates include poly-hydroxyllated compounds ranging in size from 5-6 carbon sugars to large biopolymers (starch, cellulose), are much higher in vascular plants than in algae (Cowie and Hedges, 1984). In mangroves, litter is primarily composed of non-nutritive complex carbohydrates that are difficult or impossible for detritivores to digest (Kofoed, 1975; Bianchi and Levinton et al., 1984). Sedimentary protein concentrations reflect the productivity of marine ecosystems and it appears to be a good descriptor of the trophic status of the benthic systems at different spatial scales (Danovaro et al., 1999; 2000; Dell' Anno et al., 2002). Lipids in sediments are derived not only from aquatic biota but also from higher plant wax. Lipids in surface sediments are abundant in eutrophic systems than in oligotrophic systems. Like proteins, it also indicates the productivity of the system (Gremare et al., 1997).

Carbohydrates, which are the main biochemical component in the present study region (Fig. 4.3), did not display significant spatial or temporal changes. CHO in the present study region could be supplied from mangroves since it represents 65% of organic carbon in mangrove wood (Opsahl and Benner, 1999). The adsorption to clay minerals and less susceptibility to enzymatic degradation (Fabiano and Danovaro, 1998; Turley and Lochte, 1990) leads to the accumulation of complex polymers (Khodse et al., 2007) in the sedimentary organic matter. The higher concentration at Pazhayangadi may be due to the restricted tidal activity as well as clayey texture prevailed at this station. Pre-monsoon was characterised by low concentration of carbohydrate at Pazhayangadi, Kunjimangalam and Kadalundi. Carbohydrate concentration in mangroves is affected by salt stress (Mrunalini and Chavan, 2011) which may be reflected in sediment samples also.

The concentration of sedimentary proteins is a key parameter to define the trophic characteristics of the system (Danovaro et al., 1999). Sewage input might be resulted in the accumulation of protein at Pappinissery while sandy nature at Kunjimangalam leads to intense diagenesis. The tidal flushing can restrict the accumulation of protein to a certain extent (Ding and Henrichs, 2002) at Thalassery and Kadalundi. The low PRT concentration in monsoon might be due to its intense leaching from sediment, since it is more labile than CHO. Total lipids showed significant spatial variations (P value<0.01) but did not display any seasonal variations. Pappinissery and Pazhayangadi showed comparatively higher lipid concentration indicates their productive nature. Pre-monsoon characterised by lower lipid (except Pappinissery and Kadalundi) and protein (except Thalassery and Pappinissery) concentration which could be due to higher extracellular enzymatic activities induced by high temperature and faster organic matter degradation rates (Dell'anno et al., 2008).

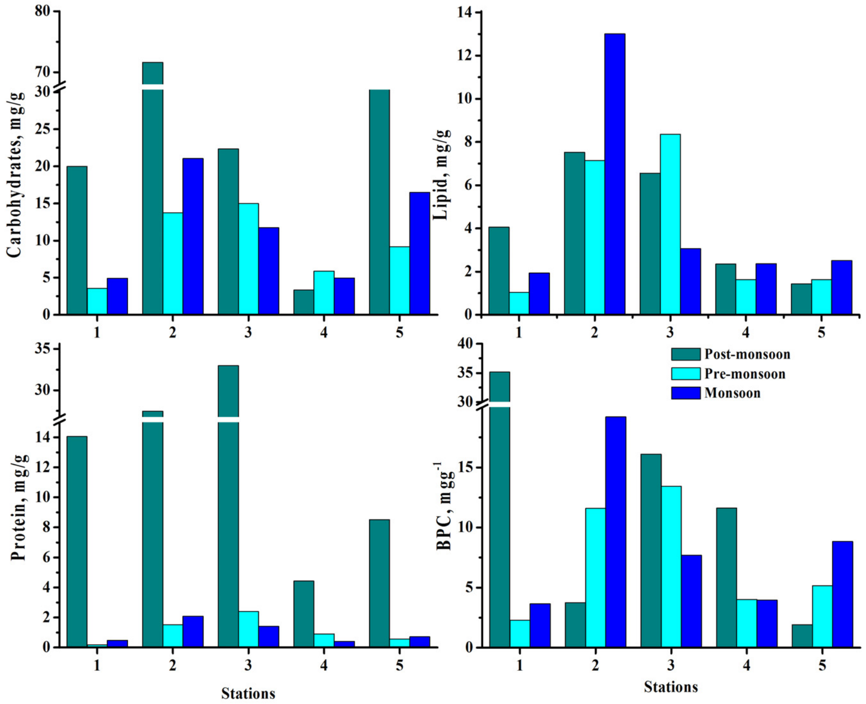

Fig. 4.3: Variation of biochemical composition in the present study region

The biopolymeric carbon (BPC) displayed significant spatial variation (P value<0.05) and showed much lower concentration in pre-monsoon except for Pappinissery and higher concentration in post-monsoon. Recent studies have shown that the BPC results are less conservative than TOC and better describe the nutritional status of a given ecosystem (Dell'Anno et al., 2002; Vezzulli et al., 2003). In the present study carbohydrates predominated in the BPC pool followed by lipids and proteins. Carbohydrates contribute 35.59 to 91.05 % in post monsoon, 44.64 to 70.94 % in pre-monsoon and 43.85 to 74.61 % in monsoon to the total BPC pool. Lipids contribute 7.01 to 47.06 %, 23.64 to 46.62 % and 21.39 to 50.82 % in post monsoon, pre-monsoon and monsoon respectively to the total BPC pool. Protein contribution to the total BPC pool was comparatively very low and was ranged from 1.93 to 17.35 %, 3.78 to 11.13 % and 3.99 to 8.95 % during post-monsoon, pre-monsoon and monsoon respectively.

Lignin has proven to be a useful chemical biomarker for vascular plant inputs to estuarine/coastal margin sediments (Gardner and Menzel, 1974; Hedges and Parker, 1976; Goñi and Hedges, 1992; Hedges et al., 1997; Louchouaran et al., 1997; Bianchi et al., 1999, 2002). Lignins are a group of macromolecular heteropolymers (600–1000 kDa) found in the cell wall of vascular plants that are made up of phenylpropanoid units (Sarkanen and Ludwig, 1971; de Leeuw and Largeau,1993) and tannins can make up as much as 20% of leaf and bark tissue (Kelsey and Harmon, 1989; Benner et al., 1990).

Pappinissery and Pazhayangadi exhibited comparatively higher concentration of tannin and lignin reflecting thick mangrove vegetation in this region (Fig. 4.4). Except Pappinissery, all other stations showed lower concentration of T & L in pre-monsoon, and it indicate lower contribution from litter falling. The positive correlation (Table 4.4) of T & L with all parameters except silt and BPC, indicates their fate or diagenetic alteration is identical. Microbial decomposition is generally slow in mangrove sediments because most of the detritus is of poor nutritious value and contains high levels of retarding compounds (Kristensen, 2008) such as tannins, lignin and structural carbohydrates (cellulose), which inhibits microbial degradation (Lee et al., 1990; Benner et al., 1990; Marchand et al., 2005). While cellulose and lignin can readily be degraded in oxic environments, these compounds are only degraded slowly under anoxic conditions (Kristensen, 2008).

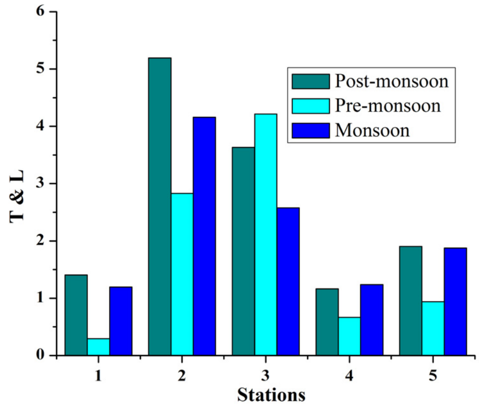

Fig. 4.4: Variation of Tannin and Lignin in the study region

Dell'Anno et al., 2002, defined hypertrophic, eutrophic and meso-oligotrophic condition according to their protein and carbohydrate concentration for the application to estuarine systems. Concentration of protein & carbohydrate for hyper-trophic, Eutrophic and meso-oligotrophic systems were > 4 & > 7 mg /g, 1.5 to 4 mg/g & 5 to 7 mg/g, < 1.5 mg /g & < 5 mg/g respectively. According to this classification post-monsoon is hypertrophic at all stations. During pre-monsoon and monsoon Pappinissery and Pazhayangadi were eutrophic and all others were meso-oligotrophic.

The protein to carbohydrate ratio is used as an index to determine the origin of materials present in sediment, and to distinguishing "fresh materials" from the aged organic matter (Cauwet, 1978; Danovaro et al., 1993; Cividanes et al., 2002). It was based on the assumption that proteins tend to be mineralized faster than carbohydrates (Newell and Field, 1983), and hence "fresh compounds" show high values for this index. Typically they are reported immediately after a micro-phytobenthic bloom (Fabiano et al., 1995) or after the deposition of freshly produced phytoplankton (Pusceddu et al., 2003). The high protein: carbohydrate in Pappinissery mangrove system may be due to the anthropogenic input of large amount of decaying meat wastes. Thalassery and Pazhayangadi mangrove stations showed higher protein: carbohydrate ratio in post monsoon season indicating large amount of recently produced organic matter. All other mangrove stations revealed protein to carbohydrate ratio less than 1.

Dominance of carbohydrate resulted in protein to carbohydrate ratio < 1, is a typical feature of detrital-heterotrophic environments (Danovaro, 1996). Similar to their study rare combination of low PRT: CHO and high BPC, were observed in the present study. Similar observation was recorded by several authors in seagrass sediments of *P. Oceanica* debris (Kenworthy and Thayer, 1984; Lawrence et al., 1989).

The lipid to carbohydrate ratio (LPD: CHO) have been used as good indices to describe the energetic (food) quality of the organic contents in the sediments (Grémare et al., 1997, 2002). The refractory fraction was higher in mangrove sediments and ratio was less than 1 at all stations. According to Joseph et al., 2008, the discrepancy between the high amounts of organic matter and its low nutritional value suggests that these ecosystems behave as a detrital trap for aged organic matter. As in the case of PRT: CHO, contribution of carbohydrate by mangrove leaf litter is the main factor controlling the LPD: CHO ratio. TOC showed positive correlation with all parameters, which may be due to their identical character of processes or the same fate i.e., they origin from mangrove leaf litter.

4.3.3 Chlorophyll pigments

In shallow coastal ecosystems, most of the primary production can be accounted for micro-phytobenthos and/or macro-algae rather than phytoplankton (Barranguet et al., 1996; Lucas et al., 2000). Although chlorophyll a is ubiquitous, chlorophyll b occurs primarily in green algae and vascular plants and chlorophyll c is characteristically found in diatoms and dinoflagellates (Emerson and Hedges, 2008). Phytopigment concentrations in the sediment are tracer of the amount of organic matter produced by photosynthesis, so that chlorophyll-a content (degradation half-life of 4-120 days) can be used as a descriptor of the trophic state and productivity of most estuarine and shallow coastal systems (Cahoon and Cooke, 1992; Lucas et al., 2000; Manini et al., 2001). In the present study, Chl *a* concentration was comparable to the high productive areas. Chl *a*, Chl *b* and Chl *c* was positively correlated with clay content (R^2 = 0.72, 0.76 and 0.65 respectively, n=15) and negatively correlated with sand content (R^2 = -0.61, -0.701 and -0.56 respectively, n=15). The preference of chlorophyll pigments in muddy sediment might be due to higher concentrations of inorganic nutrients which could help the productivity of the system (Sanil Kumar, 2009).

Alongi, 1994 reported lower intensity of microalgae in mangrove ecosystems due to light limitation. In the present study area, during pre-monsoon for it represents summer season higher quantity of light radiations are available. Pappinissery, Pazhayangadi and Kunjimangalam reported higher concentration of pigments during pre-monsoon season (Fig. 4.5) due to higher light penetration (Karlsson et al., 2009) and lower turbulence. The similar results were noticed in the water column. In Thalassery and Kadalundi hydrodynamic condition play a major role in controlling their distribution, since epipelic diatoms are easily re-suspended when exposed to conditions of high hydrodynamic energy (Delgado et al., 1991; De Jonge and Van Beusekom, 1995). At Kadalundi, Chl a concentration was higher during post-monsoon and monsoon which might be due to greater input of marine-derived organic matter. Due to the intense upwelling caused by the south west monsoon and the reversed circulation and mixed-layer deepening created by the north east monsoon, higher primary production is expected in Lakshadweep Sea (Qasim, 1982). The ratio of chlorophyll-a to pheopigment content expresses the fraction of metabolically active autotrophic component (Plante-Cuny and Bodoy, 1987) and was found to be less than one in the present study. Low values of this ratio could be due to higher rate of sedimentation from the overlying water column or greater fraction of detritus in primary material (Dell'Anno et al., 2002).

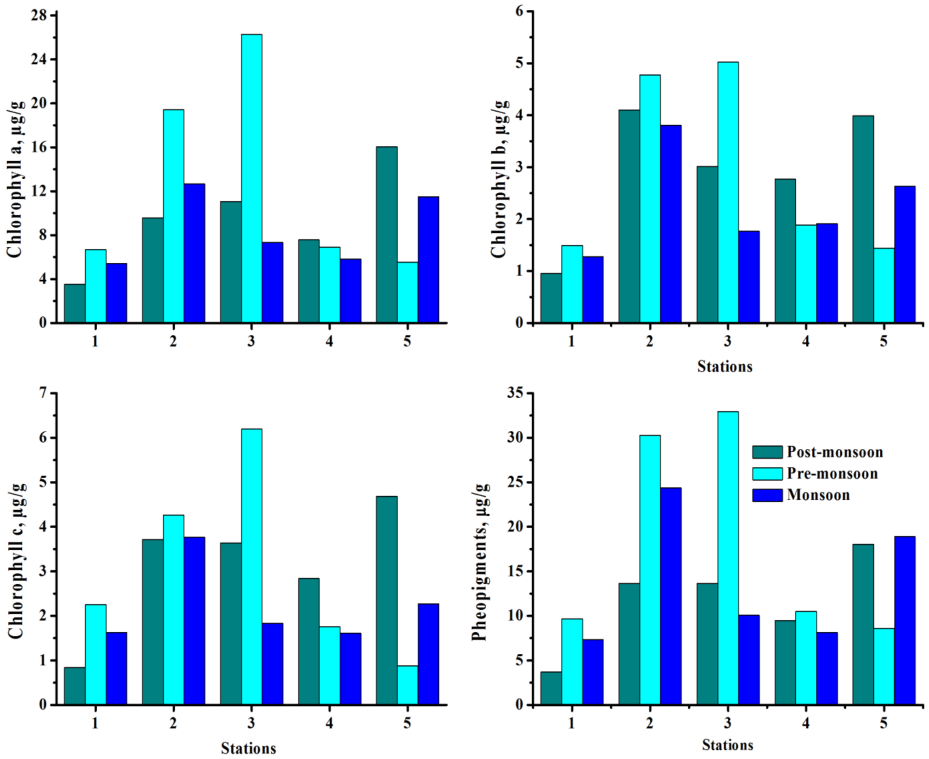

Fig. 4.5: Variation of chlorophyll pigments in the present study region

Low correlation between $\delta^{13}C_{org}$ and TOC to TN ratio (TOC/TN is already discussed in chapter 3) in the present study (Fig. 4.6) indicate that TOC /TN could not be used to evaluate the source of OM (Ogrinc et al., 2005). The plot between $TOC/TN_{corrected}$ and $\delta^{13}C$ again indicated low correlation (Fig. 4.6), insinuating contribution from microbial biomass (Liu et al., 2006; Gao et al., 2012).

The correlation analysis between $\delta^{13}C$ and Chl a (Fig. 4.6) give the inference that, a contribution of benthic primary produced organic matter to the surface sediment was feeble compared to terrestrial input (Gireeshkumar et al., 2013). It has been reported in the literature that sewage has no significant impact on the carbon isotopes and the system have strong self-purification capacities (Liu et al., 2006).

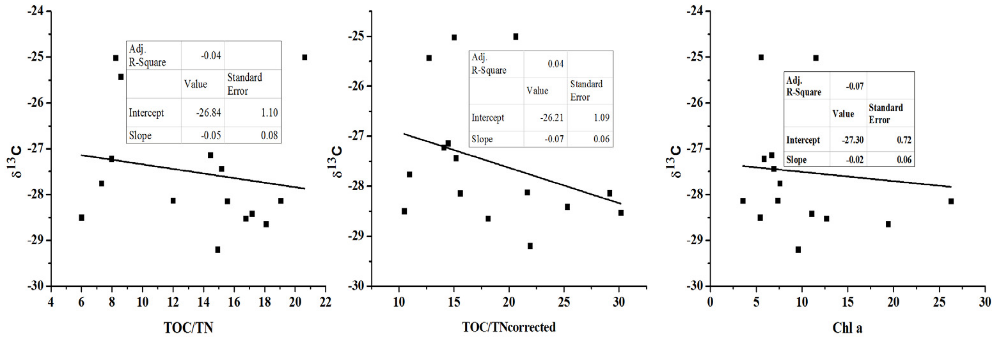

Fig. 4.6: Scatter plot of a) TOC/TN vs. $\delta\ ^{13}C$ b) $TOC/TN_{corrected}$ vs. $\delta\ ^{13}C$ c)Chl a vs. $\delta\ ^{13}C$

4.4 Factor Analysis

The effect of natural and anthropogenic flux was evaluated using factor analysis with varimax rotation which can give indication to the significance of processes. Diagenesis, allochthonous and autochthonous additions and sorption/desorption are the possible biogeochemical processes that can operate on the organic matter in aquatic systems. The redox reactions are mediated by sedimentary micro-organisms and the redox element such as sulphur, organic carbon and nitrogen can be used as their suitable indicators. The sorption/desorption processes can be explained by their relation with grain size (Joseph et al., 2008).

Table 4.5: Total Variance Explained for biochemical parameters using principal component analysis

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	9.445	62.966	62.966	9.445	62.966	62.966	6.602	44.011	44.011
2	1.944	12.961	75.927	1.944	12.961	75.927	3.041	20.275	64.286
3	1.194	7.963	83.890	1.194	7.963	83.890	2.940	19.603	83.890
4	.911	6.075	89.964						
5	.575	3.830	93.795						
6	.451	3.008	96.803						
7	.210	1.400	98.203						
8	.112	.744	98.947						
9	.080	.533	99.480						
10	.039	.262	99.742						
11	.025	.169	99.911						
12	.008	.056	99.967						
13	.005	.032	100.000						

It was found that 83.89% of the total variance (Table 4.5 and 4.6) was explained by all the three factors, and major processes operating in the system were litter addition, diagenesis and geochemical process. Positive loading in each of the components is reflected in the system as removal in the diagenesis and addition in the case of diagenesis and litter addition.

In the present study positive loading can be seen in all the three component for TOC, T &L, LPD and TN which definitely implies the character of the mangrove litter where general diagenetic composition will be comparatively lower due to the resistance of the mangrove litter to any such process. The positive loading showed on second and third components emphasis this view. The first factor contributed 44.01 % of the total variance and showed positive loading on clay, Chl a, Phaeopigments and TS apart from the above four parameters and negative loading on sand. This factor confirms importance of litter addition with minor addition from primary production in the mangrove ecosystem. This factor also gives the inference that geochemical processes like adsorption also have a role in these systems. Second factor reflected 20.28% to the total variance with positive loading on silt, CHO and PRT while third factor explains 19.60% of the total variance with positive loading on TS and negative loading on $\delta^{13}C$.

Table 4.6: Factor loading for principal component analysis using varimax rotation for biochemical analysis

	Component		
Parameters	**1**	**2**	**3**
SOC	.591	.526	.538
Sand	-.718	-.363	-.088
Clay	.727	.351	.200
Silt	.472	.667	-.325
CHO	.317	.758	.231
LPD	.477	.430	.698
PRT	-.082	.778	.374
Chl a	.953	.115	.109
Pheopigments	.984	.075	.074
T & L	.517	.564	.563
TN	.681	.482	.500
TS	.705	.114	.548
$\delta^{13}C$	-.050	-.103	-.917

4.5 Conclusions

Source characterisation of the organic matter in the selected mangrove ecosystem is successfully investigated by employing stable carbon isotopes, biochemical composition and pigment analysis. Organic matter in surface sediments is mainly derived from mangrove detritus with the substantial contribution from in situ production. For these systems, two end member mixing model, which was helpful for determining the contribution of terrestrial organic matter, ranged from 38.24 % to 73.80 %. The terrestrial organic matter contribution at Pazhayangadi and Pappinissery was established by stable carbon analysis and two end member mixing models. The correlation analysis of elemental ratio versus stable isotopic carbon indicates substantial contribution of microbial biomass. Kadalundi and Thalassery, owing to its close proximity of Lakshadweep Sea, are rich in marine-derived organic matter. Chlorophyll pigments were higher during pre-monsoon except for Thalassery and Kadalundi. The distribution of biopolymers such as carbohydrate, lipids and protein indicated the accumulation of aged or microbially modified organic matter. PCA analysis showed that litter addition, primary production and diagenesis are the major processes affecting the source, fate and preservation of organic matter in surface sediments of these mangrove systems.

Reference

Alongi, D. M., 1994. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. Hydrobiologia, 285, 19–32.

Augley, J., Huxham, M., Fernandes, T. F., Lyndon, A. R., Bury, S., 2007. Carbon stable isotopes in estuarine sediments and their utility as migration markers for nursery studies in the Firth of Forth and Forth Estuary Scotland. Estuarine Coastal and Shelf Science, 72, 648–656.

Bala Krishna Prasad, M., Ramanathan, A. L., 2009. Organic matter characterization in a tropical estuarine-mangrove ecosystem of India: Preliminary assessment by using stable isotopes and lignin phenols. Estuarine, Coastal and Shelf Science, 84, 617-624.

Barranguet, C., Plante-Cuny, M. R., Alivon, E., 1996. Microphytobenthos production in the Gulf of Fos, French Mediterranean coast. Hydrobiologia, 333, 181-193.

Belicka, L. L., Macdonald, R. W., Harvey, H. R., 2009. Trace element and molecular markers of organic carbon dynamics along a shelf – basin continuum in sediments of the western Arctic Ocean. Marine Chemistry, 115, 72-85.

Benner, R., Weliky, K., Hedges, J. I., 1990. Early diagenesis of mangrove leaves in a tropical estuary: Molecular-level analyses of neutral sugars and lignin-derived phenols. Geochimica et Cosmochimica Acta, 54, 1991–2001.

Bianchi, A. J. M., Levinton, J. S., 1984. The importance of mi- croalgae, bacteria and particulate organic matter in the somatic growth of Hydrobia totteni. Journal of marine Research, 42, 431–443.

Bianchi, T., Pennock, J., Twilley, R. R., 1999. Biogeochemistry of Gulf of Mexico Estuaries. John Wiley and Sons, New York.

Bianchi, T., Mitra, S., McKee, B. A., 2002. Source of terrestrially derived organic carbon in lower Mississippi River and Louisiana shelf: implications for differential sedimentation and transport at the coastal margin. Marine Chemistry, 77, 211–273.

Bouillon, S., Chandra Mohan, P., Sreenivas, N., Dehairs, F., 2000. Sources of suspended organic matter and selective feeding by zooplankton in an estuarine mangrove ecosystem as traced by stable isotopes. Marine Ecology Progression Series, 208, 79–92.

Bouillon, S., Koedam, N., Raman, A.V., Dehairs, F., 2002a. Primary producers sustaining macro-invertebrate communities in intertidal mangrove forests. Oecologia, 130, 441–448.

Bouillon, S., Raman, A. V., Dauby, P., Dehairs, F., 2002b. Carbon and nitrogen stable isotope ratios of sub-tidal benthic invertebrates in an estuarine mangrove ecosystem (Andhra Pradesh, India). Estuarine Coastal and Shelf Science, 54, 901–913.

Boutton, R. W., 1991. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. In: Coleman DC, Fry B (eds) Carbon isotope techniques. Academic, New York.

Cahoon, L. B., Cooke, J. E., 1992. Benthic microalgal production in Onslow Bay, North Carolina, USA. Marine Ecology Progress Series, 84, 185-196

Carlier, A., Riera, P., Amouroux, J. M., Bodiou, J. Y., Gremare, A., 2007. Benthic trophic network in the Bay of Banyuls-sur-Mer (northwest Mediterranean, France): an assessment based on stable carbon and nitrogen isotopes analysis. Estuarine Coastal and Shelf Science, 72, 1– 15.

Cauwet, G., 1978. Organic chemistry of sea water particulates: concepts and developments. Oceanologica Acta, 1, 99–105.

Cifuentes, L. A., Eldridge. P. M., 1998. A mass- and isotope-balance model of DOC mixing in estuaries. Limnology and Oceanography, 43, 1872–1882.

Cividanes, S., Incera, M., Lopez, J., 2002. Temporal variability in the biochemical composition of sedimentary organic matter in an intertidal flat of the Galician coast (NW Spain). Oceanologica Acta, 25, 1–12.

Colombo, J. C., Silverberg, N., Gearing, J. N., 1996. Lipid biogeochemistry in the Laurentian Trough I. Fatty acids, sterols and aliphatic hydrocarbons in rapidly settling particles. Organic Geochemistry, 25, 211-225.

Cowie, G. L., Hedges, J. I., 1984. Carbohydrate sources in a coastal marine environment. Geochimica et Cosmochimica Acta, 48, 2075–2087.

Danovaro, R., 1996. Detritus-bacteria-meiofauna interactions in a seagrass bed (Posidonia oceanica) of the NW Mediterranean. Marine Biology, 127, 1–13.

Danovaro, R., Dell'Anno, A., Fabiano, M., 2001. Bioavailability of organic matter in the sediments of the Porcupine Abyssal Plain (NE Atlantic). Marine Ecology Progression Series, 220, 25–32.

Danovaro, R., Fabiano, M., Della Croce, N., 1993. Labile organic matter and microbial biomasses in deep-sea sediments (Eastern Mediterranean Sea). Deep Sea Research, 5, 953–965.

Danovaro, R., Fabiano, M., 1996. Meiofaunal production in sub- tidal sediments of the Ligurian Sea: potential contribution of bacteria and microphytobenthos to the food demand. In: Albertelli G, De Maio A, Picazzo M (eds) Proceedings 11th Conference of the Italian Association of Oceanology and Limnology AIOL, Genoa.

Danovaro, R., Gambi, C., Manini, E., Fabiano, M., 2000. Maiofauna response to a dynamic river plume front. Marine Biology, 137, 359–370.

Danovaro, R., Marrale, D., Della Croce, N., Parodi, P., Fabiano, M., 1999. Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic–benthic coupling. Journal of Sea Research, 42, 117–129.

De Jonge, V. N., Van Beusekom, J. E. E., 1995. Wind- and tide- induced resuspension of sediments and microphytobenthos from tidal flats in the Ems estuary. Limnology Oceanography, 40, 766–778.

de Leeuw, J. W., Largeau, C., 1993. A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation. In: Engel, M.H., Macko, S.A. (Eds.), Organic Geochemistry, Principles and Applications, Topics in Geobiology. Plenum Press, New York.

Deegan, L. A., Garritt, R. H., 1997. Evidence for spatial variability in estuarine food webs. Marine Ecology Progress Series, 147, 31-47.

Delgado, M., De Jonge, V. N., Peletier, H., 1991. Experiments on resuspension of natural microphytobenthos populations. Marine Biology, 108, 321–328.

Dell'Anno, A., Meib, M. L., Pusceddu, A., Danovaro. R., 2008. Biochemical composition and early diagenesis of organic matter in coastal sediments of the NW Adriatic Sea influenced by riverine inputs. Chemistry and Ecology, 24, 75–85.

Dell'Anno, A., Mei, M. L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Marine Pollution Bulletin, 44, 611-622.

Ding, X., Henrichs, S., 2002. Adsorption and desorption of proteins and polyaminoacids by clay minerals and marine sediments. Marine Chemistry, 77, 225–237.

Dubois, S., Savoye, N., Grémare, A., Plus, M., Charlier, K., Beltoise, A., Blanchet, H., 2012. Origin and composition of sediment organic matter in a coastal semi-enclosed ecosystem: An elemental and isotopic study at the ecosystem space scale. Journal of Marine Systems, 94, 64-73.

Duke, N. C., 1990. Phenological trends with latitude in the mangrove tree Avicennia marina. Journal of Ecology, 78, 113–133.

Dunn, R. J. K., Welsh, D. T., Teasdale, P. R., Lee, S. Y., Lemckert, C. J., Meziane, T. 2008. Investigating the distribution and sources of organic matter in surface sediment of Coombabah Lake (Australia) using elemental, isotopic and fatty acid biomarkers. Continental Shelf Research, 28, 2535-2549.

Emerson, S., Hedges, J., 2008. Chemical oceanography and the marine carbon cycle. Cambridge University Press.

Fabiano, M., Danovaro, R., Fraschetti, S., 1995. A three-year time series of elemental and biochemical composition of organic matter in subtidal sediments of the Liguranian Sea (Northwestern Mediterranean). Continental Shelf Research, 15, 1453–1469.

Fabiano, M., Danovaro, R., 1994. Composition of organic matter in sediments facing a river estuary (Tirrenian Sea): relationships with bacteria and microphytobenthic biomass. Hydrobiologia, 277, 71–84.

Fabiano, M., Danovaro, R., 1998. Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the Ross Sea (Antarctica). Applied and environmental microbiology, 64, 3838-3845.

Fabiano, M., Danovaro, R., Fraschetti, S., 1995. A three-year time series of elemental and biochemical composition of organic matter in subtidal sediments of the Ligurian Sea (northwestern Mediterranean). Continental Shelf Research, 15, 1453–1469.

Fourqurean, J. W., Schrlau, J. E., 2003. Changes in nutrient content and stable isotope ratios of C and N during decomposition of seagrasses and mangrove leaves along a nutrient availability gradient in Florida Bay, USA. Chemistry and Ecology, 19, 373–390.

Fry, B., Sherr, E. B., 1984. δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. 27, 13–47.

Gao, X., Yang, Y., Wang, C., 2012. Geochemistry of organic carbon and nitrogen in surface sediments of coastal Bohai Bay inferred from their ratios and stable isotopic signatures. Marine pollution bulletin, 64, 1148-1155.

Gao, Y. Z., Giese, M., Lin, S., Sattelmacher, B., Zhao, Y., Brueck, H., 2008. Belowground net primary productivity and biomass allocation of a grassland in Inner Mongolia is affected by grazing intensity. Plant and Soil, 307, 41-50.

Gardner, W. S., Menzel, D. W., 1974. Phenolic aldehydes as indicators of terrestrially derived organic matter in the sea. Geochimica et Cosmochimica Acta, 38, 813-822.

Gearing, J. N., 1988. The use of stable isotope ratios for tracing the nearshoree offshore exchange of organic matter. In: Jansson, B.-O. (Ed.), Lecture Notes on Estuaries Studies, vol. 22. Springer-Verlag, Berlin.

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, 239–45.

Goñi, M. A., Hedges, J. I., 1992. Lignin dimers—structures, distribution, and potential geochemical applications. Geochimica et Cosmochimica Acta, 56, 4025–4043.

Gonneea, M. E., Paytana, A., Herrera-Silveira, J., 2004. Tracing organic matter sources and carbon burial in mangrove sediments over the past 160 years. Estuarine Coastal and Shelf Science, 61, 211–227.

Grant, J., Hargrave, B. T., 1987. Benthic metabolism and the quality of sediment organic carbon. Biological oceanography, 4, 243–264.

Grémare, A., Amouroux, J. M., Charles, F., Dinet, A., Riaux-Gobin, C., Baudart, J. C., Albert, P., 1997. Temporal changes in the biochemical composition and nutritional value of the particulate organic matter available to surface deposit-feeders: a two year study. Marine Ecology Progression Series, 150, 195–206.

Grémare, A., Medernach, L., DeBovee, F., Amouroux, J. M., Vetion, G., Albert, P., 2002. Relationships between sedimentary organics and benthic meiofauna on the continetal shelf and the upper slope of the Gulf of Lions (NW Mediterranean). Marine Ecology Progression Series, 234, 85–94.

Haines, E. B., Montague, C. I., 1979. Food sources of estuarine invertebrates analysed using $^{13}C/^{12}C$ ratios. Ecology, 60, 48–56.

Hedges, J. I., Keil, R. G., Benner, R., 1997. What happens to terrestrial organic matter in the ocean? Organic geochemistry, 27, 195-212.

Hedges, J. I., Parker, P. L., 1976. Land-derived organic matter in surface sediments from the Gulf of Mexico. Geochemica et Cosmochimica Acta, 53, 2659–2673.

Hsieh, M., 2006. Geochemical characterization of organic matter in Victoria Harbour sediments, Hong Kong. PhD thesis submitted to University of Oklahoma, Japan.

Hu, J., Peng, P., Jia, G., Mai, B., and Zhang, G., 2006. Distribution and sources of organic carbon, nitrogen and their isotopes in sediments of the subtropical Pearl River estuary and adjacent shelf, Southern China. Marine Chemistry, 98, 274-285.

Imgraben, S., Dittmann, S., 2008. Leaf litter dynamics and litter consumption in two temperate South Australian mangrove forests. Journal of sea research, 59, 83-93.

Jia, G., Peng, P., 2003. Temporal and spatial variations in signatures of sedimented organic matter in Lingding Bay (Pearl estuary), southern China. Marine Chemistry, 82, 47-54.

Joseph, M. M., Ratheesh Kumar, C. S., GireeshKumar, T. R., Renjith, K. R., Chandramohanakumar, N., 2008. Biogeochemistry of surficial sediments in the intertidal systems of a tropical environment. Chemistry and Ecology, 24, 247- 258.

Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L., Jansson, M., 2009. Light limitation of nutrient-poor lake ecosystems. Nature, 460, 506-509.

Kelsey, R. G., Harmon, M. E., 1989. Distribution and variation of extractable total phenols and tannins in the logs of four conifers after 1 year on the ground. Canadian Journal of Forest Research, 19, 1030-1036.

Kenworthy, W. J., Thayer, G. W., 1984. Production and decom- position of the roots and rhizomes of seagrasses Zostera marina and Thalassia testudinum in temperate and subtropical marine ecosystems. Bulletin of Marine Sciences, 35, 364–379.

Khodse, V. B., Fernandes, L., Gopalakrishna, V. V., Bhosle, N. B., Fernandes, V., Matondkar, S. G. P., Bhushan, R., 2007. Distribution and seasonal variation of concentrations of particulate carbohydrates and uronic acids in the northern Indian Ocean. Marine Chemistry, 103, 327–346.

Kofoed, L. H., 1975. The feeding biology of Hydrobia ventrosa (Montagu). I. The assimilation of different components of the food. Journal of Experimental Marine Biology and Ecology, 19, 233-241.

Kristensen, E., Bouillon, S., Dittmar, T., Marchand, C., 2008. Organic carbon dynamics in mangrove ecosystems: a review. Aquatic Botany, 89, 201–219.

Lawrence, J. M., Boudouresque Ch, F., Maggiore, F., 1989. Proximate costituents, biomass and energy in Posidonia oceanica (Potamogetonaceae). Marine Ecology, 10, 263-270.

Lee, K. H., Moran, M. A., Benner, R., Hodson, R. E., 1990. Influence of soluble components of red mangrove (Rhizophora mangle) leaves on microbial decomposition of structural (ligno- cellulosic) leaf components in seawater. Bulletin of Marine Sciences, 46, 374–386.

Lehmann, M. F., Bernasconi, S. M., Barbieri, A., McKenzie, J. A., 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. Geochimica et Cosmochimica Acta, 66, 3573–3584.

Leopold, A., Marchand, C., Deborde, J., Chaduteau, C., Allenbach, M., 2013. Influence of mangrove zonation on CO_2 fluxes at the sediment–air interface (New Caledonia). Geoderma, 202, 62-70.

Lepoint, G., Nyssen, F., Gobert, S., Dauby, P., Bouquegneau, J. M., 2000. Relative impact of a seagrass bed and its adjacent epilithic algal community in consumer diets. Marine Biology, 136, 513–518.

Liu, S., Huang, H., Huang, L., Lian, J., Long, A., Li, T., 2006. Ecological response of phytoplankton to the operation of Daya Bay nuclear power station. Marine Environmental Science, 25, 9-13.

Louchouarn, P., Lucotte, M., Canuel, R., Richard, L. P., Gagné, J. P., 1997. Sources and early diagenesis of lignin and bulk organic matter in recent sediments from the Lower St. Lawrence Estuary and the Saguenay Fjord, Canada. Marine Chemistry, 58, 3–26.

Lucas, C. H., Widdows, J., Brinsley, M. D., Salkeld, P. N., Herman, P. M. J., 2000. Benthic-pelagic exchange of microalgae at a tidal flat. 1. Pigment analysis. Marine Ecology Progress Series, 196, 59–73.

Manini, E., Danovaro, R., Fabiano, M., 2001. Benthic–pelagic coupling in frontal system areas of the northern Adriatic sea: analysis of the carbon budgets. Chemistry and Ecology, 18, 1–9.

Marchand, C., Disnar, J. R., Lallier–Verges, E., Lottier, N., 2005. Early diagenesis of carbohydrates and lignin in mangrove sediments subject to variable redox conditions (French Guiana). Geochimica et Cosmochimica Acta, 69, 131–142.

Mayer, L. L., Schick. L, Sawyer Plante C, Jumars, P. A., Shelf, R. L., 1995 Bioavailable amino acids in sediments: a biomimetic kinetic- based approach. Limnology and Oceanography, 40, 511–520.

Meyers, P. A., 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Organic geochemistry, 27, 213-250.

Meyers, P. A., 2003 Applications of organic geochemistry to paleolimnological reconstructions: A summary of examples, from the Laurentian Great Lakes; Organic Geochemistry, 34, 261–289.

Mrunalini, N. D., Chavan N. S., 2011. Seasonal variation in organic constituents from different parts of *Cynometra Iripa Kostel*. Plant sciences feed, 1, 6- 9

Muzuka, A. N. N., Shunula, J. P., 2006. Stable isotope compositions of organic carbon and nitrogen of two mangrove stands along the Tanzanian coastal zone. Estuarine Coastal Shelf Sciences, 66, 447–458.

Newell, R. C. Field J. G, 1983. The contribution of bacteria and detritus to carbon and nitrogen flow in a benthic community. Marine Biology Letters, 4, 23–36.

O'Leary, M. H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553–567.

Ogrinc, N., Fontolan, G., Faganeli, J., Covelli, S., 2005. Carbon and nitrogen isotope compositions of organic matter in coastal marine sediments (the Gulf of Trieste , N Adriatic Sea): indicators of sources and preservation, Marine Chemistry, 95, 163-181.

Opsahl, S., Benner, R., 1999. Characterization of carbohydrates during early diagenesis of five vascular plant tissues. Organic Geochemistry, 30, 83–94

Ostrom, P. H., Fry, B., 1993. Sources and cycling of organic matter within modern and prehistoric food webs. In: Engel, M.H., Macko, S.A. (Eds.), Organic geochemistry. Plenum, New York.

Pancost, R. D., Boot, C. S., 2004. The paleoclimatic utility of terrestrial biomarkers in marine sediments. Marine Chemistry, 92, 239–261.

Penha-lopes, G., Kristensen, E., Flindt, M., Mangion, P., Bouillon, S., Paula, J., 2010. The role of biogenic structures on the biogeochemical functioning of mangrove constructed wetlands sediments – A mesocosm approach. Marine Pollution Bulletin, 60, 560-572.

Peterson, B. J., Howarth, R. W., Garritt, R. H., 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science, 227, 1361–1363.

Pinnegar, J. K., Polunin, N. V. C., 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia, 122, 399–409.

Plante-Cuny, M. R., Bodoy, A., 1987. Biomasse et production primaire du phytoplancton et du microphytobenthos de deux biotopes sableux (Golfe de Fos, France). Oceanologica Acta, 10, 223–237.

Pusceddu, A. A., Dell, A., Danovaro, R., Manini, E., Sara, V., Fabiano, M., 2003. Enzymatically hydrolysable protein and carbohydrate sedimentary pools as indicators of the trophic state of detritus sink systems: a case study in the Mediterranean coastal lagoon. Estuaries, 26, 641–650.

Qasim, S. Z., 1982. Oceanography of the northern Arabian Sea. Deep-Sea Research, 29A, 1041–1068.

Ranjan, K., Routh, J., Ramanathan, A. L., Klump, J. V., 2011. Elemental and stable isotope records of organic matter input and its fate in the Pichavaram mangrove – estuarine sediments (Tamil Nadu, India). Marine Chemistry, 126, 163-172.

Rice, D. L., Hanson, R. B., 1984. A kinetic model for detritus nitrogen : role of the associated bacteria in nitrogen accumulation. Bulletin of Marine Science, 35, 326-340.

Riera, P., Richard, P., 1996. Isotopic determination of food sources of Crassostrea gigas along a trophic gradient in the estuarine bay of Marennes-Ole´ron. Estuarine Coastal Shelf Science, 42, 347–360.

Rossi, F., Como, S., Corti, S., Lardicci, C., 2001. Seasonal variation of a deposit-feeder assemblage and sedimentary organic matter in a brackish basin mudflat (Western Mediterranean, Italy). Estuarine, Coastal and Shelf Science, 53, 181-191.

Rossi, F., Lardicci, C., 2002. Role of the nutritive value of sediment in regulating population dynamics of the deposit-feeding polychaete Streblospio shrubsolii. Marine Biology, 140, 1129-1138.

Rundegren, N., Loader, N. J., Hammarlund, D., 2003. Stable carbon isotope composition of terrestrial leaves: inter- and intraspecies variability, cellulose and whole-leaf tissue difference and potential for climate reconstruction. Journal of Quaternary Science, 18, 583-590.

Sackett, W. M., Thompson, R. R., 1963. Isotopic organic composition of recent continental derived clastic sediment of eastern Gulf Coast, Gulf of Mexico. Bulletin - American Association of Petroleum Geologists, 47, 525–531.

Sanilkumar M. G., 2009. Microalgae in the southwest coast of india. Thesis submitted to Cochin University of Science And Technology.

Sarkanen, K. V., Ludwig, C. H., 1971. Lignins. Occurence, Formation, Structure and Reactions. Wiley-Inter science, New York.

Schultze, D. J., Calder, J. A., 1976. Organic carbon $^{13}C/^{12}C$ variations in estuarine sediments. Geochimica et Cosmochimica Acta, 40, 381–385.

Schulz, H. D., Zabel, M., 2006. Marine geochemistry, Vol. 2, Springer, Berlin.

Tenore, K. R., Cammen, L., Findlay, S. E. G., Phillips, N., 1982. Perspectives of research on detritus: do factors controlling the availability of detritus to macroconsumers depend on its source? Journal of Marine Research, 40, 473–490.

Tesi, T., Miserocchi, S., Goñi, M. A., Langone, L., Boldrin, A., Turchetto, M., 2007. Organic matter origin and distribution in suspended particulate materials and surficial sediments from the western Adriatic Sea (Italy). Estuarine, Coastal and Shelf Science, 69, 225–245.

Thornton, S. F., McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. Estuarine, Coastal and Shelf Science, 38, 219-233.

Turley, C. M., Lochte, K., 1990. Microbial response to the input of fresh detritus to the deep-sea bed. Palaeogeography, Palaeoclimatology, Palaeoecology, 89, 3-23.

Usui, T., Nagao, S., Yamamoto, M., Suzuki, K., Kudo, I., Montani, S., Noda, A., Minagawa, M., 2006. Distribution and sources of organic matter in surficial sediments on the shelf and slope off Tokachi, western North Pacific, inferred from C and N stable isotopes and C/N ratios. Marine Chemistry, 98, 241–259.

Vander Zanden, M. J., Rasmussen, J. B., 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography, 46, 2061–2066.

Velimirov, B., 1986. DOC dynamics in a Mediterranean seagrass system.Marine Ecology Progression Series, 28, 21–41.

Verlencar, X. N., Singbal, S. Y. S., Parulekar, A. H., 2006. Biochemical components of the benthic regions in Antarctic waters. Technical Report 1, National Institute of Oceanography, Goa.

Vezzulli, L., Marrale, D., Moreno, M., Fabiano, M., 2003. Sediment organic matter and meiofauna community response to long-term fish-farm impact in the Ligurian Sea (Western Mediterranean). Chemistry and Ecology, 19, 431-440

Vizzini, S., Benedetto, S., Thang, D. C., Mazzola, A., 2005. Spatial variability of stable carbon and nitrogen stable isotope ratios in a Mediterranean coastal lagoon. Hydrobiologia, 550, 73–82.

Vizzini, S., Mazzola, A., 2003. Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of primary producers and consumers in a western Mediterranean coastal lagoon. Marine Biology, 142, 1009-1018.

Vizzini, S., Mazzola, A., 2006. Sources and transfer of organic matter in food webs of a Mediterranean coastal environment: evidence for spatial variability. Estuarine Coastal Shelf Science, 66, 459–467.

Vizzini, S., Sara, G., Michener, R. H., Mazzola, A., 2002. The role and contribution of the seagrass Posidonia oceanica (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. Acta Oecologica, 23, 277–285.

Wang, X., Chen, R. F., Berry, A., 2003. Sources and preservation of organic matter in Plum Island salt marsh sediments (MA , USA): long-chain n-alkanes and stable carbon isotope compositions. Estuarine, Coastal and Shelf Science, 58, 917-928.

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Chapter 5

HYDROCARBONS AS BIOMARKERS

Contents

5.1 Introduction
5.2 Results and Discussion
5.3 Principal Component Analysis
5.4 Conclusions

5.1 Introduction

The quantity and quality of organic matter preserved in sediments varies greatly depending on the nature of the material rendered and on the depositional environment. In addition, there is temporal and spatial variation in the inputs of organic matter to these environments. The knowledge of sources, reactivity, and fate of organic matter is essential to understand the role of coastal systems in global biogeochemical cycles (Simoneit, 1978; Hedges and Keil, 1995; Bianchi and Canuel, 2001). Stable carbon isotopic ratios, elemental ratios and biochemical composition were employed in mangrove ecosystems to infer the organic carbon sources of the sediments (Machiwa, 2000; Kuramoto and Minagawa, 2001). However, these bulk organic matter techniques were not successful in complex ecosystems like mangroves, where signatures from multiple sources get overlapped (Cloern et al., 2002; Joseph et al., 2008). Therefore application of chemical biomarkers has been widespread (Bianchi and Canuel, 2001) and this chapter deals with the use of hydrocarbons (n-alkanes, n-alkenes, branched and cyclic alkanes and hopanes) as biomarkers to trace the sources of organic matter from the mangrove ecosystems of Northern Kerala Coast.

Aliphatic hydrocarbons such as n-alkanes and n-alkenes have been successfully used, to distinguish among algal, bacterial, and terrestrial sources of carbon in coastal systems (Yunker et al., 1991, 1993, 1995; Canuel et al., 1997) and for the diagnosis of paleoenvironments (Pearson and Obaje, 1999). Among short chain alkanes, n-alkanes with 15–19 carbon atoms are synthesized by aquatic bacteria and algae (Cranwell et al., 1987) and mid- chain n-C21 to n-C25 compounds are produced from submerged or floating plants (Baas et al., 2000; Ficken et al., 2000). Long-chain n-alkyl compounds with odd carbon are predominant component in epicuticular waxes of vascular plant leaves (Eglinton and Hamilton, 1967), and their concentration may vary with species as well as with environmental conditions (Dodd et al., 1999). Versteegh et al., (2004) and Mead et al., (2005) found that the most abundant hydrocarbon in *Rhizophora* leaf surface as C29 n- alkane. Rafii et al. (1996) and Dodd et al. (1998) reported high abundance of C28 n-alkane in *Avicennia* and *Rhizophora* from French Guiana and C31 from West Africa.

These variations in n-alkane homologue distributions are meaningful when expressed using different indices such as carbon preference index (CPI), Average chain length (ACL), pristane/phytane, n-C17/pristane and n-C18/phytan. It helps for identification of sources of organic matter (Volkman et al. 1992; Commendatore and Esteves, 2004; Zaghden et al., 2005; Hu et al., 2009). CPI is the ratio of the sum of odd over even numbered n-alkanes (Schefuβ et al., 2003) and ACL describes the average number of carbon atoms per molecule based on the abundance of the odd-numbered higher plant-derived alkanes (Poynter and Eglinton, 1990; Boot et al., 2006; Jeng, 2006).

Branched and cyclic hydrocarbons have also been used as paleomarkers, to interpret past changes in organic matter sources (Rowland and Robson, 1990; Meyers, 2003). In ancient sediments and petroleum, a series of mid-chain branched monomethyl alkanes accompanied by dimethyl alkanes have been found (Klomp, 1986b). Derivatives of phytol such as pristane (C19 isoprenoid hydrocarbon) and phytane (C20 isoprenoid hydrocarbon) have been used as indicators of herbivorous grazing (Blumer et al., 1964). The highly branched isoprenoids (HBI) can be used as environmental and palaeo-environmental indicators (Rowland et al., 2001; Kohnen et al., 1992) and its widespread occurrence in marine and lacustrine sediments are reported by several authors (Rowland and Robson, 1990; Hird et al., 1992).

5.2 Results and Discussion

Spatial and seasonal variation of aliphatic hydrocarbon in the mangrove ecosystem of northern Kerala Coast was depicted in the Fig. 5.1, 5.2, 5.3 and 5.4. n-Alkanes in the range from C11 to C33 were reported from the surface sediments in the selected mangrove ecosystems. Total aliphatic hydrocarbon ranged from 10.26 to 42.70 $\mu g g^{-1}$ (avg. 28.93±13.27 $\mu g g^{-1}$), 67.99 to 221.42 $\mu g g^{-1}$ (avg. 143.63 ± 73.48 $\mu g g^{-1}$) and 17.41 to 61.17 $\mu g g^{-1}$ (avg. 30.15 ± 17.90 $\mu g g^{-1}$) in post-monsoon, pre-monsoon and monsoon respectively. Pre-monsoon showed higher concentration of alkanes in the present study.

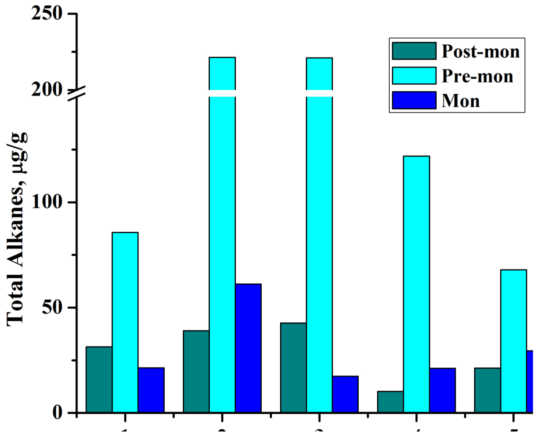

Fig. 5.1: The variation of total alkanes in the study region

Kadalundi and Pazhayangadi in pre-monsoon showed three modes of n-alkane distribution viz, (1) n-C14 to C18 with a peak at 17, (2) n-C20 to C26 with no odd/even carbon preference, (3) odd preference from n-C28 to C33. In pre-monsoon, Thalassery and Pappinissery also showed three modes of distribution- C12 to C19 with a peak at C17, C20-C24 with no odd/even preference, C25-33 with strong odd preference. But Kunjimangalam showed no odd or even preference up to n-C24. On seasonal basis monsoon season showed no odd over even preference up to C22, except for Pazhayangadi which showed even carbon preference and then C23-C33 showed strong odd preference. It is found that long chain n-alkanes (C25-C33) showed strong odd over even preference throughout the study.

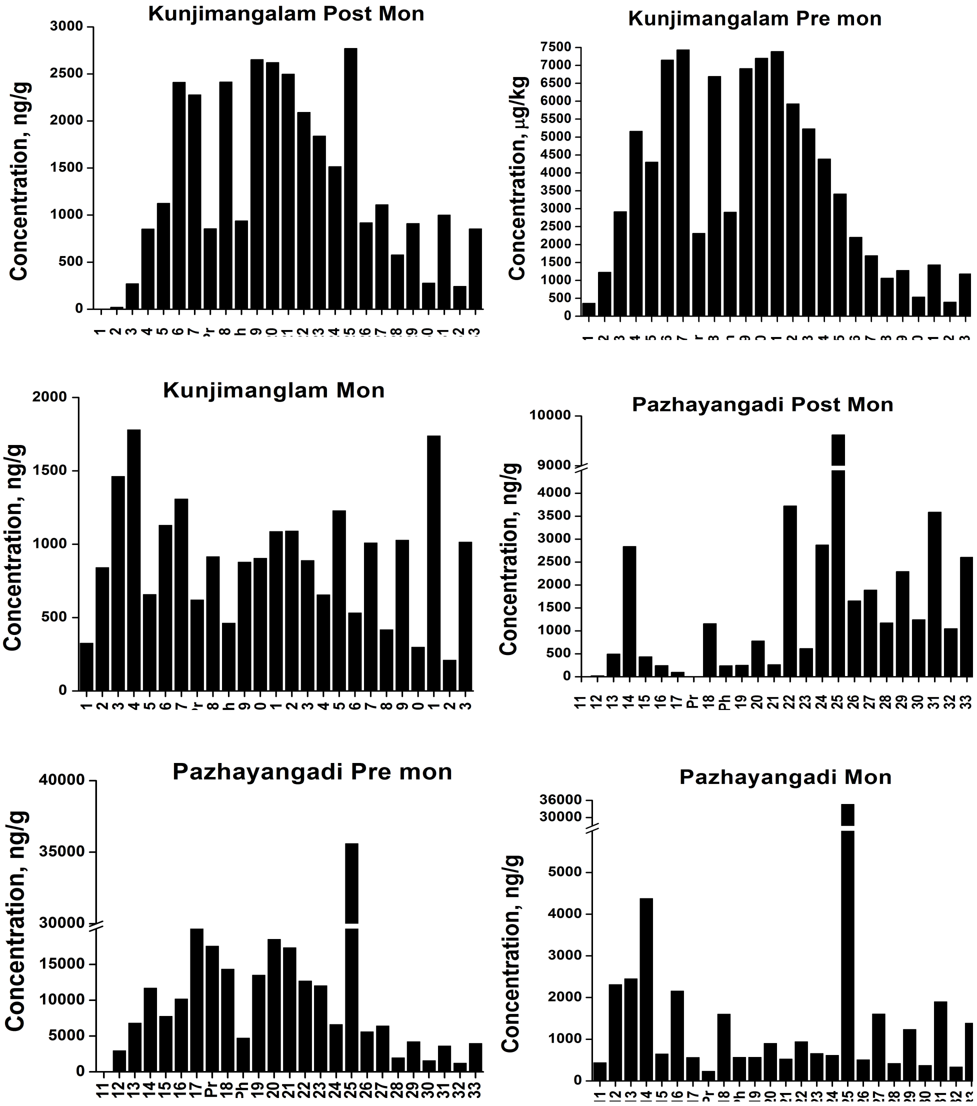

Fig. 5.2: n-Alkane distribution for the sediment sample from Pazhayangadi and Kunjimangalam

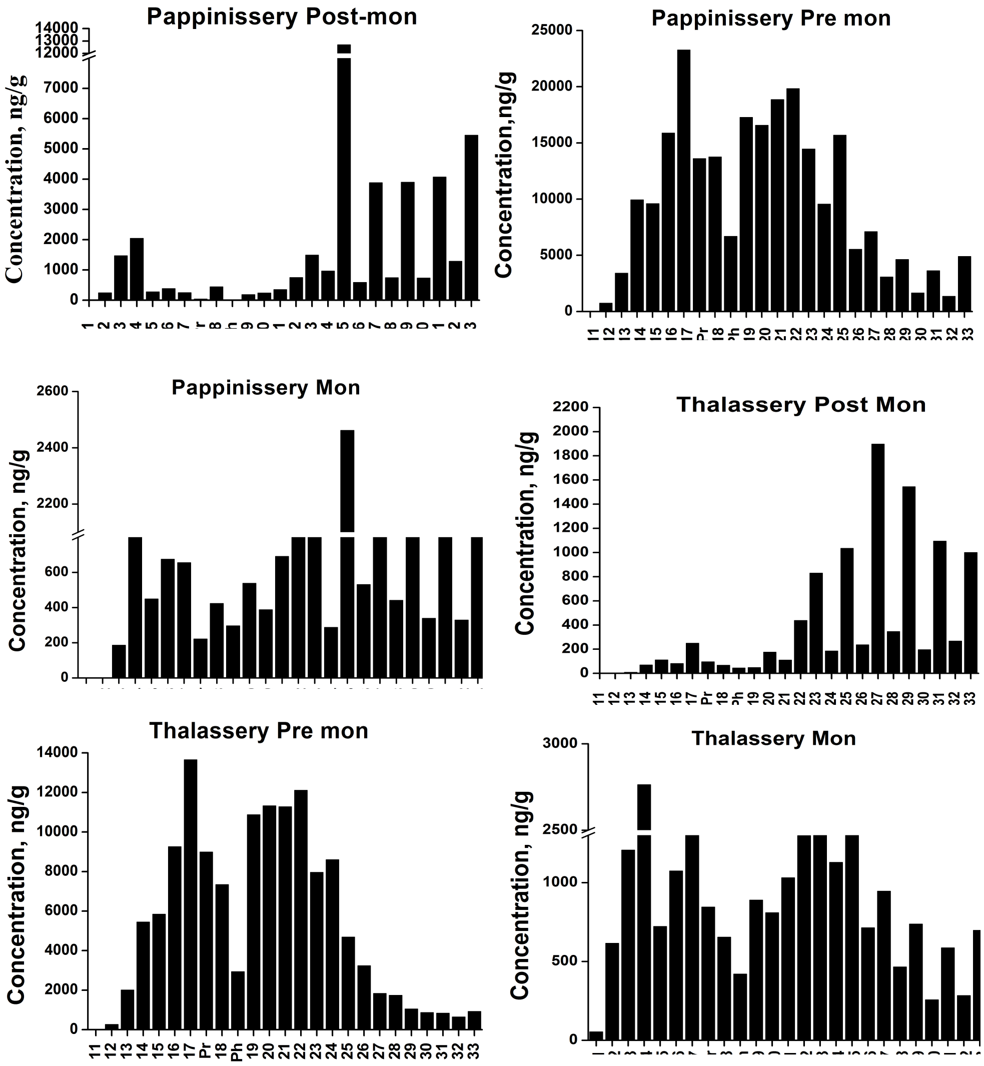

Fig. 5.3: n-Alkane (C_{11}-C_{33}) distribution for the sediment sample from Pappinissery and Thalassery

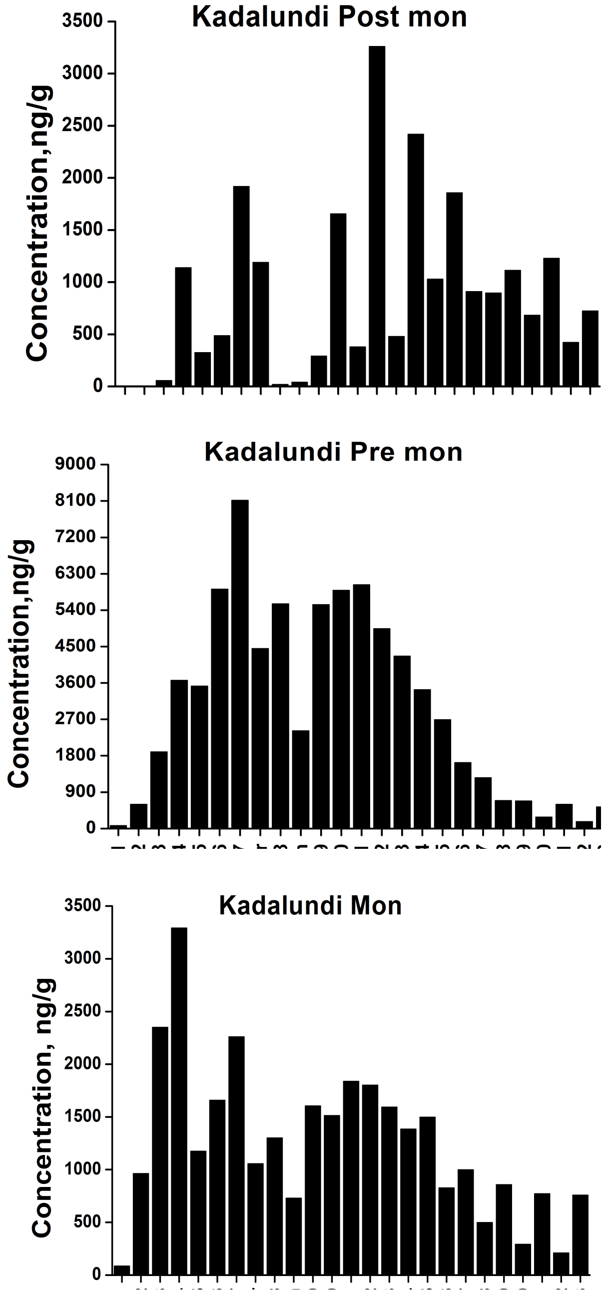

Fig. 5.4: n-Alkane distribution (C11 to C33) for the sediment sample from Kadalundi

Table 5.1: Variation of n-alkane indices in the present study region

	Post-mon				Pre-mon				mon			
	Max	**Min**	**Avg**	**Stdv**	**Max**	**Min**	**Avg**	**Stdv**	**Max**	**Min**	**Avg**	**Stdv**
CPI Long	7.72	0.92	3.89	2.72	3.99	0.94	1.97	1.21	3.71	1.82	3.08	0.75
CPI short	0.85	0.18	0.55	0.25	0.97	0.88	0.94	0.03	0.88	0.42	0.72	0.18
Pristane/Phytane	28.50	ND	6.32	12.43	3.71	0.80	2.29	1.13	2.01	0.41	1.19	0.63
C17/Pristane	6.81	ND	2.74	2.52	3.22	1.30	1.91	0.75	2.96	1.71	2.27	0.46
C18/Phy	4.77	ND	1.86	1.91	3.03	2.06	2.44	0.37	2.82	1.43	1.91	0.55
ACL	28.88	27.77	28.25	0.52	27.95	26.54	27.31	0.53	29.10	25.75	27.98	1.29
TAR	16.74	0.50	7.92	6.92	0.32	0.12	0.23	0.09	2.69	0.52	1.59	1.04
C31/C19	23.02	0.38	12.84	10.30	0.27	0.08	0.17	0.08	3.35	0.48	1.81	1.23
TOM	44.23	9.64	23.36	13.42	6.95	3.05	5.06	1.68	25.37	7.74	14.06	7.38
MOM	19.31	1.66	7.77	7.67	25.26	19.91	22.90	2.23	17.06	2.90	11.42	5.50
LMW/HMW	0.89	0.12	0.35	0.32	1.50	0.96	1.21	0.23	1.22	0.36	0.76	0.39

Different indices were calculated and are given in Table 5.1. CPI for higher alkanes, a proxy for identifying contribution of terrestrial vs. other input was calculated and is ranged from 0.92 to 7.72 (avg. ± stdv:3.89±2.72) in post-monsoon, 0.94 to 3.99(avg. ± stdv:1.97± 1.21) in pre-monsoon and 1.82 to 3.71 (avg. ± stdv:3.08 ± 0.75) in monsoon. Ratio of pristane/phytane varied from ND to 28.50 (avg. ± stdv:-6.32 ± 12.43), 0.80 to 3.71(avg. ± stdv:2.29± 1.13), 0.41 to 2.01(avg. ± stdv:1.19± 0.63) in post monsoon, pre monsoon and monsoon respectively. Ratio of C17 to Pristane ranged from ND to 6.81 (avg. ± stdv:2.74 ±2.52; post monsoon), 1.30 to 3.22 (avg. ± stdv: 1.91 ± 0.75; pre monsoon) and 1.71 to 2.96 (avg. ± stdv:2.27 ± 0.46; monsoon). Similarly C18/Phytane was also calculated and ranged from ND to 4.77 (avg. ± stdv:- 1.86 ± 1.91) in post-monsoon, 2.06 to 3.03 (avg. ± stdv: 2.44 ± 0.37) in pre-monsoon and 1.43 to 2.82 (avg. ± stdv:1.91± 0.55) in monsoon. Average chain length (ACL) varied from 27.77 to 28.88 (avg. ± stdv: 28.25± 0.52) in post-monsoon season, 26.54 to 27.95 (avg. ± stdv:27.31± 0.53) in pre-monsoon season and 25.75 to 29.10 (avg. ± stdv:27.98 ±1.29) in monsoon season. Terrigenous to Aquatic Ratio (TAR) ranged from 0.50 to 16.74 (avg. ± stdv: 7.92± 6.92), 0.12 to 0.32 (avg. ± stdv: 0.23 ±0.09), 0.52to 2.69 (avg. ± stdv:- 1.59 ±1.04) in post-monsoon, pre-monsoon and monsoon season respectively. Ratio of C31 to C19 varied from 0.38 to 23.02 (avg. ± stdv:-12.84 ± 10.30; post monsoon), 0.08 to 0.27 (avg. ± stdv: 0.17 ± 0.08; pre monsoon) and 0.48 to 3.35 (avg. ± stdv: 1.81 ± 1.23; monsoon). Terrestrial Organic Matter (TOM) was characterised by lower values in pre-monsoon and was ranged from 9.64 to 44.23 (avg. ± stdv: 23.36±13.42), 3.05 to 6.95 (avg. ± stdv:5.06 ±1.68), 7.74 to 25.37 (avg. ± stdv: 14.06±7.38) in post-monsoon, pre-monsoon and monsoon respectively. Marine Organic Matter (MOM) ranged from 1.66 to 19.31(avg. ± stdv: 7.77±7.67) in post-monsoon, 19.91 to 25.26 (avg. ± stdv: 22.90±2.23) in pre-monsoon and 2.90 to 17.06 (avg. ± stdv: 11.42 ± 5.50) in monsoon with higher values in pre-monsoon season.

Several mono-methyl, dimethyl and tri-methyl branched alkanes were detected from the study are listed in Appendix. Hopanes were also identified by monitoring ions in n-alkane at m/z=191. The 17α(H),21β(H)-hopanes that range from C27 to C34 (C28 absent) were detected in the present study (Table 5.2). In the present study, ratios such as 22S/(22S + 22R) and Ts/(Ts+Tm) were calculated using % composition of hopanes.

Table 5.2 Hopane identified in the present study

Name	Abbrevation
17α(H),21β(H)-tetrakishomohopane (C34αβ 22S)	C34αβ 22S
17α(H),21β(H)-trishomohopane (C33αβ 22R)	C33αβ 22R
17α(H),21β(H)-trishomohopane (C33αβ 22S)	C33αβ 22S
17α(H),21β(H)-bishomohopane (C32αβ 22R)	C32αβ 22R
17α(H),21β(H)-bishomohopane (C32αβ 22S)	C32αβ 22S
A'-Neogammacer-22(30)-ene or Hop-22(29)-ene or Diploptene	NG
Gammacerane	G
17α(H),21β(H)-homohopane (C31αβ 22R)	C31αβ 22R
17α(H),21β(H)-homohopane (C31αβ 22S)	C31αβ 22S
17β(H),21α(H)-hopane (C30βα)	C30βα
17α(H),21β(H)-hopane (C30αβ)	C30αβ
17β(H),21α(H)-30-norhopane (C29 βα)	C29 βα
Hop-13(18)ene	H13(18)
17α(H),21β(H)-30-norhopane (C29αβ)	C29αβ
17α-22,29,30-trisnorhopane	Tm
17β (H) -22,29,30-trinorhopene	Te
18α-22,29,30-trisnorhopane	Ts

5.2.1 n-alkanes as biomarker

Long-chain n-alkanes, which are relatively resistant to degradation with a predominant odd chain carbon (nC25–C33) are abundant in epi-cuticular waxes of vascular plant (Collister et al., 1994; Rieley et al., 1991). Hence these compounds are suitable biomarkers for higher plant sources (Cranwell, 1981). Mead et al., (2005) reported that mangroves have slightly longer mid-chain lengths compared to fresh water aquatic macrophytes and marine macrophytes (C27–C29). However, strong odd over even preference in the high molecular weight range (>C25) in present study region may also be attributed to predominant contribution from higher plant source, mainly from mangrove.

The higher relative abundance of short alkane with a peak at C15 or C17 has been used as the indicators of algal derived organic matter (Gelpi et al., 1970; Blumer et al., 1971; Meyers and Ishiwatari, 1995). Most algae have n-C15 as the major hydrocarbon, but some of them have n-C17 as principal alkane. Thalassery and Kadalundi showed comparatively higher concentration for C17 among the short chain alkanes in pre-monsoon and post-monsoon. In pre-monsoon, Pappinissery also showed predominance in C17 implying the presence of planktonic input. These results were anticipated, and is in parallel with its higher chlorophyll content. In the present study region, the odd chain n-alkanes can also be contributed from the diagenetic defunctionalisation of even-numbered alcohols, acids, and esters (Tissot and Welte, 1984).

Bacterial lipids are being suggested for the presence of the even-carbon preference for short-chain-length alkanes (Nishimura and Baker, 1986; Grimalt and Albaiges, 1987; Wu et al 2001). Such even preference with maxima at C18 and C20 has been attributed to diverse contribution of micro-organisms (Grimalt et al., 1985; Elias et al, 1997). Pazhayangadi in monsoon showed an even carbon preference for n-alkanes which can be correlated to bacterial input. Bacterial n-alkane distributions also shown to have maxima at one or two even carbon numbers in the range C14 to C22 (Davis, 1968; Jones, 1969; Han and Calvin, 1969).

Generally UCM (unresolved complex matrix) are related to petroleum input (Jeng and Huh, 2008), but are also observed in coastal marine environments away from petrogenic hydrocarbon sources (Matsumoto, 1983; Tolosa et al., 1996), which could probably be attributed to recycled organic matter (Niino and Emery, 1961; Jeng and Huh, 2008). In pre-monsoon, (except Kunjimangalam) a UCM noticeable up to C20 alkanes gives the inference of recycled organic matter by intense bacterial activity.

C14 to C20 n-alkanes without an odd/even preference are biomarkers for photosynthetic bacteria while non-photosynthetic bacteria produce n-alkanes ranging from C26 to C30 without an odd/even preference (Albro, 1976; Tanner et al., 2010). In pre-monsoon, all samples except Pazhayangadi showed short-chain alkanes without any odd/even carbon predominance indicate the presence of photosynthetic bacteria. While the samples from Kadalundi in monsoon and Thalassery in pre-monsoon gave the signatures for non-photosynthetic bacteria.

The total content of C27+C29+C31 n-alkanes have been used as terrestrial organic matter indicator (TOM), while the total content of C15+C17+C19 n-alkanes is normally considered as marine organic matter indicator (MOM) (Xing et al., 2011). Terrestrial organic matter ranged from

3.04 to 44.23% and marine organic matter ranged from 1.66 to 25.26% (Fig. 5.5). A decrease in TOM in parallel with increase in MOM was noticed in pre-monsoon, indicating the dominance of phytoplankton community during that season. In monsoon, for almost all samples TOM and MOM were less than 20 which might be due to microbial input.

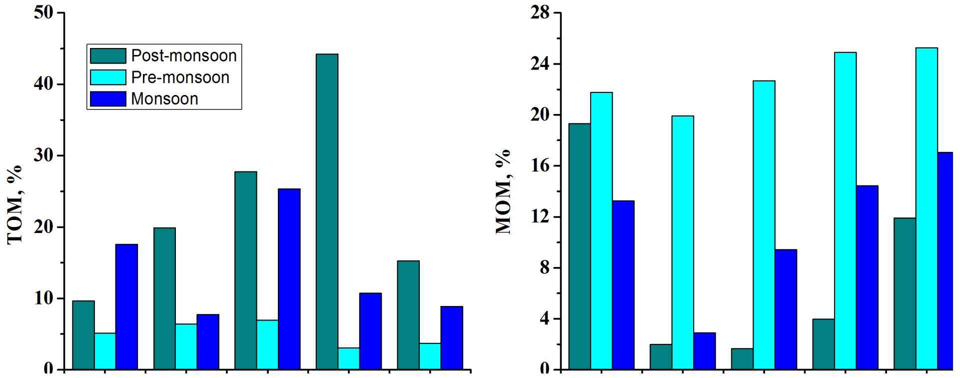

Fig. 5.5: Percentage contribution of Terrestrial organic matter (TOM) and marine organic matter (MOM) in the study area.

Origins of hydrocarbons from either terrestrial or marine environment can also be calculated by the ratio of C31 to C19 based on the assumption that C31 represent sources from terrestrial input and C19 represent the marine input (Farrington and Tripp, 1977; Boehm and Requejo, 1986; Sakari et al., 2012). In post-monsoon season except Kunjimangalam, the ratio showed higher values indicating higher terrestrial input. Conversely, in pre-monsoon the ratio was found to be less than one pointed towards the predominance of marine input (Fig. 5.6).

Ratio of LMW (sum of Low molecular weight alkanes ≤20) to HMW (sum of high molecular weight alkanes ≥21) were also calculated. According to Gearing et al., 1976, the ratio was one when input corresponds to algae, planktonic input, petroleum residues while relatively lower values were shown by sedimentary bacteria, higher plants, and marine animals (Commendatore et al., 2000). In pre-monsoon, except for Pazhayangadi (0.96), the ratio was greater than one in all samples. Kadalundi in monsoon season also showed higher value for this ratio (1.21). Thalassery (0.96) and Kunjimangalam (0.92) during monsoon season also have this ratio close to one (Fig. 5.6). These inferences point towards the predominance of planktonic input. In post-monsoon, terrestrial as well as bacterial input may be dominant.

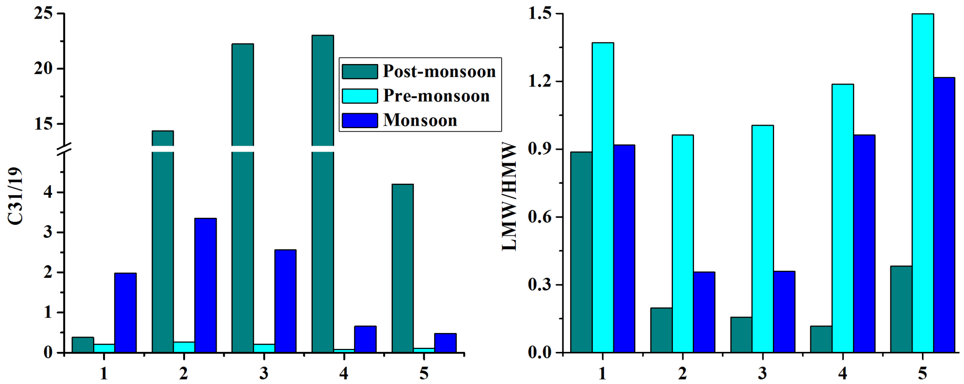

Fig. 5.6: Ratio of C31 to C19 and LMW to HMW in the selected mangrove ecosystems

5.2.1.1 Carbon Preference Index

Carbon Preference index (CPI) is a measure of homologous odd over even n-alkanes in a specified range of carbon numbers for source indication (Punyu et al., 2013) and has been utilized as a potential chemotaxonomic tool (Huang et al., 2000; Schefuβ et al., 2003). The n-alkane distribution exhibits high odd over even predominance for long-chain (C24-C32), which characterizes hydrocarbons from vascular plants versus those in petroleum and bacteria (Gearing et al., 1976; Cranwell, 1982).

CPI long chain = 1/2[(∑odd C25 to C31)/(∑even C24 to C30)+(∑odd C25 to C31)/(∑even C26 to C32)]

Hydrocarbons composed of a mixture of compounds originating from land plant material show a predominance of odd-numbered carbon chains with CPI 5-10 (Rieley et al., 1991; Hedges and Prahl, 1993). CPI values close to one are indicating higher input from marine microorganisms (bacteria and algae), recycled organic matter (Kennicutt et al., 1987; Sarkari., 2012), as well as from petroleum or those undergoing significant thermal alteration (~1; Cranwell, 1973). In organic geochemistry, the ratio is used to indicate the degree of diagenesis of straight-chain geolipids, and is a numerical representation of how much of the original biological chain length specificity is preserved in geological lipids (Meyers and Ishiwatari, 1995). Johnson and Calder (1973) found that CPI values near one indicate bacterial activity, while vascular plants have values ranging from 3 to 40 (Collister et al., 1994; Wang et al., 2003). As the distance from the shore increases, decline in CPI was noticed with the depletion of higher-plant lipid “freshness” by diagenesis (Krishnamurthy et al., 2001; Schefuß et al. 2003).

Long-chain n-alkanes with a predominance of odd carbon numbered compounds, can be considered as terrestrial input with relatively unaltered organic matter and a higher value (>3) is noted against carbon preference index (CPI). In the present study as many as six samples (Thalassery and Pappinissery in post-monsoon; Pazhayangadi in pre-monsoon; Kadalundi, Pazhayangadi, and Pappinissery in monsoon) showed value >3. According to the CPI, Pazhayangadi showed strong terrestrial signal in all seasons reflecting better preservation of mangrove signatures at this station. Pappinissery, except in pre-monsoon, also showed high CPI index season.

The CPI of higher-plant n-alkanes decreases with algal input, microbial reworking and diagenesis, and finally approaches unity during and after sedimentation (Kvenvolden, 1966; Zhang et al., 2010). For almost all samples, CPI was greater than one (Fig. 5.7). Kadalundi in post-monsoon and Thalassery in pre-monsoon showed CPI close to one. As discussed in Chapter 4, $\delta\ ^{13}C$ was found to be enriched in Kadalundi which reflect the input of marine organic matter. Lowest C/N ratio (10.86) and high chlorophyll (Chl a-16.04 $\mu g g^{-1}$) content was also reported from this station. All these inferences confirmed that the dilution of terrestrial organic matter by the contribution of marine organic matter, by its close proximal position to the Lakshadweep Sea. Thalassery mangrove ecosystem is also situated near the estuarine mouth and shows similar trends with CPI in pre-monsoon, hence the inferences are qualitatively similar to those of Kadalundi. In pre-monsoon except for Pazhayangadi, all samples have CPI <3 which should be resulted from the greater planktonic input as expected from higher chlorophyll pigments.

CPI based on short chain alkanes was also employed to determine whether the organic matter input in the study area, is from micro-organisms or petroleum (Fig. 5.7).

$$CPI_{short\ chain} = (C11 + C13 + C15 + C17 + C19 + C21) / (C12 + C14 + C16 + C18 + C20 + C22)$$

CPI values of 1 or near 1 indicate inputs from petroleum products, whereas <1 indicates inputs from microorganisms including bacteria and diatoms (Clark and Blumer, 1967; Garg and Bhosle, 2004; Ahad et al., 2011). The value was found to be less than one throughout the study, which neglected the chance of petroleum contamination. The index is comparatively higher in pre-monsoon resulted from higher planktonic input.

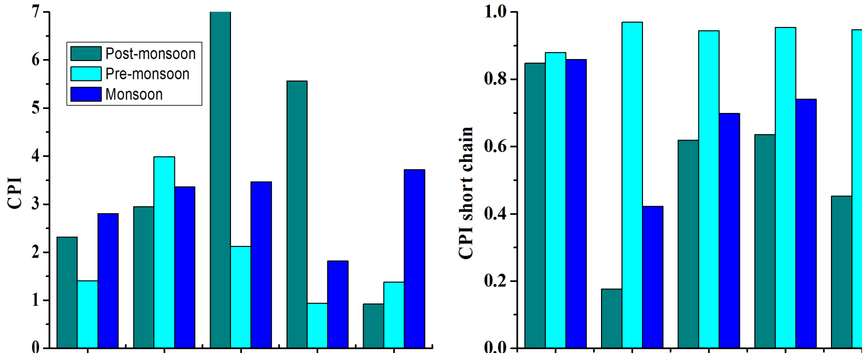

Fig. 5.7: CPI index for long and short chain alkanes

5.2.1.2 Pristane and Phytane

Acyclic isoprenoid alkanes, such as pristine (Pr) and phytane (Ph) were detected in present study, which are commonly found in lacustrine and marine environments. Generally Pristane and Phytane are considered to be geologic alteration of phytol (Didyk et al., 1978; Peters and Moldowan, 1993). Phytane which can be used as an algal source indicator formed from the phytyl side chain of chlorophyll or bacteriochlorophyll of immature sediments (Ragan and Chapman, 1978; Gireeshkumar et al., 2015) and also can be synthesised by the methanogenic and photosynthetic bacteria (Steinhauer and Boehm, 1992; Sakata et al., 1997). Archaea have also been supposed to be a source of pristane and phytane (Chappe et al., 1982). Pristane, a well-known biomarker of zooplankton (Blumer et al., 1963; Volkman et al., 1992; Gao and Chen, 2008), is produced in their diet from phytol particularly of calanoid copepods (Volkman and Maxwell, 1986; Seki et al., 2006). Pristane and Phytane could not be detected in Pazhayangadi and Pappinissery (post-monsoon) respectively.

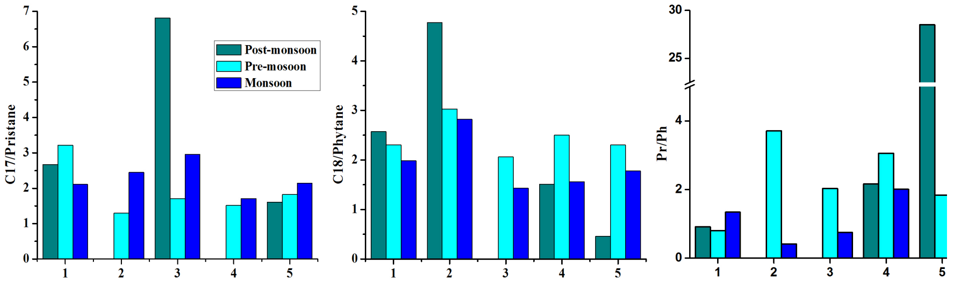

Fig. 5.8: Ratio of Pristine and Phytane in the selected study region

Pristane and phytane, which are also major constituents of crude oils, may act as good indicators of petroleum contamination (Volkman et al., 1992). The Ratios of C17/Pr and C18/Ph <1 (Fig. 5.8) indicate the contamination from crude oil and uncontaminated sediment when their value is >1. In all sampling sites C17/Pr is greater than 1. High C17/Pr ratios (≫2) are thought to be reflected by the significant contributions from algae (Readman et al., 2002), whereas low values (<1) indicated highly weathered oil (Wang et al., 1995). C18/Ph was greater than 1 in all station except Kadalundi in Post monsoon season. However it suggests the presence of degraded oil residues, though such indication must be considered with caution because of the generally low concentration of these components in these station.

Under oxidizing conditions phytol is converted to pristene then to pristane, while under anoxic conditions, it is subjected to reduction and dehydration forming Phytane (Killops and Killops, 2005; Bianchi and Canuel, 2011). Ratios of Pr/Ph can, in theory, be used to evaluate the redox state of depositional environments in sediments; however, there are many restrictions that limit its use (ten Haven et al., 1987; Gireeshkumar et al., 2015). The conventional interpretation is that Pr/Pr <1 indicates anoxic conditions, whereas Pr/Ph > 1 indicates oxic conditions. Minimum value was reported from Pazhayangadi during monsoon season indicate highly anoxic condition prevailed in the environment. Ratio was found to be less than one from 4 samples- Kunjimangalam (Post and Pre monsoon season), Pappinissery and Pazhayangadi (monsoon). The tidal action in Kadalundi contributes greater zooplankton input and maintained oxic sedimentary environment might be reason for high Pr/Ph ratio.

5.2.1.3 Average Chain Length

Average chain length (ACL), which describes the average number of carbon atoms per molecule based on the abundance of the odd carbon numbered higher plant n-alkanes (Poynter and Eglinton, 1990). ACL may vary among plant species, suggesting that taxonomy exert the major control on n-alkane distributions. For e.g., leaf lipids derived from grasslands may on average have longer chain lengths than do leaf lipids from plants in forests (Cranwell, 1973; Eglinton and Eglinton, 2008). It has been suggested that plants produce longer-chain compounds in warmer climates. A few reported works on the response of ACL in plants to changing environmental conditions are available. Gagosian and Peltzer (1986) observed that dusts derived from warmer tropical sources have long-chain n- alkanes while dusts from cooler temperate sources may have relatively more short-chain n-alkyl components. Similarly, a positive correlation between ACL in marine sediments and estimated sea-surface temperatures proposes that ACLs could serve as a proxy for continental temperature (Rinna et al., 1999). It has been found that latitude have an important role in the n-alkane distribution of higher plants (Poynter et al., 1989; Poynter and Eglinton, 1990). According to Jeng, 2006, there is a change of ~0.04 ACL units while crossing through 1° latitude i.e., model carbon number depletes with higher latitudes. Further, the distribution of ACL has been linked to the geographical distribution of fluvial and aeolian inputs and source regions (Poynter and Eglinton, 1990). In other words, for coastal marine sediments, ACL in a given area can be considered as a constant if all these factors are the same. The ACL could be lowered if there is an addition of petro-genic hydrocarbons to sediments containing biogenic hydrocarbons alone. In this study, we explored the possibility of applying ACL in this aspect.

Average Chain Length (ACL) $=\Sigma[C_i]i/\ \Sigma\ [C_i]$

where i is the odd carbon number from C23-33

The ACL for the present study ranged from 25.75 to 29.10 (Fig. 5.9). Gireeshkumar et al., 2015 studied n-alkane distribution in Cochin estuary, and ACL in the range 21 to 28 were reported. In the present study ACL was higher than 27 in almost all stations indicating the preservation of mangrove derived organic matter. Pre-monsoon characterised by lowest ACL in Pappinissery, Thalassery and Kadalundi due to planktonic input.

5.2.1.4 Terrigenous to Aquatic Ratio

The terrigenous/aquatic ratio (TAR) evaluates the terrigenous inputs versus aquatic inputs. Although it may over-represent the absolute amounts from terrigenous sources, this ratio is valuable for determining changes in relative contributions of organic matter from land and aquatic flora (Meyers, 1997).

Terrigenous to aquatic ratio (TAR) = $(C27 + C29 + 31)/\ (C15 + C17 + C19)$

High values are thought to be diagnostic of non-marine sources, whereas lower values are encountered in all kinds of marine sedimentary environments (Moldowan et al., 1985). The ratio was adopted in the present study to estimate the relative contribution of terrigenous and marine sources to the sediment. In post-monsoon, except for Kunjimangalam and Kadalundi, TAR values (> 2.0) showed that the terrigenous input was predominant (Fig. 5.9). TAR value was less than two at Kadalundi in all seasons which indicate dominance of marine input. All stations during pre-monsoon season showed TAR values <1 reflecting the dominant planktonic input. This season was also associated with high chlorophyll content. The decrease in TAR index in monsoon at Thalassery and Kadalundi also reflect the dominance of algal signatures. It is evident that TAR index are in good agreement with chlorophyll studies suggesting planktonic production in pre-monsoon.

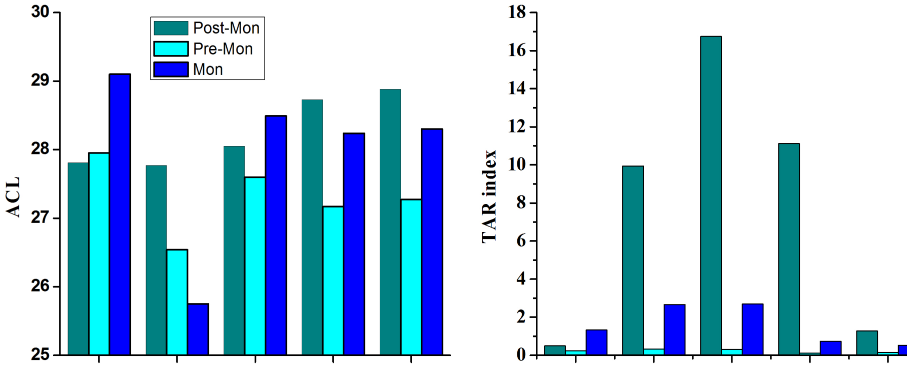

Fig 5.9: Average Chain Length and Terrigenous to Aquatic Ratio calculated in the study region

5.2.2 Alkenes

Series of alkenes from nC16 to C26, with even predominance having Cmax at C16 and C18 have been observed in previous studies (Cranwell, 1984; Aboul-Kassim and Simmoneit, 1995; Fubara et al., 2012). Predominance of even chained alkenes in the range C12:1 to C18:1 in recent sediments may be derived from unsaturated fatty acids, (Simoneit, 1984) and that of higher even numbered n-alkenes may be related to terrestrial plants. In addition to n-alkanes, there were several odd chain n- alkenes (C15:1, C17:1, C19:1, C21:5, C21:6) likely to be derived from phytoplankton (Damste et al., 1999). Alkenes in the range C18:1 to C22:1 can also be linked with various sources such as marine organisms, terrestrial plants and anthropogenic activities. Ekpo et al., 2012 reported that the concentration of C18:1 to C24:1 could be linked with mangrove vegetation.

In post-monsoon, except for Kunjimangalam alkene showed even predominance in parallel with alkane up to C22 (Fig. 5.10). Similar trends were observed in monsoon also. In pre-monsoon, peak area of alkenes was higher than that of alkanes at Thalassery, Pappinissery and Kunjimangalam. Pazhayangadi showed higher alkene abundance during monsoon.

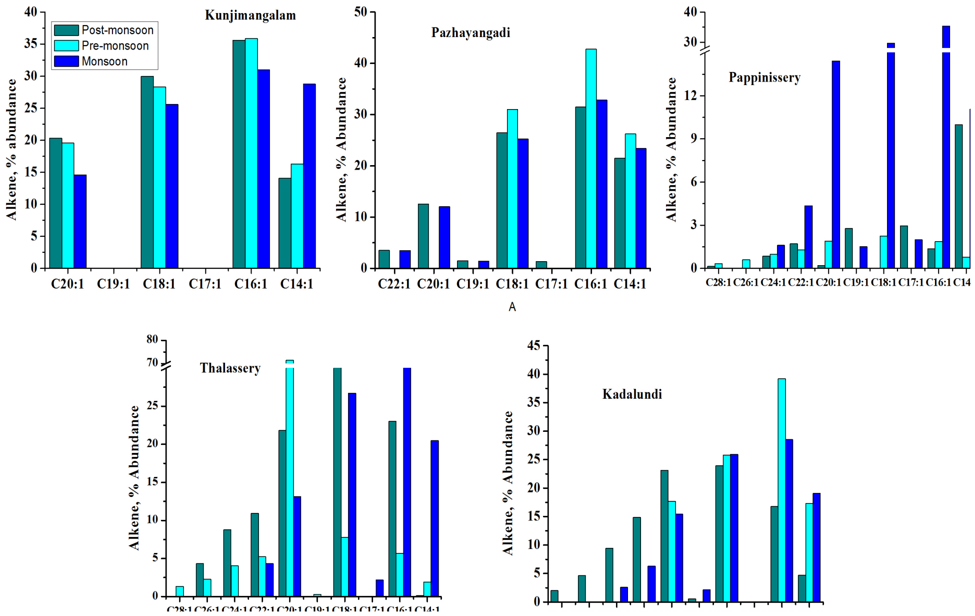

Fig. 5.10: % Abundance of alkenes in the present study region

Even n-alkenes in the range C22:1 to C32:1, synthesized by defunctionalisation of alcohols and fatty acids, are the main components of higher plant waxes (Cranwell, 1984; Aboul-Kassim and Simoneit, 1995). Hence, the presence of higher even alkenes in the presence study indicates terrestrial organic matter input. Heptadecene (C17:1) and nonadecene (C19:1) were detected in lower concentration suggesting the freshwater phytoplankton origin (Saliot et al., 1991).

5.2.3 Branched Alkanes and N-Alkyl Cyclohexanes

Branched alkanes, such as monomethyl alkanes (MMA) and dimethyl alkanes (DMA) are common in geological samples. These compounds have been identified in modern and Holocene microbial mats (Shiea et al., 1990; Kenig et al., 1995; Kenig, 2000), ancient sediments (Summons, 1987; Summons et al., 1988a,b; Summons and Walter, 1990; Hold et al., 1999; Audino et al., 2001) and crude oils (Jackson et al., 1986; Fowler and Douglas, 1987; Kissin, 1987; Warton et al.,1997), however the source organisms for these compounds are not well understood. For monomethyl alkanes, several origin have been suggested and some of them are (i) direct biological contributions of branched alkanes (Fowler and Douglas, 1987) (ii) diagenetic products formed from the transformation of functionalized lipid precursors such as carboxylic acids (Summons, 1987; Summons et al., 1988b) (iii) products of long-term equilibration of a limited range of isomers (Hoering, 1980; Klomp,1986b) (iv) acid-catalyzed (e.g. clays) products of alkenes formed by thermal cracking (Kissin, 1987)

Among the various mid-chain branched mono-methyl alkanes, synthesised by Cyanobacteria, 7- and 8-methyl heptadecanes are the most common (Han et al., 1968; Robinson and Eglinton, 1990). Also, monomethyl alkanes with long-chain branching derived from various plants have been detected in previous studies (Nelson, 1978). In petroleum and ancient sediments, they occurred as a group of structural isomers of the possible branching positions over a range of carbon numbers (C12-C30). Finally significant abundances of methyl- alkanes up to C28 without apparent branching preference, has been suggested a lipid input to sediments from prokaryotic microorganisms (Robinson and Eglinton, 1990). This result is in good agreement with the present study due to the presence of monomethyl alkanes up to C28 without any branching preference and it may be originated from prokaryotic micro-organisms or diagenetic transformation.

Dimethyl alkanes were found as the predominant hydrocarbons in the microbial mats. Some of the previous reports on dimethyl alkanes are from the desulfurized polar fraction of an Oligocene anhydrite (Adam, 1991), and Cenomanian and Turonian black shales of Canada (Simons et al., 2003). Recently, pseudo-homologous families of ethyl branched alkanes were identified in ancient sediments and oils (Kenig et al., 2001; Warton et al., 1997, 1998), but their origin also remains unclear, though a bacterial origin is preferred (Kenig et al., 2001). 2,4 dimethyl dodecane was also detected in the present study.

Trimethyl alkanes such as Dodecane, 2,6,10- trimethyl-; Tridecane, 2,6,10- trimethyl- and Pentadecane, 2,6,10- trimethyl- were found in the present study region. These acyclic isoprenes were reported from the core sediments of California Borderland and Florida Straits (Vella and Holzer, 1992). 2,6,10-trimethyltetradecane has been isolated from sulfolobus genus that belongs to Archaea domain (Holzer et al., 1979). Several researchers proposed Archaea bacterial lipids as the significant source of acyclic isoprenoids (Volkman and Maxwell, 1986; Aquilina et al., 2010).

The homologous series of n-alkyl cyclohexane (base peak-m/z 83) ranging from heptyl cyclohexane to heptadecyl cyclohexane have been reported by several authors, but in the present study as many as ten among them were identified; they are Heptadecyl Cyclohexane (Hepta DCH), Hexadecyl cyclohexane (Hexa DCH), Pentadecyl cyclohexane (Penta DCH), Tetradecyl cyclohexane (Tetra DCH), Tridecyl cyclohexane (Tri DCH), Dodecyl cyclohexane (Do DCH), Undecyl cyclohexane (Un DCH), Decyl cyclohexane (Decyl CH), Octyl cyclohexane (Octyl CH) and Heptyl cyclohexane (Heptyl CH). n-Alkyl cyclohexanes found in the present study may be possibly derived from the diagenetic alteration of algal (Fowler et al., 1986) and bacterial lipids (Fowler and Douglas, 1984; Bhattacharya et al., 2014). In the present study, except for Kunjimangalam, it can be observed from Fig. 5.11 that the percentage abundance was high for Octyl cyclohexane. In Pre-monsoon, higher percentage abundance for lower homologous was observed except in Pappinissery and Thalassery.

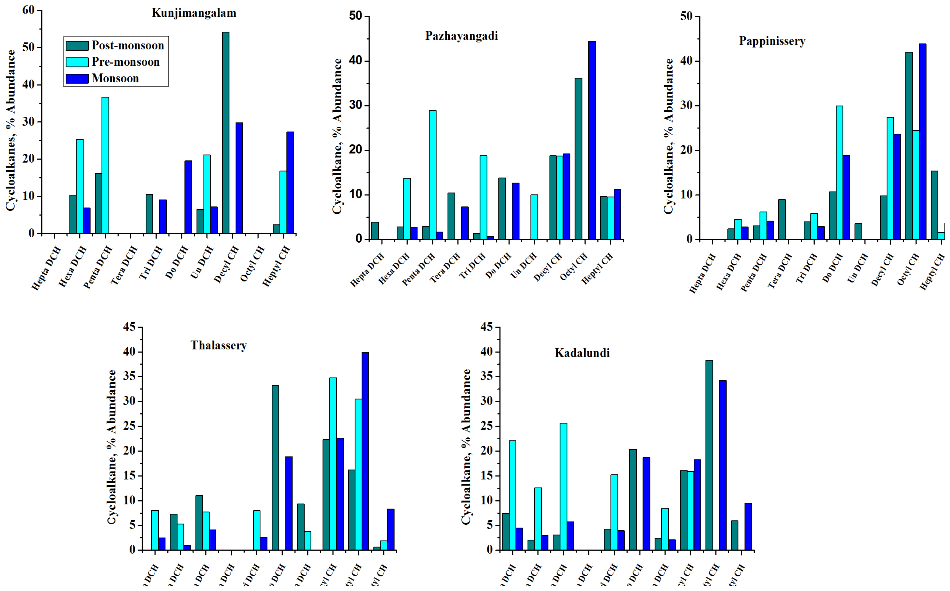

Fig. 5.11: % Abundance of n-alkyl cyclohexane in the study region

Pyrene (C16), a Polyaromatic hydrocarbon (PAH) has prominent m/z values at 200, 201, 202, 203 in the mass spectral data and was found from Pappinissery in post-monsoon (NIST MS Library). It could be formed from the incomplete combustion of organic matter such as wood and fossil fuels (Blumer, 1975; Killops and Massoud, 1992), therefore, referred to as pyrolytic products. Pyrene is listed as a priority pollutant by the U.S. Environmental Protection Agency. PAHs are extremely harmful because they exhibit toxicity and mutagenicity even at very low concentrations and have a high tendency to bind with natural organic matter (Simpson et al., 2005). Its low water solubility and strong affinity for organic matter (Sims and Overcash, 1983; Mackay et al., 1992) contribute to its persistence in sediments.

5.2.4 Hopanes

Hopanoids, maintain membrane fluidity in prokaryotes, used as a bacterial marker for their presence in geological materials (Belin, 2009). Hopanes can be detected from sedimentary rock records, were their taxonomic and/or physiological signals are preserved over billions of years (Pearson and Rusch, 2009). Naturally occurring hopanoids show stereochemistry 17β(H),21β(H), which is thermodynamically unstable, and it's the diagenesis and catagenesis can be resulted in the 17α(H),21β(H)-hopanes and 17β(H),21α(H)-moretanes (Philp, 1985; Ourisson et al., 1979). Of the four possible hopane stereoisomers, namely 17β(H),21β(H) (living organism), 17α(H),21α(H) (not present in fossil fuels), 17β(H),21α(H) (pyrolyzates-also called moretanes), 17α(H),21β(H) (natural fossil fuels), out of which the last two are the most stable ones (Seifert, 1978).

17 hopanes were identified from the mangrove ecosystems under observation. Among them 17α-22,29,30-trisnorhopane (Tm) and 17β (H) 22,29,30-trinorhopene (Te), 17α(H),21β(H)-30-norhopane (29 αβ), 17α(H), 21β(H)-homohopane (C31αβ 22S), 17α(H),21β(H)-homohopane (C31αβ 22R) and Diploptene were widespread. Diploptene (17β(H),21β(H)-hop-22(29)-ene) can be related to cyanobacteria, methylotrophic bacteria and certain species of ferns. The extended hopanes or homohopanes (C31-C35) related to the bacteriohopanepolyols and lower hopane pseudohomologs related to C30 precursors such as Diploptene and Diplopterol (Peters and Moldowan, 1991). Hopanoids undergo net reduction to hopanes during diagenesis. In some of the previously reported works, hopanes have been correlated to petrogenic origin, but in the present study the main input is from microbial as well as its diagenetic transformation products. Diploptene is produced in situ by bacteria using carbon sources (Ourisson et al., 1979, 1987; Ourisson and Rohmer, 1992; Yunker et al., 1994) available in the mangrove ecosystem. Among 22S and 22R doublets in the range from C31 to C35 the biologically produced hopane precursors carry 22R configuration that undergo epimerisation favouring a slight preference for the 22S epimer (Philp, 1985; Peters and Moldowan, 1993; Aboul-kassim and Simonit, 1996).

Generally C31- or C32-homohopane results are used for calculations of the 22S/(22S + 22R) ratio. In the present study, the ratio for C31 hopane is in the range 0.43 to 0.60 while that of C32 hopane it is 0.39 to 0.49. Predominance of the C31 homohopane in the present study region might be due to the exposure to raised oxygen levels (Boreham et al., 1994). Periodic aeration caused by tidal rhythms, bioturbation activities and root nodules of mangrove micro-pores may support this predominance. Tm is less stable (more maturable) than Ts (Peters and Moldowan, 1993), so the ratio of Ts/(Ts+Tm) has been used as a maturity index thermodynamically, and was ranged from 0.35 to 0.53 in the present study. The ratio of Tm/Ts was greater than one in Kunjimangalam, Pazhayangadi and Kadalundi in pre-monsoon season indicating preferential degradation of Tm (Peters and Moldowan, 1993). Higher abundance of hopanes was observed in Pappinissery and Pazhayangadi in pre-monsoon, Thalassery in post-monsoon, Kadalundi in monsoon. An abundance of hopanoid can be correlated with the increase in hopane producing bacteria which increases in the events of anoxia (Dahl et al., 1993).

5.3 Principal Component Analysis

Principal component analysis is used to classify hydrocarbons (n-alkanes from C11 to C33 along with pristane and phytane, alkenes, n-alkyl cyclohexanes) according to their primary sources (e.g. terrigenous, marine). It identifies the variables that co-vary and thereby give geo-chemical relationships existing in the study region (Table 5.3 and 5.4).

The first five PCs account for the 74.81 % of the total variance in the data set and reflect the most important geochemical features (Table 5.3 and 5.4). The first principal component (PC1) explained 30.03 % and PC2 explained 12.54 % of the total variability of the data. PC1 is basically defined by short chain and mid-chain alkane i.e., n-C14 to C24 along with C26, C28, pristane and phytane. This factor is contributed from planktonic as well as microbial derived organic matter. Contribution of microbial component in this factor gives the inference that recycling of organic matter by bacteria is active in this ecosystem. n-alkanes from bacterial derived organic matter is found to be co-exist with marine-derived organic matter, indicated that the main bacterial substrate was phytoplankton rather than terrestrial component

Table 5.3: Total Variance Explained for hydrocarbons using principal component analysis

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	16.30	33.96	33.96	16.30	33.96	33.96	14.41	30.03	30.03
2	7.77	16.19	50.15	7.77	16.19	50.15	7.19	14.98	45.01
3	5.58	11.63	61.78	5.58	11.63	61.78	6.02	12.54	57.56
4	5.45	11.36	73.14	5.45	11.36	73.14	4.23	8.82	66.37
5	2.83	5.90	79.04	2.83	5.90	79.04	4.05	8.44	74.81
6	2.45	5.10	84.14	2.45	5.10	84.14	3.23	6.72	81.54
7	1.95	4.07	88.21	1.95	4.07	88.21	2.19	4.55	86.09
8	1.51	3.15	91.36	1.51	3.15	91.36	2.14	4.47	90.56
9	1.19	2.47	93.83	1.19	2.47	93.83	1.57	3.27	93.83

PC2 showed high positive loading on C25, C27 and C29-C33. The contribution of high molecular weight n-alkanes with odd-numbered carbon predominance (n-C27, 29, 31, 33) is characteristic of cuticular waxes of terrestrial plants i.e input from mangrove ecosystem. PC3 showed loadings on some n-alkyl cyclohexanes and alkene C22:1 and PC4 on short-chain alkanes (<C12). n-Alkyl cyclohexanes are diagenetic products and their formation may be controlled by many factors other than the concentration of their precursor compound, hence they formed a separate group. PC5 accounts for even alkenes >C22, which can be inferred from mangrove origin. Diagenetic alterations for alkene are comparatively high so interpretations based on their % abundance may not give correct inferences.

Table 5.4 Factor loading for Hydrocarbons Principal component analysis using varimax rotation

	Component				
	1	2	3	4	5
C8	-0.26	-0.14	0.33	0.60	-0.08
C9	0.07	-0.02	0.43	0.12	0.48
C10	-0.04	-0.15	0.09	0.69	0.16
C11	0.22	-0.35	-0.03	0.83	0.10
C12	0.61	0.12	-0.25	0.66	0.13
C13	0.68	0.23	-0.15	0.57	-0.06
C14	0.72	0.30	0.00	0.35	-0.16
C15	0.97	0.13	0.03	0.06	-0.13
C16	0.97	0.03	0.00	0.14	-0.01
C17	0.96	-0.05	-0.11	-0.13	-0.04
C18	0.81	0.22	0.02	0.29	-0.09
C19	0.97	0.04	0.01	0.06	-0.16
C20	0.97	0.09	0.11	-0.08	-0.14
C21	0.97	0.08	-0.04	0.02	-0.14
C22	0.85	0.27	0.24	-0.16	-0.27
C23	0.89	0.29	-0.06	-0.08	0.01
C24	0.82	0.27	0.25	-0.04	-0.24
C25	0.32	0.72	0.06	0.18	0.31
C26	0.81	0.37	0.18	-0.18	-0.27
C27	0.33	0.89	-0.06	-0.12	0.18
C28	0.67	0.59	0.27	-0.18	-0.18
C29	0.06	0.98	0.01	-0.06	0.08
C30	0.40	0.79	0.25	-0.11	-0.18
C31	-0.09	0.94	0.09	0.18	-0.02
C32	0.16	0.93	0.17	-0.11	-0.01
C33	0.02	0.98	0.01	0.09	0.05
Pri	0.87	-0.19	-0.26	-0.16	0.12
Phy	0.81	-0.18	0.08	0.00	-0.23
C28:1	-0.28	0.21	0.04	0.35	0.69
C26:1	-0.50	0.12	0.00	0.02	0.83
C24:1	-0.20	0.04	0.33	0.11	0.88

C22:1	-0.20	0.14	0.64	0.17	0.64
C20:1	-0.37	-0.02	0.04	0.47	0.25
C19:1	0.47	-0.15	0.55	0.20	0.15
C18:1	0.14	0.10	-0.20	0.18	0.00
C17:1	0.20	0.18	0.59	-0.14	-0.28
C16:1	0.27	0.06	-0.22	0.20	-0.20
C14:1	0.50	0.05	-0.06	0.28	-0.60
Heptadecyl-CH	-0.23	0.28	0.00	0.80	0.06
Hexadecyl-CH	0.01	-0.16	-0.95	-0.13	-0.11
Pentadecyl-CH	0.07	-0.04	-0.72	0.09	-0.04
Tetradecyl-CH	0.44	0.13	0.38	0.10	-0.06
Tridecyl-CH	0.11	0.16	-0.31	0.11	-0.20
Dodecyl- CH	-0.16	-0.14	0.81	-0.32	0.17
Undecyl- CH	-0.26	-0.18	-0.88	-0.04	0.07
Decyl-CH	0.02	0.20	0.35	-0.10	0.10
Octyl- CH	-0.02	0.15	0.85	0.09	0.35
Heptyl-CH	0.24	0.06	0.14	-0.15	-0.20

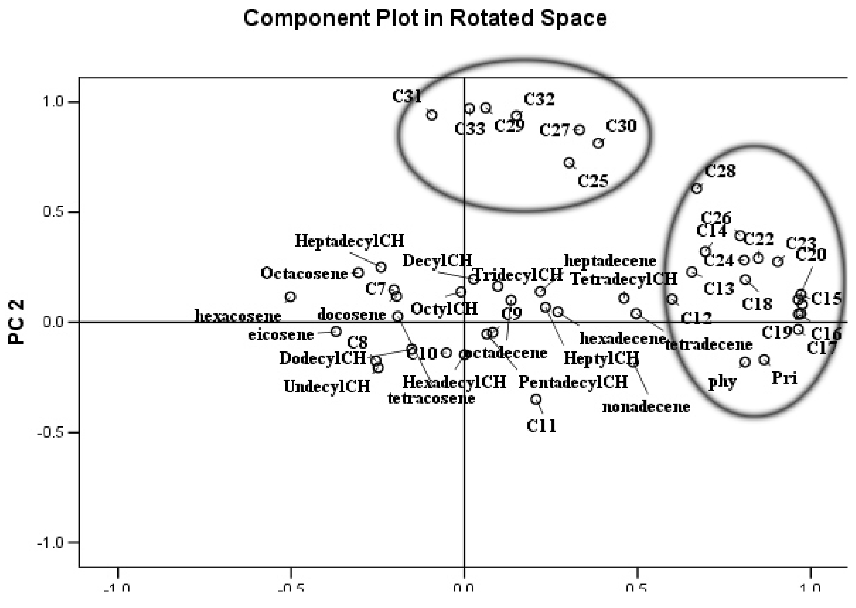

Fig. 5.12: Plot of PC1 verses PC2

The plot of PC1 versus PC2 is shown in Fig. 5.12. From the figure, it can be inferred that a group was formed by higher alkanes which indicated the terrestrial input. Separation between even and odd alkanes uptoC24 was not distinct, showing the input from planktonic algae and bacteria. C11 was obviously separated by PC1 and PC2 partly attributable to the degradation of some marine bacteria (Grimalt and Albaiges, 1987), specific species of algae (Kang et al., 2000) and possible petroleum-derived hydrocarbon inputs (Harji et al., 2008). Alkenes and n-alkyl cyclohexanes lie in the centre of the PCA plot suggesting its formation from diagenesis.

5.4 Conclusions

Source characterisation of organic matter from the surface sediments of mangrove ecosystems of northern Kerala coast was carried out by employing hydrocarbons as biomarkers. Various ratios using odd and/or even preferences and specified range of carbon numbers in the hydrocarbon chain were calculated to draw various source indications. The predominant contribution from higher plant source, chiefly from mangrove input could be observed from the strong odd over even preference in the long chain n-alkanes (>C25). Further, a characteristic dominance of terrestrial input can be confirmed in post-monsoon, using the Terrigenous/Aquatic ratio (TAR > 2.0) except for Kunjimangalam and Kadalundi. Pappinissery, except in pre-monsoon and Pazhayangadi, throughout the study showed strong terrestrial signal from Carbon preference index (CPI) and it could be due to the thick vegetation and/or clayey nature of the sediment. CPI based on short-chain alkanes were found to be less than one in the present study, confirming the appreciable input from microorganisms and absence of petroleum contamination. Significant contribution from intense bacterial activity in pre-monsoon (except Kunjimangalam) was also accounted by Unresolved Complex Matrix (UCM) found up to C20 alkanes.

In pre-monsoon, dominant planktonic input in the organic matter was accounted for strong odd over even predominance of lower alkanes, TAR (Terrestrial Aquatic ratio) <1, decrease in TOM (Terrestrial organic matter) in parallel with increase in MOM (Marine Organic matter), lower CPI (except Pazhayangadi) and higher chlorophyll content; Kadalundi and Thalassery was associated with marine organic input and non-photosynthetic bacterial signatures were obtained for these station in pre-monsoon. The tidal action at Kadalundi contributes greater zooplankton input inferred from Pr/Ph ratio.

The 17α(H),21β(H)-hopanes that range from C27 to C34 (with C28 absent) were detected in the present study. 17α-22,29,30-trisnorhopane (Tm), 17β(H)–22,29,30-trinorhopene (Te), 17α(H),21β(H)-30-norhopane (29 αβ), 17α(H),21β(H)-homohopane (C31αβ 22S), 17α(H),21β(H)-homohopane (C31αβ 22R) and Diploptene were the mostly detected. The PCA (Principal Component Analysis) showed terrestrial, planktonic algae, as well as bacterial input as the major source of organic matter in the present study; and the plot also suggested n-alkyl cyclohexanes was formed by diagenesis. From the biomarker data sets of aliphatic hydrocarbons, varying contribution from terrestrial, marine and bacterial derived organic matter were identified in these mangrove ecosystems.

References

Aboul-kassim, T. A. T., Simoneit, B. R. T., 1996. Lipid geochemistry of surficial sediments from the coastal environment of Egypt I . Aliphatic hydrocarbons - characterization and sources. Marine Chemistry, 54, 135-158.

Aboul-Kassim, T. A., Simoneit, B. R., 1995. Aliphatic and aromatic hydrocarbons in particulate fallout of Alexandria, Egypt: sources and implications. Environmental science & technology, 29, 2473-2483.

Adam, P., 1991. Nouvelles structures organo-soufrees d'interet geochimique: Aspects moleculaires et macro moleculaires. Ph.D. Thesis, Universite Louis Pasteur, Strasbourg.

Ahad, J. M. E., Ganeshram, R. S., Bryant, C. L., Cisneros- Dozale, L. M., Ascough, P. L., Fallick, A. E., Slater, G. L., 2011. Sources of n-alkanes in an urbanized estuary: Insights from molecular distributions and compound- specific stable and radiocarbon isotopes. Marine Chemistry, 126, 1–4.

Albro, P. W., 1976. Bacterial waxes. In Chemistry and biochemistry of natural waxes, Elsevier Amsterdam.

Aquilina, A., Knab, N. J., Knittel, K., Kaur, G., Geissler, A., Kelly, S. P., Fossing, H., et al., 2010. Organic Geochemistry Biomarker indicators for anaerobic oxidizers of methane in brackish-marine sediments with diffusive methane fluxes. Organic Geochemistry, 41, 414-426.

Audino, M., Grice, K., Alexander, R., Boreham, C. J., Kagi, R. I., 2001. Unusual distribution of monome- thylalkanes in Botryococcus braunii-rich samples: origin and significance. Geochimica et Cosmochimica Acta, 65, 1995–2006.

Baas, M., Pancost, R., van Geel, B., Damsté, J. S. S., 2000. A comparative study of lipids in Sphagnum species. Organic Geochemistry, 31, 535-541.

Belin, G. K., 2009. Investigation of Hopanoid Biomarkers in Lake Sediments by GC-MS and RP-HPLC-APCI-MS. Journal of Chemistry, 6, 77-88.

Bhattacharya, S., Dutta, S., Dutta, R., 2014. Molecular fossils in Cretaceous condensate from western India. Journal of Earth System Science, 123, 923-934.

Bianchi, T. S., Canuel, E. A., 2001. Organic geochemical tracers in estuaries. Organic Geochemistry, 32, 451.

Bianchi, T.S., Cannuel, E.A., 2011. Chemical Biomarkers in Aquatic Ecosystems. Princeton University Press, USA.

Blumer M., 1975. Curtisite, Idrialite and Pendletonite, polycyclic aromatic hydrocarbon minerals: their composition and origin. Chemical Geology, 16, 245-256.

Blumer, M., Guillard, R. R. L., Chase, T., 1971. Hydrocarbons of marine phytoplankton. Marine Biology, 8, 183.

Blumer, M., Mullin, M. M., Thomas, D. W., 1963. Pristane in zooplankton. Science, 140, 974.

Blumer, M., Mullin, M. M., Thomas, D. W., 1964. Pristane in the marine environment. Helgolaender Wissenschaftliche Meeresuntersuchungen, 10, 187-201.

Boehm, P. D., Requejo, A. G., 1986. Overview of the recent sediment hydrocarbon geochemistry of Atlantic and Gulf Coast outer continental shelf environments. Estuarine, coastal and shelf science, 23, 29-58.

Boot, C. S., Ettwein, V. J., Maslin, M. A., Weyhenmeyer, C. E., Pancost, R. D., 2006. A 35,000 year record of terrigenous and marine lipids in Amazon fan sediments. Organic Geochemistry, 37, 208-219.

Boreham, C. J., Summons, R. E., Roksandic, Z., Dowling, L. M., Hutton, A. C., 1994. Chemical, molecular and isotopic differentiation of organic facies in the Tertiary lacustrine Duaringa oil shale deposit, Queensland, Australia. Organic Geochemistry, 21, 685-712.

Canuel, E. A., Freeman, K. H., Wakeham, S.G., 1997. Isotopic compositions of lipid biomarker compounds in estuarine plants and surface sediments. Limnology and Oceanography, 42, 1570-1583.

Chappe, B., Albrecht, P., Michaelis, W., 1982. Polar lipids of archaebacteria in sediments and petroleums. Science, 217, 65-66.

Clark, R. C., Blumer, M., 1967. Distribution of n-paraffins in marine organisms and sediments. Limnology and Oceanography, 12, 79-87.

Cloern, J. E., Canuel, E. A., Harris, D., 2000. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnology and oceanography, 47, 713-729.

Collister, J. W., Rieley, G., Stern, B., Eglinton, T., Fry, B., 1994. Compound specific $\delta^{13}C$ analyses of leaf lipids from plants with differing carbon dioxide mechanisms. Organic Geochemistry, 21, 619–627.

Commendatore, M. G., Esteves, J. L., 2004. Natural and anthropogenic hydrocarbons in sediments from the Chubut River (Patagonia, Argentina). Marine Pollution Bulletin, 48, 910-918.

Commendatore, M. G., Esteves, J. L., Colombo, J. C., 2000. Hydrocarbons in coastal sediments of Patagonia, Argentina: levels and probable sources. Marine Pollution Bulletin, 40, 989-998.

Cranwell, P. A., 1981. Diagenesis of free and bound lipids in terrestrial detritus deposited in a lacustrine sediment. Organic Geochemistry, 3, 79-89.

Cranwell, P. A., 1982. Lipids of aquatic sediments and sedimenting particulates. Progress in lipid research, 21, 271-308.

Cranwell, P. A., 1984. Lipid geochemistry of sediments from Upton Broad, a small productive lake. Organic Geochemistry, 7, 25-37.

Cranwell, P. A., Eglinton, G., Robinson, N., 1987. Lipids of aquatic organisms as potential contributors to lacustrine sediments—II. Organic Geochemistry, 11, 513-527.

Cranwell, P. A., 1973. Chain-length distribution of n-alkanes from lake sediments in relation to post-glacial environmental change. Freshwater Biology, 3, 259–265.

Dahl, J., Moldowan, J. M., Sundararaman, P., 1993. Relationship of biomarker distribution to depositional environment: Phosphoria Formation, Montana, USA. Organic geochemistry, 20, 1001-1017.

Damste, J. S. S., De Leeuw, J. W., 1990. Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: state of the art and future research. Organic Geochemistry, 16, 1077-1101.

Damsté, J. S. S., Schouten, S., Rijpstra, W. I. C., Hopmans, E. C., Peletier, H., Gieskes, W. W., Geenevasen, J. A., 1999. Structural identification of the C 25 highly branched isoprenoid pentaene in the marine diatom Rhizosolenia setigera. Organic Geochemistry, 30, 1581-1583.

Davis, J. B., 1968. Paraffinic hydrocarbons in the sulfate-reducing bacterium Desulfovibrio desulfuricans, Chemical Geology, 3, 155–160, 1968.

Didyk, B. M., Simoneit, B. R. T., Brassell, S. C., Eglinton, G., 1978. Organic geochemical indicators of palaeoenvironmental conditions of sedimentation. Nature, 272, 216–222.

Dodd, R. S., Blasco, F., Rafii, Z. A., Torquebiau, E., 1999. Mangroves of the United Arab Emirates: ecotypic diversity in cuticular waxes at the bioclimatic extreme. Aquatic Botany, 63, 291-304.

Dodd, R. S., Rafii, Z. A., Fromard, F., Blasco, F., 1998. Evolutionary diversity among Atlantic coast mangroves. Acta Oecologica, 19, 323–330.

Eglinton, G., Hamilton, R. J., 1967. Leaf epicuticular waxes. Science, 156, 1322-1335.

Eglinton, T. I., Eglinton, G., 2008. Molecular proxies for paleoclimatology. Earth and Planetary Science Letters, 275, 1–16.

Ekpo, B. O., Oyo-Ita, O. E., Oros, D. R., Simoneit, B. R., 2012. Distributions and sources of polycyclic aromatic hydrocarbons in surface sediments from the Cross River estuary, SE Niger Delta, Nigeria. Environmental monitoring and assessment, 184, 1037-1047.

Elias, V. O., Simoneit, B. R., Cardoso, J. N., 1997. Even n-alkane predominances on the Amazon shelf and a Northeast Pacific hydrothermal system. Naturwissenschaften, 84, 415-420.

Farrington, J. W., Tripp, B. W., 1977. Hydrocarbons in western North Atlantic surface sediments. Geochimica et Cosmochimica Acta, 41, 1627-1641.

Ficken, K. J., Li, B., Swain, D. L., Eglinton, G., 2000. An n-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. Organic geochemistry, 31, 745-749.

Fowler, M. G., Abolins, P., Douglas, A. G., 1986. Monocyclic alkanes in Ordovician organic matter. Organic Geochemistry, 10, 815-823.

Fowler, M. G., Douglas, A. G., 1984. Distribution and structure of hydrocarbons in four organic- rich Ordovician rocks. Organic Geochemistry, 6, 105-114.

Fowler, M. G., Douglas, A. G., 1987. Saturated hydrocarbon biomarkers in oils of Late Precambrian age from Eastern Siberia. Organic Geochemistry, 11, 201-213.

Fubara, E. P., Ekpo, B. O., Ekpa, O. D., Marynowski, L., 2012. Predominances and source implications of even n-alkenes in surface sediments from coastal areas of Niger Delta, Nigeria. International Journal of Basic and Applied Sciences, IJBAS-IJENS, 12, 127101.

Gagosian, R. B., Peltzer, E. T., 1986. The importance of atmospheric input of terrestrial organic material to deep sea sediments. Organic Geochemistry, 25, 79–96.

Gao, X., Chen, S., 2008. Petroleum pollution in surface sediments of Daya Bay, South China, revealed by chemical fingerprinting of aliphatic and alicyclic hydrocarbons. Estuarine, Coastal and Shelf Science 80, 95-102.

Garg, A., Bhosle, N. B., 2004. Abundance of macro-algal OM in biofilms: Evidence from n-alkane biomarkers. Bio-fouling, 20, 155–165.

Gearing, P., Gearing, J. N., Lytle, T. F., Lytle, J. S., 1976. Hydrocarbons in 60 northeast Gulf of Mexico shelf sediments: a preliminary survey. Geochimica et Cosmochimica Acta, 40, 1005-1017.

Gelpi, E., Schneider, H., Mann, J., Oro T., 1970. Hydrocarbons of geochemical significance in microscopic algae. Phytochemistry 9, 603-612.

Gireeshkumar, T. R., Deepulal, P. M., Chandramohanakumar, N., 2015. Distribution and sources of aliphatic hydrocarbons and fatty acids in surface sediments of a tropical estuary south west coast of India (Cochin estuary). Environmental Monitoring and Assessment, 187, 1-17.

Grimalt, J., Albaiges, J., 1987. Sources and occurrence of C12-C22 n-alkane distributions with even carbon-number preference in sedimentary environments. Geochimica et Cosmochimica Acta, 51, 1379-1384.

Grimalt, J., Albaigés, J., Al-Saad, H. T., Douabul, A. A. Z., 1985. n-Alkane distributions in surface sediments from the Arabian Gulf. Naturwissenschaften, 72, 35-37.

Han, J., Calvin, M., 1969. Hydrocarbon distribution of algae and bacteria, and microbiological activity in sediments. Proceedings of the National Academy of Sciences, 642, 436-443.

Han, J., McCarthy, E. D., Calvin, M., Benn, M. H., 1968. Hydrocarbon constituents of the blue-green algae *Nostoc muscorum, Anacystis nidulans, Phormidium luridum and Chlorogloea frischtii.* Journal of the Chemical Society C, 2785–2791.

Harji, R. R., Yvenat, A., Bhosle, N. B., 2008. Sources of hydrocarbons in sediments of the Mandovi estuary and the Marmugoa harbour, west coast of India. Environment International, 34, 959–965.

Hedges, J. I., Keil, R. G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Marine chemistry, 49, 81-115.

Hedges, J. I., Prahl, F. G., 1993. Early diagenesis: consequences for applications of molecular biomarkers. In: Engel,M.H., Macko, S.A. (Eds.), Organic Geochemistry: principles and applications. Plenum Press, New York.

Hird, S. J., Evens, R., Rowland, S. J., 1992. Isolation and characterisation of sedimentary and synthetic highly branched C20 and C25 monoenes. Marine Chemistry, 37, 117-129.

Hoering, T. C., 1980. Monomethyl, acyclic hydrocarbons in petroleum and rock extracts. Carnegie Carnegie Institution of Washington Year Book, 80, 389.

Hold, I. M., Schouten, S., Jellema, J., Sinninghe Damste, J. S.,1999. Origin of free and bound mid-chain methyl alkanes in oils, bitumens and kerogens of the marine, Infracambrian Huqf Formation (Oman). Organic Geochemistry, 30, 1411– 1428.

Holzer, G., Oro, J., Tornabene, T. G., 1979. Gas chromatographic-mass spectrometric analysis of neutral lipids from methanogenic and thermoacidophilic bacteria. Journal of Chromatography, 186, 795-809.

Huang, Y., Dupont, L., Sarnthein, M., Hayes, J. M., Eglinton, G., 2000. Mapping of C4 plant input from North West Africa into North East Atlantic sediments. Geochimica et Cosmochimica Acta, 64, 3505-3513.

Hu, L. M., Guo, Z. G., Feng, J. L., Yang, Z. S., Fang, M., 2009. Distributions and sources of bulk organic matter and aliphatic hydrocarbons in surface sediments of the Bohai Sea, China. Marine Chemistry, 113, 197–211.

Jackson, M. J., Powell, T. G., Summons, R. E., Sweet, I. P., 1986. Hydrocarbon shows and petroleum source rocks in sediments as old as 1.7 x 109 years. Nature, 322, 727-729.

Jeng, W. L, Huh, C. A., 2008. A comparison of sedimentary aliphatic hydrocarbon distribution between East China Sea and southern Okinawa Trough. Continental Shelf Research, 28, 582-592.

Jeng, W. L., 2006. Higher plant n-alkane average chain length as an indicator of petrogenic hydrocarbon contamination in marine sediments, Marine Chemistry, 102, 242-251.

Johnson, R. W., Calder, J. A., 1973. Early diagenesis of fatty acids and hydrocarbons in a salt marsh environment. Geochimica et Cosmochimica Acta, 37, 1943–1955.

Jones, J. G., 1969. Studies on lipids of soil micro-organisms with particular reference to hydrocarbons. Journal of General Microbiology, 59, 145-152.

Joseph, M. M., Ratheesh Kumar, C. S., GireeshKumar, T. R., Renjith, K. R., Chandramohanakumar, N., 2008. Biogeochemistry of surficial sediments in the intertidal systems of a tropical environment. Chemistry and Ecology, 24, 247- 258.

Kang, Y., Mai, B., Huang, X., Zhang, G., Sheng, G., Fu, J., 2000. Primary study on status of organic pollution in surface sediments of the Pearl River Delta. Acta Science Circumstantiae, 20, 164-170.

Kenig, F., 2000. C 16–C 29 homologous series of monomethylalkanes in the pyrolysis products of a Holocene microbial mat. Organic geochemistry, 31, 237-241.

Kenig, F., Simons, D. J. H., Anderson, K. B., 2001. Distribution and origin of ethyl-branched alkanes in a Cenomanian transgressive shale of the Western Interior Seaway (USA). Organic geochemistry, 32, 949-954.

Kenig, F., Sinninghe Damstė, J. S., Dalen, A. C. K., Rijpstra, A. Y., Huc, A. Y., de Leeuw, J. W., 1995. Occurrence and origin of monomethylalkanes, dimethylalkanes, and trimethylalkanes in Modern and Holocene cyanobacterial mats from Abu-Dhabi, United-Arab-Emirates. Geochimica et Cosmochimica Acta, 59, 2999–3015.

Kennicutt, M. C., Barker, C., Brooks, J. M., DeFreitas, D. A., Zhu, G. H., 1987. Selected organic matter source indicators in the Orinoco, Nile and Changjiang deltas. Organic Geochemistry, 11, 41–51.

Kvenvolden, K. A., 1966. Molecular distributions of normal fatty acids and paraffins in some lower Cretaceous sediments. Nature, 209, 573–577.

Killops, S. D., Massoud, M. S., 1992. Polycyclic aromatic hydrocarbons of pyrolytic origin in ancient sediments: evidence for Jurassic vegetation fires. Organic geochemistry, 18, 1-7.

Killops, S., Killops, V., 2005. Organic Geochemistry Second Edition. Blackwell Publishing, USA.

Kissin, Y. V., 1987. Catagenesis and composition of petroleum: origin of n-alkanes and isoalkanes in petroleum crudes. Geochimica et Cosmochimica Acta, 51, 2445–2457.

Klomp U. C., 1986a. The chemical structure of a pronounced series of iso-alkanes in South-Oman erudes. In Advances in Organic Geochemistry 1985(Edited by Leythacuser D. and Rulikötter J.), Pergamon Journals, Oxford.

Klomp, U. C., 1986b. The chemical structure of a pronounced series of iso-alkanes in South Oman crudes. Organic Geochemistry, 10, 807-814.

Kohnen, M. E. L., Schouten, S., Sinninghe Damst, J. S., de Leeuw, J. W., Merrit, D. A., Hayes, J. M., 1992 Recognition of paleaobiochemicals by a combined molecular sulfur and isotope geochemical approach. Science, 256, 358-362.

Krishnamurthy, R.V., Machavaram, M., Baskaran, M., Brooks, J., Champ, M. A., 2001. Organic carbon flow in the Ob, Yenisey Rivers and Kara Sea of the Arctic region. Marine Pollution Bulletin, 42, 726–732.

Kuramoto, T., Minagawa, M,. 2001. Stable carbon and nitrogen isotopic characterization of organic matter in a mangrove ecosystem on the south western coast of Thailand. Journal of oceanography, 57, 421-431.

Machiwa, J. F., 2000. δ^{13}C signatures of flora, macrofauna and sediment of a mangrove forest partly affected by sewage wastes. Tanzania Journal of Science, 26, 15-27.

Mackay, D., Paterson, S., Shiu, W. Y., 1992. Generic models for evaluating the regional fate of chemicals. Chemosphere, 24, 695-717.

Matsumoto, G., 1983. Comparative study on organic constituents in polluted and unpolluted inland aquatic environments V. Organic carbons and hydrocarbons in sediments. Water Research, 17, 823-830.

Mead, R., Xu, Y., Chong, J., Jaffé, R., 2005. Sediment and soil organic matter source assessment as revealed by the molecular distribution and carbon isotopic composition of n-alkanes. Organic Geochemistry, 36, 363–370.

Meyers, P. A., 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Organic Geochemistry, 27, 213–250.

Meyers, P. A., 2003. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. Organic Geochemistry, 34, 261–289.

Meyers, P. A., Ishiwatari, R., 1995. Organicmatter accumulation records in lake sediments. In: Lerman, A., Imboden, D.M., Gat, J.R. (Eds.), Physics and Chemistry of Lakes. Springer, Berlin.

Moldowan, J, M., Seifert, W. K., Gallegos, E. J., 1985. Relationship between petroleum composition and depositional environment of petroleum source rocks. Bulletin of American Association of Petroleum Geology, 69, 1255-1268.

Nelson, D. R., 1978. Long-chain methyl-branched hydrocarbons: occurrence, biosynthesis, and function. Advances in insect physiology, 13, 1-33.

Niino, H., Emery, K. O., 1961. Sediments of shallow portions of East China Sea and South China Sea. Geological Society of America Bulletin, 72, 731-762.

Nishimura, M., Baker, E. W., 1986. Possible origin of n-alkanes with a remarkable even-to-odd predominance in recent marine sediments. Geochimica et Cosmochimica Acta, 502, 299-305.

Ourisson, G., Albrecht, P., Rohmer, M., 1979. The hopanoids. Pure and Applied Chemistry, 51, 709-729.

Ourisson, G., Rohmer, M., Poralla, K., 1987. Prokaryotic hopanoids and other polyterpenoid sterol surrogates. Annual Reviews Microbiology, 41, 301-333.

Ourisson, G., Rohmer, M., 1992. Hopanoids. 2. Biohopanoids: a novel class of bacterial lipids. Accounts of Chemical Research 25, 403–408.

Pearson, A., Rusch, D. B., 2009. Distribution of microbial terpenoid lipid cyclases in the global ocean metagenome. The ISME journal, 3, 352-363.

Pearson, M. J., Obaje, N. G., 1999. Onocerane and other triterpenoids in Late Cretaceous sediments from the Upper Benue Trough, Nigeria: tectonic and palaeoenvironmental implications. Organic Geochemistry, 30, 583-592.

Peters, K. E., Moldowan, J. M., 1993. The Biomarker Guide. Interpreting Molecular Fossils in Petroleum and Ancient Sediments. Prentice Hall, Englewood Cliffs, NJ.

Peters, K. E., Moldowan, J. M., 1991. Effects of source, thermal maturity, and biodegradation on the distribution and isomerization of homohopanes in petroleum. Organic Geochemistry, 17, 47-51.

Philp, R. P., 1985. Biological markers in fossil fuel production. Mass Spectrometry Reviews, 4, 1-54.

Poynter, J.G., Eglinton, G., 1990. Molecular composition of three sediments from hole 717C: The Bengal Fan. In: Cochran, J.R., Stow, D.A.V., et al. (Eds.), Proceedings of the Ocean Drilling Program Scientific Results,116, 155–161.

Poynter, J. G., Farrimond, P., Robinson, N., Eglinton, G., 1989. Aeolian-derived higher plant lipids in the marine sedimentary record: links with paleoclimate. In: Leinen, M., Sarnthein, M. (Eds.), Paleoclimatology and Paleometeorology: Modern and Past Patterns of Global Atmospheric Transport. Dordrecht (Kluwer Academic), Hingham, Mass, 435–462.

Punyu, V. R., Harji, R. R., Bhosle, N. B., Sawant, S. S., Venkat, K., 2013. n-Alkanes in surficial sediments of Visakhapatnam harbour , east coast of India. Journal of Earth System Science, 122, 467-477.

Rafii, Z. A., Dodd, R. S., Fromard, F., 1996. Biogeographic variation in foliar waxes of mangrove species. Biochemical Systematics and Ecology, 24, 341–345.

Ragan, M. A., Chapman, D. J., 1978. A biochemical phylogeny of the protests. Academic Press, New York.

Readman, J. W., Fillmann, G., Tolosa, I., Bartocci, J., Villeneuve, J, P, Catinni, C., Mee, L. D., 2002. Petroleum and PAH contamination of the Black Sea. Marine Pollution Bullettin, 44, 48–62.

Reiley, G., Collier, R. J., Jones, D. M., Eglinton, G., Eakin, P. A., Fallick, A. E., 1991. Sources of sedimentary lipids deduced from stable carbon-isotope analysis of individual compounds. Nature, 353, 425-427.

Rinna, J., Gqntner, U., Hinrichs, K., Mangelsdorf, U., van der Smissen, K., Rullkftter, J. H., 1999. Temperature related molecular proxies: degree of alkenone unsaturation and average chain length of n-alkanes. In: West, G.J., Buffaloe, L. (Eds.), Proceedings of the Sixteenth Annual Pacific Climate (PACLIM) Workshop, 183–192.

Robinson, N., Eglinton, G., 1990. Lipid chemistry of Icelandic hot spring microbial mats, 15, 291-298.

Rowland, S. J., Robson, J. N., 1990. The widespread occurrence of highly branched acyclic C 20, C 25 and C 30 hydrocarbons in recent sediments and biota—a review. Marine Environmental Research, 30, 191-216.

Rowland, S. J., Belt, S. T., Wraige, E. J., Massé, G., Roussakis, C., Robert, J. M., 2001. Effects of temperature on polyunsaturation in cytostatic lipids of Haslea ostrearia. Phytochemistry 56, 597–602.

Sakari, M., S., Zakaria, M. P., Lajis, N. H., Mohamed, C. A. R., Abdullah, M. H., 2012. Reconstruction of aliphatic hydrocarbons history and sources from sedimentary record of the Johor Strait, Malaysia. Coastal Marine Science, 35, 142-152.

Sakata, S., Hayes, J. M., McTaggert, A. R., Evans, R. A., Lekrone, K. J., Togasaki, R. K., 1997. Carbon isotopic fractionation associated with lipid biosynthesis by a cyanobacterium: relevance for interpretation of biomarker records. Geochimica et Cosmochimica Acta, 24, 5379–5389.

Saliot, A., Laureillard, J., Scribe, P., Sicre, M. A., 1991. Evolutionary trends in the lipid biomarker approach for investigating the biogeochemistry of organic matter in the marine environment. Marine Chemistry, 36, 233–248.

Schefuß, E., Ratmeyer, V., Stuut, J. B. W., Jansen, J. F., Damsté, J. S. S., 2003. Carbon isotope analyses of n-alkanes in dust from the lower atmosphere over the central eastern Atlantic. Geochimica et Cosmochimica Acta, 67, 1757-1767.

Seifert, W. K., 1978. Steranes and terpanes in kerogen pyrolysis for correlation of oils and source rocks. Geochimica et Cosmochimica Acta, 42, 473-484.

Seki, O., Yoshikawa, C., Nakatsuka, T., Kawamura, K., 2006. Fluxes , source and transport of organic matter in the western Sea of Okhotsk : Stable carbon isotopic ratios of n-alkanes and total organic carbon, Deep-Sea Research I, 53, 253-270.

Shiea, J., Brassell, S. C., Ward, D. M., 1990. Midchain branched monomethyl and dimethyl alkanes in hot-spring cyanobacterial mats – a direct biogenic source for branched alkanes in ancient sediments. Organic Geochemistry, 15, 223–231

Simoneit, B. R. T., 1984. Hydrothermal effects on organic matter--high versus low temperature components. In Advances in Organic Geochemistry 1983, Edited by P. A., Schenck, J. W., deLecuw and G. W. M., Lijmbach, Pergamon Press, Oxford.

Simoneit, B. R. T., 1978. The organic chemistry of marine sediments. In Chemical Oceanography (Edited by Riley J. P. and Chester R.), volume 7, Academic Press, London.

Simons, D. J. H., Kenig, F., Schröder-Adams, C. J., 2003. An organic geochemical study of Cenomanian-Turonian sediments from the Western Interior Seaway, Canada. Organic geochemistry, 34, 1177-1198.

Simpson, M. J., Chefetz, B., Deshmukh, A, P., Hatcher, P. G., 2005. Comparison of polycyclic aromatic hydrocarbon distributions and sedimentary organic matter characteristics in contaminated, coastal sediments from Pensacola Bay, Florida. Marine Environmental Research, 59, 139-163.

Sims, R. C., Overcash, M. R., 1983. Fate of polynuclear aromatic compounds (PNAs) in soil–plant systems. Brain Research Reviews, 88, 2–68.

Steinhauer, M. S., Boehm, P. D., 1992. The composition and distribution of saturated and aromatic hydrocarbons in near-shore sediments, river sediments, and coastal peat of Alaskan Beaufort Sea: implications for detecting anthropogenic hydrocarbon inputs. Marine Environmental Research, 33, 223–253.

Summons, R. E., 1987. Branched alkanes from ancient and modern sediments – Isomer discrimination by GC–MS with multiple reaction monitoring. Organic Geochemistry, 11, 281–289.

Summons, R. E., Brassell, S. C., Eglinton, G., Evans, E., Horodyski, R. J., Robinson, N., Ward, D. M., 1988a. Distinctive hydrocarbon biomarkers from fossiliferous sediment of the Late Proterozoic Walcott Member, Chuar Group, Grand-Canyon, Arizona. Geochimica et Cosmochimica Acta, 52, 2625–2637.

Summons, R. E., Powell, T. G., Boreham, C. J., 1988b. Petroleum geology and geochemistry of the Middle Proterozoic McArthur Basin, Northern Australia: III. Composition of extractable hydrocarbons. Geochimica et Cosmochimica Acta, 52, 1747–1763

Summons, R. E., Walter, M. R., 1990. Molecular fossils and microfossils of prokaryotes and protists from Proterozoic sediments. American Journal of Science 290A, 212–244.

Tanner, B. R., Uhle, M. E., Mora, C. I., Kelley, J. T., Schuneman, P. J., Lane, C. S., Allen, E. S., 2010. Comparison of bulk and compound-specific $\delta^{13}C$ analyses and determination of carbon sources to salt marsh sediments using n-alkane distributions (Maine, USA). Estuarine, Coastal and Shelf Science, 86, 283-291.

ten Haven H. L., de Leeuw J. W., Rullkotter, J., Sinninghe Damste, J. S., 1987. Can the pristane/phytane ratio be used as a palaeoenvironmental indicator? Nature, 330, 641-643.

Tissot, B. P., Welte, D. H., 1984. Petroleum formation and occurrence. Springer-Verlag, New York, NY.

Tolosa, I., Readman, J. W., Blaevoet, A., Ghilini, S., Bartocci, J., Horvat, M., 1996. Contamination of Mediterranean (Cote d'Azur) coastal waters by organotins and Irgarol 1051 used in antifouling paints. Marine Pollution Bulletin, 32, 335-341.

Vella, A. J., Holzer, G., 1992. Distribution of isoprenoid hydrocarbons and alkylbenzenes in immature sediments: evidence for direct inheritance from bacterial/algal sources. Organic Geochemistry, 18, 203-210.

Versteegh, G. J., Schefuß, E., Dupont, L., Marret, F., Damsté, J. S. S., Jansen, J. F., 2004. Taraxerol and Rhizophora pollen as proxies for tracking past mangrove ecosystems. Geochimica et Cosmochimica Acta, 68, 411-422.

Volkman J. K., Maxwell, J. R., 1986. Acyclic isoprenoids as biological markers. In Biological Markers. Edited by R. B., Johns, A Monograph.

Volkman, J. K., Holdworth, D. C., Neil, G. P., Bavor, H. J. J., 1992. Identification of natural, anthropogenic and petroleum hydrocarbons in aquatic sediments. Science of the Total Environment, 112, 203–219.

Wang, X., Chen, R. F., Berry, A., 2003. Sources and preservation of organic matter in Plum Island salt marsh sediments (MA , USA): long-chain n-alkanes and stable carbon isotope compositions, Estuarine, Coastal and Shelf Science, 58, 917-928.

Wang, Z., Fingas, M., Sergy, G., 1995. Chemical characterization of crude oil residues from an arctic beach by GC/MS and GC/FID. Environmental science & technology, 29, 2622-2631.

Warton, B., Alexander, R., Kagi, R. I., 1997. Identification of some single branched alkanes in crude oils. Organic Geochemistry, 27, 465–476.

Warton, B., Alexander, R., Kagi, R. I., 1998. The effect of maturation on the distribution of monoethylalkanes in late Cretaceous sedimentary rocks and crude oils from the Gippsland basin, Australia. Organic Geochemistry, 29, 593– 604.

Wu, Y., Zhang, J., Mi, T. Z., Li, B., 2001. Occurrence of n-alkanes and polycyclic aromatic hydrocarbons in the core sediments of the Yellow Sea. Marine Chemistry, 76, 1-15.

Xing, L., Zhang, H., Yuan, Z., Sun, Y., Zhao, M., 2011. Terrestrial and marine biomarker estimates of organic matter sources and distributions in surface sediments from the East China Sea shelf. Continental Shelf Research, 3110, 1106-1115.

Yunker, M. B., Macdonald, R. W., Whitehouse, B. G., 1994. Phase associations and lipid distributions in the seasonally ice-covered Arctic estuary of the Mackenzie Shelf. Organic Geochemistry, 22, 651-669.

Yunker, M. B., Macdonald, R.W., Cretney, W. J., Fowler, B. R., McLaughlin, F. A., 1993. Alkane, terpene, and polycyclic aromatic hydrocarbon geochemistry of the Mackenzie River and Mackenzie shelf: riverine contributions to Beaufort Sea coastal sediment. Geochimica et Cosmochimica Acta, 57, 3041–3061.

Yunker, M. B., Macdonald, R. W., Fowler, B. R., Cretney, W. J., 1991. Geochemistry and fluxes of hydrocarbons to the Beaufort Sea shelf: a multivariate comparison of fluvial inputs and coastal erosion of peat using principal components analysis. Geochimica et Cosmochimica Acta, 55, 255–273.

Yunker, M. B., Macdonald, R. W., Veltkamp, D. J., Cretney, W. J., 1995. Terrestrial and marine biomarkers in a seasonally ice- covered Arctic estuary-integration of multivariate and biomarker approaches. Marine Chemistry, 49, 1–50.

Zaghden, H., Kallel, M., Louati, A., Elleuch, B., Oudot, J., Saliot, A., 2005. Hydrocarbons in surface sediments from the Sfax coastal zone,(Tunisia) Mediterranean Sea. Marine Pollution Bulletin, 50, 1287-1294.

Zhang, J., Yu, H., Jia, G., Chen, F., 2010. Terrestrial n-alkane signatures in the middle Okinawa Trough during the post-glacial transgression : control by sea level and paleovegetation confounded by offshore transport. Geo-Marine Letters, 30, 143-150.

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Chapter 6

FATTY ACIDS AS BIOMARKERS

Contents
6.1 Introduction
6.2 Results and Discussion
6.3 Principal Component Analysis
6.4 Conclusion

6.1 Introduction

Lipid biomarkers such as n-alkanes, fatty acids, sterols etc can be used to trace seasonal and spatial variation of organic matter inputs preserved in the sedimentary record. The difference in characteristic chain lengths of n-alkanes found in marine and terrestrial plants made them befitting biomarker tool for assessing origin of organic matter in terrestrial and marine sediments. However, a few researchers have addressed the problem of introduction of sources with non-unique n-alkane compositions to the systems by significant mangrove, marine macrophyte, and/ or significant riparian–aquatic inputs. Hence, source characterisation by n-alkane chain alone in such complex environment is difficult (Sikes et al., 2009). Moreover n-alkanes can be used identification for the group of organisms (eg: terrestrial, algae, bacterial), but not with higher taxonomical specificity. The relative contributions of fatty acids in coastal environments can reveal the origins of organic materials found in the surface sediments (Carrie et al., 1998) and their use as specific biomarker for plants and micro-organisms has been widely accepted (Leveille et al., 1997). This chapter deals with the use of fatty acids as biomarkers and draws various conclusions from the sources of organic matter.

Fatty acid analysis has been widely used to assess sources of organic matter in food-webs (Saliot et al., 1991; Canuel et al., 1995). Though the multiple sources of these compound classes in the environment, including microalgae, bacteria, marine fauna and higher plants (Volkman et al., 1998), both n-alkanoic and n-alkenoic acid distributions <C19 in sedimentary mixtures have been predominantly attributed to microorganism inputs (Simoneit, 1977; Cranwell et al., 1987; Medeiros and Simoneit, 2008). Sources of terrestrial vegetation, bacterial and algal production can be identified by the presence of long chain fatty acids (>C24), odd short chain or branched fatty acids (<C18) and even short chain non-branched fatty acids (<C18) respectively. During the burial, short chain fatty acids undergo degradation diagenetically faster than long chain fatty acids (Haddad et al., 1992). In saturated fatty acids (SFA), C16:0 and C18:0 are ubiquitous in the marine environment (Parkes, 1987), and C14:0 fatty acids are present in phytoplankton especially in diatoms (Reitan et al., 1994) and to a lesser extent in dinoflagellates (Napolitano et al., 1997).

Polyunsaturated fatty acids (PUFA) are biosynthesized from saturated fatty acids by the action of desaturase enzymes, and they are very abundant in algae and plants. The position of the double bonds may be used to identify sources of organisms; generally cis configuration is a preferred in biological entities. Polyunsaturated fatty acids are represented by a simple notation scheme. For example in palmitoleic acid (cisC16:1ω7) cis refers to the stereochemistry about the C=C bond, 16 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. Linolenic acid is a polyunsaturated fatty acid (cis-C18:3ω3), in which three conjugated double bond are present and the first C=C bond occurs between C-3 and C-4, numbering from the opposite end of the acid group, and the others two are in between C-5 & C-6 and C-7 & C-8 (Killops and Killops, 2005).

Polyunsaturated fatty acids are important structural components of the cells that confer membrane fluidity and selective permeability (Hyne et al., 2009). The PUFAs are labile in nature (Smith et al., 1983) and due to its labile nature it presence may indicate recent detritus inputs (Shaw and Johns, 1985; Carrie et al., 1998; Dunn et al., 2008). PUFAs are normally linked with phytoplankton, for example; 20:5ω3, is a typical diatom fatty acids (Colombo et al., 1996); 18:2ω6, 18:3ω3 and 18:3ω6 have been used as markers of green macro-algae (Meziane and Tsuchiya, 2000; Bachok et al., 2009); and 22:6ω3 usually indicates dinoflagellate origin (Colombo et al., 1996; Budge and Parrish, 1998; Carrie et al., 1998). An estimate of the meiofaunal input can be made based on the concentration of 18:2ω6.

Microbial biomarkers (indicators of microbial populations) such as odd chain, branched and hydroxy fatty acids can be interpreted (both quantitatively and qualitatively) in terms of in situ microbial biomass. Among these hydroxy fatty acids are widely distributed in nature and their occurrence correlate with environments enriched in bacteria (Parker et al., 1982). Also iso methyl branched fatty acids (e.g., iC15) in which the branch point is on the penultimate carbon and anteiso-methyl (e.g., aC15) branched fatty acids have their branch point on the antepenultimate carbon atom (second from the fatty acid end), can be correlated to microbial biomass.

Table 6.1: List of fatty acids used as biomarker and their source in sediments

Fatty acids	Source
i15:0, a15:0, 15:0, 16:1ω5, a17:0, i17:0, 17:0, 16:1ω9, 18:1ω7, 18:1ω5, i19:0, a19:0	Most of bacteria (Jeffries, 1972;Vestal and White,1989)
i- + aC15 -, i- + aC 17, 10-Me-C 16 -Fatty acid	Bacteria (sulfate reducers) (Edlund et al., 1985; Kaneda, 1991;Taylor and Parkes, 1983)
BrFA C13-17	general bacteria indicator(Perry et al., 1979)
10Me18:0	Actinomycetes (Vestal and White, 1989)
16:0, 18:1ω9, 18:2ω6, 18:3ω6, 18:3ω3	Fungi (Vestal and White, 1989)
16:1ω8c, 18:1ω8c	Methanotrophic bacteria (Bowman et al.,1991)
10Me16:0, cy18:0 (ω7,8)	Desulfobacter (Coleman et al., 1993; Dowling et al., 1986)
i17:1ω7c, i15:1ω7c, i19:1ω7c	Desulfovibrio (Taylor and Parkes, 1983)
17:1ω6, 15:1	Desulfobulbus (Taylor and Parkes, 1983)
i17:1(9), 10Me16:0	Sulfate-reducing bacteria (Londry et al.,20004; Orphan et al., 2001)
Cy19:0	Sulfur-oxidising bacteria (Kneif , 2003)
Cy17:0	Iron-oxidising bacteria (Kneif , 2003)
11Me16:0	Nitrate-oxidising bacteria (Lipski et al., 2001)
Cy15:1	Clostridia (Vestal and White, 1989)
16:2ω4, 18:2 ω6, 18:3ω6, 18:3ω3	Cyanobacteria (Vestal and White, 1989), Kenyon et al., 1972)
16:1ω3t, 16:4ω1, 20:5ω3, 20:5ω5	Diatoms (Vestal and White, 1989; Parrish et al.,2000)
18:1ω9, 16:1ω13t, 18:3ω3, 18:2ω6	Green algaeof the classes of Chlorophyceae and Prasinophyceaea (Dustan et al.,1992; Volkman et al., 1998)
2PUFA, 20PUFA	algae (Killops and Killops, 1993; Volkman, 1986), zooplankton (Lee et al., 1971)
18PUFA, 16PUFA	algae, diatoms tend to have more 16PUFA relative to 18 PUFA non-diatom algae 18PUFA (Volkman et al., 1989)
LCFA	Epi-cuticular pant waxes, minor source: microalgae, bacteria(Eglinton and Hamilton, 1967)

To identify the presence of bacteria, Gillan et al., 1983 used a number of specific FAs: i-14 : 0, i-15 : 0, a-15 : 0, i-16 : 0, Me-16 : 0, i-17 : 0, a- 17 : 0, cy- 17 : 0, i-19 : 0, a-19 : 0, Me-18 : 0, 18 : 1ω11. Analyses of sedimentary monoenoic fatty acids can be used to indicate the microbial abundance and community structure in diverse marine sediments. There are two approaches usually used to extract microbial lipids: (i) polar phospholipid fatty acid (PLFA) analysis and (ii) total fatty acid methyl ester (total FAME) analysis. Total fame analysis was carried out in the present study.

This chapter investigated the use of fatty acids as biomarkers for the source identification of five mangrove ecosystems in the northern Kerala coast.

6.2 Results and Discussion

6.2.1 General Behaviour of Fatty Acids

Total 118 fatty acids were identified from the present study which includes saturated straight chain fatty acids (SFA), Branched fatty acids (BrFA), Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA), hydroxy fatty acids and dicarboxylic acids. TFA (Total Fatty acids) was calculated as sum of all fatty acids (excluding dicarboxylic acids) and was ranged from 38.74$\mu gg^{-1}$to 144.64 μgg^{-1}in post-monsoon, 42.09 μgg^{-1} to 580.49μgg^{-1} in pre-monsoon and 6.25 μgg^{-1} to 147.65 μgg^{-1} in monsoon (Fig. 6.1). Palmitic acid (C16) was the major contributor to the TFA and was ranged from 10.49 to 29.49 % to the total fatty acid pool. Major fatty acid indices are shown in Table 6.2. SFA (Saturated straight chain Fatty Acids) varied from 19.50 μgg^{-1} to 80.56 μgg^{-1}, 23.91 μgg^{-1} to 335.78 μgg^{-1}, 4.00 μgg^{-1} to 87.56 μgg^{-1}in post-monsoon, pre-monsoon and monsoon respectively. SFA represented 31.83 to 63.94 % of the total fatty acids pool. SCFA (Short Chain Fatty acids) also showed marked variation in the study region and ranged from 16.20 μgg^{-1} to 60.30 μgg^{-1} in post-monsoon, 16.88 μgg^{-1} to 196.62 μgg^{-1} in pre-monsoon and 2.61 μgg^{-1} to 48.48 μgg^{-1} in monsoon and its contribution to the TFA was between 23.61 and 57.56%. TFA, SCFA and SFA followed almost similar trend in all stations except Kadalundi and showed higher values in pre-monsoon and lower values in monsoon. In Kadalundi, lower value was associated in pre-monsoon. LCFA (Long Chain Fatty acids),which contributed 5.10 to 30.60 % to the TFA pool, was ranged from 3.30 μgg^{-1} to 35.72 μgg^{-1} in post-monsoon, 7.02 μgg^{-1} to 139.16 μgg^{-1} in pre-monsoon and 1.39 μgg^{-1} to 39.07 μgg^{-1} in monsoon, with its higher values in pre-monsoon at Pazhayangadi, Pappinissery and Thalassery. In Kunjimangalam, higher concentration was observed in post-monsoon and lower in monsoon and the opposite behaviour was observed in Kadalundi. Among saturated straight chain fatty acids, SCFA is dominant over LCFA. C16 contributed more to the SCFA and its contribution to SCFA was ranged from 19.27 to 54.23%.

Branched fatty acids (BrFA), contributed 6.39 to 24.85% to the TFA pool and it ranged from 6.11μgg^{-1} to 27.88 μgg^{-1}, 4.75 μgg^{-1} to 46.05 μgg^{-1}, 0.98 μgg^{-1} to 14.18 μgg^{-1}in post-monsoon, pre-monsoon and monsoon season respectively (Fig. 6.2).

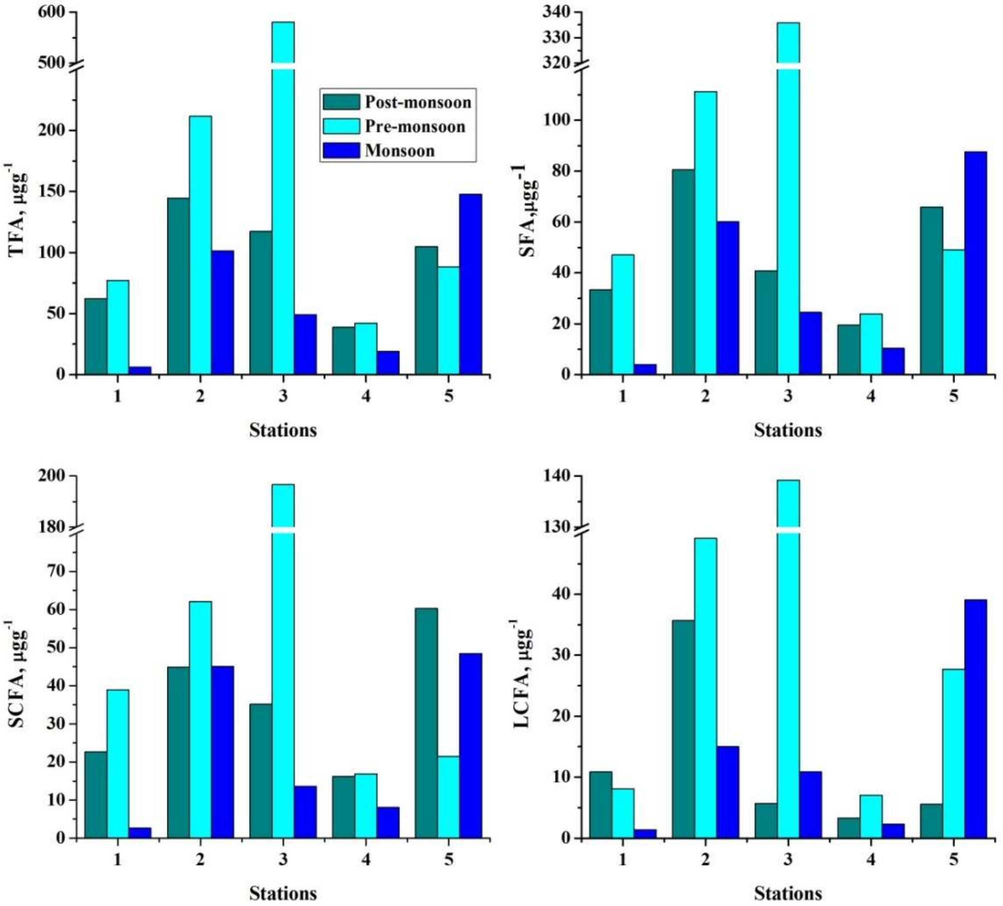

Fig. 6.1: Variation of TFA, SFA, SCFA and LCFA in the present study region

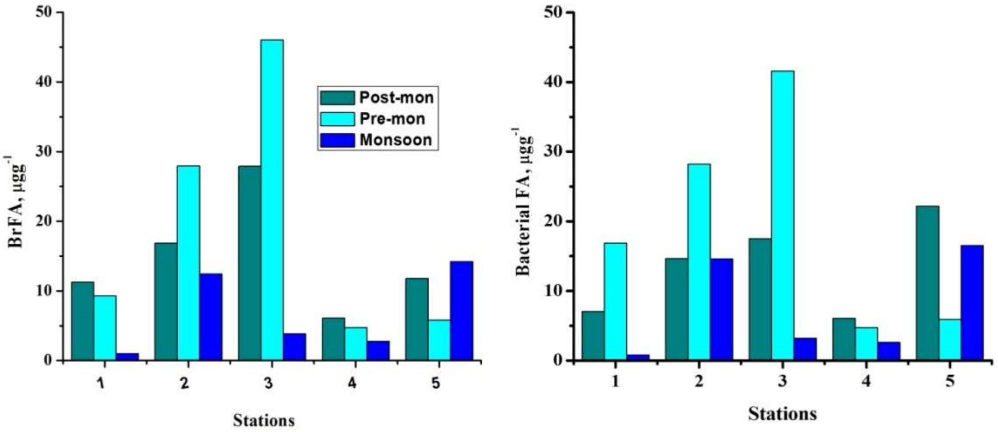

Fig. 6.2: Variation of Branched and Bacterial fatty acids in the present study

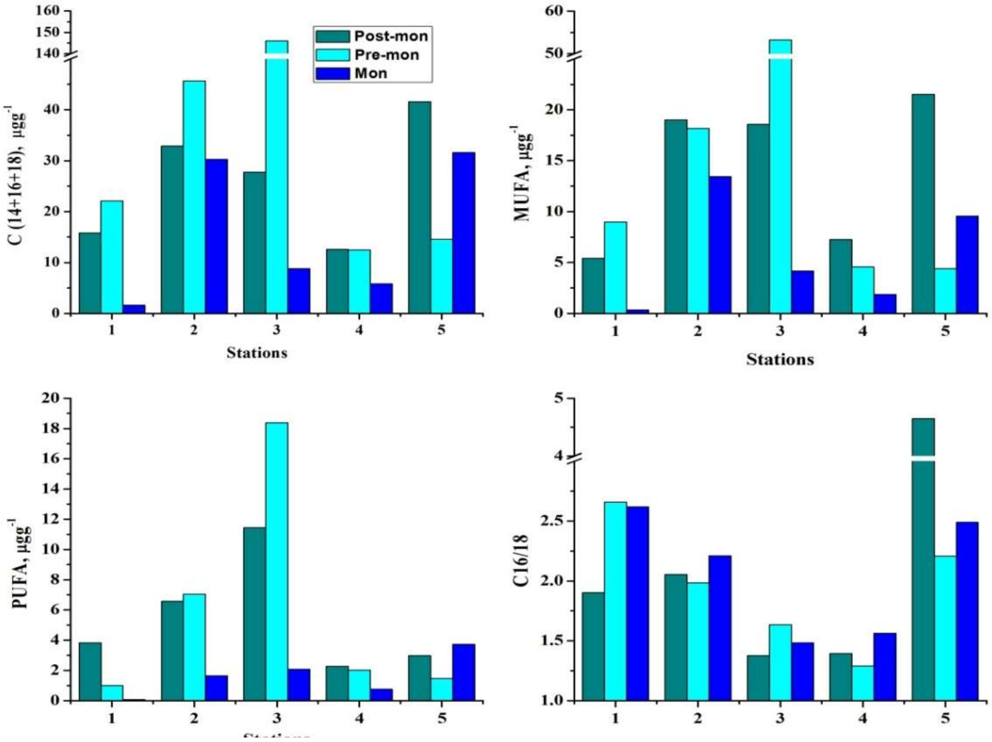

Fig. 6.3: Variation of Marine fatty acids, MUFA, PUFA and C16/18 in the present study region

Table 6.2: Variation of Indices calculated from fatty acids in the present study

	Max	Min	Avg	Stdv
TFA	580.49	6.25	119.36	138.71
SFA	335.78	4.00	66.25	80.39
BrFA	46.05	0.98	13.46	12.16
MUFA	53.22	0.35	12.70	13.12
PUFA	18.38	0.07	4.35	4.89
SCFA	196.62	2.61	42.19	46.58
LCFA	139.16	1.39	24.07	35.18
Marine FA	146.04	1.60	29.97	34.66
C16:1/16	0.46	0.04	0.19	0.13
C18:1/16:1	10.43	0.40	3.29	2.91
Bacterial FA	41.58	0.80	13.49	11.14
C16/C18	4.65	1.29	2.10	0.84
CPIshort	0.43	0.07	0.16	0.09
C16/18	10.94	4.12	5.74	1.58
SCFA/LCFA	10.85	0.77	3.12	2.68

MUFA (Mono Unsaturated Fatty Acids) varied from 5.43μgg^{-1} to 21.52μgg^{-1} in post-monsoon, 4.40μgg^{-1} to 53.22 μgg^{-1} in pre-monsoon and 0.35 μgg^{-1}to 13.45 μgg^{-1} in monsoon (Fig. 3) and its contribution to TFA pool lies between 4.86 and 20.54 %. Contribution of Poly Unsaturated Fatty Acids (PUFA) to the TFA was comparatively lower (1.19 to 10.20 %) in the present study and its concentration ranged from 2.27 μgg^{-1} to 11.44 μgg^{-1}, 1.00 μgg^{-1} to 18.38 μgg^{-1} and 0.07 μgg^{-1} to 3.72 μgg^{-1} in post-monsoon, pre-monsoon and monsoon respectively. Marine fatty acids, which represents the sum of C14+C16+C18, ranged from 12.58 μgg^{-1} to 41.61 μgg^{-1} in post-monsoon, 12.49 μgg^{-1} to 146.04 μgg^{-1} in pre-monsoon and 1.60 μgg^{-1} to 31.61μ gg^{-1} in monsoon and it contributed 16.12 to 39.72 % to the total fatty acid pool. Bacterial fatty acids included odd fatty acids<C22 along with iso and anteiso C15 and C17 and was ranged from 6.02 to 22.18μgg^{-1}, 4.73 to 41.58 μgg^{-1}, 0.80 to 16.52 μgg^{-1} during post-monsoon, pre-monsoon and monsoon which contributed 6.51 to 21.84% to the TFA. It appeared that, the results for TFA, SFA, Marine fatty acids and SCFA were qualitatively similar, that is showed higher concentration in (Pappinissery, Kunjimangalam and Pazhayangadi) in pre-monsoon and lower concentration in monsoon.

C18:1/C16:1 showed comparatively higher value at Thalassery in monsoon and was ranged from 0.40 to 3.26 in post-monsoon, 1.14 to 9.64 in pre-monsoon and 1.41 to 10.43 in monsoon. Similarly C16:1/C16 was also calculated and fall in the range 0.12 to 0.46, 0.05 to 0.27 and 0.04 to 0.26 in post-monsoon, pre-monsoon and monsoon respectively. CPI (10-20) was also calculated and was less than one for all samples.

Kunjimangalam showed much lower concentration for fatty acids and TFA ranged from 6.25 (in monsoon) to 77.12μgg^{-1} (in pre-monsoon). A total of 58 fatty acids were identified during pre-monsoon while 56 followed by 44 fatty acids were identified in post-monsoon and monsoon respectively. PUFA, LCFA and BrFA showed comparatively higher concentration in post-monsoon and lower in monsoon. C16 has major contribution to the TFA in all season and showed lower contribution in post-monsoon (14.04 to 17.62%). C14 (9.29 to 13.02 %) and C15 (2.51 to 19.11 %) showed a considerable contribution to the SFA pool and was higher in pre-monsoon. For C26 (2.89 to 6.14 %) and C28 (3.40 to 6.13 %) contribution to SFA was higher in post-monsoon while for C24 (3.74 to 11.51 %) and C30 (2.93 to 8.19 %) it was higher in monsoon.

A total of 76 fatty acids were identified from Pazhayangadi in post-monsoon while number of fatty acids identified in pre-monsoon and monsoon were 59 and 49 respectively. In Pazhayangadi, TFA ranged from 101.33 to 211.44 μgg^{-1} and showed higher concentration in pre-monsoon. SFA concentration ranged from 60.11 to 111.22μgg^{-1} which is mainly contributed by SCFA (55.66 to 75.01 %). MUFA showed comparatively higher concentration in post-monsoon (12.26 to 16.39 μgg^{-1}) while PUFA contributed 1.51 to 4.37 % to the total fatty acid pool. Concentration of bacterial fatty acids ranged from 14.59 to 28.21μgg^{-1} while that of marine fatty acids ranged from 30.24 to 45.68μgg^{-1}. Ratio C18:1/16:1 is greater than one in all samples. C16 contributed 27.27 to 34.89 % to the SFA pool while that of C14 it was 7.52 to 9.03 %. C24, C26, C28 and C30 contributed 6.65 to 10.18 %, 4.16 to 10.23 %, 4.44 to 9.81% and 4.81 to 11.34 % respectively.

Appreciable higher concentration and diversity of fatty acids were observed in Pappinissery. Number of fatty acids identified in post-monsoon, pre-monsoon and monsoon were 84, 75 and 63 respectively. Pappinissery in

Pre-monsoon recorded exceptionally higher fatty acid concentration. TFA ranged from 49.01 to 580.49µgg^{-1}. SFA varied from 24.48 to 335.78 µgg^{-1} which is mainly contributed by SCFA (52.55 to 83.99 %). BrFA, MUFA and PUFA ranged from 3.85 to 46.05 µgg^{-1}, 4.16 to 53.22 µgg^{-1} and 2.08 to 18.38µgg^{-1} respectively. Bacterial fatty acids also showed comparatively higher concentration in pre-monsoon and lies between 6.55 and 15.61 %. Marine fatty acids contributed 17.14 to 25.16 % to the TFA in the study region. Contribution of C16 to SFA fluctuated between 24.20 and 54.23 % while that of C18 was in the range 5.87 to 8.93%. Concentration of higher fatty acids like C24, C26, C28 and C30 was ranged from 2.22 to 32.35µgg^{-1}, 0.44 to 19.43µgg^{-1}, 0.59 to 22.64 µgg^{-1} and 1.17 to 44.47 µgg^{-1} respectively which showed higher input in pre-monsoon and lower input in post-monsoon.

Fatty acid diversity was low in Thalassery in which 49, 56 and 55 fatty acids were identified in post-monsoon, pre-monsoon and monsoon respectively. In Thalassery, TFA ranged from 19.14 to 42.09 µgg^{-1} with higher concentration in pre-monsoon and lower concentration in monsoon. SFA, mostly contributed by SCFA (70.62 to 83.07 %), showed much higher concentration in pre-monsoon, and was ranged from 10.36 to 23.91µgg^{-1}. Higher concentration of BrFA, MUFA, PUFA, and Marine fatty acids were recorded in post-monsoon and were ranged from 3.85 to 6.11 µgg^{-1}, 1.86 to 7.26 µgg^{-1}, 0.75 to 2.27 µgg^{-1} and 5.83 to 12.58 µgg^{-1}respectively. Compared to other stations, the ratio of C18:1 to 16:1 was much higher in Thalassery (monsoon season) which lies between 2.01 to 10.43. SCFA such as C14, C16 and C18 contributed 8.14 to 11.53%, 36.41 to 44.80%, 6.49 to 8.17 % respectively to the SFA. Higher fatty acids were comparatively low at Thalassery and recorded higher concentration in pre-monsoon and lower concentration in monsoon. The contribution of C24, C26, C28 and C30 to the SFA pool ranged from 3.38 to 4.99 %, 2.39 to 3.50 %, 4.44 to 8.69 and 3.49 to 7.94 % respectively.

A total of 46 fatty acids were identified from Kadalundi in post-monsoon while 55 and 57 were identified in pre-monsoon and monsoon. TFA ranged from 90.52 to 147.65 μgg^{-1} and recorded higher concentration in monsoon and lower in pre-monsoon. SFA, mainly contributed by SCFA (43.56 to 91.56%), except for pre-monsoon and was ranged from 49.08 to 87.56μgg^{-1}. BrFA also showed lower concentration during pre-monsoon and was ranged from 5.78 to 14.18 μgg^{-1}. MUFA, SCFA, Marine fatty acids and Bacterial fatty acids showed higher concentration in post-monsoon and were ranged from 4.40 to 21.52 μgg^{-1}, 21.38 to 60.30 μgg^{-1}, 14.59 to 41.61 μgg^{-1} and 5.89 to 22.18μgg^{-1} respectively. LCFA and PUFA displayed higher concentration in monsoon and varied from 5.56 to 39.07 $\mu gg^{-1}$and 1.47 to 3.72 μgg^{-1}. Ratio of C18:1 to 16:1 fatty acids ranged from 0.40 to 3.47 which displayed lower value in post-monsoon and higher value in pre-monsoon. Ratio of SCFA to LCFA ranged from 0.77 to 10.85 with lower value in pre-monsoon and higher value in pre-monsoon. Contribution of C14, C15 and C16 to SFA was ranged from 0.13 to 11.99%, 2.02 to 17.80% and 19.27 to 46.91 respectively. LCFA contribution to SFA pool was less in post-monsoon and was dominated by the contribution of C30 in pre-monsoon and monsoon. Contribution of C24, C28, C28 and C30 ranged from 2.29 to 8.94%, 1.57 to 6.65%, 1.55 to 8.65% and 1.75 to 25.90% respectively.

Generally, n-fatty acids from algae and bacteria have higher abundance of short chain compounds (C12 to C20) with a maximum at C16, whereas those in higher plant waxes show carbon numbers>C22 with a maximum at

C24 or C26(Eglinton and Calvin, 1967; Simoneit, 1978; Gao et al., 2008). Much higher concentration of Palmitic acid (C16) was found in all samples. C16 is the most abundant Saturated straight chain fatty acid (SFA) in mangrove leaves (Sassen, 1977; Mfilinge et al., 2003, 2005; Hall et al., 2006) which indicates the degradation state, since the concentration of SFA in detritus declines constantly with age (Mfilinge et al., 2003). C16 is a dominant fatty acid in bacterial as well as marine organisms and also can be contributed by sewage discharge (Quemeneur and Marty, 1992). The CPI index, for short chain fatty acids, was found to be very low and was ranged from 0.07 to 0.43 indicated the dominance of even chain fatty acids.

In Kunjimangalam, abundance of fatty acids were higher in pre-monsoon and lower in monsoon. The dominance of SCFA with comparatively higher concentration of odd chain and bacterial fatty acids in pre-monsoon indicated higher microbial activity at higher temperature. These indices recorded much lower concentration in monsoon indicated lower activity of microbial community. Low planktonic production due to reduced salinity, low temperature, pH and high turbidity and resuspension events during monsoon seasons (Rejil, 2013) might be other factors affecting the fatty acid composition. MUFAs also showed higher concentration in pre-monsoon. Concentration of LCFA and PUFA were found to be higher in post-monsoon and lower in monsoon. In post-monsoon, deciduous mangrove leaf contributed to LCFA, but its concentration was lower than SCFA in all season. Even though pre-monsoon period characterised by higher chlorophyll, PUFAs were not so much high due to its greater susceptibility to degradation (Smith et al., 1983). Retention of fatty acids was much lower at Kunjimangalam due to its sandy texture.

In Pazhayangadi indices like TFA, SFA, BrFA, MUFA, PUFA, SCFA, LCFA, Bacterial fatty acids and marine fatty acids showed lower concentration in monsoon (except SCFA and Bacterial fatty acids) and higher concentration in pre-monsoon. These results indicated active growth of microbial as well as planktonic community in pre-monsoon and their growth was least in monsoon. Pre-monsoon season is characterised by the clayey texture at Pazhayangadi which reinforces the organic matter accumulation.

The abundance and concentration of fatty acids were much higher at Pappinissery, reflecting the diversity and abundance of organisms in the study region. In this station, almost all indices showed higher concentration in pre-monsoon and lower concentration in monsoon. Dissolved Oxygen in the water column may play an important role in sediment organic matter composition so that lesser aerobic processes or benthic activity exist during periods of anoxia/hypoxia (Diaz and Rosenberg, 1995; Zimmerman and Canuel, 2001). In Pappinissery it was found that the amount of fatty acids was high at pre-monsoon; this was in line with depleted dissolved oxygen in the water column which inhibits organic matter degradation by aerobic processes in synchronous with an increase in anaerobic process.

Fatty acid diversity was low at Thalassery which might be either due to sandy texture of the sediments that helps to the aerobic oxidation of organic matter or due to the low diversity of organisms. The amount of saturated fatty acids was higher in pre-monsoon season, while that of others were high in post-monsoon. In Thalassery Chl a, MUFAs and PUFAs were comparatively higher in post-monsoon which indicates more planktonic input in the season.

Kadalundi in pre-monsoon characterised by low concentration of TFA, SFA, BrFA, MUFA, PUFA, Bacterial and Marine fatty acids which could be due to sandy nature of the sediment. Low chlorophyll content and PUFA was noticed in pre-monsoon indicating lower planktonic input. Since all indices were lower in pre-monsoon it can be inferred that fatty acid concentration in Kadalundi is mainly attributed to planktonic input. The presence of lower concentrations of inorganic nutrients in sandy sediments may decrease the phytoplankton production (Sanilkumar, 2009).

MUFA, SCFA, Bacterial and marine fatty acids reported higher values in post-monsoon reflecting active growth of planktonic as well as bacterial derived organic matter in post-monsoon. In the monsoon and post-monsoon texture was characteristically silt (42.15%, 42.25% respectively in monsoon and post-monsoon) and clay (40.42%, 37.91% respectively in monsoon and post-monsoon) in equal proportion which supports planktonic production as well as organic matter retention.

6.2.2 Multimethyl Branched fatty acids:

Dodecanoic Acid, 3,7,11-Trimethyl-, Methyl Ester, Tridecanoic Acid, 4,8,12-Trimethyl-, Methyl Ester, Tetradecanoic Acid, 5,9,13-Trimethyl-, Methyl Ester, Hexadecanoic Acid, 3,7,11,15-Tetramethyl-, Methyl Ester and Heneicosanoic Acid, 2,5-Dimethyl-, Methyl Ester were identified from the study area. Multimethyl branched acids are abundant in cell wall lipids of Mycobacteria (Minnikin, 1982; Minnikin et al., 2002). 3,7,11,15-Tetramethyl-Hexadecanoic Acid (phytanic acid), which is found in zooplankton (Rontani et al., 1999) as a diagenetic product of chlorophyll (Schlüter et al., 2002). Phytanic acid is formed in animal tissues by oxidation of phytol to phytenic acid (only encountered in tissues under artificial feeding conditions, followed by reduction (Avigan and Blumer, 1968). In the present study, phytanic acid was detected from two samples, from Kunjimangalam in pre-monsoon and monsoon. Freshwater sponges contain polymethyl branched fatty acids such as 4,8,12-trimethyltridecanoic acid and have chemo-taxonomical significance for both marine and freshwater sponges (Dembitsky et al., 2003). 4,8,12-Trimethyltridecanoic acid, metabolic products of the dietary phytol (Prahl et al., 1984) is also common in fish and other marine organisms (Ackman and Hooper, 1968). Also, multimethyl branched acids are found in bacteria.

Multimethyl branched acids are also dominant in the uropygial waxes of the birds preen gland that serves to waterproof the feathers (Jacob and Ziswiler, 1982). The precise composition of this varies from species to species, but all are characterized by high concentrations of branched-chain fatty acids (and alcohols). A common pattern is to find series such as 2,4-, 2,6-, 2,8- and 4,6-dimethyl, and so forth, with 2,4,6-, 2,4,8- and 2,6,8-trimethyl, and 2,4,6,8-tetramethyl fatty acids. 2,4,6-Trimethyl Nonanoic Acid is detected from Kunjimangalam in post-monsoon.

6.2.3 Bacterial Fatty Acids

Bacteria occur in all present day sedimentary environments including polar, deep-sea, anoxic-basin, intertidal, salt marshes, mangrove and lacustrine sediments (Gong and Hollander, 1997; Dunn et al., 2008). The fatty acid composition of microorganisms is known to vary in response to temperature changes (Sushchik et al., 2003; Teoh et al., 2004), irradiance conditions (Brown et al., 1996) or with growth phase (Brown et al., 1996; Gugger et al., 2002), but in most cases the changes are limited to variations in relative abundance.

Findlay and Dobb., 1993 classified the microorganisms based on their fatty acid composition into four distinct functional groups, namely, micro-eukaryotes (polyunsaturated fatty acids), aerobic prokaryotes (monounsaturated fatty acids), gram-positive and other anaerobic bacteria (saturated and branched fatty acids in the range of C14 to C16), and Sulphate Reducing Bacteria and other anaerobic bacteria (saturated and branched fatty acids in the range of C16 to Cl9). Gram-negative bacteria produces mainly 16:1ω7, cy17:0, 18:1ω7, and cy19:0 while Gram-positive bacteria produces i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0, and a18:0.

Mangroves contribute organic matter to the environment, mainly through leaf litter production (Ashton et al., 1999; Hogarth, 1999; Lee, 1999). Earlier reports suggested that microbial processing convert mangrove litter into nutrient-rich food for fishes (Odum, 1971; Lee et al., 1990; Kathiresan and Bingham, 2001; Kathiresan and Rajendran, 1998, 1999, 2000, 2004, 2007; Ashton et al.,1999). Composition of sedimentary fatty acids changes significantly during decomposition of mangrove leaves by microbial colonization i.e., a change is often occurred from saturated straight chain to monounsaturated fatty acids followed by branched fatty acids(Alikunhi et al., 2010).

Odd chain fatty acids and branched chain fatty acids such as iso- and anteiso-homologue of C15 and iso-homologue C17 carbon atoms are commonly produced by bacteria, and can be used as bacterial biomarkers (Volkman et al., 1980; Goosens et al., 1989; Haddad et al., 1992; Meyers and Ishiwatari, 1993; Rajendran et al., 1997). Bacterial biomarkers were substantially higher in pre-monsoon, except for Kadalundi and Thalassery, indicated relatively higher abundance of microbial community induced by high temperature. The C_8 to C_{10} fatty acids were present in the study region and their probable origin is from bacteria (Hayashi and Takii, 1977). The fatty acid iC12 was detected from Pappinissery in post-monsoon and monsoon season, but in lower concentration. Also iC13 (0.06 to 0.58 μgg^{-1}) and aC13 (0.01 to 0.44 μgg^{-1}) was detected with much lower concentrations from the study region. In all stations, except Thalassery in pre-monsoon and Kunjimangalam in monsoon, iC13 detected the presence of aC13 was noticed from six observations (Pappinissery in all seasons, Kunjimangalam in post-monsoon and pre-monsoon and in Thalassery during monsoon season).

Saturated and branched fatty acids in the range of C14 to C16 are present in gram reducing and other anaerobic bacteria. Branched chain fatty acids such as iC14, iC15, aC15 and iC16 were detected in all samples. Concentration of iC14, iC15, aC15 and iC16 ranged from 0.03 to 3.04μgg^{-1}, 0.20 to 14.74μgg^{-1}, 0.15 to 8.34μgg^{-1} and 0.10 to 5.65μgg^{-1} respectively and recorded much higher concentration in Pappinissery (pre-monsoon). Only 3 samples showed iC14 concentration greater than one μgg^{-1}, Pappinissery during post-monsoon (1.65 μgg^{-1}) and pre- monsoon (3.04 μgg^{-1}) along with Pazhayangadi during pre-monsoon (2.22 μgg^{-1}).

iC15 showed comparatively lower concentration (>3 μgg^{-1}) in seven observations, i.e., Pazhayangadi in all seasons, Pappinissery in post-monsoon and pre-monsoon and Kadalundi in post-monsoon and monsoon. All those samples (except for Kadalundi) which showed higher concentration of iC15 was also characterised by higher abundance of aC15 (> 2 μgg^{-1}). It has been reported that the fatty acid biomarkers iso-C15:0, anteiso-C15:0 and C15:0 are abundant in sulphate reducing bacteria (Taylor and Parkes, 1983; Dowling et al., 1986;Vainshtein et al., 1992; Kohring et al., 1994) and gram-positive bacteria

(Kanede, 1991; Dunn et al., 2008). C15 was greater than 4 μgg^{-1} in 5 observations which includes Kadalundi (11.72 μgg^{-1}) in post-monsoon, Kunjimangalam (8.98 μgg^{-1}), Pazhayangadi (6.42 μgg^{-1}) and Pappinissery (5.07 μgg^{-1}) in pre-monsoon and Pazhayangadi in monsoon (4.15 μgg^{-1}).

iC16 was detected in all observation and was ranged from 0.10 to 5.65 μgg^{-1}. Concentration was greater than two in Pazhayangadi during pre-monsoon (3.62 μgg^{-1}), Pappinissery during post-monsoon (2.89 μgg^{-1}) and pre-monsoon season (5.65 μgg^{-1}), and Kadalundi during monsoon (2.61 μgg^{-1}) season. Even though, fatty acid profile of bacteria with a branched-chain lipid type is affected by its growth conditions; branched fatty acids in the range C17-19 indicate the presence of sulphate reducing bacteria and anaerobic bacteria. Sulfate-reducing bacteria decompose organic matter and produced sulfide that precipitates black iron-sulfide or pyrite (Stal, 2000; Dijkman et al., 2010). Sulfide serves as an electron donor for anoxygenic photosynthesis by purple sulfur bacteria and sulfur oxidizing bacteria by aerobically and anaerobically (Caumette et al., 1994; Wieland et al., 2003; Dijkman et al., 2010).In the present study iC17 and aC17 were detected in all stations and was ranged from 0.05 to 4.29 μgg^{-1} and 0.13 to 3.49 μgg^{-1} respectively. Both fatty acids displayed higher concentration in Pappinissery and Pazhayangadi in pre-monsoon. Other two C17 branched fatty acids were also detected from the present study. One among them was widely distributed (0.05 to 3.66 μgg^{-1}) while the other detected in 4 samples (Pappinissery in post-monsoon and monsoon along with Pazhayangadi and Kunjimangalam during post-monsoon). Branched C18 fatty acids were also detected from 5 samples which include Pazhayangadi in post-monsoon, Pappinissery in post-monsoon and pre-monsoon, Thalassery in post-monsoon and monsoon. C19 branched fatty acid detected in low concentration and was ranged from 0.01 to 0.85 μgg^{-1} and recorded comparatively higher at Pappinissery in pre-monsoon.

Gram-negative community was characterised by higher abundance of anteiso- and iso- branched FAME relative to non-branched FAME (Ibekwe and Kennedy, 1999; Navarrete et al., 2000). iC15/C15 was greater than one in most of the samples (except Kunjimagalam in post-monsoon, Kadalundi in pre-monsoon and Pazhayangadi in monsoon). a-C15 also very high except four samples(Kadalundi in all the three seasons, and Kunjimangalam in pre-monsoon and monsoon). iC17/C17 was greater than one in Pappinissery in all seasons, Kunjimangalam and Pazhayangadi in post-monsoon. In Pappinissery, both iC17/C17 and aC17/C17 was very high in monsoon season.

Mfilinge et al., 2005 carried out leaf degradation experiment in the mangrove forest located in the northern part of Okinawa Island in southern Japan and they reported that an increase in BrFAs, MUFAs and BFAs (odd-BrFAs + 18:1ω7) bacterial markers (Jeffries, 1972; Volkman et al., 1980) after 45 days during winter and 17 days during summer. This degradation corresponds to significant increases in total FAs and total lipid in the detritus of both species, suggesting that bacterial activity plays a significant role in lipid and fatty acid transformation. In the present study fatty acids are comparatively higher during pre-monsoon (except Kadalundi) which was expected to be contributed by bacterial colonization induced by higher temperature.

MUFAs can also give the indications of bacterial community in the study region. Along with iC15 and aC15 occurrence of sulfur-reducing bacteria can also be demonstrated by the presence of MUFA (C17:1; Taylor and Parkes, 1983, 1985; Parkes and Calder, 1985). C17:1, may be a useful biomarker for *Desulfobulbus*-type bacteria in marine systems (Taylor and Parkes, 1985) and was detected from Pappinissery in post-monsoon and monsoon, Kadalundi post-monsoon, Pazhayangadi in monsoon and Kunjimangalam in pre-monsoon. C18:1ω7c a bacterial marker, has been found in high amount in purple sulfur bacteria (Grimalt et al., 1992), Type II methane-oxidizers (Naganuma et al., 1996), Gram-negative bacteria (Ratledge and Wilkinson, 1988; Dijkman et al., 2010) and in small amounts are also present in cyanobacteria and diatoms. C18:1ω7c was detected from 10 observations with much lower concentration (< 1μgg^{-1}) except Pappinissery in pre-monsoon and its concentration ranged from 0.12 to 4.27 μgg^{-}1.

The increase in cyclopropyl fatty acids can be associated with cell age, nutrient deprivation, and/or anaerobiosis (Kieft et al., 1994) typically produced when microorganisms move from the logarithmic to the stationary phase of growth (Poerschmann et al., 2005). Fatty acids derived from cyclopropane (cy-17:0, cy-19:0) are typical for anaerobic organisms (Salomonová et al., 2003). *Desulfobacter spp* showed high levels of cyclopropyl fatty acids, including two isomers of both methylene hexadecanoic (cy-l7:0) and methylene heptadecanoic (cy-l8:0) acids (Dowling et al., 1986). Cy-C17:0, was detected from 10 observations and was ranged from 0.06 to 1.11 μgg^{-1}. Higher concentration was detected from Pazhayangadi in post-monsoon, while others showed appreciably lower concentration (<1 μgg^{-1}). The cy-C18 has been found in a wide range of bacterial species of many different types, both Gram-negative and Gram-positive, from strict anaerobes to obligate aerobes (Rontani et al., 2005), could not identify from the present study region. Both cy-C18 and its MUFAs showed similar splitting in mass spectrum, so either cy-C18 was absent in the study region or it could not be distinguished from mass spectrum of MUFA. Cy-19:0 fatty acid has been observed as a major lipid component of *Brevundimonas sp*. (Lipski and Altendorf, 1997), purple bacteria such as *Rhodobacter sp* and *Ectothiorhodospira sp*. (Grimalt et al., 1992) and the sulfur- and iron-oxidising bacteria Acidthiobacillus sp. (Kneif et al., 2003). Cy-19:0 was detected in all observations except Kadalundi in pre-monsoon and Pappinissery in monsoon and was ranged from 0.03 to 2.23 $\mu g g^{-1}$.

α-hydroxy acids and β-hydroxy acids <C20 was detected in the present study region, in which 2-OHC16 was most widely distributed (9 out of 15 observations). Higher hydroxy fatty acids are detected in Pappinissery in post-monsoon and pre-monsoon. Along with iso and anteiso acids of C15 and C17, the presence of β-hydroxy acids or 3-hydroxy acids is the most reliable indication of bacterial activity (Qi et al., 1997; Green et al., 2002). 3-hydroxy acids <C20 are characteristic of cell walls Gram-negative bacteria (Weckesser et al., 1979; Goossens et al., 1986) and those between C8 and C26 were found in cyanobacteria (bacteria Gram-negative), and certain microalgae (Eglinton et al, 1968; Goossens et al., 1986; Mendoza et al., 1987). Hydroxy fatty acids may also result from oxidation of aerobic unsubstituted acids in the water column (Johns and Onder, 1975; Boon et al., 1977; Kawamura and Ishiwatari, 1984). Sphingomonadaceae are generally characterized by the presence of sphingolipids and 2-hydroxyacids and it lack 3-hydroxyacids (Rontani et al., 2005).

3-OH-14:0 fatty has been isolated from waterborne Gram-negative pathogens (Poerschmann et al., 2005) and was detected in four samples with comparatively low concentration (0.11 to 0.66 μgg^{-1}). Its presence was detected in Kunjimangalam during post-monsoon and pre-monsoon and Pazhayangadi during pre-monsoon and monsoon. Water biofilms including Pseudomonas have 3-OH-10:0 and 3-OH-12:0 fatty acids (Poerschmann et al., 2005), both are detected from Pappinissery in post-monsoon and pre-monsoon.

Bacterial populations have been reported to be positively correlated with increase in organic matter (Carrie et al., 1998; Dunn et al., 2008) which stimulate microbial metabolism, sediment oxygen demand and nutrient regeneration within surface sediments (Pfannkuche, 1993; Slomp et al., 1993; Boon et al., 1999). So, appreciably high abundance of bacterial fatty acids accompanied by higher amount of sedimentary organic matter in Pappinissery and Pazhayangadi was in line with the observation of previous studies (Boon et al., 1999; Carrie et al., 1998; Dunn et al., 2008). An increase in concentration of branched fatty acid and MUFAs are indicative of significant bacterial production (Gillan and Hogg, 1984) in the present study region. So from the results of bacterial fatty acids, it can infer the prevalence of bacterial re-working which leads to an efficient recycling of mangrove and algal material of the sedimentary organic matter.

6.2.4 Algal and Zooplankton Signals

Mangrove ecosystems are highly productive and a total of 72 species of microalgae (Bacillariophycea, Chlorophyceae, Cyanophyceae, and Dinophycea, in which Bacillariophyceae) and 80 species microphytobenthos (blue green algae, green algae and dinoflagellates-diatom was the major group) has been identified from six mangrove station around Cochin area, with a mean primary productivity 2.258 gC/m^3/day and an average seasonal primary production in the range 0.36 to 3.3 gC/m^3/day (Rejil, 2013). Earlier reports in

Pitchavaram mangrove waters of Tamilnadu showed a seasonal average in the range 0.36 to 3.3 gC/m^3/day, while in Dharmadam estuarine waters it was 0.24 to 1.14 gC/m^3/day (Rejil, 2013). So, higher phytoplankton production can be expected in the present study also.

Marine fatty acids are calculated as the sum of the most abundant marine homologues n-C14, n-C16 and n-C18 and follow the same trend as TFA and SFA. But in the present study Palmitic acid (16:0) is the major contributor to the SCFA and is originated from marine, bacterial as well as terrestrial sources. Also, C16, and C18 fatty acids are less source-specific than mono- and polyunsaturated fatty acids. These fatty acids can also be formed from biogeochemical reduction of unsaturated fatty acids which limit their biomarker potential (Birgel et al., 2004). Since there are many other sources for C16 and C18 than marine sources, it can be inferred that calculation of marine fatty acids could not only give the correct prediction as phytoplankton or algal contribution. However, C14:0 is reliably derived from primary production, on account of its high abundance in diatoms (Volkman et al., 1989; Viso and Marty, 1993).

The fatty acid markers have been used to distinguish amongst taxonomic classes of microalgae (Claustre et al., 1989). 18:4ω3, 20:5ω3 and 22:6ω3 are the predominant polyunsaturated fatty acids (PUFAs) in microalgae (Viso and Marty, 1993) in which 18:4ω3 was absent in the present study region. C18:1ω9 have used as biomarker for brown algae; the Cis form present in all the stations (0.19 to 22.19 μgg-1). An appreciably higher concentration of C18:1ω9 found in Pappinissery and Pazhayangadi in post-monsoon and pre-monsoon, may be accounted for (>5μgg-1) the abundance of brown algae.

Myristoleic acid (C14:1), a general biomarker for cyanobacteria (Caudales and Wells, 1992; Caudales et al., 1993), was detected from Pappinissery and Thalassery in post-monsoon (0.13 µgg-1). Priya and Niranjana, 2012 reported that C14:1 was isolated from *Aegiceras Corniculatum*.

Vascovsky et al., (1996) reported that the major fatty acids in green macro algae are 18:2ω6 and 18:3ω3, among them 18:2ω6 was detected in all the samples (0.03 to 7.60 μgg^{-1}). Comparatively higher concentration of 18:2ω6 was observed in the sediment samples from Pappinissery during in post-monsoon (3.41 μgg^{-1}) and pre-monsoon (7.60 μgg^{-1}). Fatty acid 18:2ω6, 18:4 may be related with cyanobacteria, heterocystous cyanobacteria respectively, but the later was not found in the present study (Vargas et al., 1998). 18:3ω3 was detected in as many as 7 out of 15 observations and was ranged from 0.11 to 4.59 μgg^{-1}. Pappinissery and Pazhayangadi in post - monsoon and pre-monsoon season and Thalassery in all seasons 18:3ω3 were among them. Further elevated concentration of 18:3ω3 (>1 μgg^{-1}) was recorded in, Pappinissery (post-monsoon, 1.86 µgg-1 and pre-monsoon, 4.59 µgg-1) and Pazhayangadi (pre-monsoon, 2.15 µgg-1).

Flagellated algae contain 22:6ω3, with much fairly lower amounts of 16:1ω7 and 20:5ω3 (Joseph, 1975; Bachok et al., 2009). However, 22:6ω3 was less abundant in the present study and was detected in Pappinissery (0.05 µgg-1) in post-monsoon. The fatty acid 18:4ω3 is also typical of dinoflagellates (Mansour et al., 1999) could not be detected in the present study.

Myristic acid (C14), a diatom marker (Reitan et al., 1994) also present in dinoflagellates to a lesser extent (Napolitano et al., 1997), was found to be much higher in concentration in pre-monsoon and lower concentration in monsoon (except Thalassery and Kadalundi). Post-monsoon period was characterised by higher C14 (Fig. 6.4) concentration at Thalassery and Kadalundi. These results were in synchronous with the chlorophyll concentration found for the sample in that season. Concentration of C14 was greater than one in all samples except for Kunjimangalam in monsoon and was much higher (>10 µgg-1) in two observations (Pappinissery and Pazhayangadi during pre-monsoon). This may be accounted for the fact that Pappinissery and Pazhayangadi were associated with clayey texture, higher sedimentary chlorophyll and organic carbon in pre-monsoon. The presence of higher concentrations of inorganic nutrients in muddy sediment might enhance the phytoplankton production (Sanil Kumar, 2009) in these stations.

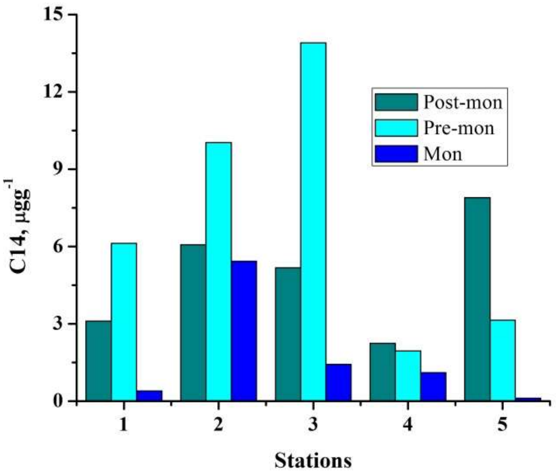

Fig. 6.4: Variation of C14 in the present study region

The PUFAs such as 20:5ω3 and 20:4ω3 have been used as diatom markers (Dunstan et al., 1994; Pond et al., 1998). 20:5ω3 detected in all samples indicating contribution from diatom. The concentration was fairly high (> 2 µgg-1) in as many as six observations, Pappinissery and Pazhayangadi in post-monsoon and pre-monsoon, Kunjimangalam in post-monsoon and Kadalundi in monsoon. In as many as ten samples presence of fatty acid 20:4ω3 was detected, but concentration was much lower in the present study (0.07 to 0.79µgg-1). It was not detected from the samples at Kunjimangalam and Kadalundi (in pre-monsoon and monsoon), and Thalassery in monsoon, In addition to 20:5ω3 and 20:4ω3 , 16:1ω7 has been used as diatom marker but can also be synthesised by dinoflagellates, prymnesiophytes and haptophytes (Viso and Marty, 1993; Hu et al., 2006). All samples showed the presence of 16:1ω7 and its concentration were appreciable, fluctuating between 0.07 and 11.95 µgg-1 in the present study.

Fatty acid based proxies such as C16:1/C16:0, ∑C16/∑C18 and C20:5/C22:6 have been used to distinguish between phytoplankton taxonomic classes such as diatoms and dinoflagellates (Budge and Parrish, 1998; Ramos et al., 2003). The ratio of 16:1/16:0 is usually used to distinguish between diatom and dinoflagellate (Budge and Parrish, 1998). However, due to the higher susceptibility of unsaturated fatty acids to the biological and chemical degradation during the sedimentation, the ratio of 16:1/16:0 in surface sediments was well below 1 (Birgel et al., 2004) in the present study. ∑C16 to ∑C18 ratio (> 2) have also been used as diatoms marker (Parrish et al., 2000) and the ratio was higher than 1.0 in all cases and had the maximum value of 4.65. The ratio was greater than two in as many as seven observations, which includes samples from Kadalundi in all seasons, Pazhayangadi and Kunjimangalam in monsoon. This apparent disagreement between the two diatom indicators is most likely attributed to an interference of C16:0 fatty acid and degradation of unsaturated fatty acids.

Another biomarker ratio, 20:5ω3 to 20:4ω6 was used for the prediction (>10) of red algae (Khotimchenko and Vaskovsky, 1990; Alikunhi et al., 2010). The ratio was greater than 10 at Kunjimangalam in post-monsoon, Pappinissery in monsoon which may indicate the presence of red algae at these stations.

16:1ω7/18:1ω9 is also used to distinguish between diatom as well as flagellated algae (Tolosa et al., 2004), based on the dominance of 16:1ω7 in diatoms (Volkman et al., 1989; Viso and Marty, 1993; Dustan et al., 1994). The ratio was greater than one in three observations, Kadalundi (post-monsoon), Kunjimangalam (pre-monsoon) and Pazhayangadi (monsoon) indicated the dominance of diatoms in the system. C18:1/C16:1 was fairly high at Thalassery during monsoon season, may be attributed to contribution of planktonic species other than the diatoms, since the latter species are known to be enriched in the C16 homologues (Dunstan et al., 1993). Ratio of C18:1 to C16:1 was less than one in Kadalundi during post-monsoon indicated that the planktonic input was dominated by diatoms.

In bacteria and fecal pellets, 18:1ω9/18:1ω7 is generally < 1, while it is usually >1 in phytoplankton and animal tissues (Thoumelin et al., 1997; Tolosa et al., 2004). In the present study 18:1ω7 detected from as many as ten observations, and in that samples the ratio was greater than one. This result indicated the dominance of phytoplankton in the study region.

C20:1, C22:1ω9, C24:1 MUFAs were considered to be zooplankton markers (Wakeham et al., 1997; Falk-Petersen et al., 2002; Sañé et al., 2011). C24:1 detected in 2 observations, at Pappinissery in post-monsoon (0.99 μgg-1) and at Kadalundi in pre-monsoon (0.72 μgg-1). Two C20:1 MUFAs were detected from the study region. C20:1ω9 were detected from 3 out of

15 observations, Pappinissery (0.07 μgg-1) and Pazhayangadi (0.21 μgg-1) during post-monsoon and Thalassery during pre-monsoon (0.05 μgg-1). The other MUFA was detected only from Pazhayangadi (0.05μgg-1) in post-monsoon. Erucic acid (C22:1ω13) was detected in six samples, (Kunjimangalam and Pazhayangadi in post-monsoon, Pappinissery in Post-monsoon and pre-monsoon along with Kadalundi in pre-monsoon and monsoon) and was ranged from 0.01 to 0.76 μgg-1. An isomer of C22:1 was also detected in the present study region. Its presence was noticed at Pappinissery in post-monsoon (1.14 μgg-1) and at Thalassery (0.07 μgg-1) and Kadalundi in monsoon (0.71 μgg-1).

Concentrations of zooplankton markers were moderately low at all stations except Pappinissery in post-monsoon and Kadalundi in pre-monsoon and monsoon. Kadalundi mangrove ecosystem which is proximate to the Lakshadweep Sea may contribute zooplankton markers to the system.

Dissolved oxygen super-saturation was noticed in the water column in pre-monsoon season but could not be detected from fatty acid biomarker. These PUFAs associated with phytoplankton would not be expected to be preserved in the soils and sediment in their original amounts, so that diagnostic PUFAs were absent or depleted within the sediment record (Carrie et al., 1998; Dunn et al., 2008). This liability results from their rapid losses by bacterial degradation and/or zooplankton grazing (Alfaro et al., 2006; Hu et al., 2006; Dunn et al., 2008). The proportion of bacterial fatty acids (i.e., iso and anteiso branched fatty acids) was appreciably higher in the present study, implying that microbial processes were active. These observations suggest that marine products like PUFAs have been significantly degraded (Harvey et al., 1987; Harvey and Macko, 1997), leaving behind the more resistant markers.

6.2.5 Vascular Plant Signals

The long chain fatty acids (LCFA) such as C24:0, C26:0 and C28:0, are considered as typical vascular plant biomarkers (Alfaro et al., 2006; Hall et al., 2006). Mangroves are characterized by high Cl6 and C18 members as well as C18:1 diacid (Sassen, 1977; Shaw and Johns, 1985; Mfilinge et al., 2003, 2005; Hall et al., 2006). Mfilinge et al., (2003) suggested that the amount of C16:0 fatty acids in mangrove leaves may be an indicator of degradation state since the concentration of SAFA in detritus declines constantly with age. In the present study, among long chain fatty acids >C20 which are derived from waxy leaf coatings showed predominance for even chain fatty acids over odd chain fatty acids. Except for Kadalundi, concentration of LCFA was found to be higher in pre-monsoon season. These LCFAs preserved in mangrove sediments and nearby ecosystems such as estuarine and oceanic region for a long period without undergoing further transformation, make them an effective mangrove biomarker (Mfilinge et al., 2005).

The ratio, SCFA to LCFA (Fig. 6.5) was greater than one for all samples except for Kadalundi in pre-monsoon. It was found that the amount of LCFA was much lower than short chain fatty acids in these stations. SCFA in the present study region was dominated by C16 followed by C18 which can be contributed by marine plankton, mangrove plants and bacteria. Meziane et al., 2007 studied fatty acid composition of six mangrove species such as *Kandelia obovata*, *Aegiceras corniculatum, Avicennia marina, K. obovata*, *Rhizophora stylosa* and *Bruguiera Gymnorrhiza* and found that total amount of LCFAs was <10%. According to their study other fatty acids such as C16:0, C18:1ω9, C18:2ω6 and C18:3ω3 were contributed up to 76–89% of the total fatty acid content in most cases. It was also pointed out that the fatty acid concentration varied according to local growing conditions as well as other chemical and structural adaptations. The ratio of SCFA to LCFA which was found to be greater than one may be attributed by the lower contribution of LCFA with respect to SCFA was in line with Meziane et al., 2007.

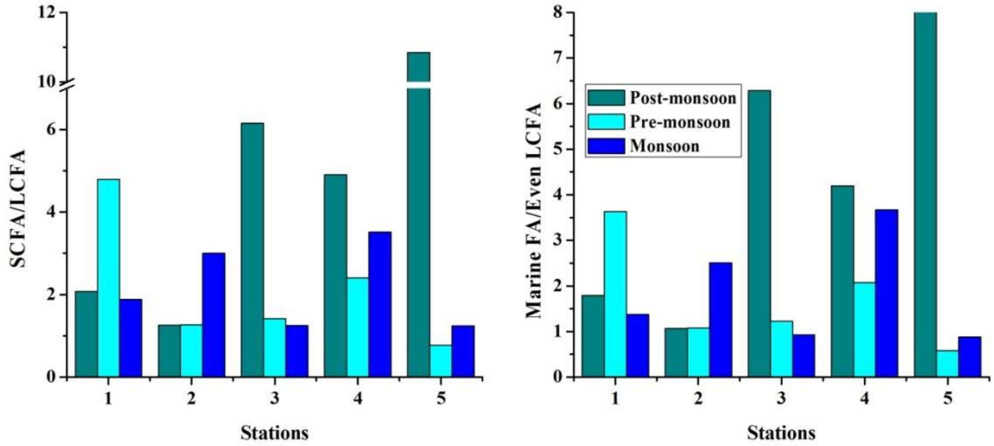

Fig. 6.5: Variation of SCFA to LCFA and Marine to even LCFA in the present study region

The ratio of total input of marine fatty acids (C14:0+16:0+18:0) to total input of even-numbered LCFAs (C22:0+24:0+26:0+28:0+30:0) were also calculated and was less than one in 4 observations (Kadalundi (pre-monsoon and monsoon), Pappinissery (monsoon) and Pazhayangadi (post-monsoon) which can be used as an indicator of aquatic vs. terrestrial organic matter sources (Meyers, 1997; Waterson, 2005).

The fatty acids C18:3ω6 and C18:3ω3 have been used as markers of green algae (Dunstan et al., 1992; Napolitano et al., 1997; Meziane and Tsuchiya, 2000), however, recent fatty acid analyses revealed that they also constitute dominant fatty acids in mangrove leaves (Sassen, 1977; Hall et al., 2006; Meziane et al., 2007). PUFAs like C18:3ω6 could not be detected in the present study, while C18:3ω3 were found in eight out of 15 observations. The high content of polyunsaturated fatty acids (PUFA), in particular 18:2ω6 and 18:3ω3, has been identified as useful biomarkers of mangrove leaves in estuarine food chains (Sassen, 1977; Hall et al., 2006; Meziane et al., 2007). The presence of 18:2ω6 was noticed in all samples and its concentration was varied from 0.03 to 7.59 $\mu gg^{-1}$and Except for Pappinissery, all stations showed higher concentration in post-monsoon followed by pre-monsoon season. Highest leaf litter fall is in between December and March, and highest fruit fall season is in between December and January (Duke, 1990; Imgraben and Dittmann, 2008) may be attributed to increase in concentration of these PUFAs.

The presence of α,ω-dicarboxylic acids of higher fatty acids have been previously detected in the sedimentary environment, originated from higher-plants and see grass (Shaw and Johns, 1985). A part of higher molecular weight fatty acids (C20-C32) undergoes β- and ω-oxidative degradation, even though they are diagenetically stable (Kawamura and Ishiwatari, 1984). The long chain dicarboxilic acids such as Eicosanebioic Acid, Tetracosaedioic Acid, Pentacosaedioic Acid, Hexacosaedioic Acid (α, ω-Dicarboxyacid) and Heptacosaedioic Acid (α, ω-Dicarboxyacid) were detected in the present study is of higher plant origin either by direct input or by diagenetic process.

2-hydroxyalkanoic acids (α-hydroxy fatty acids,) ranging from C24–C28, may be formed by cutin-derived hydroxy fatty acids (Goñi and Hedges, 1990; Crow et al., 2009) or from microbial metabolism of higher plant wax (Findlay and White, 1983). In the present study 2-OH fatty acids in the range C23 to C28 was detected indicating its origin from mangroves either by direct input or by microbial modifications.

6.3 Principal Component Analysis

A total of 119 fatty acids were identified in the present study, so it is very difficult to extract information from complex mixtures of compounds. Mangroves are most productive and complex ecosystem for their associated flora and fauna include bacteria, fungi, algae, lichens, zooplankton, benthos, birds, reptiles and mammals. In such complex systems, multivariate statistical techniques are very useful for extracting maximum information (Mudge and Norris, 1997; Mudge et al., 1998). PCA was performed on the data set and the results are detailed in Tables 6.3, 6.4 and 6.5. A total of 97.45% of variance was explained by all the nine factors in which first four factors explains 81.63%.

Table 6.3 Total variance explained by principal component analysis using Varimax rotation

Component	**Initial Eigen values**			**Extraction Sums of Squared Loadings**			**Rotation Sums of Squared Loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	39.81	49.76	49.76	39.81	49.76	49.76	35.13	43.92	43.92
2	11.07	13.83	63.59	11.07	13.83	63.59	10.94	13.68	57.60
3	8.60	10.75	74.34	8.60	10.75	74.34	10.86	13.58	71.18
4	7.10	8.87	83.21	7.10	8.87	83.21	8.36	10.45	81.63
5	3.43	4.29	87.50	3.43	4.29	87.50	3.71	4.64	86.26
6	3.15	3.93	91.43	3.15	3.93	91.43	3.47	4.33	90.59
7	2.08	2.61	94.03	2.08	2.61	94.03	2.22	2.78	93.37
8	1.73	2.16	96.19	1.73	2.16	96.19	2.18	2.73	96.10
9	1.01	1.26	97.45	1.01	1.26	97.45	1.08	1.35	97.45
10	0.80	1.00	98.45						
11	0.70	0.88	99.32						
12	0.37	0.47	99.79						

Table 6.4 Factor loading for Principal component analysis using varimax rotation for fatty acids

		C8	C9	C10	C11	C10-2OH	C10-3OH	iC12	C12	iC13	aC13	C13	C12-2OH	iC14	C14:1	C14
Components	1	-0.05	-0.02	0.50	-0.26	0.61	0.77	0.00	0.51	0.78	0.78	0.43	0.91	0.83	-0.09	0.65
	2	0.63	-0.14	0.70	0.56	0.29	0.02	-0.07	0.74	0.57	0.08	0.85	0.11	0.46	-0.06	0.74
	3	-0.26	-0.09	-0.40	-0.19	-0.17	0.43	0.93	-0.15	0.00	0.12	-0.04	0.08	0.26	0.75	0.07
	4	0.10	0.83	0.07	0.56	0.18	0.15	-0.09	0.09	-0.10	-0.20	0.21	-0.20	-0.07	-0.07	0.09
		iC15	aC15	C15	C14-3OH	iC16	C16:1	C16:1ω7	C16:1*	C16:1*	C16	C17:1	bC17	bC17	iC17	aC17
	1	0.76	0.79	0.29	0.22	0.82	-0.05	0.47	0.74	0.53	0.72	0.85	0.74	-0.03	0.84	0.90
	2	0.59	0.46	0.91	0.32	0.47	0.31	0.83	0.52	0.61	0.64	0.02	0.54	-0.10	0.50	0.32
	3	0.20	0.32	-0.06	-0.16	0.26	-0.07	0.08	0.01	0.22	0.07	0.11	0.18	0.20	0.11	0.26
	4	0.05	0.03	-0.08	-0.20	-0.01	0.92	0.05	-0.32	0.46	0.08	0.03	0.26	0.11	0.16	0.04
		C17:1ω7	Cy-C17	C17	C16-2OH	cisC18:2ω6	cisC18:3ω6	cisC18:1ω9	C18:1*	C18:1ω11	C18:1*	C18	C17-3OH	C17-2OH	bC19*	bC19*
	1	-0.33	-0.22	0.69	0.72	0.76	0.87	0.83	0.81	0.92	-0.07	0.89	0.92	0.01	-0.10	0.91
	2	0.82	0.55	0.68	-0.16	0.35	0.07	0.37	0.48	0.17	-0.20	0.41	0.10	-0.02	0.26	0.15
	3	-0.01	0.41	0.02	0.31	0.33	0.35	0.19	0.19	0.08	-0.05	0.02	0.12	0.97	-0.10	0.15
	4	-0.16	0.59	0.08	0.25	0.08	0.03	0.15	0.04	-0.08	0.84	0.03	-0.22	-0.09	-0.06	0.14
		C19:2	Cy-C19	C19	C20:4	C20:5	C19-3OH	C20:2	C20:1ω11	C20:1*	C20	iC21	C21:2	C21	C22:6	C21-2OH
	1	0.85	0.54	0.78	0.63	0.68	0.08	0.08	0.02	0.08	0.95	0.41	0.16	0.93	0.02	-0.06
	2	0.03	0.40	0.05	0.54	0.15	0.05	0.05	0.04	0.05	0.17	0.10	0.00	0.04	0.07	-0.05
	3	0.14	0.46	-0.03	0.26	0.44	-0.01	-0.01	0.93	-0.01	-0.02	-0.05	0.75	-0.26	0.99	-0.10
	4	-0.16	0.31	0.23	0.14	0.23	0.99	0.99	0.28	0.99	-0.10	0.89	0.32	0.11	-0.08	-0.08
		C21-3OH	C22:1ω11	C22:1ω9	C22	C23	C22-2OH	C24:1	C24	C23-3OH	C25	C24-2OH	C26	C25-2OH	C27	iC28
	1	0.78	0.01	0.41	0.83	0.95	0.98	0.01	0.92	0.94	0.97	0.90	0.86	0.93	0.85	-0.04
	2	0.02	0.04	-0.11	0.05	0.19	0.02	-0.03	0.18	0.04	0.16	0.06	0.19	-0.02	0.17	-0.06
	3	0.36	0.98	0.00	-0.09	-0.16	-0.04	0.77	-0.09	-0.26	-0.02	0.07	-0.26	-0.21	-0.32	0.82
	4	-0.17	-0.08	0.07	0.06	-0.03	0.00	-0.10	0.14	-0.04	0.03	0.07	0.29	0.01	0.04	-0.15

PC1 explained 43.92% of the total variance and was contributed by hydroxy fatty acids (except 3 OH-C14, 3-OH C17), Branched fatty acids (iC13, aC13, iC14, iC15, aC15, iC16, iC17, aC17), SCFAs (C14 to C22), LCFAs, MUFAs (C16:1, C17:1, C18:1ω9, C18:1ω11, C18:1), PUFAs(C20:5, 20:4, C19:2, cisC18:3ω6, cisC18:2ω6) and Cy - C19. Mangrove biomarkers suchas LCFAs and PUFAs (cisC18:3ω6, cisC18:2ω6) and diatom markers such as C20:5, 20:4 fatty acids were included in this component. Fatty acids such as iso and anteiso fatty acids, MUFA (C18:1ω11) and Cy-C19 are biomarkers for bacteria, which is attributed to colonisation of bacterial community. So in total, PC1 represents recycling of mangrove derived organic matter by bacterial community.

PC2 explained 13.68% of the total variance contributed by SCFA's (C8, C10, C12, C13, C14, C15, C16 and C17) and MUFAs (C16:1, 16:1ω7, C17:1ω7). MUFAs and SCFAs are indicators the bacterial contribution. PC1 and PC2 showed loading on C16 indicates that it is contributed by bacterial, mangroves as well as planktonic input.

Table 6.5 Factor loading for Principal component analysis using varimax rotation for fatty acids

		C26-2OH	C28	C29	aC30	C30
Components	1	0.90	0.88	0.91	0.02	0.87
	2	-0.07	0.17	0.14	0.07	0.11
	3	-0.19	-0.26	-0.24	0.99	-0.18
	4	0.04	0.22	0.28	-0.08	0.17

PC3 contributed 13.58% to the total variance and showed loading on iC12, C14:1, 2-OHC17, C20:1ω11, C21:2, C22:6, C22:1ω11, C24:1, br-C28 and 30. Myristoleic acid (C14:1) may be related to the cyanobacterial input while zooplankton input can be correlated with C20:1, C22:1 and C24:1. PC4 explained 10.45% of the total variance and showed loading on C9, C11, C16:1, Cy-C17, C18:1, C20:2 and C20:1. This factor can also be correlated with bacterial input by the presence of Cy-C17 and MUFAs. C20:1, a zooplankton marker also showed loading in this component.

The figure shows (Fig. 6.6) plot between PC1 and PC2 in which 4 clusters were identified. Mangrove fatty acids are clustered together at the right-hand side end of the plot. Also, it can be seen from the figure that Branched fatty acids along with C16 and C18 located near the vascular signals indicating the association of bacterial, as well as mangrove derived organic matter. Fatty acid biomarkers of Flagellated algae, zooplankton and cyanobacteria are formed another cluster which lies almost in the centre of the plot.

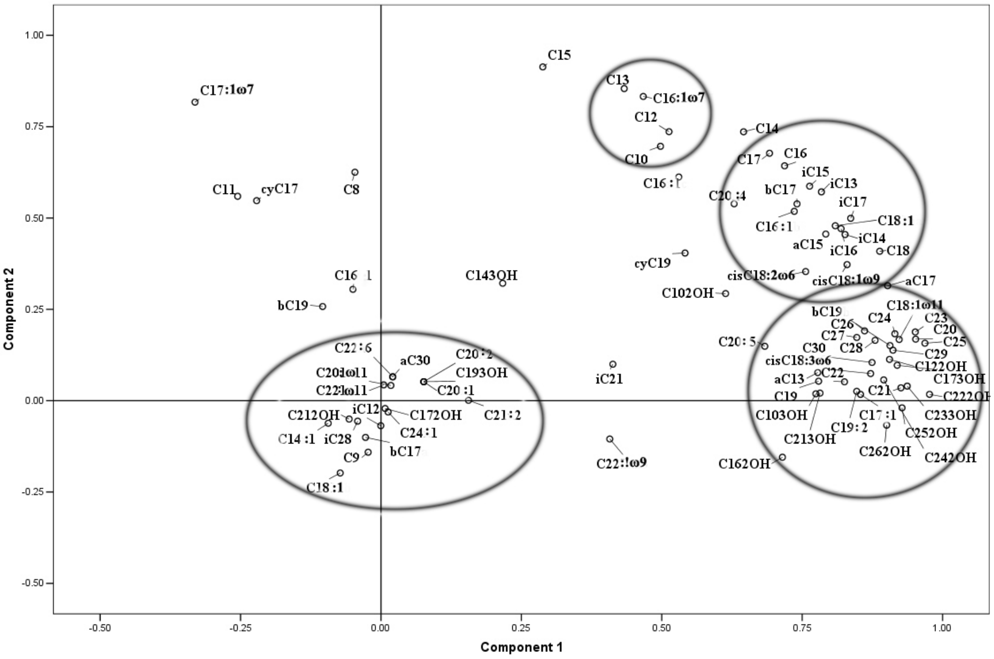

Fig. 6.6: Plot between PC1 and PC2

6.4 Conclusion

In the present study, SCFA/LCFA was found to be greater than one does not imply low input from vascular plants indicate lower contribution of LCFA to the study region. Palmitic acid (16:0) is contributed by mangroves, marine, as well as bacterial sources overwhelming the higher fatty acid signatures from mangroves is also contributing factor to the low ratio. Pappinissery was associated with higher fatty acids during pre-monsoon period which might be due to the depletion of dissolved oxygen in the water column, which inhibit organic matter degradation by aerobic processes in synchronous to an increase in anaerobic process. Grain size also has a role in the distribution of organic matter. At Kunjimangalam and Kadalundi (pre-monsoon) and to a lesser extend at Thalassery sediment is sandy in texture which is impervious to formation of organic matter. Pre-monsoon is characterised by the higher fatty acids contributed by increased microbial activity at higher temperature and increased algal production by greater light penetration except in Thalassery and Kadalundi.

The concentrations of PUFAs were lower in pre-monsoon except Pappinissery and Pazhayangadi due to its greater susceptibility to degradation by the active microbial community. Ratio of C18:1 to 16:1 showed the dominance of diatom in Kadalundi during post-monsoon and all other stations showed phytoplankton input other than diatoms. Multimethyl branched fatty acids were detected in the present study region derived from bacterial fatty acids (eg: halophilic Bacillus species), uropygial waxes of the birds preen gland and by the oxidation of phytol in animal tissues. An increase in branched fatty acid concentrations and MUFAs are indicative of bacterial networking in the present study region. The biomarkers of Desulfobulbus-type bacteria, methane-oxidizing bacteria, sulfur-reducing bacteria, Sulfur-oxidising bacteria, Gram-negative bacteria, waterborne Gram-negative pathogens and Iron-oxidising bacteria were also detected in the present study region. Zooplankton signals are detected in high concentration at Kadalundi by the seawater influx due to its close proximity to the Arabian Sea. Algal biomarkers gave the presence of brown algae, cyanobacteria, red algae, dinoflagellates and diatoms in the study region. PUFA a general phytoplankton marker, would not necessarily be expected to be preserved in their original amounts in the present study as a result of the lability by bacterial activity. C14, a diatom marker was much higher in Pappinissery with higher concentration (except Thalassery and Kadalundi) during pre-monsoon season which was in accordance with the chlorophyll concentration. 18:2ω6 and C18:3ω3 can be contributed by algae as well as mangroves. Also, C16:1ω7 is contributed by algae as well as bacteria. The presence of higher dicarboxylic acids and hydroxy fatty acids may be contributed from higher plant origin either by direct contribution or by diagenesis.

References

Ackman, R. G., Hooper, S. N., 1968. Examination of isoprenoid fatty acids as distinguishing characteristics of specific marine oils with particular reference to whale oils. Comparative biochemistry and physiology, 24, 549-565.

Alfaro, A. C., Thomas, F., Sergent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuarine, Coastal and Shelf Science, 70, 271-286.

Alikunhi, N. M., Narayanasamy, R., Kandasamy, K., 2010. Fatty acids in an estuarine mangrove ecosystem. Revista de biología tropical, 58, 577-587.

Ashton, E. C., Hogarth, P. J., Ormond, R., 1999. Breakdown of mangrove leaf litter in a managed mangrove forest in Peninsular Malaysia. Hydrobiologia, 413, 77–88.

Avigan, J., Blumer, M., 1968. On the origin of pristane in marine organisms. Journal of Lipid Research, 9, 350-352.

Bachok, Z., Meziane, T., Mfilinge, P. L., Tsuchiya, M., 2009.Fatty acid markers as an indicator for temporal changes in food sources of the bivalve Quidnipagus palatum. Aquatic Ecosystem Health & Management, 12, 390-400.

Birgel, D., Stein, R., Hefter, J., 2004. Aliphatic lipids in recent sediments of the Fram Strait/Yermak Plateau (Arctic Ocean): composition, sources and transport processes. Marine Chemistry, 88, 127-160.

Boon, A. R., Duineveld, G. C. A., Kok, A., 1999. Benthic organic matter supply and metabolism at depositional and non-depositional areas in the North Sea. Estuarine, Coastal and Shelf Science, 49, 747–761.

Boon, J. J., De Leeuw, J. W., Hoek, G. J., Vosja, J. H., 1977. Significance and taxonomic value of is0 and anteiso monoenoic fatty acids and branched β- hydroxy acids in Desulfovibrio desulfuricans. Journal of Bacteriology, 129, 1183-1191.

Bowman, J. P., Skerratt, J. H., Nichols, P. D., Sly, L. I., 1991. Phospholipid fatty acid and lipopolysaccharide fatty acid signature lipids in methane-utilizing bacteria. FEMS Microbiology Letters, 85, 15-21.

Brown, M. R., Dunstan, G. A., Norwood, S. J., Miller, K. A., 1996. Effects of harvest stage on the biochemical composition of the diatom Thalassiosira pseudonana. Journal of Phycology, 32, 64–73.

Budge, S. M., Parrish, C. C., 1998. Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Organic Geochemistry, 29, 1547-1559.

Canuel, E. A., Cloern, J. E., Ringelberg, D. B., Guckert, J. B., Rau, G. H., 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnology and Oceanography, 40, 67–81.

Carrie, R. H., Mitchell, L., Black, K. D., 1998. Fatty acids in surface sediment at the Hebridean shelf edge, west of Scotland. Organic Geochemistry, 29, 1583-1593.

Caudales, R., Wells, J. M., 1992. Differentiation of free-living Anabaena and Nostoc cyanobacteria on the basis of fatty acid composition. International journal of systematic bacteriology, 42, 246-251.

Caudales, R., Wells, J. M., Butterfield, J. E., Antoine, A. D., 1993. Host-related variability in fatty acid composition of symbiotic cyanobacteria isolated from the aquatic fern, azolla. In Endocytobiology V-Proceedings of the 5th International Colloquium on Endocytobiology and Symbiosis. Tubingen University Press, Tübigen, Germany.

Caumette, P., Matheron, R., Raymond, N., Relexans, J. C., 1994. Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de-Giraud, France). FEMS Microbiology Ecology, 13, 273-286.

Claustre, H., Marty, J. C., Cassiani, L., 1989. Intraspecific differences in the biochemical composition of a diatom during a spring bloom in Villefranche-Sur-Mer Bay, Mediterranean Sea. Journal of Experimental Marine Biology and Ecology, 129, 17–32.

Coleman, M. L., Hedrick, D. B., Lovley, D. R., White, D. C., Pye, K., 1993. Reduction of Fe(III) in sediments by sulphate-reducing bacteria. Nature, 361, 436–438.

Colombo, J. C., Silverberg, N., Gearing, J. N., 1996. Lipid biogeochemistry in the Laurentian Trough: I—fatty acids, sterols and aliphatic hydrocarbons in rapidly settling particles. Organic Geochemistry, 25, 211-225.

Cranwell, P. A., Eglinton, G., Robinson N., 1987. Lipids of aquatic organisms as potential contributors to lacustrine sediments--II. Organic Geochemistry, 11, 513-527.

Crow, S. E., Lajtha, K., Filley, T. R., Swanston, C. W., Bowden, R. D., Caldwell, B. A., 2009. Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Global Change Biology, 15, 2003-2019.

De Moreno, J. E. A., Pollero, R. J., Moreno, V. J., Brenner, R. R., 1980. Lipids and fatty acids of the mussel (Mytilus platensis d'Orbigny) from South Atlantic waters. Journal of Experimental Marine Biology and Ecology, 48, 263-276.

Dembitsky, V. M., Rezanka, T., Srebnik, M., 2003. Lipid compounds of freshwater sponges: family Spongillidae, class Demospongiae. Chemistry and physics of lipids, 123, 117-155.

Diaz, R. J., Rosenberg, R., 1995. Marine benthic hypoxia: a review of its ecological effects and the behaviour responses of benthic macrofauna. Oceanography and Marine Biology: an Annual Review, 33, 245–303.

Dijkman, N. A., Boschker, H. T. S., Stal, L. J., Kromkamp, J. C., 2010. Composition and heterogeneity of the microbial community in a coastal microbial mat as revealed by the analysis of pigments and phospholipid-derived fatty acids. Journal of Sea Research, 63, 62-70.

Dowling, N. J. E., Widdel, F., White, D. C., 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. Journal of general microbiology, 132, 1815–1825.

Duke, N. C., 1990. Morphological variation in the mangrove genus Avicennia in Australasia: Systematic and ecological considerations. Australian Systematic Botany, 3, 221-239.

Dunn, R. J. K., Welsh, D. T., Teasdale, P. R., Lee, S. Y., Lemckert, C. J., Méziane, T., 2008. Investigating the distribution and sources of organic matter in surface sediment of Coombabah Lake (Australia) using elemental, isotopic and fatty acid biomarkers. Continental Shelf Research, 28, 2535-2549.

Dunstan, G. A., Volkman, J. K., Barrett, S. M., Garland, C. D., 1993. Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. Journal of Applied Phycology, 5, 71-83.

Dunstan, G. A., Volkman, J. K., Jeffrey, S. W., Barrett, S. M., 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. Journal of Experimental Marine Biology and Ecology, 161, 115-134.

Dustan, G. A., Volkman, J. K., Barret, S. M., Leroi, J. M., Jeffrey, S. W., 1994. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry, 35, 155–161.

Edlund, A., Nichols, P. D., Roffey, R., White, D. C., 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from Desulfovibrio species. Journal of Lipid Research, 26, 982-988.

Eglinton, G., Calvin, M., 1967. Chemical fossils. Scientific American, *216*, 32-43.

Eglinton, G., Hamilton, R. J., 1967. Leaf epi-cuticular waxes. Science, 156, 1322–1335.

Eglinton, G., Hunneman, D. H., 1968. Gas chromatographic-mass spectrometric studies of long chain hydroxyacids--I. The constituent cutin acids of apple cuticle. Phytochemistry, 7, 313-322.

Eglinton, G., Hunneman, D. H., Douraghi-Zadeh, K., 1968. Gas chromatographic-mass spectrometric studies of long chain hydroxyacids. Tetrahedron, 24, 5929-5941.

Falk-Petersen, S., Dahl, T. M., Scott, C. L., Sargent, J. R., Gulliksen, B., Kwasniewski, S., ..., Millar, R. M., 2002. Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. Marine Ecology Progress Series, 227, 187-194.

Findlay, R. H., Dobbs, F. C., 1993. Quantitative description ofmicrobial communities using lipid analysis. In: Handbook of Methods in Aquatic Microbial Ecology (Kemp P E, SherrB F, eds.). Lewis Publisher, Boca Raton, USA

Findlay, R. H., White, D. C., 1983. Polymeric β-hydroxy alkanoates from environmental samples and Bacillus megaterium. Applied and Environmental Microbiology, 45, 71–78.

Gao, X., Chen, S., Long, A., 2008. Composition and sources of organic matter and its solvent extractable components in surface sediments of a bay under serious anthropogenic influences: Daya Bay, China. Marine Pollution Bulletin, 56, 1066– 1075.

Gillan, F. T., Hogg, R. W., 1984. A method for the estimation of bacterial biomass and community structure in mangrove-associated sediments. Journal of Microbiological Methods, 2, 275-293.

Gillan, F. T., Johns, R. B., Verheyen, T. V., Nichols, P. D., Esdaille, R. J., Bavor H. J., 1983. Monounsaturated fatty acids as specific bacterial markers in marine sediments.In Advances in Organic Geochemistry 1981 (Edited by Bjøroy M. et al).Wiley, Chichester.

Gong, C., Hollander, D. J,. 1997. Differential contribution of bacteria to sedimentary organic matter in oxic and anoxic environments, Santa Monica Basin, California. Organic Geochemistry, 26, 545–563.

Goñi, M. A., Hedges, J. I., 1990. Potential applications of cutin-derived CuO reaction products for discriminating vascular plant sources in natural environments. Geochimica et Cosmochimica Acta, 54, 3073-3081.

Goosens, H., Duren, R. R., de Leew, J. W., Schenck, P. A., 1989. Lipids and their mode of occurrence in bacteria and sediments: II. Lipids in the sediment of a stratified, freshwater lake. Organic Geochemistry, 14, 27–41.

Goossens, H., Rijpstra, W. I. C., Duren, R. R., Leeuw J. W., de Schenck P. A., 1986. Bacterial contribution to sedimentary organic matter; a comparative study of lipid moieties in bacteria and recent sediments. In Advances in Organic Geochemistry 1985 (Edited by Leythaeuser Dand Rullk6tter J.). Organic Geochemistry, 10, 683-696. Pergamon Press, Oxford.

Green, P. R., Kemper, J., Schechtman, L., Guo, L., Satkow- ski, M., Fiedler, S., Steinbuchel, A., Rehm, B. H. A., 2002. Formation of short chain length/medium chain length polyhydroxy alkanoate copolymers by fatty acid β-oxidation inhibited Ralstonia eutropha. Bio-macromolecules, 3, 208–213.

Grimalt, J. O., de Wit, R., Teixidor, P., Albaigés, J., 1992. Lipid biogeochemistry of Phormidium and Microcoleus mats. Organic Geochemistry, 19, 509-530.

Gugger, M., Lyra, C., Suominen, I., Tsitko, I., Humbert, J. F., Salkinoja-Salonen, M. S., Sivonen, K., 2002. Cellular fatty acids as chemotaxonomic markers of the genera Anabaena, Aphanizomenon, Microcystis, Nostocand Planktotrix (cyanobacteria). International Journal of Systematic and evolutionary microbiology, 52, 1007–1015.

Haddad, R. I., Martens, C. S. Farrington, J. W., 1992. Quantifying early diagenesis of fatty acids in a rapidly accumulating coastal marine sediment. Organic Geochemistry, 19, 205-216.

Hall, D., Lee, S. Y., Meziane, T., 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: Tracer transfer. Journal of Experimental Marine Biology and Ecology, 336, 42–53.

Harvey, H. R., Eglinton, G., O'Hara, S. C. M., Corner, E. D. S., 1987. Biotransformation and assimilation of dietary lipids by Calanus feeding on a dinoflagellate. Geochimica et Cosmochimica Acta, 51, 3031–3040.

Harvey, H. R., Macko, S. A., 1997. Kinetics of phytoplankton decay during simulated sedimentation: changes in lipids under oxic and anoxic conditions. Organic Geochemistry, 27, 129–140.

Hayashi, H., Takii, S., 1977. Vertical distribution and some properties of bacteria in a stored core sample from Lake Biwa. In: Hori, S. (Ed.), Paleolimnology of Lake Biwa and the Japanese Pleistocene, Kyoto University Press, Kyoto.

Hedrick, D. B., Peacock, A. D., Long, P., White, D. C., 2008. Multiply methyl-branched fatty acids and diacids in the polar lipids of a microaerophilic subsurface microbial community. Lipids, 43, 843-851.

Hogarth, P. J., 1999. The Biology of Mangroves. Oxford University Press, New York.

Hu, J., Zhang, H., Peng, P. A., 2006. Fatty acid composition of surface sediments in the subtropical Pearl River estuary and adjacent shelf, Southern China. Estuarine, Coastal and Shelf Science, 66, 346-356.

Hyne, R. V., Sánchez-Bayo, F., Bryan, A. D., Johnston, E. L., Mann, R. M., 2009. Fatty acid composition of the estuarine amphipod, Melita plumulosa (Zeidler): Link between diet and fecundity. Environmental Toxicology and Chemistry, 28, 123-132.

Ibekwe, A. M., Kennedy, A. C., 1999. Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant and Soil, 206, 151–161.

Imgraben, S., Dittmann, S., 2008. Leaf litter dynamics and litter consumption in two temperate South Australian mangrove forests. Journal of Sea Research, 59, 83-93.

Jacob, J., Ziswiler, V., 1982. The uropygial gland.Avian biology, 6, 199-324.

Jeffries, P. H., 1972. Fatty acid ecology of tidal marsh. Limnology and Oceanography, 17, 433-440.

Johns, R. B., Onder, O. M., 1975. Biological diagcnesis: dicarboxylic acids in recent sediments. Geochimica et Cosmochimica Acta,39, 129-136.

Joseph, J. D. 1975. Identification of 3,6,9,12,15-octadecapentaenoic acid in laboratory cultured photosynthetic dinoflagellates. Lipids, 10, 395–403.

Kaneda, T., 1991. Iso-fatty and anteiso-fatty acids in bacteria-biosynthesis, function, and taxonomic significance. Microbiological Reviews, 55, 288–302.

Kathiresan, K. Rajendran, N., 1999. Do decomposing leaves ofmangroves attract fishes?. Current Science, 77, 972-976.

Kathiresan, K., Rajendran, N., 2000. The effects of electric impulse on growth of Rhizophora mucronata seedlings (Rhizophorales: Rhizophoraceae). Revista de Biología Tropical, 48, 919-925.

Kathiresan, K., Bingham, B. L., 2001. Biology of mangroves and mangrove ecosystems. Advances in Marine Biology, 40, 81-251.

Kathiresan, K., Rajendran, N., 1998. Mangrove associated communities. Proceedings of Biodiversity of Gulf of Mannar Marine Biosphere Reserve,(Ed). Rajeswari M. Anand, K. Dorairaj and A. Parida.MS Swaminathan Research Foundation. Chennai.

Kathiresan, K., Rajendran, N., 2004. How to increase juvenile shrimps in mangrove waters? Wetlands Ecology and Management, 12, 179-188.

Kathiresan, K., Rajendran, N., 2007. Microbial flora associated with submerged mangrove leaf litter in India. Revista de biología tropical, 55, 393-400.

Kawamura K., Ishiwatari, R., 1984. Fatty acid geochemistry of a 200 m sediment core from Lake Biwa, Japan.Early diagenesis and paleoenvironmental information. Geochimica et Cosmochimica Acta, 48, 251-266.

Kawamura, K., Ishiwatari, R., 1984. Fatty acid geochemistry of a 200 m sediment core from Lake Biwa, Japan. Early diagenesis and paleoenvironmental information. Geochimica et Cosmochimica Acta, 48, 251-266.

Kenyon, C. N., Rippka, R., Stanier, R. Y., 1972. Fatty acid composition and physiological properties of some filamentous blue-green algae. Archiv für Mikrobiologie, 83, 216-236.

Khotimchenko, S. V., Vaskovsky, V. E., 1990. Distribution of C20 polyenoic fatty acids in red macrophytic algae. Botanica marina, 33, 525-528.

Kieft, T. L., Ringelberg, D. B., White, D. C., 1994. Changes in ester-linked phospholipids fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and Environmental Microbiology, 60, 3292– 3299.

Killops, S., Killops, V., 2005. Introduction to Organic Geochemistry. Blackwell Publishing, Oxford, USA.

Killops, S. D., Killops, V. J., 1993.An Introduction to Organic Geochemistry. Longman Scientific and Technical, UK.

Knief, C., Lipski, A., Dunfield, P. F., 2003. Diversity and activity of methanotrophic bacteria in different upland soils. Applied and Environmental Microbiology, 69, 6703–6714.

Kohring, L. L., Ringelberg, D. B., Devereux, R., Stahl, D. A., Mittelman, M. W., White, D. C., 1994. Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate- reducing bacteria. FEMS Microbiology Letters, 119, 303–308.

Lee, K. H., Moran, M. A., Benner, R., Hodson, R. E., 1990. Influence of soluble components of red mangrove (Rhizophora mangle) leaves on microbial decomposition of structural (lignocellulosic) leaf components in seawater. Bulletin of marine science, 46, 374-386.

Lee, R. F., Hirota, J., Barnett, A. M., 1971. Distribution and importance of wax esters in marine copepods and other zooplankton. In Deep Sea Research and Oceanographic Abstracts, 18, 1147-1165.

Lee, S. Y., 1999. Tropical mangrove ecology: Physical and biotic factors influencing ecosystem structure and function. Australian Journal of Ecology, 24, 355–366.

Léveillé, J. C., Amblard, C., Bourdier, G., 1997. Fatty acids as specific algal markers in a natural lacustrian phytoplankton. Journal of Plankton Research, 19, 469-490.

Lipski, A., Altendorf, K., 1997. Identification of heterotrophic bacteria isolated from ammonia-supplied experimental biofilters. Systematic and applied microbiology, 20, 448-457.

Lipski, A., Spieck, E., Makolla, A., Altendorf, K., 2001. Fatty Acid Profiles of Nitrite-oxidizing Bacteria Reflect their Phylogenetic Heterogeneity. Systematic and applied microbiology, 24, 377-384.

Londry, K. L., Jahnke, L. L., Des Marais, D. J., 2004. Stable carbon isotope ratios of lipid biomarkers of sulfate-reducing bacteria. Applied and Environmental Microbiology, 70, 745–751.

Mansour, M. P., Volkman, J. K., Jackson, A. E., Blackburn, S. I., 1999. The fatty acid and sterol composition of five marine dinoflagellates. Journal of Phycology, 35, 710-720.

Medeiros, P. M., Simoneit, B. R. T., 2008. Multi-biomarker characterization of sedimentary organic carbon in small rivers draining the North western United States. Organic Geochemistry, 39, 52-74.

Mendoza Y. A., Gula~ar, F. O., Hu Z. L., Buchs A., 1987. Unsubstituted and hydroxy substituted fatty acids m a recent lacustrine sediment. International Journal of Environmental Analytical Chemistry, 31, 107 127.

Meyers, P. A., 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Organic geochemistry, 27, 213-250.

Meyers, P. A., Ishiwatari, R., 1993. Lacustrine organic geochemistry—an overview of indicators of organic matter sources and diagenesis in lake sediments. Organic Geochemistry, 20, 867–900.

Méziane, T., Lee, S. Y., Mfilinge, P. L., Shin, P. K. S., Lam, M. H. W., Tsuchiya, M., 2007. Inter-specific and geographical variations in the fatty acid composition of mangrove leaves: implications for using fatty acids as a taxonomic tool and tracers of organic matter. Marine Biology, 150, 1103-1113.

Meziane, T., Tsuchiya, M., 2000. Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Marine Ecology Progress Series, 200, 49-57.

Mfilinge, P. L., Meziane, T., Bachok, Z., Tsuchiya, M., 2003. Fatty acids in decomposing mangrove leaves: microbial activity, decay and nutritional quality. Marine ecology progression series, 265, 97–105.

Mfilinge, P. L., Meziane, T., Bachok, Z., Tsuchiya, M., 2005. Litter dynamics and particulate organic matter outwelling from a subtropical mangrove in Okinawa Island, South Japan. Estuarine, Coastal and Shelf Science, 63, 301–313.

Minnikin, D. E., 1982. Lipids: complex lipids, their chemistry, biosynthesis and rolesC Ratledge, J Stanford (Eds.), The Biology of the Mycobacteria, Academic Press, London.

Minnikin, D. E., Kremer, L., Dover, L. G., Besra, G. S., 2002. The methyl-branched fortifications of Mycobacterium tuberculosis.Chemistry & biology, 9, 545-553.

Mudge, S. M., East, J. A., Bebianno, M. J., Barreira, L. A., 1998. Fatty acids in the Ria Formosa lagoon, Portugal. Organic Geochemistry, 29, 963-977.

Mudge, S. M., Norris, C. E., 1997. Lipid biomarkers in the Conwy Estuary (North Wales, UK): a comparison between fatty alcohols and sterols. Marine Chemistry, 57, 61-84.

Naganuma, T., Wada, H., Fujioka, K., 1996. Biological community and sediment fatty acids associated with the deep-sea whale skeleton at the Torishima Seamount. Journal of Oceanography, 52, 1-15.

Napolitano, G. E., Pollero, R. J., Gayoso, A. M., 1997. Fatty acids as Trophic markers of phytoplankton blooms in the Bahia Blanca estuary (Buenos Aires, Argentina) and trinity Bay (Newfoundland, Canada). Biochemical Systematics and Ecology 25, 739–755.

Navarrete, A., Peacock, A., Macnaughton, S. J., Urmeneta, J., Mas-Castellà, J., White, D. C., Guerrero, R., 2000. Physiological status and community composition of microbial mats of the Ebro Delta, Spain, by signature lipid biomarkers.Microbial ecology, 39, 92-99.

Odum, E. P., 1971. Fundamentals of Ecology. W. B. Saundersand Co., Philadelphia, USA.

Orphan, V. J., Hinrichs, K. U., Ussler, W. I. I. I., Paull, C. K., Taylor, L. T., Sylva, S. P., DeLong, E. F., 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Applied and Environmental Microbiology, 67, 1922-1934.

Parker, J. H., Smith, G. A., Fredrickson, H. L., Vestal, J. R., White, D. C.,1982. Sensitive assay, based on hydroxy fatty acids from lipopolysaccharide lipid A, for Gram-negative bacteria in sediments. Applied and Environmental Microbiology, 44, 1170-1177.

Parkes, R. J., 1987. Analysis of microbial communities within sediments using biomarkers. In: Fletcher, M., Gray, T.R.G., Jones, J.G. (Eds.), Ecology of Microbial Communities. Cambridge University Press, London.

Parkes, R. J., Calder, A. G., 1985. The cellular fatty acids of three strains of Desulfobulbus, a propionate-utilising sulphate-reducing bacterium. FEMS Microbiology Ecology, 1, 361-363.

Parrish, C. C., Abrajano, T. A., Budge, S. M., Helleur, R. J., Hudson, E. D., Pulchan, K., Ramos, C., 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky, P. (Ed.), The Hand- book of Environmental Chemistry, Part D, Marine Chemistry. Springer, Berlin, Heidelberg.

Perry G. J., Volkman J. K., Johns R. B., Bavor. H. J., 1979. Fatty acids of bacterial origin in contemporary marine sediments. Geochimica et Cosmochimica Acta , 43, 1715-1725.

Pfannkuche, O., 1993. Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 471N, 201W. Deep-Sea Research II, 40,135–149.

Poerschmann, J., Parsi, Z., Górecki, T., Augustin, J., 2005. Characterization of non-discriminating tetramethyl ammonium hydroxide-induced thermochemolysis– capillary gas chromatography–mass spectrometry as a method for profiling fatty acids in bacterial biomasses. Journal of Chromatography A, 1071, 99-109.

Pond, D. W., Bell, M. V., Harris, R. P., Sargent, J. R., 1998. Microplanktonic polyunsaturated fatty acid markers: a mesocosm trial. Estuarine, Coastal and Shelf Science, 46, 61-67.

Prahl, F. G., Eglinton, G., Corner, E. D. S., O'Hara, S. C. M., Forsberg, T. E. V., 1984. Changes in plant lipids during passage through the gut of *Calanus*. Journal of the Marine Biological Association of the United Kingdom, 64, 317-334.

Priya, D. P. A., Niranjana, S. C. A., 2012. Potential testing of fatty acids from mangrove *Aegiceras Corniculatum* (L.) blanco. International Journal of Pharmacy and Pharmaceutical Sciences, *3*, 569-571.

Qi, Q. S., Rehm, B. H. A., Steinbuchel, A., 1997. Synthesis of poly(β-hydroxy alkanoates) in *Escherichia coli* expressing the PHA synthase gene PhaC2 from *Pseudomonas aeruginosa*: comparison of PhaC1 and PhaC2. FEMS Microbiology Letters, 157, 155–162.

Quemeneur, M., Marty, Y., 1992. Sewage influence in a macrotidal estuary: fatty acid and sterol distributions. Estuarine, Coastal and Shelf Science, 34, 347–363.

Rajendran, N., Matsuda, O., Rajendran, R., Urushigawa, Y., 1997. Comparative description of microbial community structure in surface sediments of eutrophic bays. Marine Pollution Bulletin, 34, 26-33.

Ramos, C. S., Parrish, C. C., Quibuyen, T. A. O., Abrajano, T. A., 2003. Molecular and carbon isotopic variations in lipids in rapidly settling particles during a spring phytoplankton bloom. Organic geochemistry, 34, 195-207.

Ratledge, C.,Wilkinson, S. G., 1988. Microbial Lipids Vol. 1, Academic Press, San Diego.

Reitan, K. I., Rainuzzo, J. R. Olsen, Y., 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. Journal of Phycology 30, 972–979.

Rejil.T., 2013. Microalgal vegetation in the selected Mangrove Ecosystem of Kerala. Ph.D Thesis submitted to the Cochin University of Science and Technology.

Rontani, J. F., Bonin, P. C., Volkman, J. K., 1999. Biodegradation of free phytol by bacterial communities isolated from marine sediments under aerobic and denitrifying conditions. Applied and Environmental Microbiology, 65, 5484–5492.

Rontani, J. F., Christodoulou, S., Koblizek, M., 2005. GC-MS structural characterization of fatty acids from marine aerobic anoxygenic phototrophic bacteria. Lipids, 40, 97-108.

Saliot, A., Laureillard, J., Scribe, P., Sicre, M. A., 1991. Evolutionary trends in the lipid biomarker approach for investigating the biogeochemistry of organic matter in the marine environment. Marine Chemistry, 36, 233-248.

Salomonovả, S., Lamaḉovả, J., Rulik̛, M., Rolḉik̛, J., Cˇap, L.,…. Bednả ŗ̄, P., 2003. Determination of phospholipid fatty acidsin sediments. Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium, Chemica, 42, 39–49.

Sañé, E., Isla, E., Pruski, A. M., Bárcena, M. A., Vétion, G., DeMaster, D., 2011. Diatom valve distribution and sedimentary fatty acid composition in Larsen Bay, Eastern Antarctica Peninsula. Continental Shelf Research, 31, 1161-1168.

Sanilkumar, 2009. Microalgae in the Southwest Coast of India. Thesis submitted to the Cochin University of Science and Technology.

Sassen, R., 1977. Early diagenesis of fatty acids in mangrove peats, St Croix, U.S. Virgin Island. significance with respect to petroleum genesis. In Proceedings, Third International Coral Reef Symposium, University of Miami, 2, 136-147.

Schlüter, A., Yubero, P., Iglesias, R., Giralt, M., Villarroya, F., 2002. Short Communication, The chlorophyll-derived metabolite phytanic acid induces white adipocyte differentiation. International journal of obesity, 26, 1277-1280.

Shaw, P. M., Johns, R. B., 1985. Organic geochemical studies of a recent inner Great Barrier Reef sediment—I. Assessment of input sources. Organic Geochemistry, 8, 147–156.

Sikes, E. L., Uhle, M. E., Nodder, S. D., Howard, M. E., 2009. Sources of organic matter in a coastal marine environment: evidence from n-alkanes and their $\delta^{13}C$ distributions in the Hauraki Gulf, New Zealand. Marine Chemistry, 113, 149–163.

Simoneit, B. R. T., 1977. Diterpenoid compounds andother lipids in deep sea sediments and their geo- chemical significance. Geochimica et Cosmochimica Acta, 41, 463-476.

Simoneit, B. R. T., 1978. The organic chemistry of marine sediments. In: Riley JP, Chester R, editors. Chemical oceanography. Vol. 7, New York, Academic Press.

Slomp, C. P., Van Raaphorst, W., Malschaert, J. F. P., Kok, A., Sandee, A. J. J., 1993. The effect of deposition of organic matter on phosphorus dynamics in experimental marine sediment systems. Hydrobiologia, 253, 83–98.

Smith, D. J., Eglinton, G., Morris, R. J., 1983. The lipid chemistry of an interfacial sediment from the Peru continental shelf. Fatty acids, alcohols, aliphatic ketones and hydrocarbons. Geochimica et Cosmochimica Acta, 47, 2225–2232.

Stal, L. J., 2000. Cyanobacterial mats and stromatolites. In: Whitton, B.A., Potts, M. (Eds.), The Ecology of Cyanobacteria. Kluwer Academic Publishers, Dordrecht.

Sushchik, N. N., Kalacheva, G. S., Zhila, N. O., Gladychev, M. I., Volova, T. G., 2003. A temperature dependence of the intra- and extracellular fatty acid composition of green algae and cyanobacterium. Russian Journal of Plant Physiology, 50, 420–427.

Taylor, J., Parkes, R. J., 1983. The cellular fatty acids of the sulphate- reducing bacteria, Desulfobacter sp., Desulfobulbus sp., and Desulfovibrio desulfuricans. Journal of General Microbiology, 129, 3303–3309.

Taylor, J., Parkes, R., 1985. Identification of different populations of sulphate-reducing bacteria with marine sediment systems, using fatty acid biomarkers. Journal of General Microbiology, 131, 631-642.

Teoh, M. L., Chu, W. L., Marchant, H., Phang, S. M., 2004. Influence of culture temper- ature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. Journal of Applied Phycology, 16, 421–430.

Thoumelin, G., Bodineau, L., ….Wartel, M., 1997. Origin and transport of organic matter across the Seine estuary: Fatty acid and sterol variations. Marine Chemistry, 58, 59-71.

Tolosa, I., Vescovali, I., LeBlond, N., Marty, J. C., de Mora, S., Prieur, L., 2004. Distribution of pigments and fatty acid biomarkers in particulate matter from the frontal structure of the Alboran Sea (SW Mediterranean Sea). Marine Chemistry, 88, 103-125.

Vainshtein, M., Hippe, H., Kroppenstedt, R. M., 1992.Cellular fatty acid composition of Desulfovibrio species and its use in classification of sulfate-reducing bacteria. Systematic and Applied Microbiology, 15, 554–566.

Vargas, M. A., Rodriguez, H., Moreno, J., Olivares, H., Del Campo, J. A., Rivas, J., Guerrero, M. G., 1998. Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. Journal of phycology, 34, 812-817.

Vascovsky, V. E., Khotimchenko, S. V., Xia, B., Hefang, L., 1996. Polar lipids and fatty acids of some marine macrophytes from the Yellow Sea. Phytochemistry, 42, 1347–1356.

Vestal, J. R., White, D. C., 1989. Lipid analysis in microbial ecology. Bioscience, 535-541.

Viso, A., Marty, J., 1993. Fatty acids from 28 marine microalgae. Phytochemistry, 34, 1521-1533.

Volkman, J. K., 1986. A review of sterol markers for marine and terrigenous organic matter. Organic Geochemistry, 9, 83–99.

Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L., Gelin, F., 1998. Microalgal biomarkers: A review of recent research developments. Organic Geochemistry, 29, 1163–1179.

Volkman, J. K., Eglinton, G., Corner, E. D. S., Sargent, J. R., 1980. Novel unsaturated straight-chain C 37 to C 39 methyl and ethyl ketones in marine sediments and a coccolithophore Emiliania huxleyi. Physics and Chemistry of the Earth, 12, 219-227

Volkman, J. K., Jeffrey, S. W., Nichols, P. D., Rogers, G. I., Garland, C. D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology, 128, 219-240.

Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., Peterson, M. J., 1997. Molecular indicators of diagenetic status in marine organic matter. Geochimica et Cosmochimica Acta, 61, 5363-5369.

Waterson, E. J., 2005. Sources of sedimentary organic matter in the Mississippi River and adjacent Gulf of Mexico. PhD thesis submitted to The College of William and Mary

Weckesser, J., Drews, G., Mayer, H., 1979. Lipopolysaccharides of photosynthetic prokaryotes. Annual Review of Microbiology, 33, 215-239.

Wieland, A., Kühl, M., McGowan, L., Fourçans, A., Duran, R., Caumette, P., ... Herbert, R. A., 2003. Microbial mats on the Orkney Islands revisited: microenvironment and microbial community composition. Microbial ecology, 46, 371-390.

Zimmerman, A. R., Canuel, E. A., 2001. Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. Estuarine, Coastal and Shelf Science, 53, 319-341.

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Chapter 7

TRITERPENIODS AND FATTY ALCOHOLS AS BIOMARKERS

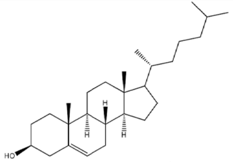
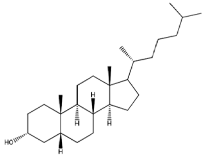
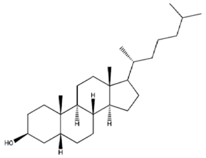
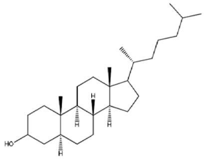
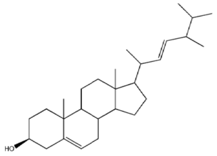
Contents

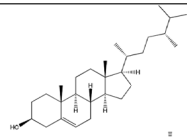
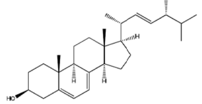
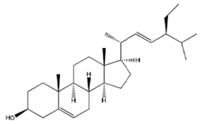
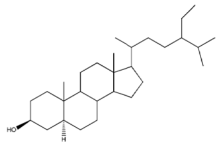
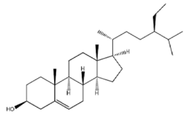
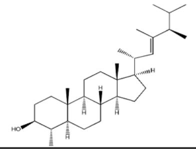
7.1 Introduction
7.2 Results
7.3 Discussion
7.4 Conclusion

7.1 Introduction

Fatty acids, especially saturated, Monounsaturated (MUFA), Polyunsaturated (PUFA), hydroxyl and branched are effective in tracing the origin of organic matter in the marine environments. However, disparities are often observed while evaluating the origin of carbon sources solely on the basis of fatty acid data. These limitations of fatty acids are the direct consequence of change in the concentrations of biomarkers, due to the degradation by heterotrophs (Smith et al., 1983). Considering these facts, multiple biomarker approach is an effective method to characterize the sources of sedimentary organic matter. In this regard, numerous organic compounds have been identified as biomarkers to evaluate organic carbon input. However, Pentacyclic triterpenoids/sterols due to their widespread distribution in the tissues of marine and terrestrial plants, structural diversity, and long-term stability (Volkman, 1986; Yunker et al., 1995; Mudge and Norris, 1997; Munoz et al., 1997) has been identified as an apt choice to full fill the vision of this thesis.

Table 7.1 Major sterol used as biomarkers

Common Name	Structure	Molecular Formula	IUPAC Name	Source
Cholesterol		C27H46O	cholest-5-en-3β-ol	Animal sterol, widely distributed in nature
epi-Coprostanol		C27H48O	Cholestan-3-ol, (3α,5β)-	Present in sewage sludge
Coprostanol		C27H48O	Cholestan-3-ol, (3β,5β)-	Human faecal marker
Cholestanol		C27H48O	chloestan-5α-ol	Diagentic product of cholesterol by microbial reduction in anaerobic condition
Brassicasterol		C28H46O	24-methylcholesta-5,22-dien- 3β-ol	Algal biomarker

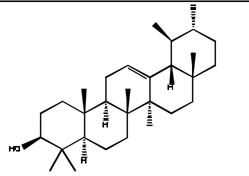
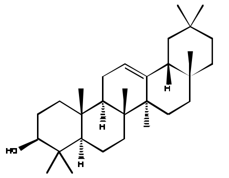
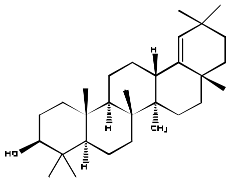
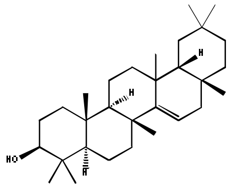
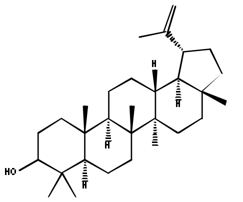
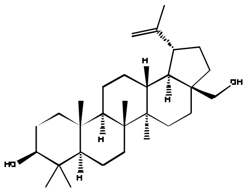
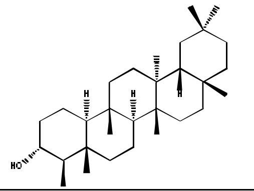
Campesterol		$C_{28}H_{48}O$	24-Methylcholest-5-en-3β-ol	Higher plant origin
Ergosterol		$C_{28}H_{44}O$	24-Methyl-5α-cholestan-3β-ol	Fungal origin
Stigmasterol		$C_{29}H_{48}O$	24- Ethylcholest-5, 22-dien-3β-ol	Higher plant origin, also synthesised by certain Algal groups
Stigmastanol		$C_{29}H_{52}O$	24-ethyl-cholestan-ol	Algal sterol biomarker, also found in reducing environments
β sitosterol		$C_{29}H_{50}O$	24-Ethylcholest-5-en-3β –ol	Higher plant origin
Dinosterol		$C_{30}H_{52}O$	4α,23,24-Trimethyl-5α-cholest-22-en-3β-ol	Dinoflagellates marker

Steroids, a modified version of triterpenoids, provide vital information regarding the flora and fauna, which contribute to the carbon budget of the marine environment (Rice et al., 1986). Structural features of steroids such as double bonds, extent of methylation in the ring, and side chain alkylation are regulated by the biosynthetic pathways (Volkman, 1986; Steudler et al., 1977). Hence, information regarding these details of the steroids/triterpenoids structures plays a vital role in unravelling the biogenic materials and food chain prevailing in the system membranes (Kulshreshtha et al., 1972; Moyna et al., 1983; Killops and Frewin, 1994; Dodd et al., 1995; Rafii et al., 1996; Wollen weber et al., 1999; Tolosa et al., 2003; Koch et al., 2003). Since, each species specifically synthesize certain sterols in response to the conditions prevailing in the environment, for example, richness of cholesterol in erigstigmatophtes, 24-methl- cholesta 5,22E –diene-3β-ol by diatoms and mixture of 4- desmethl and 4-methl sterols in dinoflagellates (Volkman, 1986). Sitosterol, Campesterol and Stigmasterol are tracers of terrestrial input (Hartmann, 1998; Moreau et al., 2002). Major sterols used as biomarkers are listed in Table 7.1.

Contribution of higher plants to organic carbon turnover of the marine environment can be easily identified and estimated using triterpenoids alcohols (ten Haven and Rullkotter, 1988). For example, C30 pentacyclic triterpenoids with six member E ring is a specific marker for angiosperm organic matter (Killops and Frewin, 1994; Otto and Wilde, 2001; Moldowan et al., 1994; Versteegh et al., 2004; Xu et al., 2006). Even then, these data should be correlated with the information deduced from other biomarkers, because, depending on the oxic and anoxic conditions prevailing in the environment, susceptibility of triterpenoid alcohols to get transformed to triterpenoid ketones, alkenes, and aromatic ketones have been observed (Simoneit, 2008). In Table 7.2

major pentacyclic triterpenoids used as higher plant biomarkers are listed.

Table 7.2 Major Pentacyclic triterpenoids of higher plant origin

Common Name	Structure	MolecularFormula
α-Amyrin		$C_{30}H_{50}O$
β-Amyrin		$C_{30}H_{50}O$
Germanicol		$C_{30}H_{50}O$
Taraxerol		$C_{30}H_{50}O$
Lupeol		C30H50O
Betulin		$C_{30}H_{50}O2$
Friedelan-3α-ol		$C_{30}H_{52}O$

The information provided by fatty alcohols also used in this chapter to distinguish between terrestrial and marine sources. Terrestrial plants biosynthesize fatty alcohols, of which long chain moieties of even chain length ranging from C22 – C32 were found in their waxy coating. Whereas, marine organism biosynthesize fatty alcohols in the range of C-14 to C-16 (Meyers and Ishiwatari, 1993). Even though, bacteria also synthesize fatty alcohols <C20, presence of odd chain length along with branching, is a unique feature which distinguish bacterial fatty alcohols from terrestrially synthesized/derived fatty alcohols by plants (Zhang et al., 2004). Fatty alcohols also give vital information regarding carbon input by zooplankton and diatoms. For example, presence of nC22:1 and nC18:1, classes of monounsaturated fatty alcohols are indicative of zooplankton and diatoms respectively (Sargent and Lee, 1975; Berge et al., 1995).

Finally, biomarkers are not only useful in delineating contribution by flora and fauna to the environment but also in assessing the load of urban pollution and waste water discharge (Vivian, 1986; Eganhouse et al., 1988; Venkatesan et al., 1998). For example, Coprostanol (5β-cholesta -3β-ol), epi-coprostanol, and 24 ethyl Coprostanol are used to delineate the impact/ contribution of human fecal matter, sewage sludge, and herbivore fecal inputs respectively (Bothner et al., 1994; Pratt et al., 2007). Hence, this chapter, multiple biomarker approach, using triterpenoids/steroids and fatty alcohols are opted to deduce the biogenic (marine versus terrestrial) and urban input (Walker et al., 1982) in the mangrove environment.

7.2 Results

7.2.1 Fatty alcohol

Analysis of Fatty alcohols in the sediment samples of five mangrove ecosystems were carried out using GC-MS and were identified by the comparison of mass spectra with literature and library data, and interpretation of mass spectrometric fragmentation patterns. Fatty alcohols in the range C12-C32 were detected in the present study. Both odd and even chains were detected, for short-chain n-fatty alcohols in the range C12-C18, and were dominated by C16 in all samples. But, even chained compounds were dominated in the case of Long chain fatty alcohols. Fatty alcohols identified from the present study are listed in Table 7.3.

At Kunjimangalam, in all season, C16 was the dominant among all short Chain n-fatty alcohols (>10 %), both odd and even chain fatty alcohols in the range C12-18 were identified in post-monsoon. However, fatty alcohols in the range C18-C26 were exclusively even chained. Moderate dominance of short chain fatty alcohols prevailed in post-monsoon season was found to be shifted to long chain fatty alcohols in pre-monsoon and monsoon. Hence, the distribution of n-fatty alcohols was seemed to be influenced by seasonal factors. In pre-monsoon both even and odd chain fatty alcohols except C15 and C23 (<C26) were detected. Pre-monsoon was characterized by higher % abundance of long-chain n-fatty alcohols such as C22 (5.92 %), C28 (11.27 %) and C30 (7.79 %). In monsoon n-fatty alcohols such as C12, C19 and C25 were absent. A higher % abundance for C28 (9.38 %) and C30 (11.06 %), that is moderate dominance of long chain compounds can also be seen in monsoon. Multi methyl branched fatty alcohols such as 6,10,14-Trimethyl-Pentadecan-2-ol and 3,7,11,15-Tetramethyl-2-Hexadecanol were also detected from this station. However, though 6,10,14-Trimethyl-Pentadecan-2-ol was detected in all the three seasons 3,7,11,15-Tetramethyl-2-Hexadecanol was identified only in monsoon season.

Table 7.3: Fatty alcohols detected in the present study

	Kunjimangalam			Pazhayangadi			Pappinissery			Thalassery			Kadalundi		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
C12	†	†		†	†				†				†		
C13	†	†	†	†	†										
C14	†	†	†	†	†	†	†	†	†		†	†			†
C15	†		†	†	†	†	†	†	†		†	†		†	†
C16	†	†	†	†	†	†	†	†	†		†	†		†	†
C17	†	†	†	†	†	†	†		†		†	†			†
C18	†	†	†	†	†	†	†	†	†		†	†		†	†
C19		†							†						
C20	†	†	†	†	†	†	†	†	†		†	†		†	†
C21		†												†	†
C22	†	†	†	†	†	†	†	†	†		†	†		†	†
c23			†			†						†		†	†
C24	†	†	†	†	†	†	†	†	†		†	†		†	†
C25		†			†							†		†	
C26	†	†	†	†	†	†	†	†	†		†	†		†	†
C28		†	†	†	†	†		†	†		†	†		†	†
C30		†	†			†		†	†		†	†		†	
C32		†	†	†								†		†	
C20:1									†						
C18:1							†								
Phytol	†	†	†	†	†	†	†	†	†		†	†		†	†
6,10,14-TrimethylL-Pentadecan-2-oL	†	†	†	†	†										
3,7,11,15-Tetramethyl-Hexadecan-2-ol			†												

1 post-monsoon 2 pre-monsoon 3 monsoon

Sediment samples from Pazhayangadi mangrove ecosystem were abundant in long-chain n-alcohols irrespective of the seasons. In Pazhayangadi C19 and C21 n-alkanols were absent. All n-Alcohols in the range C12-18 were present in post-monsoon and pre-monsoon while C12 and C13 could not be detected in monsoon. Appreciably higher % abundance was noticed for C24 (11.23 to 21.81 %), C26 (13.75 to 15.03 %) and C28 (16.81 to 21.13 %) in all seasons. 6,10,14-Trimethyl-Pentadecan-2-ol, a multimethyl branched n-fatty alcohol was also detected from Pazhayangadi in post-monsoon and pre-monsoon.

In Pappinissery, dominance of short-chain n-fatty alcohols was observed in post-monsoon, but this was shifted to long even chain n-fatty alcohols in pre-monsoon and monsoon. However, characteristic higher abundance of C16, 13.79 %, 22.33 % and 13.27 % in post-monsoon pre-monsoon and monsoon respectively, was observed. Pre-monsoon and monsoon also related with higher abundance of long even chain n-fatty alcohols like C28 and C30 (>10 %). Unsaturated fatty alcohol such as C20:1 and C18:1 was identified in monsoon and post-monsoon respectively. Abundance C20:1 is much lower while that of C18:1 much higher in the Pappinissery mangrove ecosystem.

In pre-monsoon sample of Thalassery, both odd and even chain n-fatty alcohols in the range C14 to C18 were present while only even chains were detected in the range C18 to C30. Pre-monsoon was characterized by higher abundance of C16 (21.50 %), C22 (9.77 %) and C28 (9.61 %). n-Fatty alcohols in monsoon season was predominated in long chain compounds such as C26 (11.09 %), C28 (32.97 %) and C30 (17.33 %). Fatty alcohols could not be detected from Thalassery in post-monsoon.

In Kadalundi n-fatty alcohols could not be detected in post-monsoon with the exception of negligibly lower abundance of C12. In pre-monsoon, percentage abundance of long even chain fatty alcohols such as C24 (9.19 %), C26 (8.21 %), C28 (23.58 %) and C30 (10.31 %) were higher. In monsoon, also % abundance was higher for long-chain fatty alcohols (C20-C28).

Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), of which substantial higher percentage abundance varied from 6.72 to 52.41 % in the present study, was detected in all samples except for Kadalundi and Thalassery in post-monsoon. Comparatively lower % abundance was noticed in Pazhayangadi and Thalassery in monsoon, Pappinissery in pre-monsoon and monsoon and Kadalundi in pre-monsoon (<20 %). Monsoon season was characterized by lower % abundance of phytol in all stations except Kadalundi.

7.2.2 Triterpenoids

A total of 20 triterpenoids including 13 sterols were identified in the present study region and are listed in Table 7.4 and 7.5.

From Kunjimangalam, a total of 15, 7 and 8 triterpenoids were identified in post-monsoon, pre-monsoon and monsoon respectively, out of which 11, 3 and 3 respectively were sterols. Germanicol, α- Amyrin, Betulin, Stigmastanol and Dinosterol were identified from Kunjimagalam in all the three seasons, while Cholesterol was detected in post-monsoon and pre-monsoon. Coprostanol, epi-coprostanol, Cholestanol, Brassicasterol, Campesterol, Stigmasterol and 24-ethyl delta (22) coprostanol were detected in post-monsoon and Urs-12-ene-3-one was extracted in monsoon. β- sitosterol and β-Amyrin were co-eluted in post-monsoon and monsoon contributing 28.88% and 26.25% respectively. β-Amyrin, Germanicol and Dinosterol contributed

25.88 %, 20.90% and 20.17% respectively in pre-monsoon. In monsoon Germanicol (29.57 %) and Lupeol (17.59 %) showed higher abundance.

Table 7.4: Sterols detected in the study region

	Kunjimangalam			**Pazhayangadi**			**Pappinissery**			**Thalassery**			**Kadalundi**		
	1	2	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Coprostanol	+				+		+					+		+	
Epicoprostanol	+				+			+							
24-Nor-22,23-methylene cholest-5en-3beta-ol														+	
Cholesterol	+	+		+	+		+	+		+	+	+		+	+
Chloestanol	+			+	+		+	+				+		+	+
Brassicosterol	+			+			+	+				+		+	
Campesterol	+			+			+					+		+	
Both sitosterol and amrin	+		+	+	+						+	+		+	
Stigmasterol	+			+	+		+	+		+	+	+		+	+
24-ethyl delta (22) Coprostanol	+													+	
β sitosterol										+					
Stigmastanol	+	+	+				+	+				+			+
Dinosterol	+	+	+											+	+

1: Post-monsoon, 2: Pre-monsoon, 3:Monsoon

Total number of triterpenoids and its subset sterol derivative for Pazhayangadi in post-monsoon, pre-monsoon, monsoon were found 11, 11, 5 and 6, 6, 0 respectively. Presence of α-Amyrin and β-Amyrin were detected in all the three seasons. Cholesterol, Cholestanol, Stigmasterol, Betulin and Urs-12-en-3-one were detected in post-monsoon and pre-monsoon and Taraxerol was detected in pre-monsoon and monsoon. Campesterol and Brassicasterol were detected in post-monsoon. Epi-coprostanol, and Coprostanol were detected only in the pre-monsoon. Germanicol was the highest contributor in post-monsoon and monsoon (56.92 and 55.30 %). β- sitosterol and β-Amyrin were found to be co-eluted in post-monsoon and pre-monsoon contributing 19.14 % and 75.33 % respectively. But in monsoon β- sitosterol was absent and β-Amyrin contributed 16.93 %.

Among 11, 11, 4 triterpenoids detected in Pappinissery, 6, 6, 0 were sterols in post-monsoon, pre-monsoon and monsoon respectively. Germanicol, β-Amyrin, α- Amyrin and Betulin were extracted in all the three seasons. Cholesterol, Cholestanol, Brassicasterol, Stigmasterol and Stigmastanol were detected during post-monsoon and pre-monsoon season from Pappinissery. Coprostanol and campesterol detected in post-monsoon season, cholestan-2-one and epi-Coprostanol were detected during pre-monsoon season. β-amyrin (11.63 %, 23.23 % and 24.32 % in post-monsoon, pre-monsoon and monsoon) followed by Germanicol (13.08 %, 13.37 % and 29.55 % in post-monsoon, pre-monsoon and monsoon) showed appreciable abundance in the present study. Betulin (35.71 % and 27.14 % respectively) also showed much higher abundance in post-monsoon and monsoon.

Table 7.5: Triterpenoids detected in the present study

	Kunjimangalam			**Pazhayangadi**			**Pappinissery**			**Thalassery**			**Kadalundi**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
cholestan-2-one								+							
Urs-12-en-3-one			+	+	+										
Germanicol	+	+	+	+		+	+	+	+		+			+	+
β-Amyrin		+				+	+	+	+						+
Taraxerol					+	+									
α-Amyrin	+	+	+	+	+	+	+	+	+		+	+		+	+
Lupeol						+								+	
Betulin	+	+	+	+	+		+	+	+	+	+			+	+

1: Post-monsoon, 2: Pre-monsoon, 3:Monsoon

Total number of triterpeniods detected in Thalassery in post-monsoon, pre-monsoon and monsoon were 4, 6, 10 respectively, of these a total of 3, 2, 8 were sterols. Monsoon season showed the presence of Coprostanol, Cholestanol, Brassicasterol, Campesterol and Stigmastanol in Thalassery. Stigmasterol and cholesterol were detected in all the three seasons in which Stigmasterol showed considerable abundance in all the three seasons (>15 %). Betulin (13.40 to 17.70 %) showed appreciable abundance in post-monsoon and pre-monsoon while the Co-elutent peak of β-sitosterol and β-Amyrin (23.79 to 33.88 %) were showed much higher abundance in pre-monsoon and monsoon season. Germanicol (25.97 %) was also detected in pre-monsoon season with appreciable abundance.

Triterpenoids couldn’t be detected from Kadalundi during post-monsoon season. A total of 15, 9 triterpenoids were identified in pre-monsoon and monsoon respectively, of these 10, 5 were sterols. Cholesterol, Cholestanol, Stigmasterol, Germanicol, Dinosterol, Betulin and α- Amyrin were present in pre-monsoon and monsoon. Pre-monsoon showed the presence of Coprostanol, 24-Nor-22,23-methylene cholest-5en-3beta-ol, Campesterol, Brassicasterol, Lupeol and 24-ethyl delta (22) Coprostanol. Monsoon season showed the presence of stigmastanol and β- Amyrin. Major contributors were β-sitosterol and β- Amyrin (22.69 %) followed by Betulin (17.48 %) in pre-monsoon and β- Amyrin (30.36%), followed by Betulin (22.31%) and Germanicol (19.41%) in the monsoon.

7.3 Discussion

7.3.1 Fatty Alcohols as biomarker

It is generally accepted that the dominant long even chain fatty alcohols indicate vascular input (Cranwell et al., 1987). n-Fatty alcohols ranged from nC12 to nC32 with a high abundance of long chains (C22–C30) were found in the present study. Signals for vascular plant input were detected for all samples except Pappinissery in post-monsoon.

n-Alkanols in the range C14 to C16 are synthesized by marine organisms(Meyers and Ishiwatari, 1993). Short chain n-fatty alcohols in all samples showed the predominance of C16.

The presence of odd chain saturated fatty alcohols <C18 in most of the samples suggests the bacterial activity (Mudge and Norris, 1997). However, % abundance of C13 was fairly low in the present study. Branched fatty alcohols extracted in the present study i.e., 6,10,14-Trimethyl-Pentadecan-2-ol and 3,7,11,15-Tetramethyl-2-Hexadecanol implies the presence of bacterial inputs (Parkes, 1987).

In Kadalundi during post-monsoon, only C12 n- alcohol was detected. The C-12 n-alcohol may possibly be derived from zooplankton or bacteria feeding on zooplankton fecal pellets. n-C18:0 was present in all samples, which is the major n-fatty alcohols in herbivorous aquatic fauna (Robinson et al., 1984). The n-C20:1 monounsaturated alcohols in Pappinissery during monsoon season suggests the presence of calanoid copepods (Tolosa et al., 2003). Unsaturated n-fatty alcohols, except for C20:1, could not be detected from the study region suggesting either low contribution or degradation under oxygenated condition. The monounsaturated n-C18:1 fatty alcohol, which has been found in some species of diatoms (Berge et al., 1995) was also detected from Pappinissery in post-monsoon.

Fukushima and Ishiwatari (1984) used the L/S ratio (ratio of Long chain to short chain fatty alcohols) on the assumption that chain length distributions may reflect the input of fatty alcohols from particular source organisms. The long/short chain ratio is greater than 1.0 except for Pappinissery and Kunjimagalam in post-monsoon and for Thalassery in pre-monsoon (Fig 7.1). Predominance of longer chain compounds than the shorter chain indicated that n-fatty alcohols from mangrove vegetation dominate over other sources. Dominance of planktonic as well as microbial input resulted in to lower ratio of long chain to short chain fatty alcohols.

The short chain compounds undergo degradation faster than the longer chain compounds since bacteria preferentially utilized shorter chain compounds. The longer chain compound may also degrade to shorter chain compounds with time, however, in general, the class of alcohols >C20 are better preserved in sediments than the <C20 class.

Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), a potential marker for phototrophic organisms and can also be generated in the water column by hydrolysis of chlorophyll (Baker and Louda, 1983), digestive processes of copepods (Shuman and Lorenzen, 1975) and senescence of diatoms (Jeffrey, 1974) while it was lower in plant detritus (Nichols et al., 1982). No structural modifications occurred significantly for phytol during its passage through the copepod guts (Prahl et al., 1984). But during the biomarker extraction, saponification of the sample will release the phytol from chlorophyll into the solvent (Tolosa et al., 2003). Therefore, the phytol in the present study region also represents chlorophyll in the water column from the terrestrial and marine environment.

Phytol is present in all samples except Thalassery and Kadalundi in post-monsoon season. Its % abundance was higher in Kunjimangalam during post-monsoon and was lower in Thalassery during monsoon.

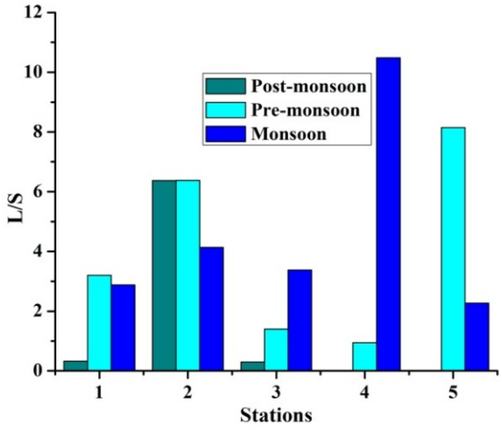


Fig 7.1: Variation of Long chain to Short chain fatty alcohols in the present study

7.3.2 Triterpenoids as vascular plant indicators

Pentacyclic triterpenoids and sterols are common chemical constituents of terrigenous plants (Kulshreshtha et al., 1972; Pant and Rastogi, 1979; Das and Mahato, 1983) and have generally been used to characterise sources of sedimentary organic matter (Volkman, 1986; Yunker et al., 1995; Mudge and Norris, 1997; Munoz et al., 1997).

Phytosterols like Sitosterol, Campesterol and Stigmasterol were detected in the present study. Even though 24-ethyl sterols have been considered as terrigenous origin, it was also detected in various algal species, such as prymnesiophytes, chlorophytes, green algae, dinoflagellates and cyanobacteria (Gagosian and Farrington, 1978; Boon et al., 1983; Volkman, 1986; Nichols et al., 1989; Volkman et al., 1990). Stigmasterol was found widely distributed (10 out of 15 observations) in the present study with significant abundance. The presence of Stigmasterol has been reported in *Avicenna* (Mahera et al., 2011), *Rhizophora* mangle leaves (Killops and Frewin, 1994) and *Barringtonia racemosa* (Sun et al., 2006). In the present study region, *Avicenna –Rhizophora* combinations are dominant species;

hence application of Stigmasterol as mangrove biomarker is justified in the present study. Higher abundance of Stigmasterol, except in Kadalundi and Thalassery in Post-monsoon was corroborated to litter fall of mangroves.

Koch et al., 2003 analysed biomarker composition of three mangrove species (*R. mangle, A. germinans and L. racemosa*) along with two herb species and found that mangrove species could be clearly identified by their biomarker composition, especially using β-sitosterol. In the present study β-sitosterol was found to be co-eluted with Pentacyclic triterpenoid β-Amyrin and the abundance of co-eluted peak was comparatively high in the present study, and may be attributed to mangrove litter addition. Pure β-sitosterol signal was observed only in Thalassery, in pre-monsoon. Campesterol (24-methylcholesterol) was found in five observations with low abundance. Campesterol, which has been considered as a higher-plant marker (Volkman, 1986), but often encountered with green algae, diatoms, dinoflagellates and mosses (Matsumoto et al., 1985). However, on account of vascular plant signals from n-alkanes and fatty alcohols, it is conceivable that these phytosterols in the present study are mainly contributed by mangrove vegetation.

A part from these sterols, triterpenoid alcohols are also abundant in mangroves (Wannigama et al., 1981) and in higher plants (some peat-forming plants; Pancost et al., 2002). Several studies of mangrove systems in South America (Koch et al., 2003; Boot et al., 2006), Florida (Killops and Frewin, 1994) and Victoria, Australia (Wannigama et al., 1981) have suggested that triterpenoid alcohols might be useful markers for identifying mangrove derived organic matter in sediments. They are diagenetically stable, if any is possible, it can retain the characteristic structures of their precursors such that their abundance in marine sediments could be used as a tracer for mangrove swamps on adjacent coasts (Killops and Frewin, 1994; Versteegh et al., 2004). Triterpenoids with a single hydroxyl group (α- and β- amyrin, germanicol and Taraxerol) are usually employed as terrestrial plant biomarkers.

Trimethyl silyl ester of Germanicol was identified using mass spectrum which shows characteristics peaks at 73, 95, 109, 177, 189, 204, 231 and 498. Germanicol, a triterpenoid compound was reported as a biomarker for organic matter derived from *Rhizophora* mangle (Koch et al., 2003; Killops and Frewin, 1994). The presence of this compound in sediments can therefore be regarded as vascular plant input especially from mangrove vegetation. Germanicol was found to be widely distributed and was detected in 11 observations with comparatively high abundance. So in the environmental condition prevailed in Kerala either Germanicol produced with appreciably higher amount or it is well preserved. From the above inference it could be assumed that Germanicol can be used as a biomarker for in the present study region.

Taraxerol is a biomarker commonly found in mangrove environments (Wannigama et al., 1981; Versteegh et al., 2004). But use of this compound as quantitative mangrove marker has been questioned (Versteegh et al., 2004; Boot et al., 2006), as it is degraded to pentacyclic ketones and alkenes without detecting significant amounts of these degradation products in the total neutral extracts from the sediments. In the present study, Taraxerol was reported only from 2 samples (Pazhayangadi pre-monsoon and monsoon) with negligible lower abundance. Since the presence of Taraxerol could not be detected in all samples its use as biomarker is restricted in the present study.

In the leaf cuticle of *Rhizophora* mangle, β-Amyrin and Taraxerol were found to be major component of epicuticular wax and cutin respectively (Killops and Frewin, 1994). α- and β-Amyrin were abundant in the mangroves and has been detected in the marine as well as freshwater sediments. β-amyrin were detected in most of the samples either as co-eluted peak or pure, hence it can be deliberately used as mangrove biomarker in the present study. Even though α- Amyrin detected in 13 samples, its abundance was fairly low, suggesting it can only be used as tracer for terrestrial input in the present study.

Koch et al., 2011, analysed for biomarker composition of three major species *R. mangle* (red mangrove), *A. germinans* (black mangrove) and *Laguncularia racemosa* (white mangrove), and lupeol detected in all three species, but it was only minor in *R. mangle*. Lupeol detected in 3 out of 15 observations that restrict its application as mangrove biomarker in the present study.

Olean-12-en-3-one (β-amyrenone) was identified from 3 samples (Pazhyangadi post-monsoon and pre-monsoon and Kunjimangalam monsoon) in the present study. β-Amyrenone and α-amyrenone are frequent constituents of higher plants, notably in epicuticular waxes and in the tree bark of angiosperms (Corbet et al.,1980; Tulloch, 1982; Otto and Simoneit, 2001). β-Amyrenone and α-amyrenone could also be formed by oxidation of the corresponding β-amyrin and α-amyrin (Corbet et al., 1980).

In the present study Betulin was identified by comparing with mass spectral data of trimethyl silyl derivative which showed major peaks at 73, 189, 203, 279, 393, 483, and 496 with M+ 1 peak at 589. Betulin an important Pentacyclic triterpenoid diol of the lupine series, has been reported in the mangroves *Rhizophora mucronata* (Ghosh et al., 1985) and *Avicennia germinans* (Koch et al., 2003). Betulin has been reported as a major constituent in the German Sea sediments and its source could be traced back to peat deposits (Volkman et al., 2000). Betulin was detected in 11 out of 15 samples. The earlier reports suggested that Betulin could be degraded quickly (Koch et al., 2005), but from the present study it can extracted from 11 samples with appreciable abundance. So we can conclude that Betulin is produced with much higher abundance in the present study region even though its use as biomarker needs further research.

β-Amyrin positively correlated with Stigmasterol (R^2=0.98,n=4) and Germanicol (R^2=0.93, n=7) indicating that source and fate of these compounds were almost similar. α-Amyrin was positively correlated with Lupeol (R^2=0.86, n=8), β-sitosterol + β- Amyrin co-eluted (R^2=0.99, n=5) peak and clay (R^2=0.74, n=13). This correlation analysis indicated that source and fate of these triterpenols and phytosterols are similar.

7.3.3 Sterols as algal biomarkers

It is widely accepted that C28-sterol 24-methylcholesta-5,22E-dien-3β-ol (brassicasterol) is a diatom biomarker, but is also detected in other algal groups (Volkman, 1986) including dinoflagellates (Goad and Withers, 1982), haptophytes and prymnesiophytes (Volkman et al., 1998). Presence of brassicasterol was detected in at least six sediment samples (Pappinissery in post-monsoon and pre-monsoon, Pazhayangadi and Kunjimangalam in post-monsoon, Thalassery in monsoon and Kadalundi in pre-monsoon). A 4-methyl sterols, 4α,23,24R-trimethyl-5α-cholest-22E-en-3β-ol (C30 sterol) termed as Dinosterol has been widely regarded as a marker for dinoflagellates (Robinson et al., 1984; Volkman et al., 1993). It is the major sterol in many dinoflagellates (approx. I1 of 18 species; Withers, 1983) with some exceptions (Amphidinium spp; Kokke et al., 1981) and is also present in diatoms in minor quantities (Volkman et al., 1993). Dinosterol was detected from Kunjimangalam in all seasons and from Kadalundi in pre-monsoon and post-monsoon. Comparatively higher % abundance of Dinosterol was detected from Kunjimangalam in pre-monsoon. Comparatively high % abundance of dinosterol could be correlated with dissolved oxygen super saturation (DGS), reported from water column produced by excessive growth of algae. So it is reasonable to suppose that DGS is produced by dinoflagellates. But, dinoflagellates abundance, which could not be observed from fatty acid composition on account of heterotrophic or aerobic utilisation, is now established.

7.3.4 Sterols as indicators of anthropogenic activities as well as redox status

Molecular biomarkers are widely used as a tool for assessing urban pollution, waste water discharge (Vivian, 1986; Eganhouse et al., 1988; Venkatesan et al., 1998) and indicator of redox status in coastal as well as marine systems.

Cholesterol is considered to be a major sterol in marine zooplankton that can be formed from the dietary alteration of phytosterols (Volkman, 1986; Volkman et al., 1987). Cholesterol was also detected in terrestrial fauna and other organisms (diatom, microbial communities, macrophytes, algae and phytoplankton) (Logan et al., 2001; Reeves and Patton, 2001), though in small amounts (Volkman, 1986).In the present study cholesterol was detected in almost all samples, but its abundance was much lower. It is widely accepted that cholesterol is most abundant and ubiquitous in the environment, due to the variety of sources present (Pratt et al., 2008). The higher concentrations of phytosterols compared to cholesterol detected in the present study region suggesting lower contribution of animal-derived detritus and fecal material, and is in good agreement with Tolosa et al., 2003,. Also, cholesterol found in lower amount in the present study, so it could not be used as biomarker due to its non-specific origin.

5α-cholestanol, a common biohydrogenation product of cholesterol (Burns et al., 2004) was detected from 8 sediment samples, but its abundance was fairly low. The ratio of stanols to sterols is indicative of the degree of degradation or microbial processes in the sediments (Killops and Killops, 2013). Cholestanol to Cholesterol ratio was ranged from 0.45 to 10.70 in the present study. The ratio showed greater than one in as few as two samples, Kunjimangalam in post-monsoon (1.63) and Pappinissery in pre-monsoon (10.70). Dissolved oxygen was found to be negatively correlated with Cholestanol (R^2=-0.82, n=8), hence it could be inferred that Cholestanol is an anoxic indicator in the present study.

Stigmastanol, generally an algal biomarker, was also found in reducing environments (Pratt et al., 2007), and was detected from seven observations. These include Pappinissery in pre-monsoon and monsoon, Thalassery and Kadalundi in monsoon and Kunjimangalam throughout the seasons. Though Stigmasterol was absent at Kunjimangalam during pre-monsoon and monsoon season, stigmastanol present. Either algal input or faster degradation of its sterol counterpart due to sandy nature of sediment could be assumed in the present study. Stigmastanol to Stigmasterol ratio was considerably low in the present study (0.29-0.95). Various ratios employed in the present study are given in Table 7.6. Stigmastanol and Stigmasterol were found to be positively correlated

(R^2=0.97, n=5) suggesting the mechanism of Stigmastanol formation is more closely related to reducing environment than algal production.

Following Killops and Killops (2005), the concentrations of three stanols (cholestanol, campestanol and stigmastanol) to their corresponding sterol concentrations (cholesterol, campesterol, stigmasterol) was calculated to assess the reducing nature of the system, ranging from 0.11 to 1.97, with three samples showing the ratio >1 (Pappinissery in pre-monsoon, Kunjimangalam in pre-monsoon and post-monsoon). From the above observation it can be inferred that a reducing environment was prevailed in these stations.

The presence of 5β stanols (Coprostanol and Epi-Coprostanol) in sediments is often taken as evidence for the presence of fecal derived organic matter (Nishimura, 1982). Coprostanol (5β-cholestan-3β-ol) is a C_{27} stanol formed from the biohydrogenation of cholesterol (cholest-5en-3β-ol) in the gut of higher animals and birds; hence this compound can be used as a biomarker human fecal matter and domestic waste in the environment (Walker et al., 1982; Bothner et al., 1994). In the present study, Coprostanol was detected in five samples (post-monsoon-Pappinissery and Kunjimangalam, pre-monsoon-Pazhayangadi and Kadalundi, monsoon-Thalassery) though in lower abundance. Epi-coprostanol (5β-cholestan-3α-ol) can be used as an indicator for non- point sources of sterols (Maldonado et al., 2000) but also indicate anoxic mud flats (Reeves and Patton, 2001). In the present study, Epi-coprastanol was observed for the sample taken from Pappinissery and Pazhayangadi in pre-monsoon and Kunjimangalam in post-monsoon. Anoxicity in the water column was noticed in Pappinissery in pre-monsoon due to dumping of poultry waste which is a non-point source to the mangrove ecosystem.

Table 7.6: Sterol ratios calculated from %abundance

	Kunjimangalam			**Pazhayangadi**			**Pappinissery**			**Thalassery**			**Kadalundi**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Cholestanol/Cholesterol	1.63			0.49	0.57		0.81	10.70				0.45		0.50	0.79
Coprostanol/epi-coprostanol	1.72				0.83										
Coprostanol/Epi-coprostanol	0.37				0.26		0.36	1.53				1.06		1.11	0.00
Coprostanol+Epi-Coprostanol/Dinosterol	0.28													0.75	0.00
Coprostanol/Cholestanol	0.08				0.32		0.19					0.57		0.26	0.00
Coprostanol/Cholesterol	0.12				0.18		0.15					0.26		0.13	0.00
Coprostanol/cholesterol+Cholestanol	0.05				0.12		0.08					0.18		0.09	0.00
stigmastanol/sterol	0.95						0.51	0.88				0.29			0.95
Total stanol/sterol	1.04	1.71		0.11	0.22		0.59	1.97				0.32		0.25	0.89

Marine mammalian feces also contain Coprostanol and Epi-Coprostanol but have higher percentage of epi-coprostanol (Venkatesan and Santiago, 1989; Venkatesan and Kaplan., 1990). In the present study both were detected from Pazhayangadi in pre-monsoon and Kunjimagalam in post-monsoon. The ratio of Coprostanol to Epi-Coprostanol close to or larger than one (greater proportion of coprostanol) attributed to human wastes and was 0.83 for Pazhayangadi in pre-monsoon and 1.72 for Kunjimagalam in post-monsoon. Percentage abundance of Coprostanol and Epi-Coprostanol were very of low in the present study region indicated lower contribution of sewage pollution to the mangrove ecosystems. Coprostanol/cholesterol threshold is 0.2 which indicates natural cholesterol degradation in aerobic environments (Pratt et al., 2007). The total Cholest-5β-stanols (Coprostanol+epi-coprostanol) to Dinosterol ratio was very low in the present study region indicates appreciably lower contribution of sewage discharges (Venkatesan and Kaplan, 1990).

The major human biomarker ratio 0.7 (Coprostanol to Cholestanol ratio) initially proposed by Grimalt et al., 1990 is thought to be too high to be useful in tropical regions because of the higher water temperatures and faster degradation rates in these climate zones by a study conducted in Malaysia and Vietnam (mean surface water temperatures of 29.4 and 28.6 °C respectively (Isobe et al., 2002). Pratt et al., 2007 redefined the ratio threshold as 0.3 which indicates the origin of 5α-stanols from human fecal material. But the temperature in the present study region is generally is in the range 28^0C to 33^0C (Rejil, 2013), so chances are there for higher degradation rate of sterols. 5β (H) stanols can also formed under highly reducing conditions and its presence can be considered as an indicative of reducing condition

(Venkatasan and Santiago, 1989). Both Coprostanol and Cholestanol were detected only from five samples (Pappinissery and Kunjimangalam during post-monsoon, Pazhayangadi and Kadalundi during pre-monsoon, Thalassery monsoon). Fairly higher values of this ratio were obtained for the samples from Pazhayangadi (0.32) and Thalassery (0.57), indicating human faeces contamination.

24-ethyl delta (22) coprostanol was detected from Kadalundi and Kunjimangalam in pre-monsoon and post-monsoon respectively. The compounds were identified by comparison with mass spectral data of silyl derivative and showed major peaks at 257, 346, 353, 374, 396, 486. Reduction of sterols to stanols can be occurred both in sediments and in particulate matter in the water column in the early stage of diagenesis by microbially mediated process under anaerobic conditions, (Wakeham, 1989). This transformation proceeds via ketone (stanone) intermediates or by the direct reduction of the C=C bond and yields predominantly 5α-stanols (Mackenzie et al., 1982), with much lower amounts of 5β-stanols. However, 5α - stanols (e.g. cholestanol) can also reach the sediment, from partial heterotrophic transformation of algal sterols and from direct inputs from marine organisms such as diatoms (Volkman et al., 1990). Cholest-2-one was detected from Pappinissery during pre-monsoon season.

The elevated triterpenoids in Pazhayangadi and Pappinissery during pre-monsoon is consistent with the increased organic carbon content at this station and reflects a better preservation of organic matter in the anoxic regime (Venkatesan and Kaplan, 1990).

7.4 Conclusion

Phyto-sterols like Stigmasterol, Campesterol and β-sitosterol were detected in the present study region, among them Stigmasterol was widely detected. The presence of other Triterpenoids like Taraxerol, Lupeol, Germanicol, β-Amyrin, α-Amyrin and Betulin were also identified in the present study region which are higher plant indicators, derived mainly from mangroves. In the present study Germanicol, Stigmasterol, α-Amyrin and Betulin can be used as biomarker for mangroves since it was extracted with appreciable concentration. Along with these, algal sterols like Dinosterol and Brassicasterol were also detected indicating the presence of diatoms and dinoflagellates. Dinosterol was detected with higher abundance in Kunjimangalam during pre-monsoon season and can be correlated with DO super-saturation noticed in the water column. Cholesterol was detected from all samples with low abundance indicating lower input from zooplankton. Transformation products of cholesterol such as Coprostanol, epi-coprostanol and Cholestanol were detected with low % abundance, and are generally used as the indicators of the human fecal marker, sewage marker and anaerobic reduction product respectively. Cholestanol was negatively correlated with DO, and hence can be used as an anoxic indicator in the present study. Sterol to stanol ratios was higher in Pappinissery during pre-monsoon season indicate anoxic character of the systems which allows better preservation of organic matter. Phytol was detected in almost all samples with higher concentration contributed both from the living material and those released during alkaline hydrolysis involved in the biomarker extraction. Both short chains, as well as long chain n-fatty alcohols, were detected indicated the inputs from micro-organism as well as terrestrial sources. The long/short chain ratio is greater than 1.0 in most of the samples indicating dominant input from mangrove plants.

Reference

Baker, E. W., Louda, J. W., 1983. Thermal aspects in chlorophyll geochemistry. In Advances in Organic Geochemistry 1981. Wiley Chichester.

Berge, J. P., Gouygou, J. P., Dubacq, J. P., Durand, P., 1995. Reassessment of lipid composition of the diatom, Skeletonemacostatum.Phytochemistry, 39, 1017–1021.

Boon J. J., Hines H., Burtingame A. L..Klok J., Rijpstra W. I. C., Leeuw J. W. de, Edmunds K. E., Eglinton G., 1983. Organic geochemical studies of Solar Lake laminated cyanobacterial mats. In Advances in Organic Geochemistry 1981 (Edited by Bjoroy M. et al.), Wiley, New York.

Boot, C. S., Ettwein, V. J., Maslin, M. A., Weyhenmeyer, C. E., Pancost, R. D., 2006. A 35,000 year record of terrigenous and marine lipids in Amazon Fan sediments. Organic Geochemistry, 37, 208–219.

Bothner, M. H., Takada, H., Knight, I. T., Hill, R. T., Butman, B., Farrington, J. W., ...Grassle, J. F., 1994. Sewage contamination in sediments beneath a deep-ocean dump site off New York. Marine Environmental Research, 38, 43-59.

Brassell, S. C., Eglinton, G., Maxwell, J. R., 1983. The geochemistry of terpenoids and steroids. Biochemical Society Transactions, 11, 575-586.

Burns, K. A., Greenwood, P., Benner, R., Brinkman, D., Brunskill, G., Codi, S., Zagorskis, I., 2004.Organic biomarkers for tracing carbon cycling in the Gulf of Papua (Papua New Guinea).Continental shelf research, 24, 2373-2394.

Corbet, B., Albrecht, P., Ourisson, G. J., 1980. Photo- chemical or photomimetric fossil terpenoids in sediments and petroleum. Journal of American Chemical Society, 102, 1171-1173.

Cranwell, P. A., Eglinto, G., Robinson, N., 1987. Lipids of aquatic organisms as potential contributors to lacustrine sediments--II. Organic Geochemistry, 11, 513-527.

Das, M. C., Mahato, S. B., 1983. Review: Triterpenoids. Phytochemistry, 22, 1071–1095.

Dodd, R. S., Fromard, F., Rafii, Z. A., Blasco, F., 1995. Biodiversity among West African Rhizophora: foliar wax chemistry. Biochemical Systematics and Ecology, 23, 859–868.

Eganhouse, R. P., Olaguer, D. P., Gould, B. R., Phinney, C. S., 1988. Use of molecular markers for the detection of municipal sewage sludge at sea. Marine environmental research, 25, 1-22.

Fukushima, K., Ishiwatari, R., 1984. Acid and alcohol compositions of wax esters in sediments from different environments. Chemical Geology, 47, 41-56.

Gagosian, R. B., Farrington, J. W., 1978. Sterenes in surface sediments from the southwest African shelf and slope. Geochimica et Cosmochimica Acta, 42, 1091-1101.

Ghosh, A., Misra, S., Dutta, A.K., Choudhury, A., 1985. Pentacyclic triterpenoids and sterols from seven species of mangrove. Phytochemistry, 24, 1725–1727.

Gillan, F. T., Hogg, R. W., Drew, E. A., 1984. The sterol and fatty- acid compositions of 7 tropical seagrasses from North Queensland, Australia. Phytochemistry, 23, 2817–2821.

Goad, L. J., Withers, N., 1982. Identification of 27-nor-(24R)-24-methylcholesta-5, 22-dien-3β-ol and brassicasterol as the major sterols of the marine dinoflagellates Gymnodinium simplex. Lipids, 17, 853–8.

Grimalt, J. O., Ferna´ndez, P., Bayona, J. M., Albaige´s, J., 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environmental Science and Technology 24, 357-363.

Hartmann, M. A., 1998. Plant sterols and the membrane environment. Trends in Plant Science, 3, 170–175.

Isobe, K. O., Tarao, M., Zakaria, M. P., Chiem, N. H., Minh, L. Y., Takada, H., 2002. Quantitative application of fecal sterols using gas chromatography-mass spectrometry to investigate fecal pollution in tropical waters: Western Malaysia and Mekong Delta, Vietnam. Environmental science & technology, 36, 4497-4507.

Jeffrey, S. W., 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Marine biology, 26, 101–110.

Killops, S. D., Frewin, N. L., 1994. Triterpenoid diagenesis and cuticular preservation. Organic Geochemistry, 21, 1193–1209.

Killops, S. D., Killops, V. J., 2013. Introduction to organic geochemistry. John Wiley & Sons.

Killops, S., Killops, V., 2005. Introduction to Organic Geochemistry, 2nd edn. Geofluids, 5, 236-237.

Koch, B. P., Harder, J., Lara, R. J., Kattner, G., 2005. The effect of selective microbial degradation on the composition of mangrove derived Pentacyclic triterpenols in surface sediments. Organic Geochemistry, 36, 273-285.

Koch, B. P., Rullkötter, J., Lara, R. J., 2003. Evaluation of triterpenols and sterols as organic matter biomarkers in a mangrove ecosystem in northern Brazil. Wetlands Ecology and Management, 11, 257–263.

Koch, B. P., Souza Filho, P. W., Behling, H., Cohen, M. C., Kattner, G., Rullkötter, J., ... Lara, R. J., 2011. Triterpenols in mangrove sediments as a proxy for organic matter derived from the red mangrove (Rhizophora mangle). Organic Geochemistry, 42, 62-73.

Kokke, W. C. M. C., Fenical W., Bohlin L., Djerassi, C., 1981. Sterol synthesis by cultured zooxanthellac: implications concerning sterol metabolism in the host- symbiotic association in Caribbean gorgonians. Comparative Biochemistry and Physiology - Part B, 68, 281-287.

Kulshreshtha, M. J., Kulshreshtha, D. K., Rastogi, R. P., 1972. The triterpenoids. Phytochemistry, 11, 2369–2381

Logan, G. A., Hinman, M. C., Walter, M. R. M., Summons, R. E., 2001. Biogeochemistry of the 1640 Ma McArthur River (HYC) lead–zinc ore and host sediments, Northern Territory, Australia. Geochimica et Cosmochimica Acta, 65, 2317– 2336.

Mackenzie, A. S., Brassell, S. C., Eglinton, G., Maxwell J. R., 1982. Chemical fossils: the geological fate of steroids. Science 217, 491-504.

Mahera, S. A., Ahmad, V. U., Saifullah, S. M., Mohammad, F. V., Ambreen, K., 2011. Steroids and triterpenoids from grey mangrove Avicennia marina. Pakistan Journal of Botany, 43, 1417-1422.

Maldonado, C., Venkatesan, M. I., Phillips, C. R., Bayona, J. M., 2000. Distribution of trialkylamines and coprostanol in San Pedro shelf sediments adjacent to a sewage outfall. Marine Pollution Bulletin, 40, 680-687.

Matsumoto, source hi, Kanda, Matsumoto holds Suites, Emulgenki, kann the inter, Hikaruro Silicone., 1985. Hydrocarbons, sterols and hydroxy acids in Antarctic mosses. Antarctic data, 87, 23-31.

Meyers, P. A., Ishiwatari, R., 1993. Lacustrine organic geochemistry: an overview of indicators of organic matter sources and diagenesis in lake sediments. Organic Geochemistry, 20, 867– 900.

Moldowan, J. M., Dahl, J., Huizinga, B. J., Fago, F. J., Hickey, L. J., Peakman, T. M., Taylor, D. W., 1994. The molecular fossil record of oleanane and its relation to angiosperms. Science, 265, 768–771.

Moreau, R. A., Whitaker, B. D., Hicks, K. B., 2002. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Progress in Lipid Research 41, 457–500.

Moyna, P., Heinzen, H., Laborde, E., Ramos, G., 1983. Epicuticular wax of Colletiaparadoxa. Phytochemistry, 22, 1283–1285.

Mudge, S. M., Norris, C. E., 1997. Lipid biomarkers in the Conwy Estuary (NorthWales, U.K.): a comparison between fatty alcohols and sterols. Marine Chemistry, 57, 61–84.

Muñoz, D., Guiliano, M., Doumenq, P., Jacquot, F., Scherrer, P., Mille, G., 1997. Long term evolution of petroleum biomarkers in mangrove soil (Guadeloupe). Marine Pollution Bulletin, 34, 868–874.

Nichols, P. D., Palmisano, A., Rayner, M. S., Smith, G. A., White, D. C., 1989. Changes in the lipid composition of Antarctic sea-ice diatom communities during a spring bloom: an indication of community physiological status. Antarctic Science, 1, 133-140.

Nichols, P. D., Klumpp, D. W., Johns, R. B., 1982. Lipid components of the seagrasses *Possidonia australis* and *Heterozostera tasmanica* as indicators of carbon source. Phytochemistry, 21, 1613–1621.

Nishimura, M., 1982. 5β-isomers of stanols and stanones as potential markers of sedimentary organic quality and depositional paleoenvironments. Geochimica et Cosmochimica Acta, 46, 423-432.

Otto A., Simoneit B. R. T., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz formation, Saxony, Germany. Geochimica et Cosmochimica Acta, 65, 3505-3527.

Otto, A., Wilde, V., 2001.Sesqui-, di-, and triterpenoids as chemosystematic markers in extant conifers—a review. Botanical Review, 67, 141–238.

Pancost, R. D., Baas, M., van Geel, B., Sinninghe-Damste´, J., 2002. Biomarkers as proxies for plant inputs to peats: an example from a sub-boreal ombrotrophric bog. Organic Geochemistry, 33, 675–690.

Pant, P., Rastogi, R. P., 1979. The Triterpenoids. Phytochemistry, 18, 1095–1108

Parkes, R. J., 1987. Analysis of microbial communities within sediments using biomarkers. In: Ecology of Microbial Communities, SGM 41. Cambridge University Press, London.

Prahl, F. G., Eglinton, G., Corner, E. D. S., O'Hara, S. C. M., Forsberg, T. E. V., 1984. Changes in plant lipids during passage the gut of Calanus. Journal of the Marine Biological Association of the United Kingdom, 64, 317–334.

Pratt, C., Warnken, J., Leeming, R., Arthur, J. M., Grice, D. I., 2007. Detection of intermittent sewage pollution in a subtropical, oligotrophic, semi-enclosed embayment system using sterol signatures in sediments. Environmental science & technology, 41, 792-802.

Pratt, C., Warnken, J., Leeming, R., Arthur, M. J., Grice, D. I., 2008. Degradation and responses of coprostanol and selected sterol biomarkers in sediments to a simulated major sewage pollution event: A microcosm experiment under sub-tropical estuarine conditions. Organic Geochemistry, 39, 353-369.

Rafii, Z. A., Dodd, R. S., Fromard, F., 1996. Biogeographic variation in foliar waxes of mangrove species. Biochemical Systematics and Ecology, 24, 341–345.

Reeves, A. D., Patton, D., 2001. Measuring change in sterol input to estuarine sediments. Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere, 26, 753-757.

Rejil.T, 2013. Microalgal vegetation in the selected Mangrove Ecosystem of Kerala. Thesis submitted to the Cochin University of Science and Technology.

Rice, A. L., Billett, D. S. M., Fry J., John W. G., Lampitt, R. S., Mantoura, R. F. C., Morris, R. J., 1986. Seasonal deposition of phytodetritus to the deep-sea floor. Royal Society of Edinburgh, 88B, 265-279.

Robinson, N., Eglinton, G., Brassel, S. C., Cranwell. P. A., 1984. Dinoflagellates origin for sedimentary 4α-methyl steroids and 5α(H)-stanols. Nature, 308, 439-442.

Sargent, J. R., Lee, R. F., 1975. Biosynthesis of lipids in zooplankton from Saanich Inlet, British Columbia, Canada. Marine Biology, 31, 15–23.

Shuman, F. K., Lorenzen, C. J., 1975. Quantitative degradation of chlorophyll by a marine herbivore. Limnology and Oceanography, 20, 580–586.

Simoneit, B. R., 2008. Natural products as biomarker tracers in environmental and geological processes. Selected Topics in the Chemistry of Natural Products, 77-126.

Smith, D. J., Eglinton, G., Morris, R. J., 1983. The lipid chemistry of an interfacial sediment from the Peru continental shelf. Fatty acids, alcohols, aliphatic ketones and hydrocarbons. Geochimica et Cosmochimica Acta, 47, 2225–2232.

Steudler, P. A., Schmitz, F. J., Ciereszko, L.S., 1977. Chemistry of coelenterates. Sterol composition of some predator–pray pairs on coral reefs. Comparative Biochemistry and Physiology, B 56, 385–392

Sun, H. Y., Long, L. J., Wu, J., 2006. Chemical constituents of mangrove plant Barringtoniaracemosa. Journal of Chinese medicinal materials, 29, 671-672.

ten Haven H. L., Rullkrtter J., 1988. The diagenetic fate of taraxer-14-ene and oleanene isomers. Geochimica et Cosmochimica Acta, 52, 2543-2548.

Tolosa, I., LeBlond, N., Copin-Montégut, C., Marty, J. C., de Mora, S., Prieur, L., 2003.Distribution of sterol and fatty alcohol biomarkers in particulate matter from the frontal structure of the Alboran Sea (SW Mediterranean Sea).Marine chemistry, 82, 161-183.

Tulloch, A. P., Hoffman, L. L., 1982. Epicuticular wax of Cirsiumarvense. Phytochemistry, 21, 1639-1642.

Venkatesan, M. I., Chalaux, N., Bayona, J. M., Zeng, E., 1998. Butyltins in sediments from Santa Monica and San Pedro basins, California. Environmental pollution, 99, 263-269.

Venkatesan, M. I., Kaplan, I. R., 1990. Sedimentary coprostanol as an index of sewage addition in Santa Monica Basin, Southern California. Environmental Science & Technology, 24, 208-214.

Venkatesan, M. I., Santiago, C. A., 1989. Sterols in ocean sediments: novel tracers to examine habitats of cetaceans, pinnipeds, penguins and humans. Marine Biology, 102, 431-437.

Versteegh, G. J. M., Schefub, E., Dupont, L., Marret, F., Sinninghe Damste , J. S., Jansen, J. H. F., 2004. Taraxerol and Rhizophora pollen as proxies for tracking past mangrove ecosystems. Geochimica et Cosmochimica Acta, 68, 411-422.

Vivian, C. M. G., 1986. Tracers of sewage sludge in the marine environment: A review. Science of the total environment, 53, 5-40.

Volkman J. K., Kearney P. S, Jeffrey S. W., 1990. A new source of 4-methyl sterols and 5~(H)-stanols in sediments: prymnesiophyte microalgae of the genus Pavlova. Organic Geochemistry, 15, 489-497.

Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L., Gelin F., 1998. Microalgal biomarkers: A review of recent research developments. Organic Geochemistry, 29, 1163–1179.

Volkman, J. K., Barrett, S. M., Dunstan, G. A., Jeffrey, S. W., 1993. Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom. Organic Geochemistry, 20, 7–15.

Volkman, J. K., Rohjans, D., Rullkötter, J., Scholz-Böttcher, B. M., Liebezeit, G., 2000. Sources and diagenesis of organic matter in tidal flat sediments from the German Wadden Sea. Continental Shelf Research, 20, 1139-1158.

Volkman, J. K., Farrington, J. W., Gagosian, R. B., 1987. Marine and terrigenous lipids in coastal sediments from the Peru upwelling region at 153S: Sterols and triterpene alcohols. Organic Geochemistry, 11, 463-477.

Volkman, J. K., 1986. A review of sterol markers for marine and terrigenous organic matter. Organic Geochemistry, 9, 83–99.

Wakeham S. G., I987. Steroid geochemistry in the oxygen minimum zone of the eastern tropical North Pacific Ocean. Geochimica et Cosmochimica Acta, 51, 3051-3069.

Wakeham, S. G., 1989. Reduction of stenols to stanols in particulate matter at oxic–anoxic boundaries in sea water. Nature, 342, 787-790.

Walker, R. W., Wun, C. K., Litsky, W., Dutka, B. J., 1982. Coprostanol as an indicator of fecal pollution. Critical Reviews in Environmental Science and Technology, 12, 91-112.

Wannigama, G. P., Volkman, J. K., Gillan, F. T., Nichols, P. D., Johns, R. B., 1981. A comparison of lipid components of the fresh and dead leaves and pneumatophores of the mangrove *Avicennia marina*. Phytochemistry, 20, 659-666.

Withers, N., 1983. Dinoflagellates sterols. In Marine Natural Products (Edited by Schener E.), Academic Press, New York.

Wollen weber, E., Doerr, M., Siems, K., Faure, R., Bombarda, I., Gaydou, E. M., 1999. Triterpenoids in lipophilic leaf and stem coatings. Biochemical Systematics and Ecology, 27, 103–105.

Xu, Y., Mead, R., Jaffė, R., 2006. A molecular marker based assessment of sedimentary organic matter sources and distributions in Florida Bay. Hydrobiologia, 569, 179-192.

Yunker, M. B., Macdonald, R.W., Veltkamp, D.J., Cretney, W. J., 1995. Terrestrial and marine biomarkers in a seasonally ice-covered Arctic estuary - integration of multivariate and biomarker approaches. Marine Chemistry, 49, 1–50.

Zhang, J. Z., Fisher. C. J., Ortner, P. B. 2004. Potential availability of sedimentary phosphorus to sediment resuspension in Florida Bay. Global Biogeochemical Cycles, 18.

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Chapter 8

SUMMARY AND CONCLUSIONS

Mangrove forest biomes, which are considered as most productive and complex ecosystems on the tropical region, are largely seen in saline and intertidal sediments. These systems are very complex in the sense they receive organic matter from autochthonous production by mangroves, bacteria and phytoplankton as well as allochthonous input from tidal inundation and riverine sources. Thus, organic content of varying nature is deposited along with fine sediment and is preserved in the intertidal mangrove sediment and undergo various chemical, physical and biological process, or transformations. Hence, mangrove sediment record information about various biogeochemical, biological process prevailed in these systems. The organic carbon that is trapped by these systems in larger amount acts as a potential carbon sink; hence have a role in reducing greenhouse effect.

Even though mangroves have a major role in global carbon climate, studies on these ecosystems are limited in Indian Territory especially in northern Kerala coast that shares about 83 % of the total mangrove density of the state. Five mangrove ecosystems on the northern Kerala, namely Kunjimangalam, Pazhayangadi, Pappinissery, Thalassery and Kadalundi, were selected for the present study. These ecosystems lie in the shorelines on the Indian side of Lakshadweep Sea. The Mangrove swamps are unique ecosystem, also each mangrove ecosystem is unique in its own depository environment, variation in tidal inundations etc, from others. Temporal variation also plays an important role in the fate and diagenesis of the organic matter in these ecosystems. Thus, source characterization of organic matter in these systems has valid significance in understanding its fate and diagenesis, and was carried out by stable carbon isotopes, biochemical analysis and biomarker analysis.

Water quality parameters give the first-hand information on the on-going processes of the system. In Kunjimangalam, comparatively higher salinity, chlorophyll and DO was noticed in pre-monsoon season owing to DO super-saturation. Compared to other stations, Thalassery and Kadalundi situated in the close proximity of Lakshadweep Sea and therefore prominent tidal activity can alter the salinity of these systems. The domestic sewage in Pappinissery was also reflected in the water quality parameters in pre-monsoon resulted in the depletion of dissolved oxygen and higher concentration of NH_4^+. Phytoplankton productivity in the present study region is independent of nutrient concentration, and it is evident from the poor correlation between chlorophyll pigments and nutrients.

General sedimentary characteristics and phosphorus fractionation are used for understanding the geochemical character of the system. Sediment texture was sandy in Kunjimangalam while in Pazhayangadi it is clayey. Thalassery also lies in sandy end of tri-linear diagram. Pappinissery and Pazhayangadi, which were closed in nature and less exposed to the tidal flow and turbulence is associated with higher amount of fine sediments. Tidal rhythm modifies the sediments in Kadalundi mangrove ecosystem, thus clayey texture in monsoon and post-monsoon is get modified to the sandy texture, in pre-monsoon. Thus, inorganic and organic constituents in the sediments can be considered as a function of textural characteristics.

Fractionation of sedimentary phosphorus was used to identify redox status of the system. Fe and P was correlated positively, confirming the prevalence of Fe(OOH)≈P. Higher Fe(OOH)≈P noticed in monsoon season in Kadalundi and Kunjimangalam while for all other stations it was during post-monsoon due to the presence of an oxic layer favoring phosphorus adsorption. Conversely, lower value of Fe(OOH)≈P in pre-monsoon, indicated anoxic character promoted by alkaline conditions and subsequent charge shift of Fe-oxides from positive to negative. Significant correlation of TOC and TS displayed in pre-monsoon also supported the above fact. Higher Acid-OP in pre-monsoon except for Kadalundi is taken as an input of organic material from microalgae. Ability of sediment humic acids to bind Phosphorus increases with decreasing salinity. Hence, Alkali-OP displayed significant seasonal variation with highest concentration in monsoon season. Positive correlation of Alkali-OP showed with Fe(OOH)≈P and Fe suggest organic-metal complex formation of Fe with humic acids and phosphate by ligand exchange reactions.

Elemental ratios in combination with stable carbon isotopes, biochemical composition and pigment analysis, is used for the source characterisation of the mangrove ecosystems. Since the mangrove litter addition is the major source in the study region high ratio was expected for TOC and TN, but it ranged from 5.99 to 20.60 only. It was found that the inference of inorganic nitrogen in post-monsoon and monsoon is one of the several causes, and is corrected considering inorganic nitrogen to fall in a better range (9.60 to 27.69).

Stable carbon isotope of the surface sediments ranged from -25 to -29.19 ‰ and was found to be enriched relative to mangrove plants by the substantial planktonic contribution from in situ production as well as marine sources. The correlation analysis of elemental ratio versus stable isotopic carbon displayed low correlation which also pointed towards the substantial input from microbial biomass. Depleted $\delta^{13}C$ values were observed from Pazhayangadi followed by Pappinissery and most enriched values from Kadalundi followed by Thalassery. Terrestrial contribution calculated from two end member mixing model (F %), showed the substantial contribution of terrestrial organic matter at Pazhayangadi and Pappinissery. Higher Pheopigments compared to Chl a in post monsoon and monsoon seasons indicated the presence of more detrital matter from the surrounding environment. The distribution of biopolymers such as carbohydrate, Lipids and Protein indicated the accumulation of aged or microbially modified organic matter.

From the above observations, it can be inferred that signatures from multiple sources get overlapped so that complete source characterisation is not possible by employing water quality parameters and $\delta^{13}C$ only; therefore the application of chemical biomarkers is employed. The molecular information extracted from biomarkers, which is more specific and sensitive, helps for characterization of organic matter with multiple or complex origin. Lipids can be used as effective biomarkers of organic matter in coastal systems, which represent an important component of organic matter with diverse compound classes (e.g., hydrocarbons, fatty acids, n-alkanols, and sterols).

Strong preference for long odd chain n-alkanes showed in high molecular weight range (>C_{25}) can be attributed to predominant contribution from higher plant source, particularly from mangrove litter addition in the present study. Also, the input of intense bacterial activity was represented by the presence of Unresolved Complex Matrix (UCM) up to C_{20} alkanes (except Kunjimangalam). The source characterisation based on variations in n-alkane homologue distributions are more informative when expressed using different indices such as terrigenous/aquatic ratio (TAR), carbon preference index (CPI), Average chain length (ACL), pristane/phytane, n-C17/pristane and n- C18/Phytane. The higher terrigenous/aquatic ratio (TAR) (> 2.0) in post-monsoon (except for Kunjimangalam and Kadalundi) showed the dominance of terrestrial input. Pazhayangadi showed strong terrestrial signal from CPI in all seasons reflecting the thick vegetation and/or clayey texture of the sediment leading to the better preservation of organic matter. The Average Chain Length (ACL) for the study sites ranged between 25.75 and 29.10, and most of the samples showed higher values indicating the better preservation of mangrove derived organic matter. The 17α(H),21β(H)-hopanes that ranged from C27 to C34 (C28 absent), were detected in the present study with less prominent 17β (H), 21α (H)-hopanes. Also, n-alkyl cyclohexanes is expected to be derived from the diagenetic alteration of algal or microbial fatty acids. The PCA (Principal Component Analysis) of n-alkanes showed terrestrial, planktonic algae, as well as bacterial input are the major source of organic matter.

n-Alkanes can predict the presence of certain group of organism such as algae, bacteria, plants etc., however higher taxonomical specificity can be achieved using fatty acids. Fatty acids, especially saturated, PUFA, branched and hydroxyl, are effective in tracing the origin of organic matter in the marine, as well as terrestrial environments. Long-chain saturated fatty acids are considered more refractory than unsaturated fatty acids, and their presence, together with the long-chain n-alkanes, reveals a significant input from vascular plant-derived organic matter. But the high value in the ratio of Short chain to Long chain fatty acids (SCFA/LCFA) in the present study may be due to the production of the low amount of LCFA by mangroves. Also, LCFA Palmitic acid (16:0) is contributed by mangroves, marine as well as bacterial sources overwhelming the higher fatty acid signatures from mangroves, hence the ratio is low.

The concentrations of poly unsaturated fatty acids (PUFAs) were lower in pre-monsoon except Pappinissery and Pazhayangadi due to its greater susceptibility to degradation by the active microbial community. Multimethyl branched fatty acids were detected in the present study region derived from bacterial fatty acids, uropygial waxes of the birds preen gland and by the oxidation of phytol in animal tissues. An increase in branched fatty acid concentrations and MUFAs are the indicatives of bacterial reworking in the present study region. The biomarkers of Desulfobulbus-type bacteria, methane-oxidizing bacteria, sulfur-reducing bacteria, Sulfur-oxidising bacteria, Gram-negative bacteria, waterborne Gram-negative pathogens and Iron-oxidising bacteria were also detected in the present study region. Algal biomarkers gave the presence of brown algae, cyanobacteria, red algae, dinoflagellates and diatoms in the study region. The presence of higher dicarboxylc acids and hydroxy fatty acids may be contributed from higher plant origin either by direct contribution or by diagenesis. PUFA a general phytoplankton marker, would not necessarily be expected to be preserved in their original amounts in the present study as a result of the liability by intensive bacterial activity.

Pentacyclic triterpenoids/sterols due to their widespread distribution in the tissues of marine and terrestrial plants, structural diversity, and long term stability fit their use as biomarkers. Phyto-sterols such as Stigmasterol, Campesterol and β-sitosterol and algal sterols such as Dinosterol and Brassicasterol were detected from the present study region. Also, presence of triterpenoids like Taraxerol, Lupeol, Germanicol, β-Amyrin, α-Amyrin, Betulin and Friedelan-3α-ol were also identified from the present study region indicating higher plant origin, derived mainly from mangroves. In the present study Germanicol, Stigmasterol and Betulin can be effectively used as biomarker for mangroves since they can be extracted from surface sediments with an appreciable amount.

Cholesterol was detected from all samples with low abundance indicating lower input from zoo-plankton. Stereoisomeric products formed from the biological and anaerobic reduction of cholesterol such as Coprostanol, epi-coprostanol and Cholestanol, which are indicators of the human fecal marker, sewage marker and anaerobic reduction product of cholesterol respectively, were detected with low % abundance. Cholestanol was found to be negatively correlated with dissolved oxygen, pointed to the fact it was an anoxic indicator in the present study region. Phytol was detected in almost all samples with higher concentration contributed both from the living material and those released during alkaline hydrolysis involved in the biomarker extraction. Both short chains as well as long-chain n-fatty alcohols were detected, indicating the inputs from micro-organism, as well as terrestrial sources. The long/short chain ratio is greater than 1.0 in most of the samples indicated dominant input from mangrove plants.

Specific information's such as dominance of planktonic input in Kadalundi and Thalassery, anthropogenic input in Pappinissery, excess Dinoflagellate growth in Kunjimangalam can be successfully explained using biomarker approach.

The degree to which organic matter preserved can be varied across mangrove systems, with a higher reliance on 'closed' systems where more of the organic production is retained and less exchange of material with adjacent systems is useful. Thalassery and Kadalundi situated in the close proximity of the Lakshadweep Sea and are open to tidal inundations and organic matter is entered and takes away into the system. In these stations, semi-diurnal tidal action is in the range 0.09 to 1.48 m, which creates conditions unfavourable to soil development, by remobilizing the organic particles such as leaf litter and nutrients further downstream. Tidal influx modified textural characteristics in Kadalundi, for which silt and muddy sand was dominant in post-monsoon, and was shifted to the sandy texture in pre-monsoon. Pre-monsoon is characterised by lower TOC, TN, TS, BPC, Al-OC, Chlorophyll pigments and fatty acids concentrations in Kadalundi and Thalassery due to the sandy texture. But the effect was less prominent at Thalassery. Thus, grain size played a major role in the distribution of organic matter in these stations.

In Kadalundi and Thalasseri, Chl a concentration was higher in post-monsoon and monsoon which may be due to greater input of marine-derived organic matter. Higher primary production produced in the Lakshadweep Sea is exchanged by tidal influx to the system. TOC/TN ratio at Thalassery in all seasons and Kadalundi in post-monsoon and monsoon showed predominance of planktonic input. Most enriched values of $\delta^{13}C$, higher chlorophyll pigment, and lower values of terrestrial derived organic matter calculated from two end member mixing model (F %) in Kadalundi in post-monsoon and monsoon also vindicate the clear contribution of marine organic matter.

Biomarker study also justified the dominant marine input of Kadalundi and Thalassery. From n-Alkane markers and various ratios employed in the concentration of various combination of short chain, long chain, odd, even, specific chain alkanes, predominance of planktonic input was identified. Dominance of algal alkane (C_{17}), non-photosynthetic bacteria (C_{26} to C_{30} showed no odd/even carbon predominance), high ratio of sum of Low chain (n-alkanes ≤ 20) to long-chain alkanes (≥ 21), CPI (Carbon preference index) ≈ 1 and lower TAR value are the observation which helped to deduce predominance of planktonic input in Thalassery and Kadalundi in post-monsoon and monsoon. Fatty acids in Kadalundi during Post-monsoon period was characterised by higher C14 concentration and $16{:}1\omega7/18{:}1\omega9$ along with low ratio of C18:1 to C16:1 that could be used as indicators of diatom dominated system. Fatty acid biomarkers of zooplankton and dinoflagellates, sterol biomarker for dinoflagellates (Dinosterol) can also be extracted from the system.

Anthropogenic intervention at Pappinissery, which received large quantity of domestic garbage and poultry waste resulting in the reducing environment, and recorded elevated concentration of nutrients (especially NH_4^+) and low DO. Also, bioturbation activities, that produce reducing sediment micro-pores resulting in the build-up of ammonium by the process of anaerobic ammonification, were active. Total Lipids, n-alkanes, n-alkyl cyclohexanes, fatty acids and sterols were also recorded higher concentration in pre-monsoon. Dissolved Oxygen in the water column may play a role in sediment OM composition so that minimum aerobic processes and benthic activity during the periods of anoxia/hypoxia in synchronous to an increase in anaerobic process results in a better preservation of organic matter. Bacterial fatty acids including those for anaerobic bacteria (eg: sulphate reducing bacteria) such as iC14, iC15, aC15 and iC16 iC17 and aC17, C17, C17:1, C18:1ω7c, β-hydroxy acids or 3-hydroxy acid were detected with comparatively higher concentration indicating active bacterial reworking. Cholestanol to Cholesterol and Total Sterols to Stanols ratios were recorded highest value at Pappinissery also indicated the reducing condition prevailed at this station. Epi-coprostanol (5β-cholestan-3α-ol) a non-point source indicator, a useful anoxic indicator, was also detected in the station. Moreover, the sample was characterised by clayey sediment texture, higher TOC and TN content. The increased net primary production and sewage pollution can also contribute organic matter to the system.

The Dissolved Oxygen Supersaturation (DGS-132 %) was noticed from the water column at Kunjimangalam in pre-monsoon. The station was also characterized by higher chlorophyll content, a slight depletion in $\delta^{13}C$ and F%, and all these results could be correlated to DGS. Dinosterol, a dinoflagellates biomarker was also detected with high % abundance. It could be also correlated with DGS and hence it can be expected to be produced by excessive dinoflagellates growth. But such dinoflagellates abundance could not observed form fatty acid composition. Since the process arose in the water column, dinoflagellates PUFAs does not necessarily become a part of the sediment record due to heterotrophic or aerobic utilisation. This result also substantiates the use of multi-marker approach used in the present study.

Complete process in the system could not be explained with the use of a single biomarker. Fatty alcohols and n-alkanes can effectively describe contribution of terrestrial organic matter and to some extent algal input. Fatty acids give better inference about bacterial, algal and zooplankton signals, even though, some of the PUFAs degraded by heterotrophic utilisation. Sterols provided better understanding of terrestrial signals, sewage pollution and DGS in the present study region. Thus, Biomarkers which used in the present study are proved successful, for obtaining signatures of individual organisms or groups of organisms, or of certain environmental processes.

In the present study fingerprinting of the mangrove ecosystem using elemental, stable carbon isotopes and biochemical composition along with multi-marker approach are used as a powerful tool to establish correct relationships between various processes. It could account for the various processes like tidal inundation, spatial and temporal variation, DGS, reducing environment, microbial activities prevailed in the study area. The study, which explores formation, transport, transformation of aged organic matter and recent changes in the surface sediment due to the anthropogenic inventions, can be useful for understanding global carbon climate and better policy making in the similar ecosystems. Biomarkers proposed other than in Indian Territory were also employed since reported works are very limited in Indian scenario. But environmental modifications in metabolites concentration are common, and the present study could not account for such environmental modifications, and is open for further research.

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Appendices

Appendix1: Variation of water quality parameters in the present study region

	post monsoon					pre monsoon					monsoon				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
pH	7.20	7.40	7.10	7.30	7.20	8.00	7.90	7.20	8.00	8.00	7.81	8.03	8.05	7.82	7.89
Salinity, ppt	8.21	7.67	4.26	9.25	4.26	35.97	33.97	29.31	34.30	34.70	0.73	0.71	0.24	11.14	26.64
DO, mg/L	3.84	4.90	2.86	5.31	6.41	9.34	2.38	0.51	5.09	5.26	3.76	6.86	5.22	5.88	7.35
Alkalinity, mg CaCO3/L	49.50	79.20	44.55	49.50	54.45	138.23	128.53	167.33	121.25	123.68	43.65	25.22	37.83	83.42	22.31
Nitrite, μM/L	0.15	0.28	0.22	0.22	0.55	0.59	0.29	0.61	0.70	0.99	0.42	0.33	0.46	0.99	0.31
Nitrate, μM/L	1.37	3.65	1.94	2.79	8.43	0.29	0.85	1.31	4.33	2.34	3.14	11.68	15.67	16.81	20.79
Ammonia, μM/L	4.73	10.24	22.85	27.95	25.21	7.88	0.79	98.09	5.12	0.00	5.52	48.45	80.36	69.33	7.09
Phosphate, μM/L	0.59	0.83	0.59	0.93	1.37	3.57	3.13	6.56	2.89	1.32	11.11	14.92	14.39	9.98	15.07
Silicate, μM/L	17.78	12.12	28.60	7.33	47.79	16.80	44.72	106.60	21.74	15.55	44.98	80.76	71.14	76.86	80.76
TDN, μM/L	34.55	53.35	36.75	42.50	63.69	91.99	38.19	102.78	32.74	10.26	138.88	186.50	179.88	124.78	188.38
TDP, μM/L	2.96	4.31	8.61	5.92	7.54	3.67	3.56	6.65	2.95	1.53	21.96	21.43	21.15	10.04	22.88
Chl a, μg/L	17.29	3.85	0.77	1.19	6.01	40.86	18.91	3.23	2.18	15.19	3.09	2.81	1.11	ND	0.36
Chl b, μg/L	0.09	0.17	0.38	ND	1.61	3.65	2.43	1.99	1.12	6.00	1.39	1.71	1.71	ND	ND
Chl c, μg/L	4.34	1.29	0.31	ND	1.93	13.80	6.21	3.37	1.70	7.43	0.05	2.44	0.87	ND	0.00
Phaeophytin, μg/L	31.01	24.59	4.28	4.01	25.93	22.27	10.52	1.98	1.01	7.64	3.31	23.20	1.23	ND	0.19
Mg, mg/L	269.50	174.32	251.67	540.23	65.44	1077.58	902.51	1362.80	1185.41	616.65	23.13	17.46	8.63	621.09	36.62
Ca, mg/L	41.85	95.24	172.51	98.56	21.78	164.41	453.13	300.75	148.37	196.49	14.44	9.62	8.02	108.27	14.44
Na, mg/L	1300.00	1000.00	988.85	2523.00	434.00	5300.00	7400.00	6200.00	5600.00	4000.00	150.00	110.00	50.00	2600.00	220.00
K,mg/L	41.36	58.00	58.75	105.00	18.00	170.00	320.00	270.00	200.00	160.00	6.90	6.00	3.30	130.00	10.50
Li, mg/L	0.40	0.20	0.60	1.00	0.20	1.60	3.20	3.20	2.00	2.00	ND	ND	0.20	1.40	0.20
So_4^2 mg/L	1086.50	371.82	235.24	855.86	425.25	4166.67	2466.67	1700.00	3533.33	4000.00	38.33	37.33	12.00	966.67	67.67
Iron mg/L	0.14	0.85	0.24	0.09	0.24	0.08	0.49	0.13	0.16	0.62	0.21	1.43	0.39	0.04	0.07

Appendix 2: Variation of General sedimentary characteristics and Phosphorous fractions in the present study region

	Post-monsoon					Pre-monsoon					Monsoon				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
sand, %	59.89	19.26	47.44	50.43	19.84	73	4.62	39.05	51.37	72.23	83.2	1.65	67.64	63.48	17.43
Clay, %	36.02	44.82	22.19	25.48	37.91	24.02	80.06	53.76	23.4	18.36	15.99	55.82	22.03	20.86	40.42
Silt, %	4.09	35.91	30.37	24.09	42.25	2.98	15.32	7.19	25.23	9.41	0.81	42.52	10.33	15.66	42.16
TOC, %	2.29	7.76	8.93	1.17	2.49	1.01	7.41	9.02	1.82	1.85	0.6	7.71	3.12	0.8	2.14
TN, %	0.12	0.52	0.52	0.16	0.29	0.07	0.41	0.58	0.12	0.09	0.1	0.46	0.26	0.1	0.26
TS, %	ND	0.27	1.99	0.45	0.22	ND	1.99	1.94	0.27	ND	0.21	2.23	0.54	0.15	0.18
TOC/TS	ND	28.73	4.49	2.6	11.31	ND	3.73	4.65	6.74	ND	2.85	3.46	5.77	5.3	11.9
TOC/TN	19.05	14.92	17.17	7.32	8.58	14.44	18.08	15.55	15.17	20.61	5.99	16.75	11.99	7.96	8.24
TOC/TN$_{corrected}$	10.21	23.60	20.44	27.21	11.86	14.44	18.08	15.55	15.17	20.61	12.90	19.88	27.69	9.60	13.78
Exchangable-P	4.17	3.46	6.31	1.98	2.37	5.70	6.39	9.21	4.65	10.05	4.80	5.84	6.51	12.80	14.78
Fe(OOH)≈P	90.73	468.37	284.31	263.69	508.42	101.95	202.34	204.71	36.50	154.97	176.74	351.48	197.47	235.23	557.70
CaCO3≈P	110.56	95.23	152.23	182.56	326.84	87.80	127.80	92.12	131.39	81.10	78.03	130.65	94.60	134.43	182.75
Acid-OP	35.10	89.38	93.35	49.45	113.21	105.65	194.89	159.67	90.33	71.10	93.53	118.34	53.12	55.29	70.29
Alkali-OP	19.76	333.23	184.72	76.06	370.55	77.85	189.70	57.57	111.20	85.46	329.74	850.59	158.09	145.49	905.22
Res-OP	12.44	54.47	38.63	17.66	55.42	13.90	91.85	59.11	26.00	38.29	10.79	84.32	27.82	15.28	51.12

Appendix 3: Variation of Biochemical components and Stable carbon isotopes in the present study region

	Post-monsoon					Pre-monsoon					Monsoon				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
CHO, mg/g	20.00	71.61	22.34	3.34	34.73	3.55	13.72	15.00	5.88	9.16	4.92	21.05	11.76	4.96	16.48
LPD, mg/g	4.06	7.53	6.56	2.35	1.43	1.03	7.14	8.35	1.63	1.63	1.93	13.01	3.06	2.37	2.52
PRT, mg/g	14.05	27.46	33.00	4.43	8.52	0.18	1.52	2.40	0.91	0.57	0.48	2.09	1.40	0.40	0.72
Chl a, μg/g	3.53	9.57	11.06	7.57	16.04	6.68	19.40	26.27	6.90	5.53	5.42	12.67	7.35	5.83	11.49
Chl b, μg/g	0.96	4.10	3.01	2.77	3.99	1.49	4.78	5.02	1.88	1.44	1.28	3.81	1.77	1.91	2.63
Chl c, μg/g	0.84	3.71	3.64	2.84	4.68	2.25	4.27	6.19	1.75	0.88	1.63	3.77	1.83	1.61	2.27
Pheo a, μg/g	3.71	13.64	13.63	9.47	18.03	9.68	30.26	32.94	10.48	8.59	7.33	24.38	10.07	8.11	18.91
T&L, mg/g	1.41	5.20	3.63	1.16	1.90	0.29	2.83	4.22	0.66	0.94	1.19	4.16	2.58	1.24	1.88
PRT/CHO	0.70	1.78	2.21	1.33	0.60	0.05	0.11	0.16	0.16	0.06	0.10	0.10	0.12	0.08	0.04
LPD/CHO	0.20	0.49	0.44	0.71	0.10	0.29	0.52	0.56	0.28	0.18	0.39	0.62	0.26	0.48	0.15
BPC, mg/g	11.38	35.16	14.94	3.75	15.26	2.28	11.59	13.44	4.02	5.17	3.65	19.21	7.69	3.96	8.84
Chl a/pheo	0.95	0.70	0.81	0.80	0.89	0.69	0.64	0.80	0.66	0.64	0.74	0.52	0.73	0.72	0.61
δ^{13}C, ‰	-28.13	-29.19	-28.42	-27.76	-25.43	-27.14	-28.64	-28.14	-27.44	-25.01	-28.50	-28.52	-28.12	-27.22	-25.02

Appendix 4: Branched Alkanes detected in the selected study region by GC-MS analysis

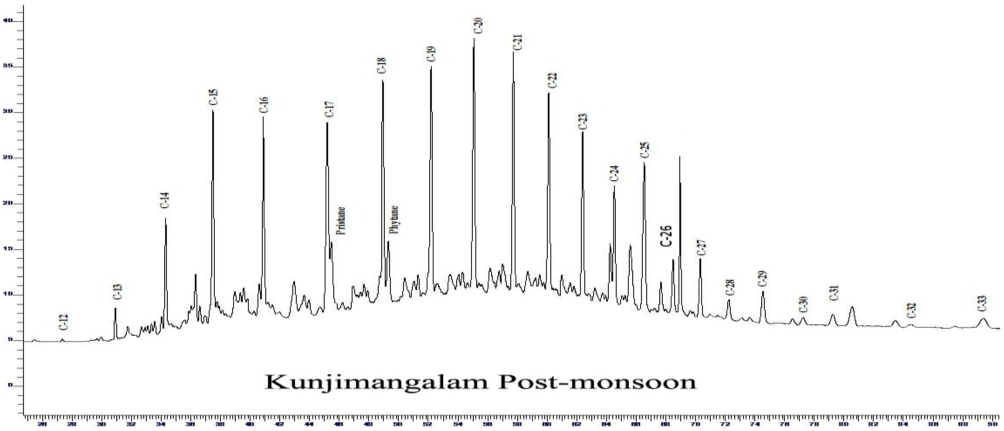
	Thalassery			Pappinissery			Pazhayangadi			Kunjimangalam			Kadalundi		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Teracosane, 10-methyl-									†						
Tetracosane, 3-methyl-					†						†			†	†
Ttetracosane, 2-methyl-					†						†			†	†
Unkown		†			†						†			†	†
Docosane, 3-methyl-					†			†		†	†			†	†
Docosnae, 2-methyl-		†			†			†		†	†			†	†
Docosane, 4-methyl-		†			†			†		†	†			†	
Docosane, 5-methyl-		†			†			†			†				
Unkown					†			†			†			†	
Henicosane, 3- methyl-					†			†			†			†	
Henicosane, 2- methyl-		†			†			†			†			†	
Henicosane, 4-methyl-		†			†			†			†			†	
Henicosane, 5-methyl-		†			†			†			†				
Henicosane, 6-methyl-											†				
Unkown		†			†			†			†			†	
Eicosane, 3-methyl-		†			†			†			†			†	†
Eicosane, 2-methyl-		†			†			†			†			†	
Eicosane, 4-methyl-		†			†						†			†	

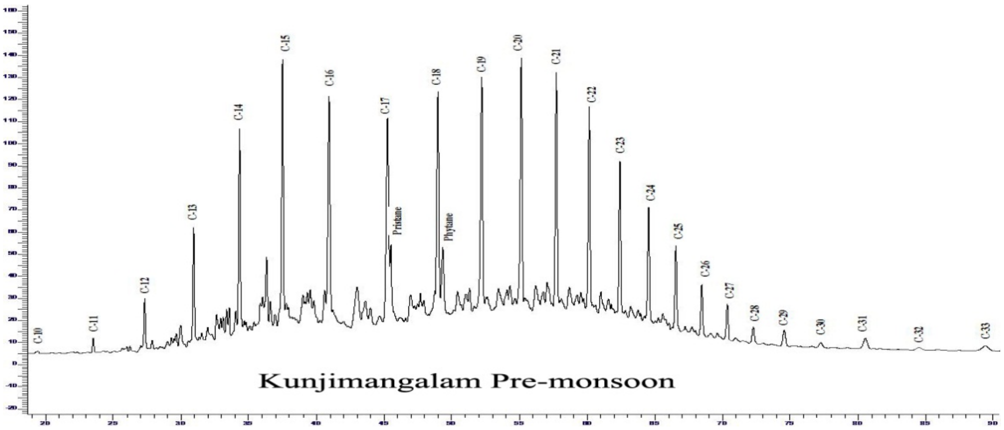
Eicosane, 5-methyl-					†										
Eicosane, 6-methyl-					†						†			†	
Eicosane, 7-methyl-														†	
Unkown		†			†			†			†			†	
Nonadecane, 3-methyl-		†			†			†		†	†			†	†
Unkown		†													
Nonadecane, 2-methyl-		†			†			†		†	†			†	†
Nonadecane, 4-methyl-		†			†						†				†
Nonadecane, 5-methyl-		†			†						†			†	
Nonadecane, 6-methyl-		†			†						†			†	†
Unkown					†			†		†	†				
Unkown														†	
Unkown					†						†			†	†
Octadecane, 3-methyl-		†			†			†		†	†			†	†
Octadecane, 2-methyl-		†			†			†		†	†	†			
Octadecane, 4-methyl-		†			†			†	†	†				†	
Octadecane, 5-methyl-		†			†			†			†			†	
Octadecane, 6-methyl		†						†			†				
Unkown	†	†			†			†		†	†			†	
Unkown							†					†			†
Heptadecane, 3-methyl-		†	†		†	†	†	†	†	†	†	†			†
Heptadecane, 2-methyl-		†	†		†	†	†	†	†	†	†	†		†	†

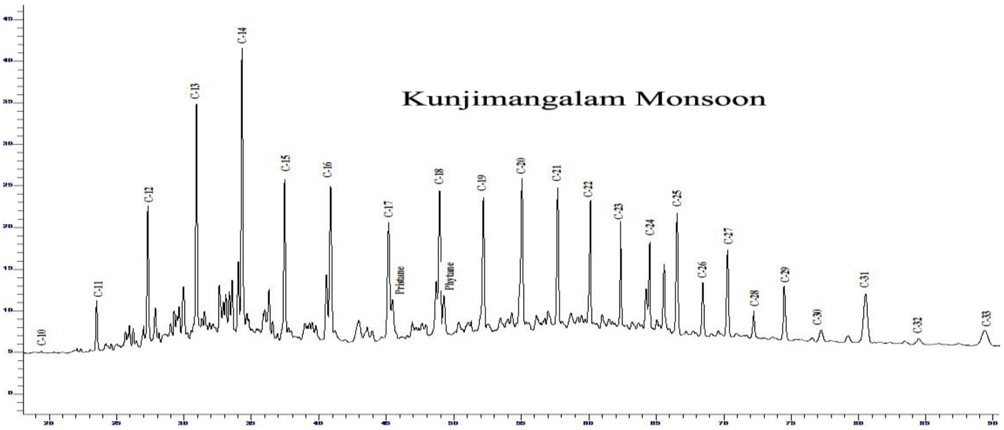
Heptadecane, 4-methyl-			†		†	†	†	†	†	†	†	†		†	†
Heptadecane, 5-methyl-		†	†		†	†	†	†	†	†	†	†		†	†
Heptadecane, 6-methyl-		†			†			†	†	†	†			†	†
Unkown		†			†			†			†			†	†
Unkown			†			†	†			†		†		†	
Unkown	†			†			†		†			†	†		†
Hexadecane, 3- methyl-		†	†		†	†		†	†	†	†			†	
Hexadecane, 2- methyl-			†			†		†		†	†	†			
Hexadecane, 4- methyl-		†			†		†	†	†		†	†		†	†
Tridecane, 9-propyl-, 8-methyl -										†					
Hexadecane, 5- methyl -		†			†	†		†		†	†	†		†	†
Pentadecane, 2,6,10-, trimethyl-		†	†		†	†	†	†	†	†	†	†	†	†	†
Hexadecane, 3-methyl-			†				†		†						
Pentadecane, 3-methyl-		†	†		†	†	†	†	†		†	†	†	†	†
Unkown										†					
Pentadecane, 2-methyl-		†	†		†	†		†			†	†	†	†	†
Pentadecane, 4-methyl-		†			†	†		†			†	†		†	†
Unkown										†					
Unkown		†	†		†	†	†	†	†	†	†	†	†	†	†
Pentadecane, 6-methyl-		†	†		†	†		†			†	†			†
Unkown		†			†	†		†		†	†	†			†
Tetradecene, 3-methyl-		†	†	†	†	†		†	†	†	†	†	†	†	†

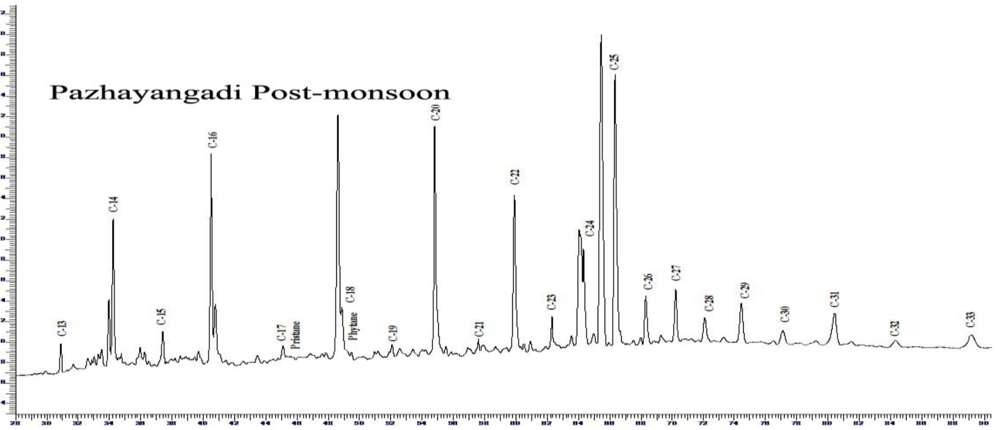
Tetradecene, 2-methyl-	+	+	+	+	+	+	+		+	+	+	+	+	+
Tridecane, 2,6,10- trimethyl-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tetradecane, 4-methyl-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tetradecane, 5-methyl-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Unkown													+	
Unkown								+						+
Unkown													+	
Dodecane, 2,6,10- trimethyl-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tridecane,3-methyl-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Unkown	+	+			+	+	+	+	+		+	+		
Tridecane, 2-methyl-			+	+						+			+	+
Tridecane, 4-methyl-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dodecane, 2,4- dimethyl-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Unkown	+	+	+		+	+	+	+	+	+	+	+	+	+
Dodecane, 5-ethyl-		+				+		+			+			+
Unkown				+			+			+			+	

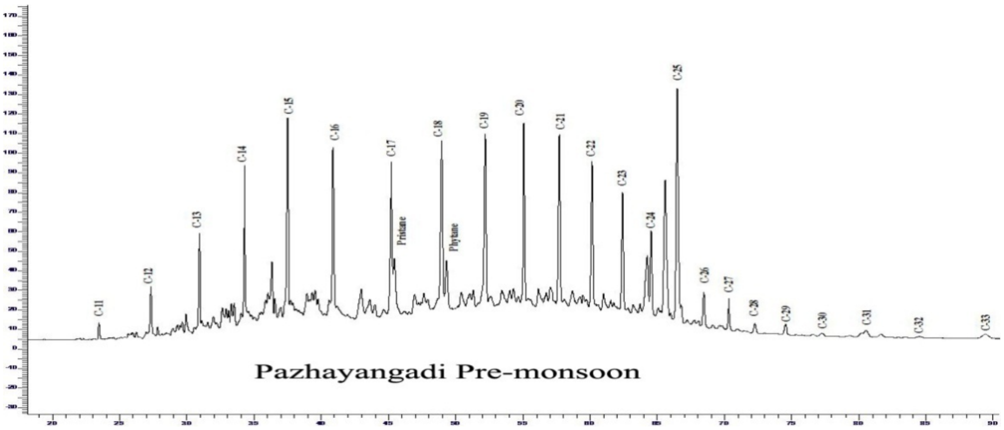
Appendix 5: Total ion Chromatogram of n-alkanes in the present study

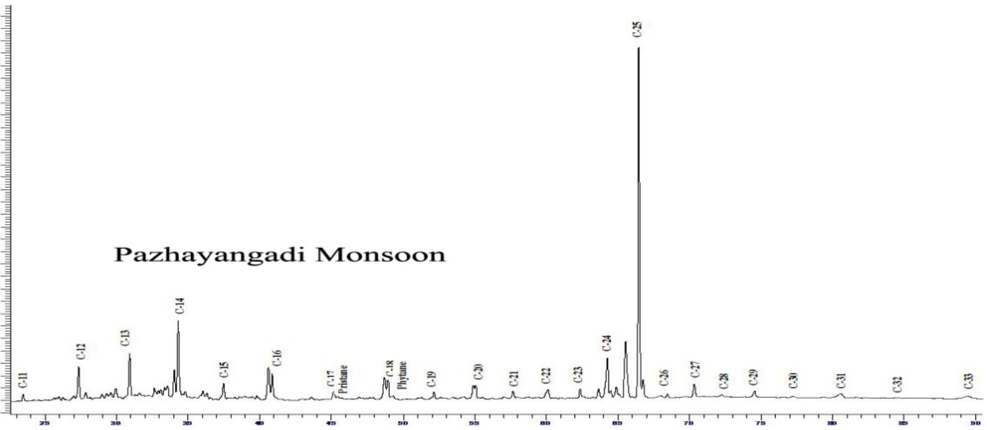


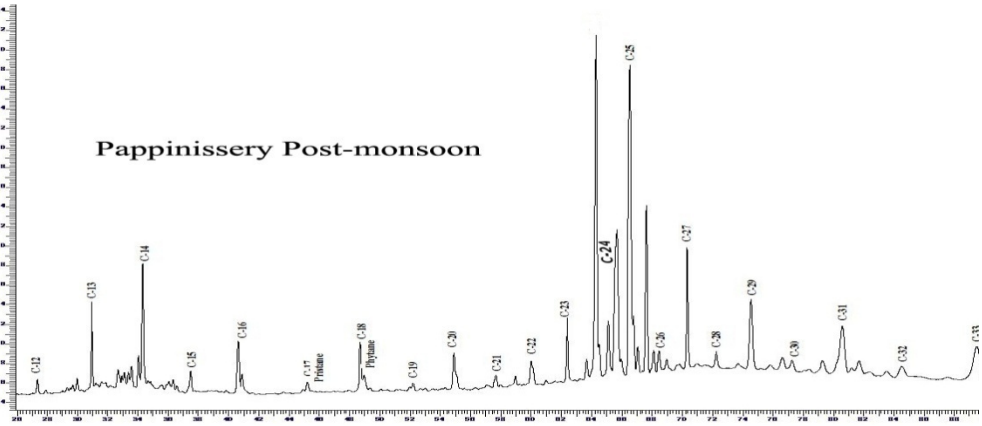


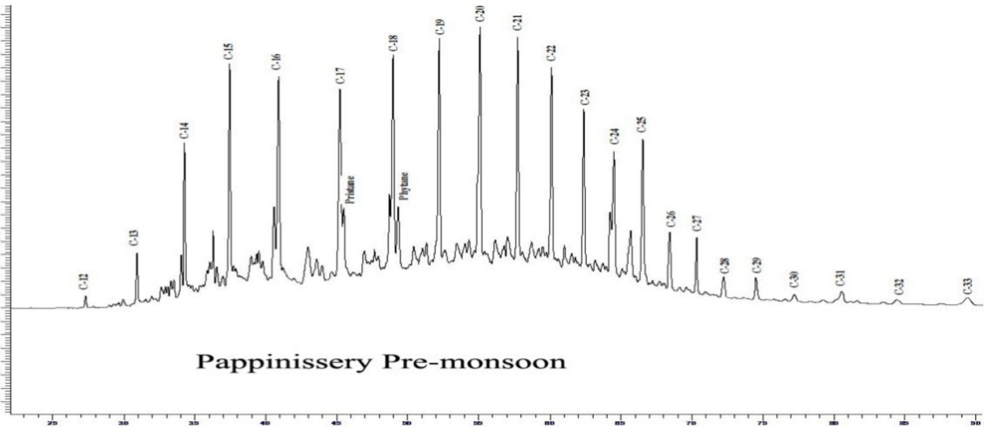


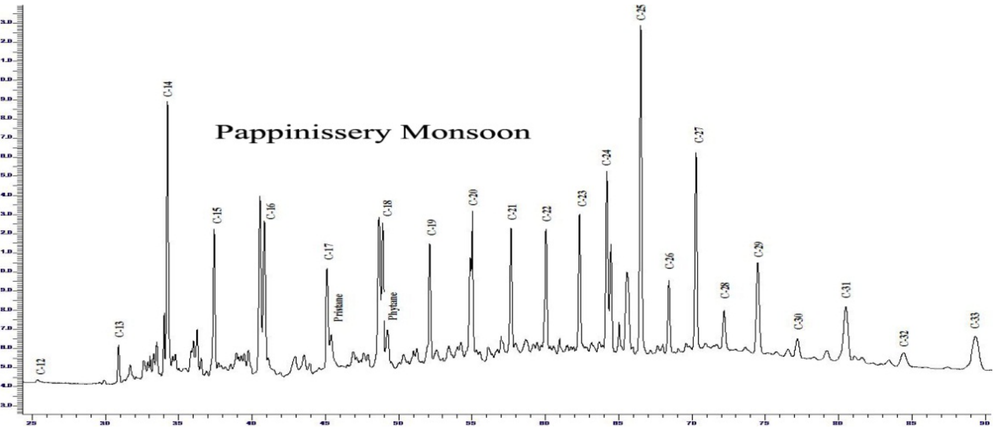

Pazhayangadi Post-monsoon
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33


C-11
C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33
Pazhayangadi Pre-monsoon


Pazhayangadi Monsoon
C-11
C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33


Pappinissery Post-monsoon
C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33


C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33
Pappinissery Pre-monsoon


C-12
C-13
C-14
Pappinissery Monsoon
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
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C-27
C-28
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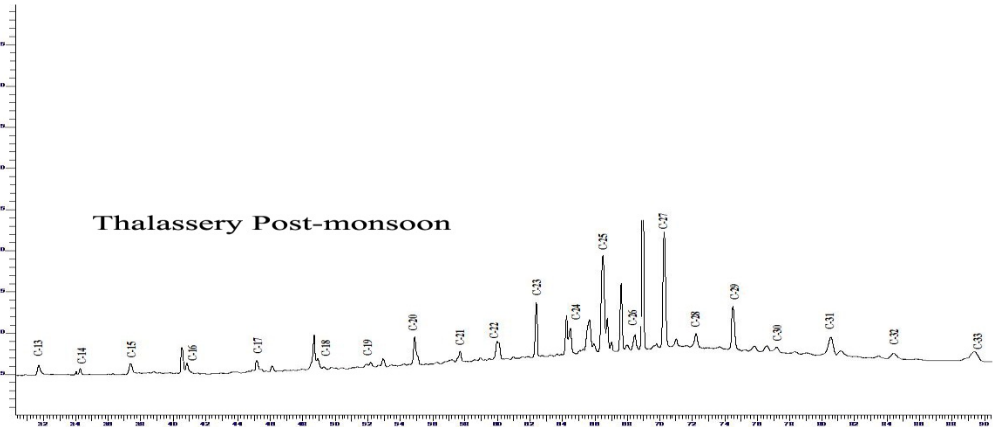
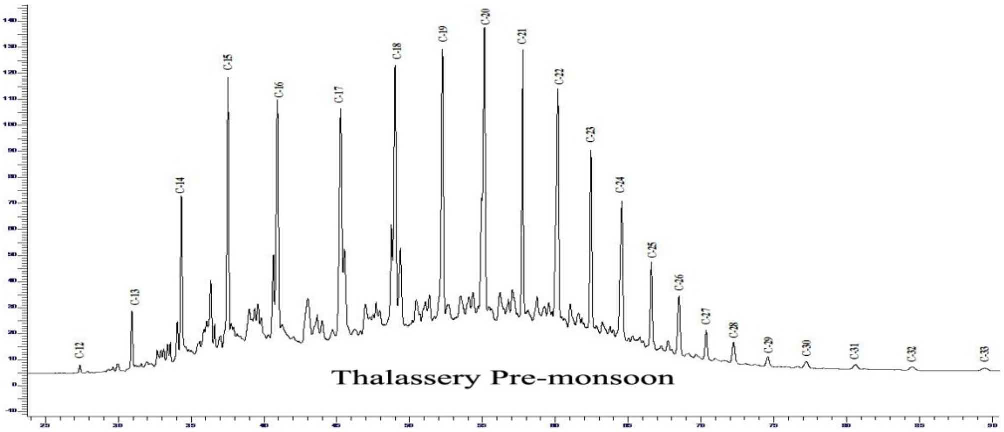
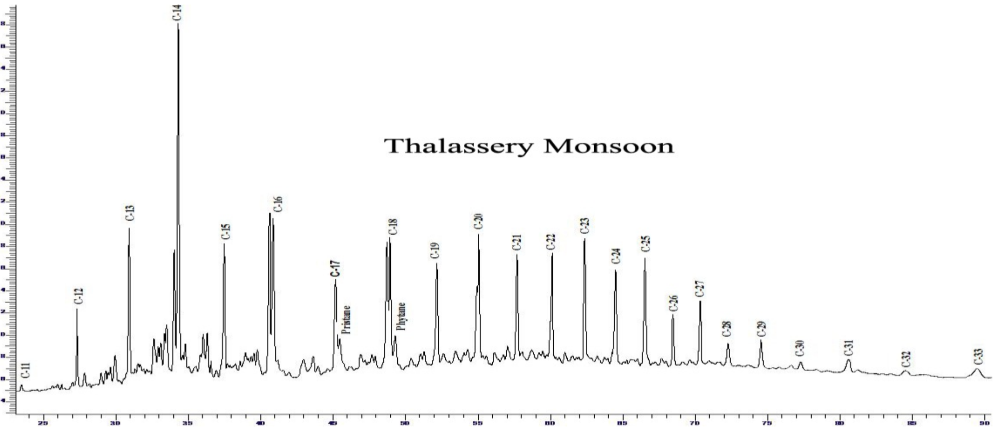
C-30
C-31
C-32
C-33


Thalassery Post-monsoon
C-13
C-14
C-15
C-16
C-17
C-18
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33


C-12
C-13
C-14
C-15
C-16
C-17
C-18
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33
Thalassery Pre-monsoon


Thalassery Monsoon
C-11
C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
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C-25
C-26
C-27
C-28
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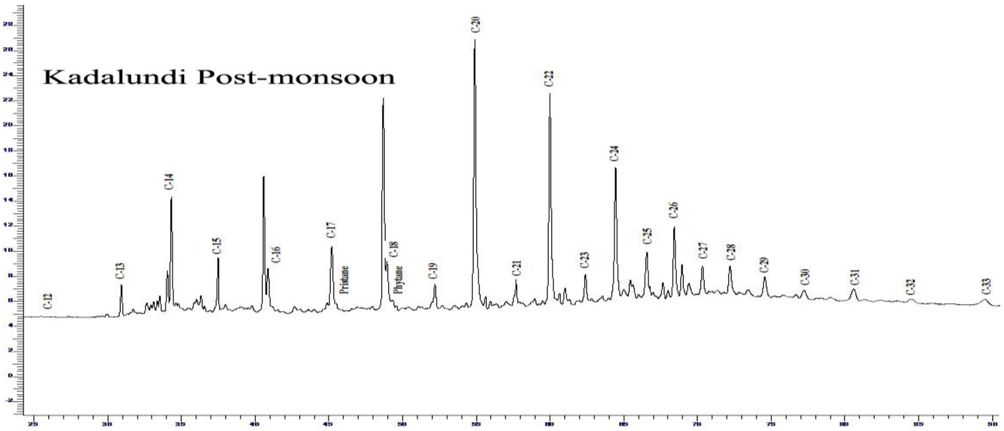
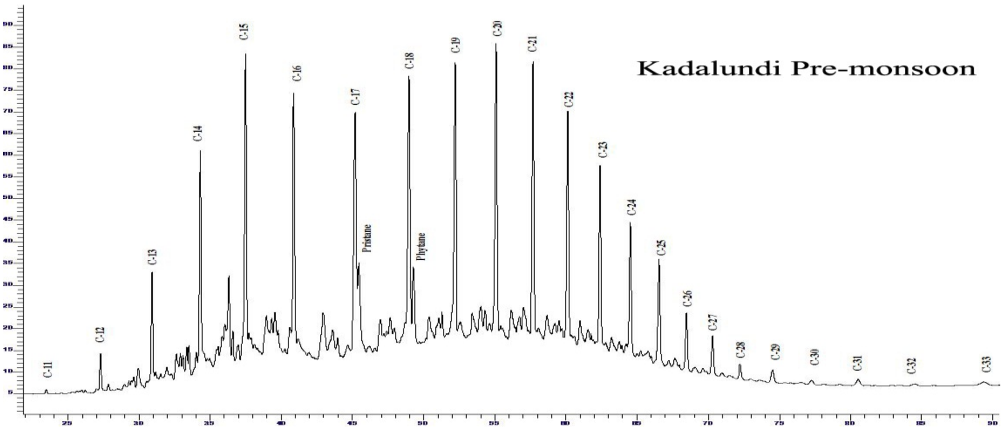
C-30
C-31
C-32
C-33


Kadalundi Post-monsoon
C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33


Kadalundi Pre-monsoon
C-11
C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
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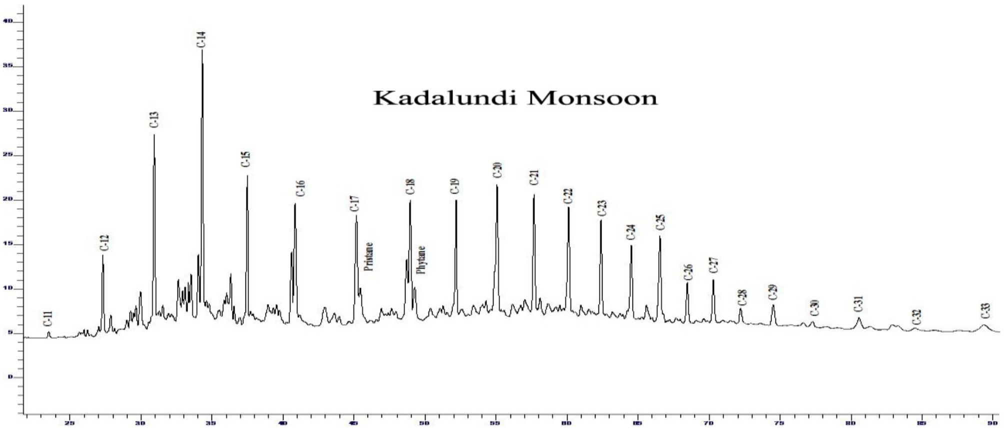
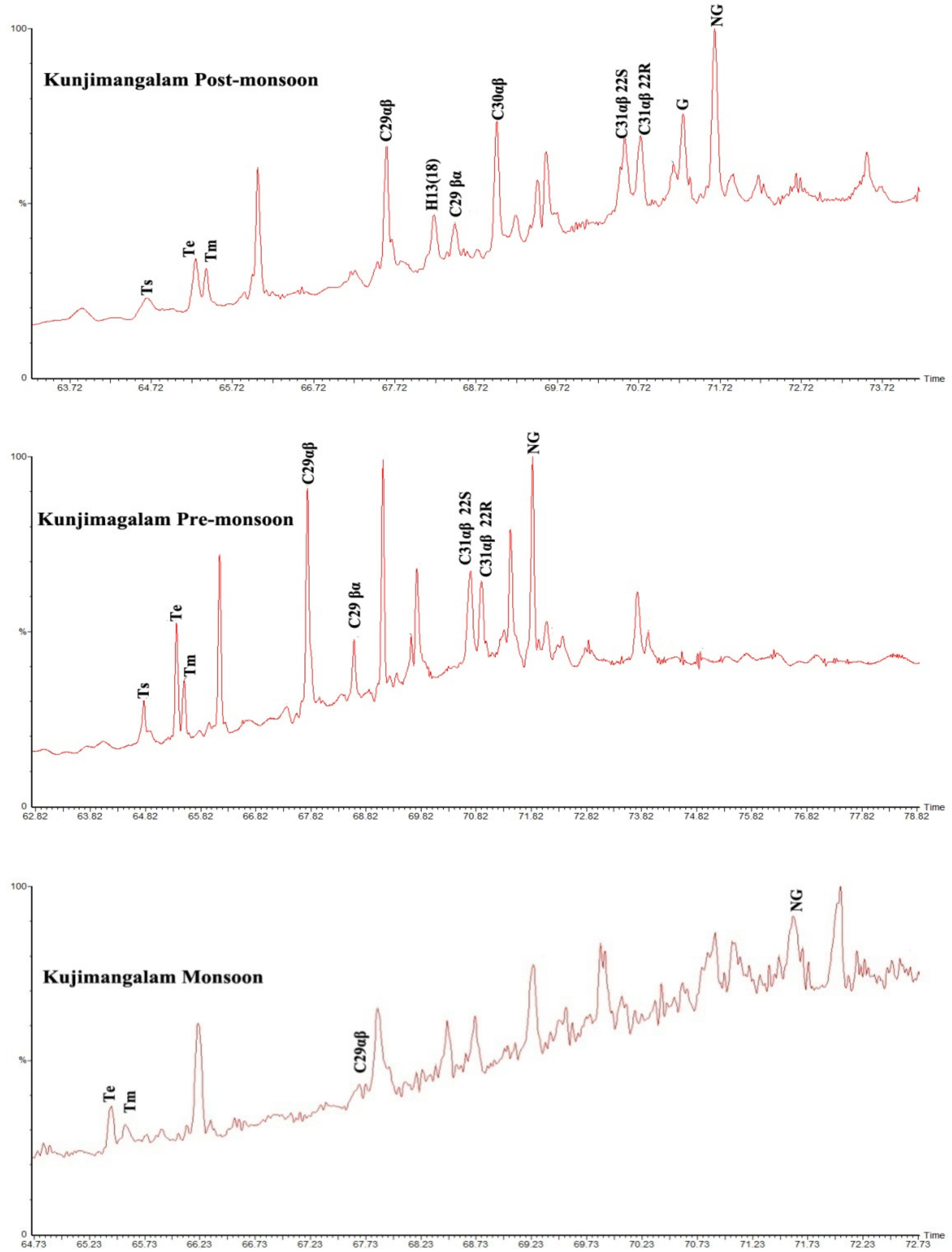
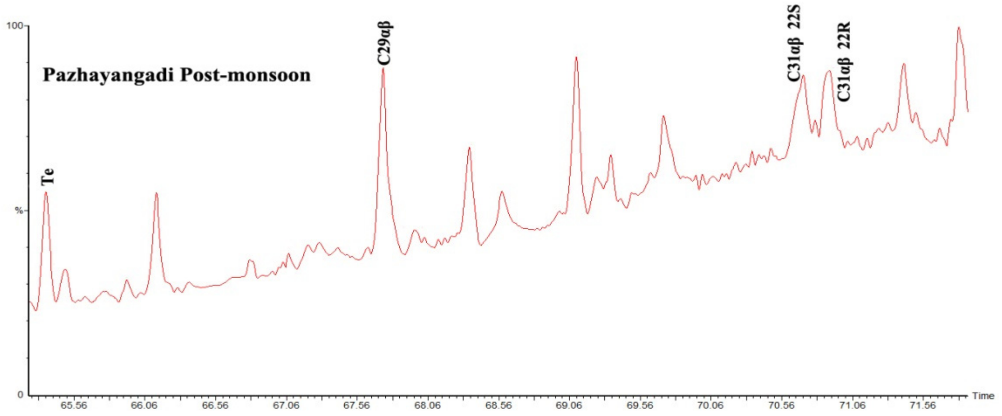
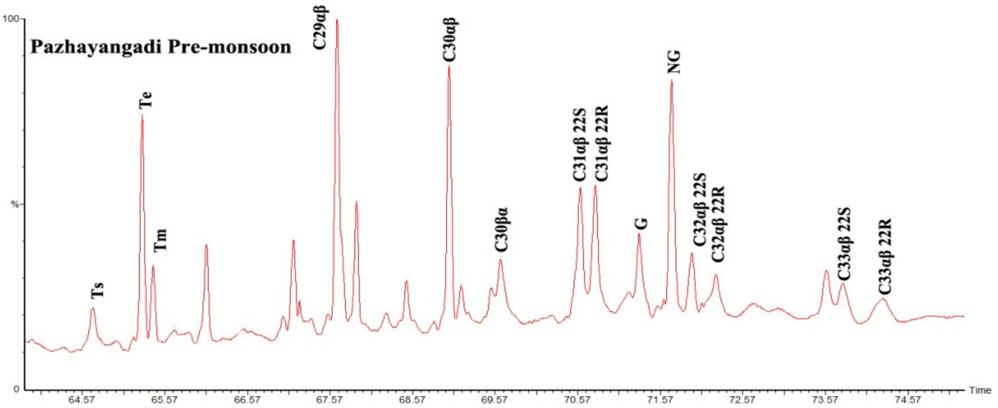
C-30
C-31
C-32
C-33

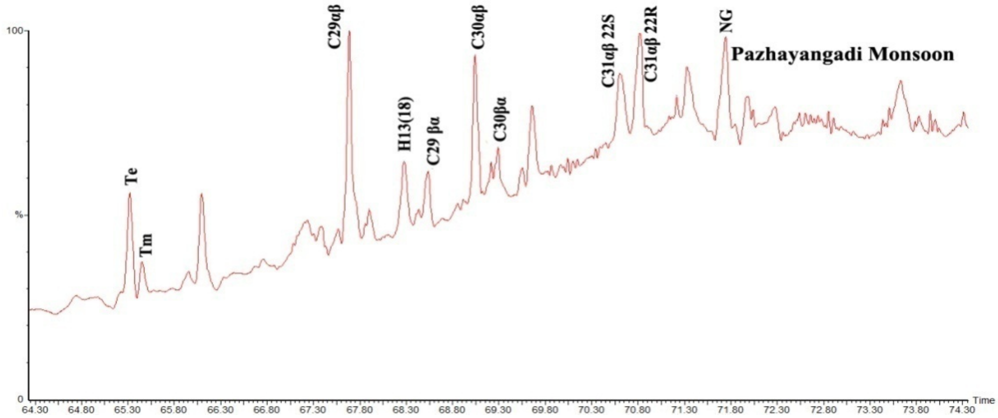

Kadalundi Monsoon
C-11
C-12
C-13
C-14
C-15
C-16
C-17
Pristane
C-18
Phytane
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
C-29
C-30
C-31
C-32
C-33

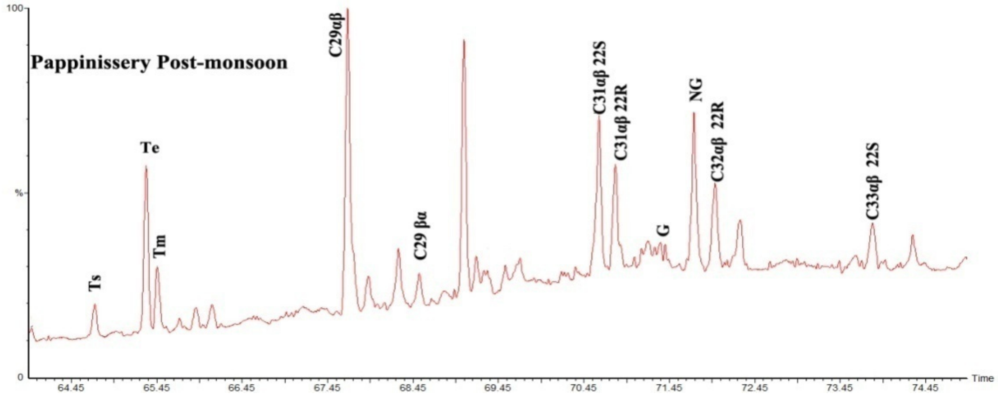
Appendix 6: Chromatogram of hopanes at m/z 191 in the present study

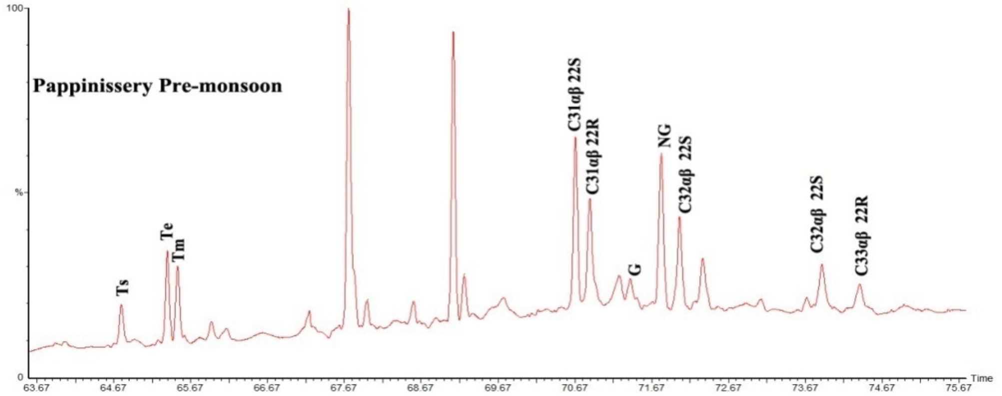


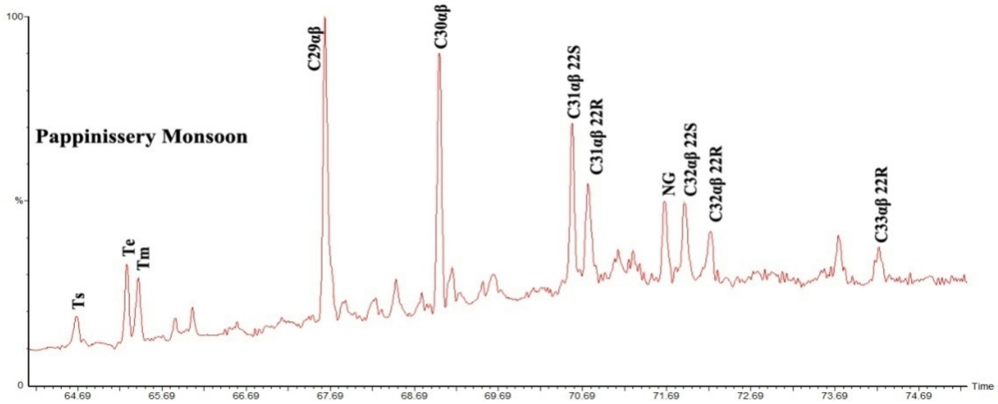

Pazhayangadi Post-monsoon
Te
C29αβ
C31αβ 22S
C31αβ 22R
100
%
0
65.56
66.06
66.56
67.06
67.56
68.06
68.56
69.06
69.56
70.06
70.56
71.06
71.56
Time

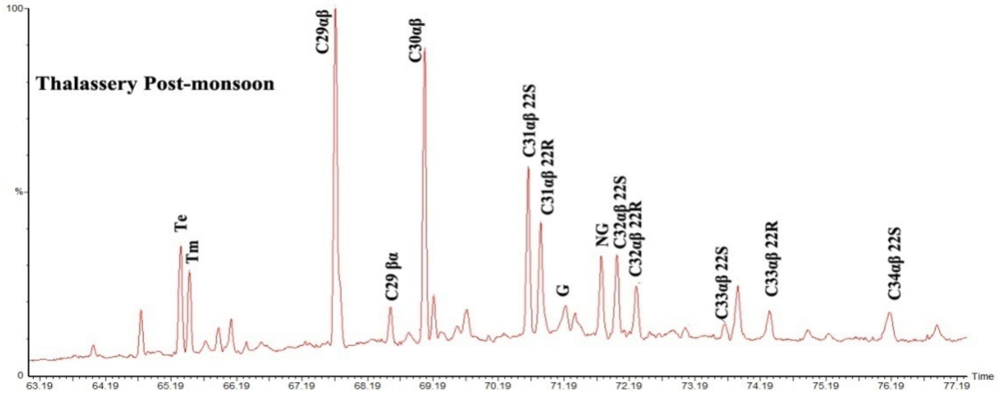

Pazhayangadi Pre-monsoon
Ts
Te
Tm
C29αβ
C30αβ
C30βα
C31αβ 22S
C31αβ 22R
G
NG
C32αβ 22S
C32αβ 22R
C33αβ 22S
C33αβ 22R
100
%
0
64.57
65.57
66.57
67.57
68.57
69.57
70.57
71.57
72.57
73.57
74.57
Time

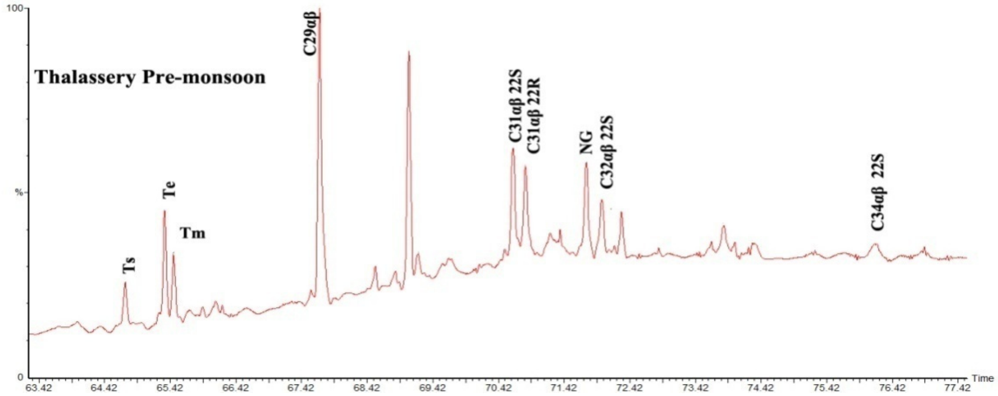

Te
Tm
C29αβ
H13(18)
C29 βα
C30αβ
C30βα
C31αβ 22S
C31αβ 22R
NG
Pazhayangadi Monsoon
100
%
0
64.30
64.80
65.30
65.80
66.30
66.80
67.30
67.80
68.30
68.80
69.30
69.80
70.30
70.80
71.30
71.80
72.30
72.80
73.30
73.80
74.30
Time

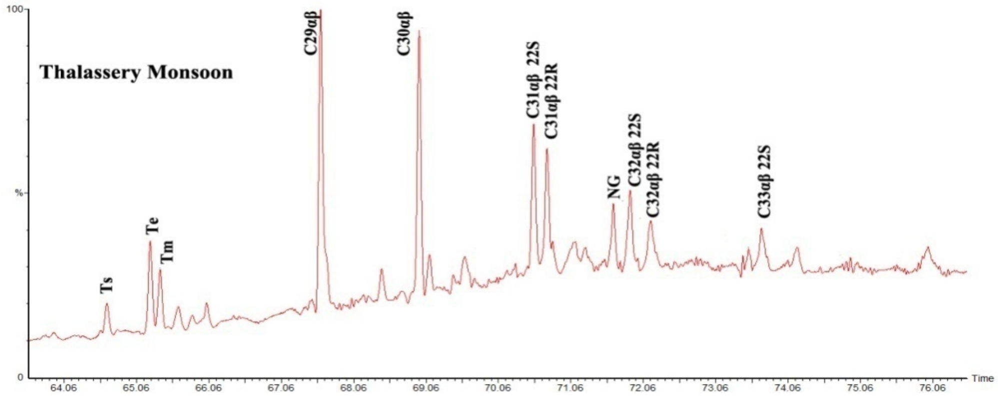

100
%
0
Pappinissery Post-monsoon
Ts
Te
Tm
C29αβ
C29 βα
C31αβ 22S
C31αβ 22R
G
NG
C32αβ 22R
C33αβ 22S
64.45
65.45
66.45
67.45
68.45
69.45
70.45
71.45
72.45
73.45
74.45
Time

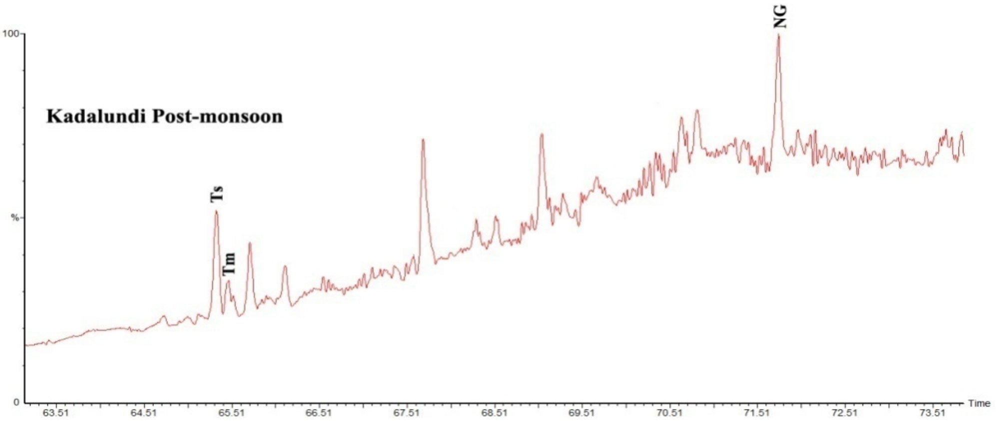

100
%
0
Pappinissery Pre-monsoon
Ts
Te
Tm
C31αβ 22S
C31αβ 22R
G
NG
C32αβ 22S
C32αβ 22S
C33αβ 22R
63.67
64.67
65.67
66.67
67.67
68.67
69.67
70.67
71.67
72.67
73.67
74.67
75.67
Time

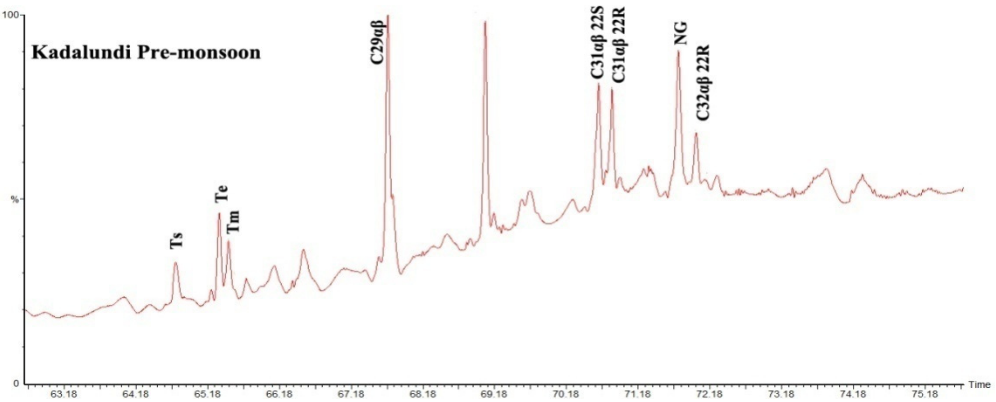

100
%
0
Pappinissery Monsoon
Ts
Te
Tm
C29αβ
C30αβ
C31αβ 22S
C31αβ 22R
NG
C32αβ 22S
C32αβ 22R
C33αβ 22R
64.69
65.69
66.69
67.69
68.69
69.69
70.69
71.69
72.69
73.69
74.69
Time

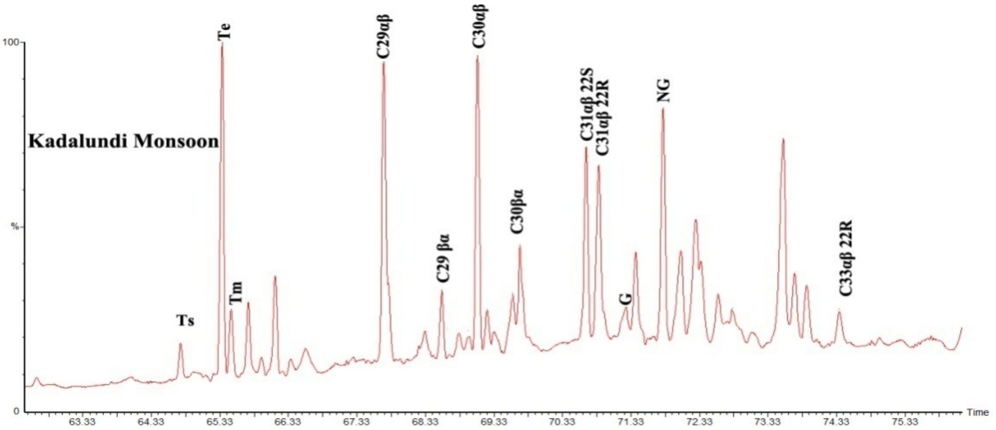

Thalassery Post-monsoon
100
%
0
Time
63.19
64.19
65.19
66.19
67.19
68.19
69.19
70.19
71.19
72.19
73.19
74.19
75.19
76.19
77.19
Te
Tm
C29αβ
C29 βα
C30αβ
C31αβ 22S
C31αβ 22R
G
NG
C32αβ 22S
C32αβ 22R
C33αβ 22S
C33αβ 22R
C34αβ 22S


Thalassery Pre-monsoon
100
%
0
Time
63.42
64.42
65.42
66.42
67.42
68.42
69.42
70.42
71.42
72.42
73.42
74.42
75.42
76.42
77.42
Ts
Te
Tm
C29αβ
C31αβ 22S
C31αβ 22R
NG
C32αβ 22S
C34αβ 22S


Thalassery Monsoon
100
%
0
Time
64.06
65.06
66.06
67.06
68.06
69.06
70.06
71.06
72.06
73.06
74.06
75.06
76.06
Ts
Te
Tm
C29αβ
C30αβ
C31αβ 22S
C31αβ 22R
NG
C32αβ 22S
C32αβ 22R
C33αβ 22S


Kadalundi Post-monsoon
NG
Ts
Tm
100
%
0
Time
63.51
64.51
65.51
66.51
67.51
68.51
69.51
70.51
71.51
72.51
73.51


Kadalundi Pre-monsoon
C29αβ
C31αβ 22S
C31αβ 22R
NG
C32αβ 22R
Ts
Te
Tm
100
%
0
Time
63.18
64.18
65.18
66.18
67.18
68.18
69.18
70.18
71.18
72.18
73.18
74.18
75.18


Kadalundi Monsoon
Te
C29αβ
C30αβ
C31αβ 22S
C31αβ 22R
NG
C30βα
C29 βα
G
C33αβ 22R
Ts
Tm
100
%
0
Time
63.33
64.33
65.33
66.33
67.33
68.33
69.33
70.33
71.33
72.33
73.33
74.33
75.33

Appendix 7: Variation of fatty acids in the present study region

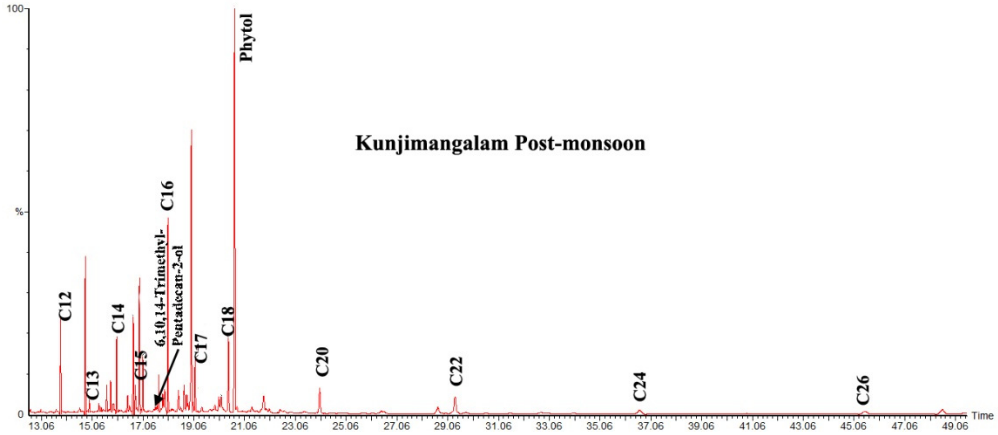
	Post-monsoon					Pre-monsoon					Monsoon				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
C8	ND	0.31	ND	ND	0.62	ND	0.69	ND	0.21	0.27	ND	0.70	ND	0.12	0.69
C9	ND	0.20	ND	ND	ND	ND	ND	ND	0.07	0.12	ND	ND	ND	0.05	0.54
C10	0.16	0.38	ND	ND	0.45	0.45	0.59	0.61	0.16	0.19	ND	0.61	ND	0.09	0.54
C 11	ND	0.13	ND	ND	0.14	0.08	ND	ND	0.02	0.07	ND	ND	ND	0.02	0.16
2-OH C10	ND	0.25	0.03	ND	0.34	ND	ND	0.70	0.10	0.17	ND	ND	ND	0.07	ND
3-OH C10	ND	0.27	0.51	ND	ND	ND	ND	0.88	ND	0.08	ND	ND	0.06	ND	ND
iC12	ND	ND	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	ND	ND
C12	1.65	2.29	1.27	0.77	2.20	3.09	2.52	3.45	1.07	1.12	0.27	3.53	0.62	0.69	3.12
ic13	0.09	0.16	0.19	0.06	0.29	0.14	0.43	0.58	ND	0.11	ND	0.24	0.06	0.06	0.32
ac13	0.04	ND	0.12	ND	ND	0.14	ND	0.44	ND	ND	ND	ND	0.01	0.02	ND
C13	0.20	0.48	0.27	0.10	0.68	0.40	0.50	0.51	0.13	0.19	0.02	0.31	0.08	0.02	0.49
3-OH C12	ND	ND	0.20	ND	ND	ND	0.71	0.94	ND	ND	ND	ND	ND	ND	ND
iC14	0.81	0.86	1.65	0.41	0.92	0.66	2.22	3.04	0.34	0.65	0.07	0.85	0.34	0.21	0.03
C14:1ω5	ND	ND	0.13	0.13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C14	3.11	6.07	5.18	2.25	7.90	6.12	10.04	13.90	1.95	3.14	0.40	5.43	1.43	1.11	0.12
iC15	2.42	4.28	5.71	1.73	4.45	2.49	8.19	14.73	1.19	1.62	0.20	3.92	1.07	0.72	4.64
aC15	1.51	2.57	4.90	1.46	1.95	1.68	6.56	8.34	1.00	1.02	0.15	2.03	0.70	0.66	2.84
C15	0.84	1.99	2.19	1.11	11.72	8.98	6.42	5.07	0.87	0.99	0.18	4.15	0.47	0.41	2.88
3-OH C14	0.11	ND	ND	ND	ND	0.24	0.66	ND	ND	ND	ND	0.24	ND	ND	ND
iC16	1.48	1.70	2.89	0.75	1.44	1.27	3.62	5.65	0.59	0.96	0.10	1.61	0.61	0.33	2.61
C16:1*	0.16	3.56	ND	0.07	0.70	0.17	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:1ω7 cis	0.85	3.46	3.45	1.79	11.95	2.88	5.06	9.48	0.19	0.54	0.08	4.45	0.70	0.07	1.31
C16:1*	0.09	ND	0.29	0.10	0.51	0.32	0.61	1.20	ND	ND	0.01	0.19	0.07	ND	0.26
C16:1*	0.17	1.89	1.03	0.40	1.05	0.31	1.24	1.30	0.23	0.19	0.01	0.80	0.11	0.09	0.39
C16	10.56	23.03	19.37	8.74	30.89	13.82	30.33	108.21	8.70	9.45	1.01	20.97	5.92	4.04	26.41
C17:1*	ND	0.11	0.13	0.06	ND	ND	ND	0.66	ND	ND	ND	ND	ND	ND	ND

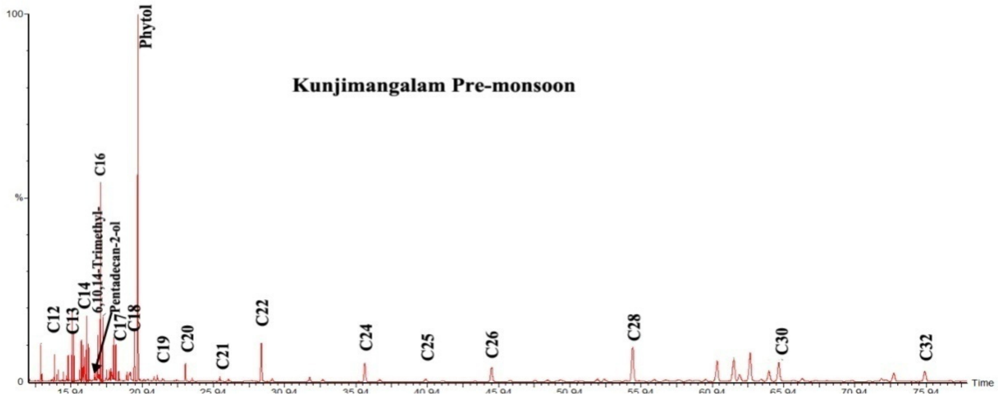
bC17**	0.58	2.33	1.76	0.58	1.07	1.01	2.25	3.66	0.60	0.34	0.05	1.68	0.25	0.29	1.00
bC17**	0.77	0.16	0.18	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	ND	ND
iC17	0.67	1.81	1.43	0.47	1.15	0.80	2.17	4.29	0.41	0.61	0.05	1.35	0.44	0.20	1.57
aC17	0.48	1.04	1.56	0.45	0.52	0.61	1.96	3.49	0.40	0.45	0.13	0.76	0.33	0.23	1.18
C17:1ω7	ND	ND	0.25	ND	1.67	1.12	ND	ND	ND	ND	ND	0.31	0.09	ND	ND
Cy-C17:0	ND	1.11	0.80	0.26	0.58	0.70	ND	ND	0.17	ND	ND	0.41	0.11	0.06	0.37
C17	0.59	1.58	1.24	0.60	1.57	1.52	2.40	3.79	0.54	0.66	0.06	1.75	ND	0.24	1.84
2-OH C16	0.35	0.63	0.62	0.20	ND	ND	ND	1.32	ND	0.47	ND	ND	0.23	0.13	1.09
bC18	ND	0.23	0.29	0.07	ND	ND	ND	0.56	ND	ND	ND	ND	ND	0.04	ND
3-OH C16	0.28	ND	0.36	ND	ND	ND	ND	0.83	ND	ND	ND	ND	ND	ND	ND
cisC18:2ω6	0.73	1.90	3.41	1.73	1.56	0.71	1.88	7.60	1.33	0.53	0.03	1.14	0.39	0.45	1.22
C18:3ω3	ND	0.69	1.86	0.38	ND	ND	2.15	4.59	0.27	ND	ND	ND	ND	0.11	ND
C18:1ω9 cis	3.31	6.39	6.24	2.78	3.42	2.20	5.45	22.19	2.41	1.89	0.19	4.12	2.41	1.27	4.19
C18:1*	0.83	2.82	4.05	1.64	2.10	1.71	5.02	13.93	1.26	0.64	0.06	3.35	0.67	0.37	1.56
C18:1ω11	ND	0.29	0.61	0.14	0.12	0.27	0.77	4.27	0.29	ND	ND	0.22	ND	ND	0.39
C18:1*	ND	0.29	ND	0.15	ND	ND	ND	ND	0.13	ND	ND	ND	0.10	ND	ND
C18	2.12	3.77	3.19	1.59	2.82	2.16	5.32	23.93	1.84	2.00	0.18	3.84	1.44	0.67	5.08
3-OH C17	ND	ND	0.58	ND	ND	0.37	0.83	3.33	ND	ND	ND	ND	0.19	ND	ND
2-OH C17	ND	ND	0.78	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.17	ND	ND
bC19**	ND	ND	ND	ND	ND	0.14	ND	ND	ND	ND	ND	ND	ND	ND	ND
bC19**	ND	0.30	0.27	0.08	ND	0.21	0.39	0.89	0.02	ND	ND	ND	ND	0.04	ND
C19:2	ND	ND	0.22	ND	ND	ND	ND	1.09	ND	ND	ND	ND	ND	ND	ND
Cy-C19:0	0.64	1.76	2.23	0.18	0.33	0.62	0.53	1.85	0.20		0.03	1.39		0.06	0.29
C19	0.33	0.57	0.23	0.10	ND	0.28	ND	1.35	0.11	0.16	0.02	0.33	0.12	0.06	0.39
C20:4ω6	0.10	0.38	0.46	0.17	0.53	ND	0.34	0.79	0.09	ND	ND	0.21	0.07	ND	ND
C20:5ω3	2.53	2.93	4.15	ND	0.89	0.29	2.18	4.32	0.32	0.94	0.04	0.30	1.32	0.19	2.13
3-OH C19	ND	0.28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:2ω9	ND	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:1ω9	ND	0.07	0.21	ND	ND	ND	ND	ND	0.05	ND	ND	ND	ND	ND	ND

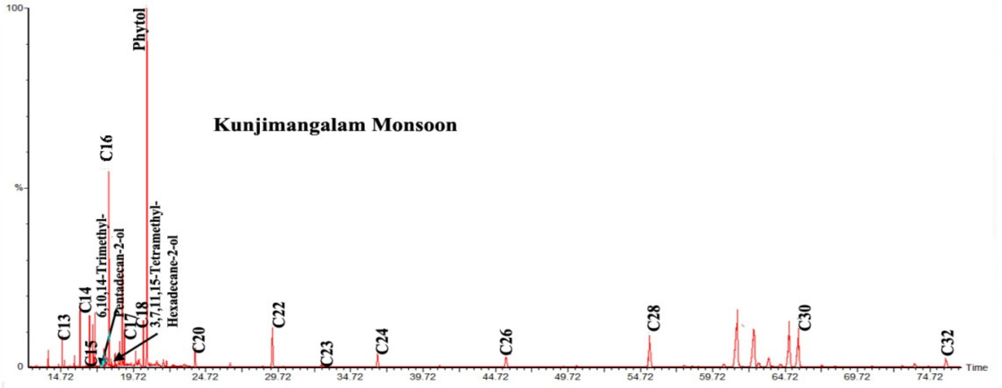
C20:1*	ND	0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20	0.82	0.87	1.04	0.34	0.52	0.78	1.59	11.41	0.37	0.68	0.12	1.04	0.89	0.14	1.47
iC21	ND	1.41	0.03	0.04	ND	0.12	0.16	0.38	0.04	ND	ND	ND	ND	ND	ND
C21:2	0.47	0.61	1.19	ND	ND	ND	0.49	ND	ND	ND	ND	ND	0.30	ND	0.37
C21	0.59	0.67	ND	0.09	ND	0.19	1.14	2.11	0.13	0.26	0.03	0.27	0.23	0.06	0.54
C22:6ω3	ND	ND	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-OHC20	ND	ND	ND	ND	ND	0.33	ND	ND	ND	1.12	0.04	ND	ND	ND	1.78
3-OHC20	ND	ND	0.37	ND	ND	ND	ND	0.82	ND	ND	ND	ND	ND	0.05	ND
C22:2ω6	ND	ND	0.10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:1ω11	ND	ND	1.14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07	0.71
C22:1ω9	0.01	0.08	0.05	ND	ND	ND	ND	0.19	ND	0.42	ND	ND	ND	ND	0.76
C22	1.63	2.51	1.20	0.51	0.79	1.02	0.52	22.28	0.71	2.08	0.31	2.16	2.39	0.32	4.22
C23	0.73	0.71	0.32	0.12	0.27	0.39	1.34	3.88	0.20	0.54	0.06	0.75	0.38	0.08	0.89
2-OHC22	0.81	1.35	0.91	0.28	ND	0.73	3.26	9.10	0.40	1.67	0.08	0.98	1.01	0.25	2.45
C24:1ω9	ND	ND	0.99	ND	ND	ND	ND	ND	ND	0.72	ND	ND	ND	ND	ND
C24	3.12	7.30	2.22	0.66	1.51	1.76	11.32	32.35	1.19	4.39	0.46	4.00	3.77	0.48	7.48
2-OH C23	0.43	0.73	ND	0.09	ND	0.36	2.44	6.07	0.20	1.20	0.05	0.70	0.63	ND	1.63
C25	0.84	1.40	0.98	0.18	0.24	0.63	2.33	8.19	0.54	1.01	0.10	1.35	0.62	0.09	0.93
2-OH C24	5.28	13.99	11.19	2.59	1.37	7.19	35.72	91.43	5.33	20.35	0.62	9.97	11.17	2.76	23.87
C26	2.06	8.24	0.44	0.47	1.03	1.36	7.70	19.43	0.84	3.26	0.19	2.50	1.83	0.27	4.82
2-OH C25	0.36	0.74	ND	ND	ND	0.31	1.89	5.91	ND	1.31	0.03	ND	0.56	ND	1.15
C27	0.15	0.58	ND	ND	0.13	0.77	1.01	2.37	0.09	0.44	0.02	0.38	0.14	0.51	0.47
iC28	2.42	ND	5.96	ND	ND	ND	ND	ND	0.16	ND	0.24	ND	ND	ND	ND
2-OHC26	ND	0.53	ND	ND	ND	ND	1.03	3.89	0.45	1.08	ND	ND	0.32	ND	ND
C28	2.05	7.02	0.59	0.87	1.02	1.60	10.91	22.64	2.08	4.25	0.18	2.67	1.56	0.48	5.78
C29	0.34	2.18	0.01	ND	0.19	0.24	1.94	5.84	0.18	0.60	0.04	0.48	0.29	0.03	0.70
aC30	ND	ND	0.90	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C30	1.58	8.30	1.17	1.01	1.16	1.38	12.61	44.47	1.90	13.20	0.33	2.89	2.30	0.36	18.00

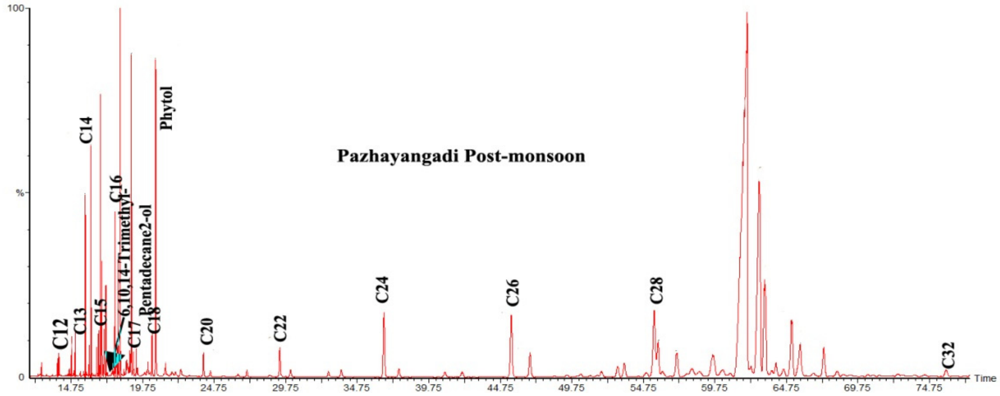
*unsaturated or cyclic fatty acid **Branching fatty acids, branching position unknown

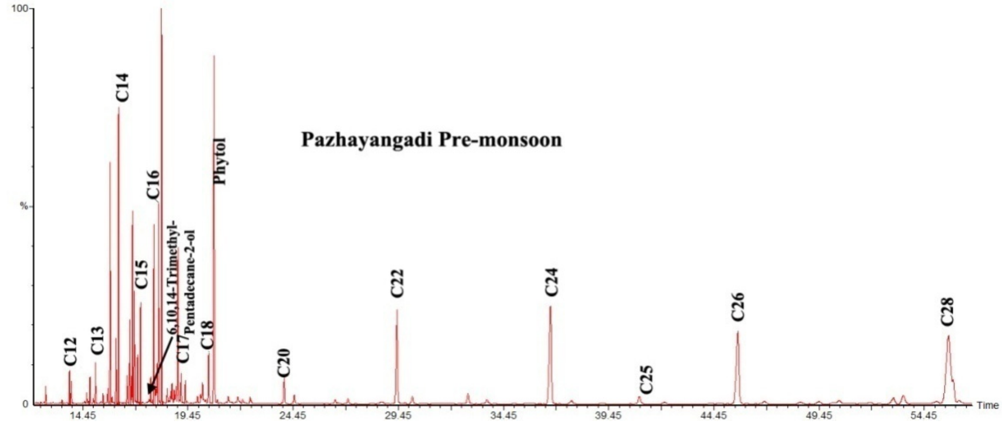
Appendix 8: Total ion Chromatogram of fatty alcohols in the present study

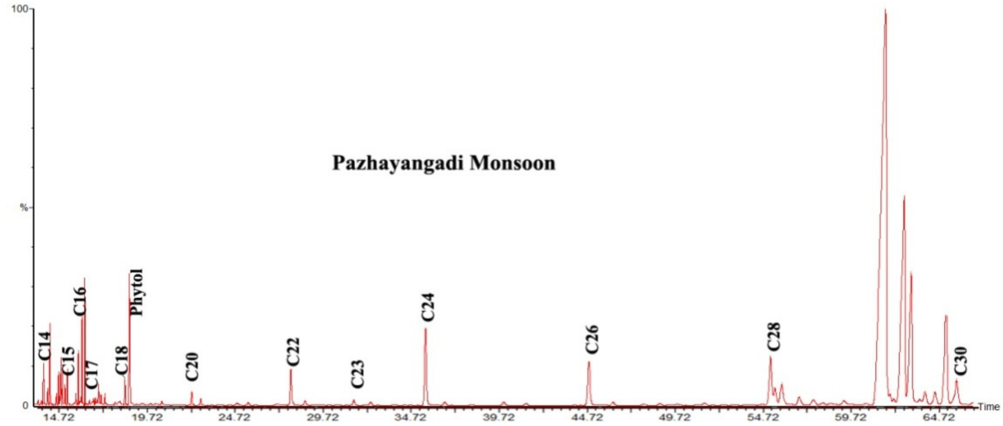


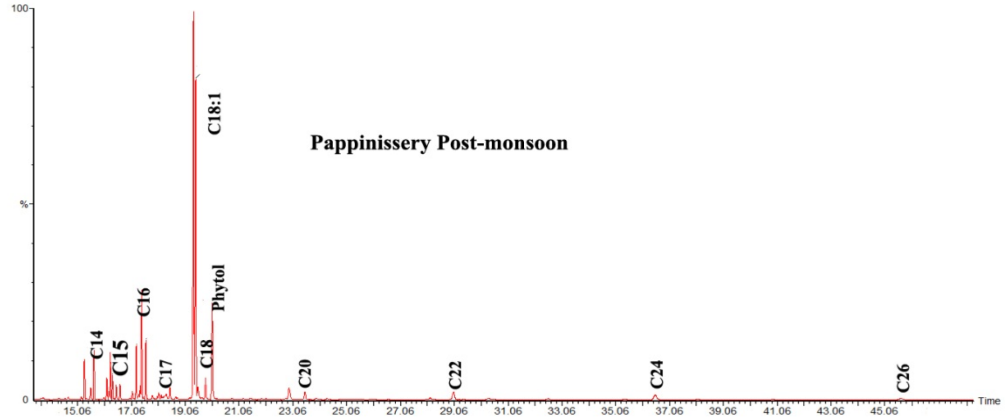


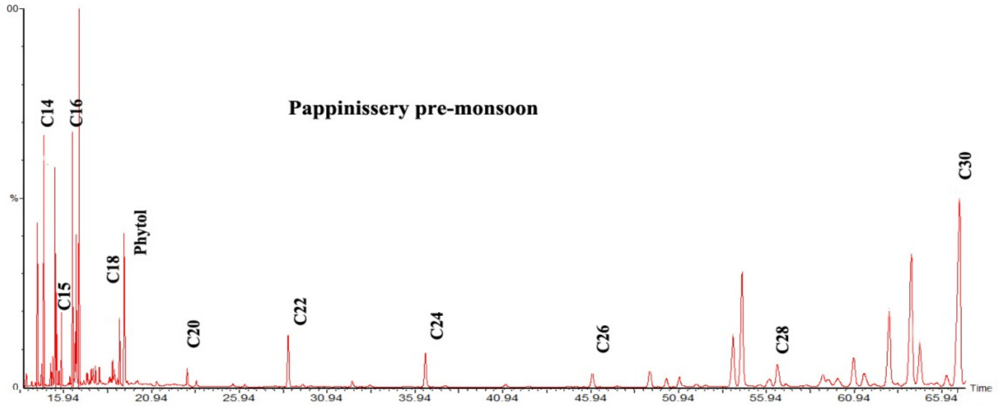


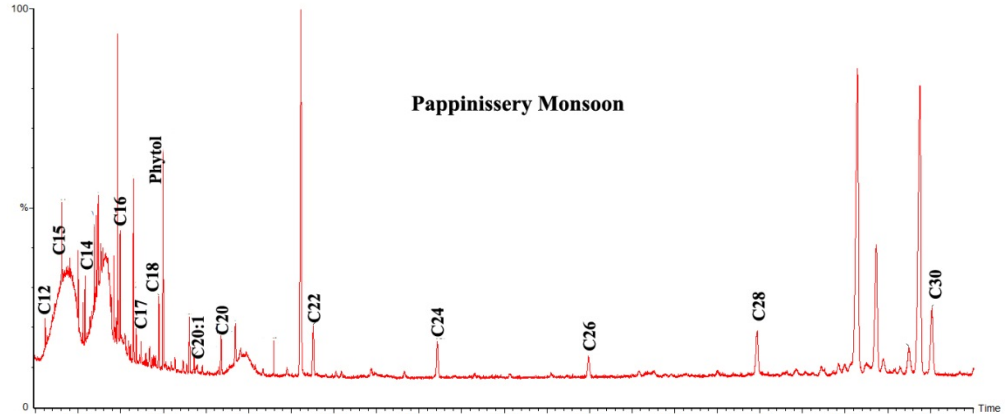

Pazhayangadi Post-monsoon
100
%
0
C12
C13
C14
C15
C16
6,10,14-Trimethyl-
Pentadecane2-ol
C17
C18
Phytol
C20
C22
C24
C26
C28
C32
14.75
19.75
24.75
29.75
34.75
39.75
44.75
49.75
54.75
59.75
64.75
69.75
74.75
Time

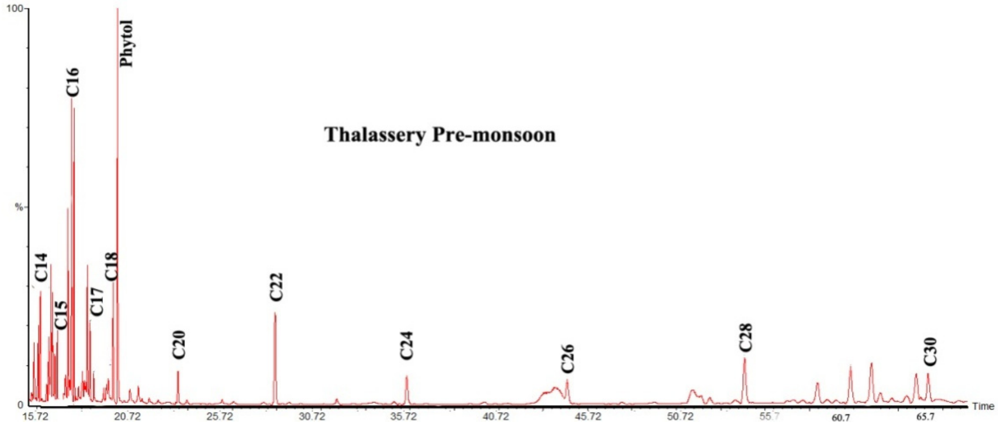

Pazhayangadi Pre-monsoon
100
%
0
C12
C13
C14
C15
C16
6,10,14-Trimethyl-
Pentadecane-2-ol
C17
C18
Phytol
C20
C22
C24
C25
C26
C28
14.45
19.45
24.45
29.45
34.45
39.45
44.45
49.45
54.45
Time

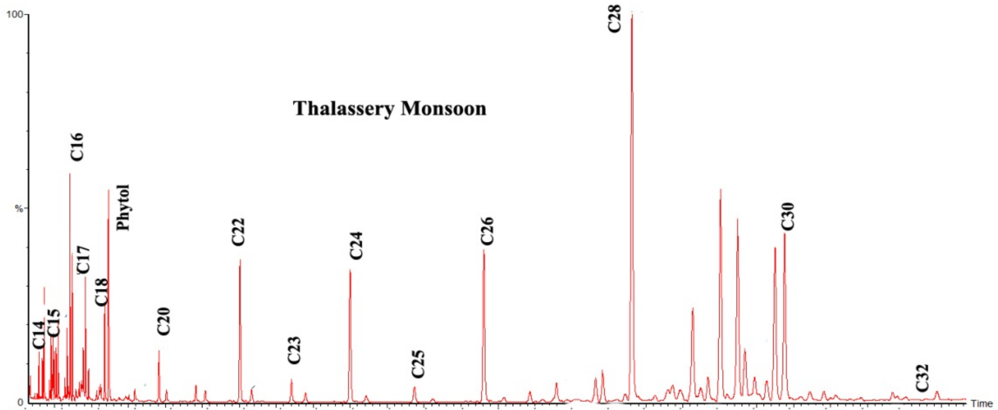

Pazhayangadi Monsoon
100
%
0
C14
C15
C16
C17
C18
Phytol
C20
C22
C23
C24
C26
C28
C30
14.72
19.72
24.72
29.72
34.72
39.72
44.72
49.72
54.72
59.72
64.72
Time

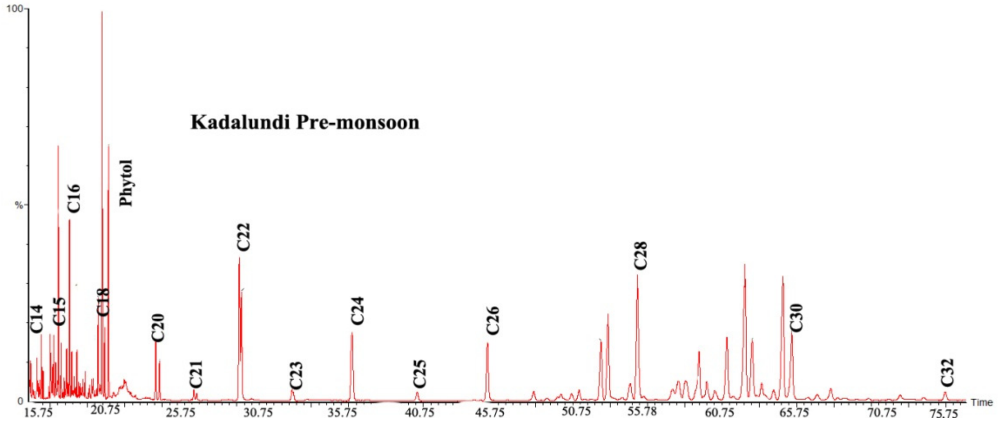

Pappinissery Post-monsoon
100
%
0
C14
C15
C16
C17
C18:1
C18
Phytol
C20
C22
C24
C26
15.06
17.06
19.06
21.06
23.06
25.06
27.06
29.06
31.06
33.06
35.06
37.06
39.06
41.06
43.06
45.06
Time

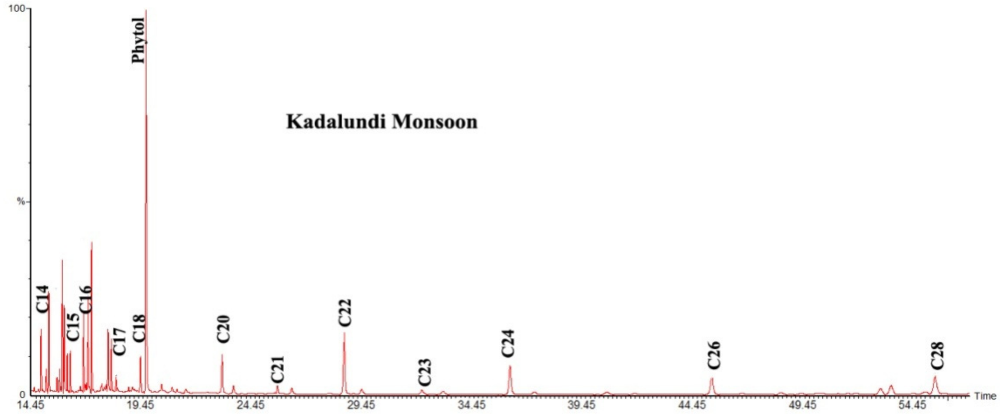

Pappinissery pre-monsoon
00
%
0
C14
C16
C15
C18
Phytol
C20
C22
C24
C26
C28
C30
15.94
20.94
25.94
30.94
35.94
40.94
45.94
50.94
55.94
60.94
65.94
Time


Pappinissery Monsoon
100
%
0
C12
C15
C14
C16
C17
C18
Phytol
C20:1
C20
C22
C24
C26
C28
C30
15.75
20.75
25.75
30.75
35.75
40.75
45.75
50.75
55.75
60.75
65.75
Time

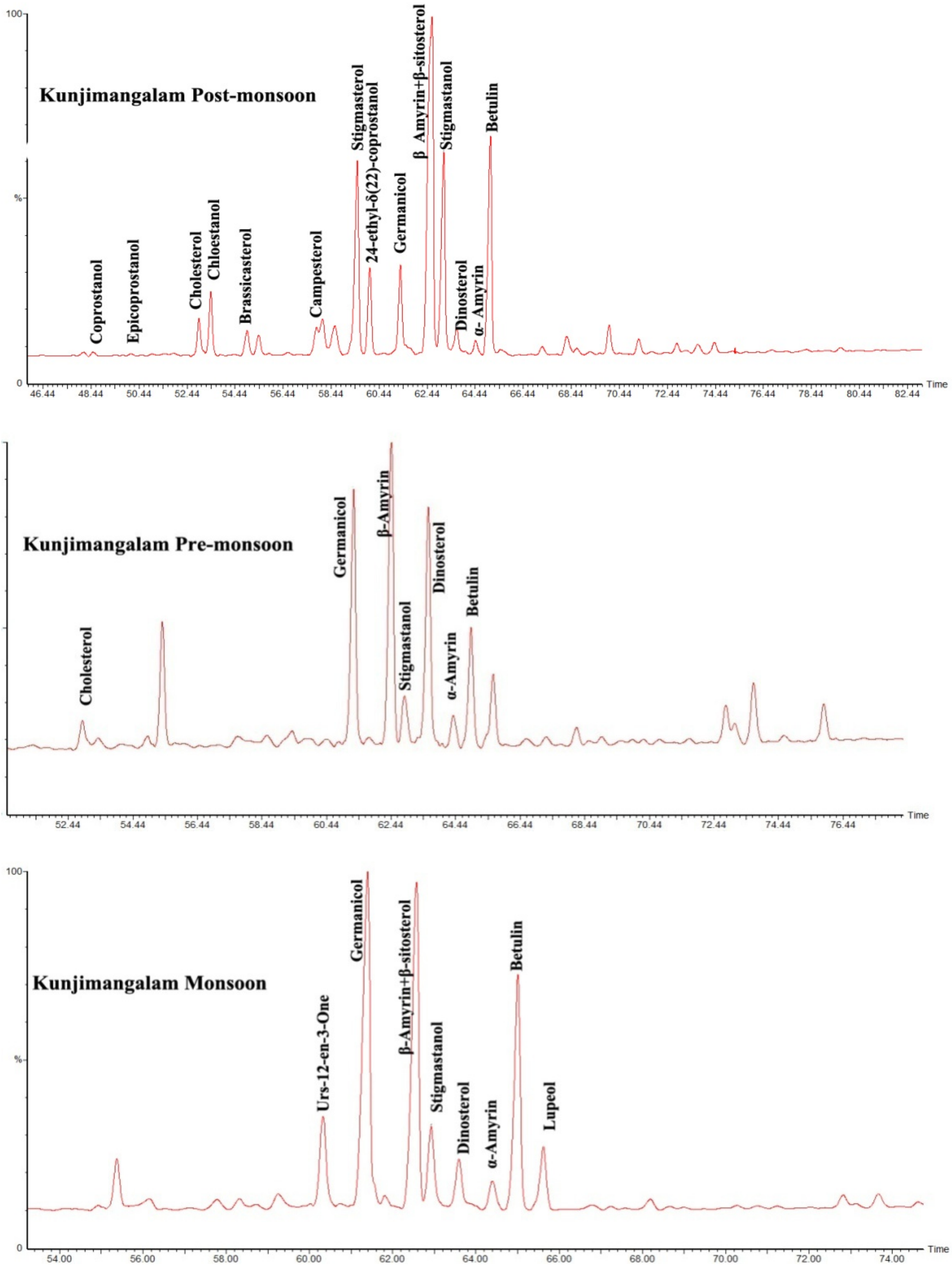

Thalassery Pre-monsoon
100
%
0
C14
C15
C16
C17
C18
Phytol
C20
C22
C24
C26
C28
C30
15.72
20.72
25.72
30.72
35.72
40.72
45.72
50.72
55.7
60.7
65.7
Time

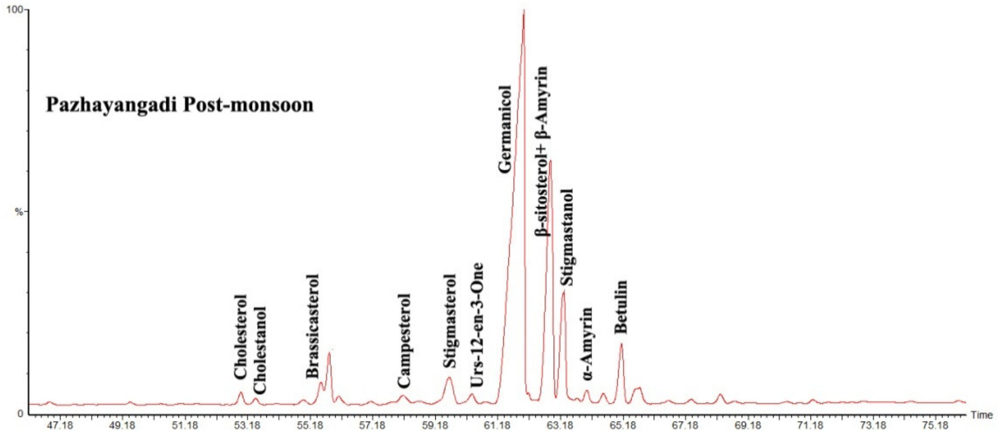

Thalassery Monsoon
100
%
0
C14
C15
C16
C17
C18
Phytol
C20
C22
C23
C24
C25
C26
C28
C30
C32
14.72
19.72
24.72
29.72
34.72
39.72
44.72
49.72
54.72
59.72
64.72
69.72
74.72
Time

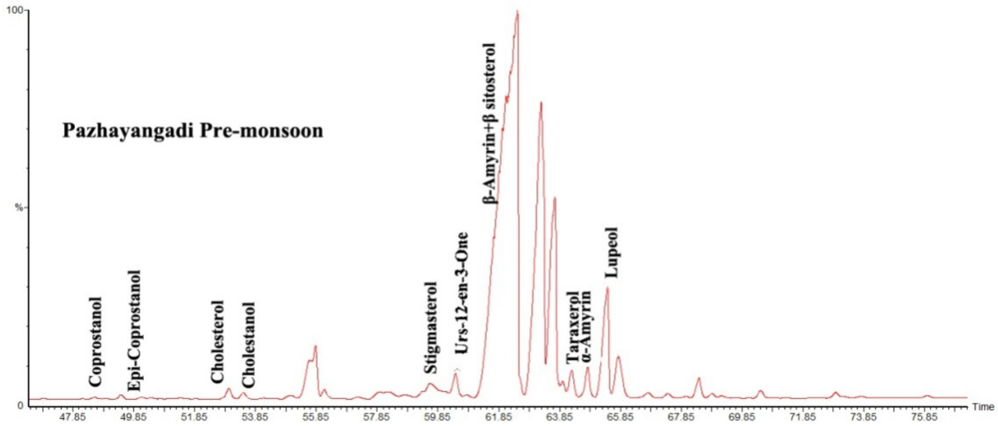

Kadalundi Pre-monsoon
100
%
0
C14
C15
C16
C18
Phytol
C20
C21
C22
C23
C24
C25
C26
C28
C30
C32
15.75
20.75
25.75
30.75
35.75
40.75
45.75
50.75
55.78
60.75
65.75
70.75
75.75
Time

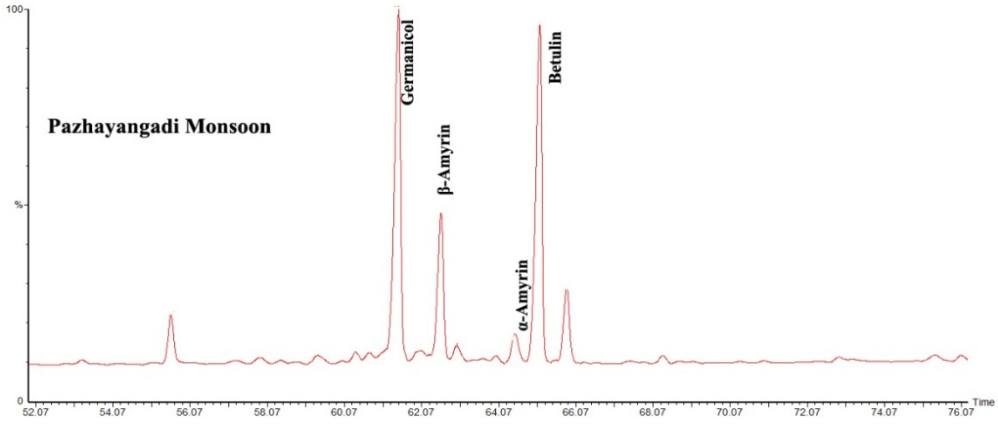

Kadalundi Monsoon
100
%
0
Phytol
C14
C15
C16
C17
C18
C20
C21
C22
C23
C24
C26
C28
14.45
19.45
24.45
29.45
34.45
39.45
44.45
49.45
54.45
Time

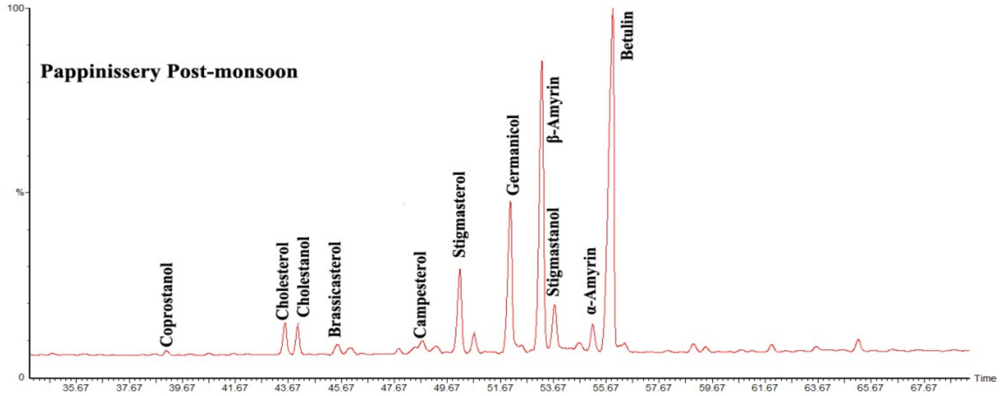
Appendix 9: Total ion Chromatogram of triterpeniods in the present study

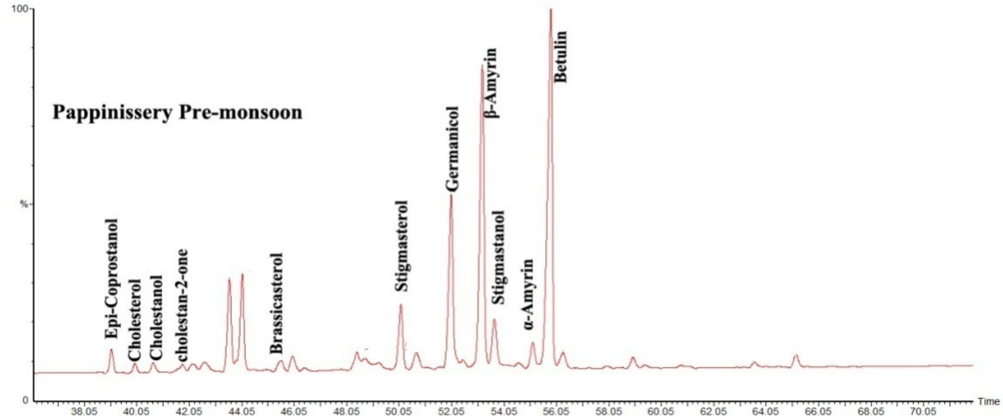


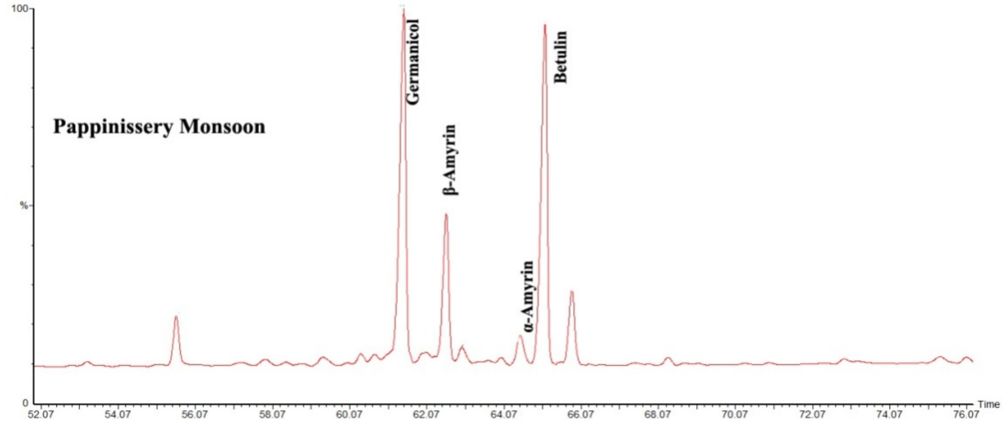

Pazhayangadi Post-monsoon
100
%
0
Cholesterol
Cholestanol
Brassicasterol
Campesterol
Stigmasterol
Urs-12-en-3-One
Germanicol
β-sitosterol+ β-Amyrin
Stigmastanol
α-Amyrin
Betulin
47.18
49.18
51.18
53.18
55.18
57.18
59.18
61.18
63.18
65.18
67.18
69.18
71.18
73.18
75.18
Time

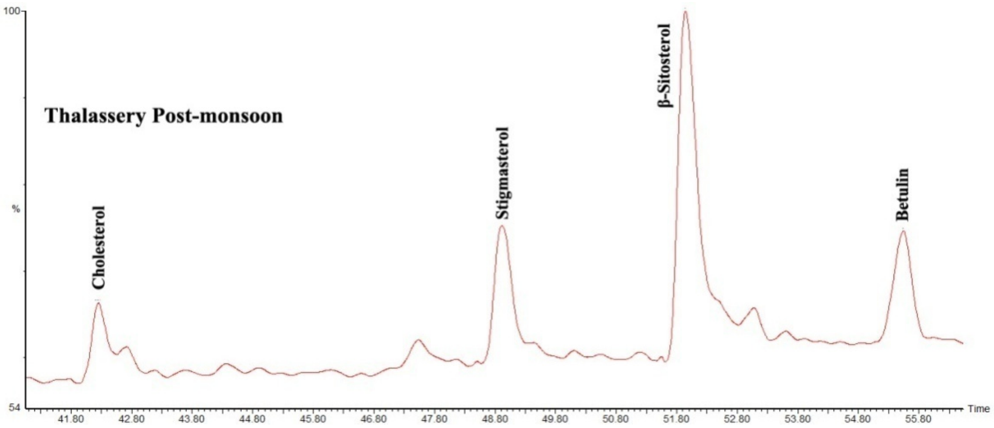

Pazhayangadi Pre-monsoon
100
%
0
Coprostanol
Epi-Coprostanol
Cholesterol
Cholestanol
Stigmasterol
Urs-12-en-3-One
β-Amyrin+β sitosterol
Taraxerol
α-Amyrin
Lupeol
47.85
49.85
51.85
53.85
55.85
57.85
59.85
61.85
63.85
65.85
67.85
69.85
71.85
73.85
75.85
Time

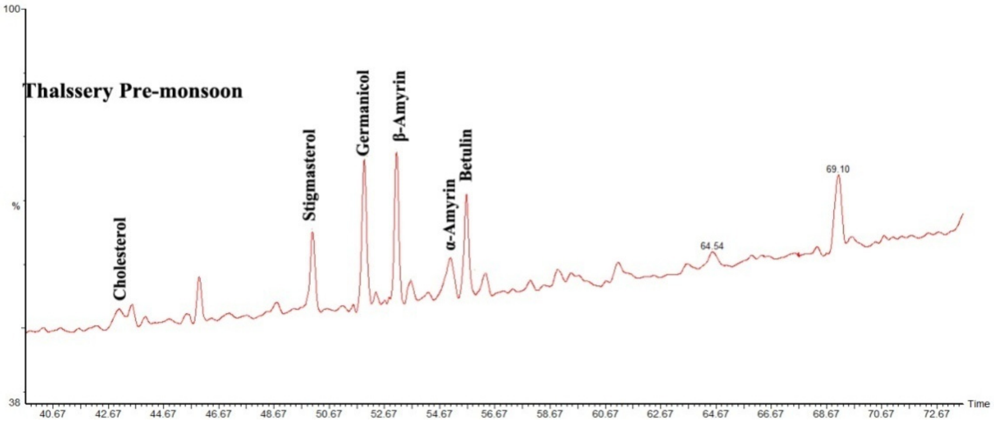

Pazhayangadi Monsoon
100
%
0
Germanicol
β-Amyrin
α-Amyrin
Betulin
52.07
54.07
56.07
58.07
60.07
62.07
64.07
66.07
68.07
70.07
72.07
74.07
76.07
Time

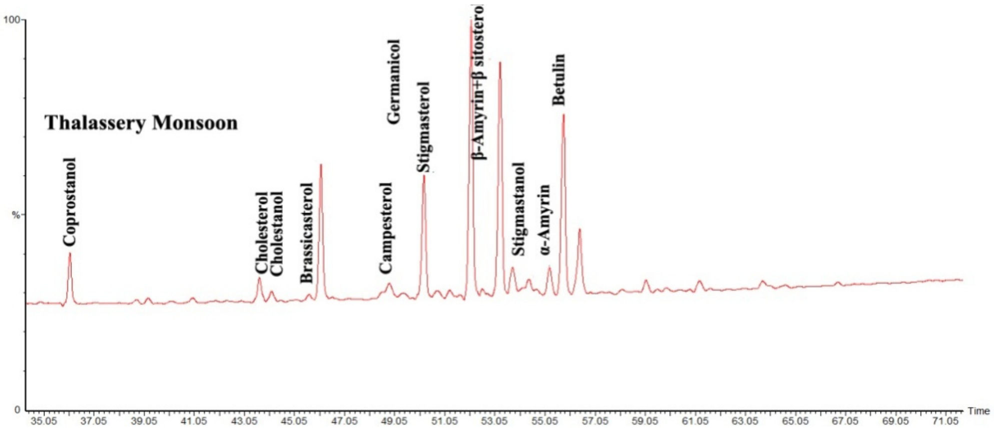

Pappinissery Post-monsoon
100
%
0
Coprostanol
Cholesterol
Cholestanol
Brassicasterol
Campesterol
Stigmasterol
Germanicol
β-Amyrin
Stigmastanol
α-Amyrin
Betulin
35.67
37.67
39.67
41.67
43.67
45.67
47.67
49.67
51.67
53.67
55.67
57.67
59.67
61.67
63.67
65.67
67.67
Time

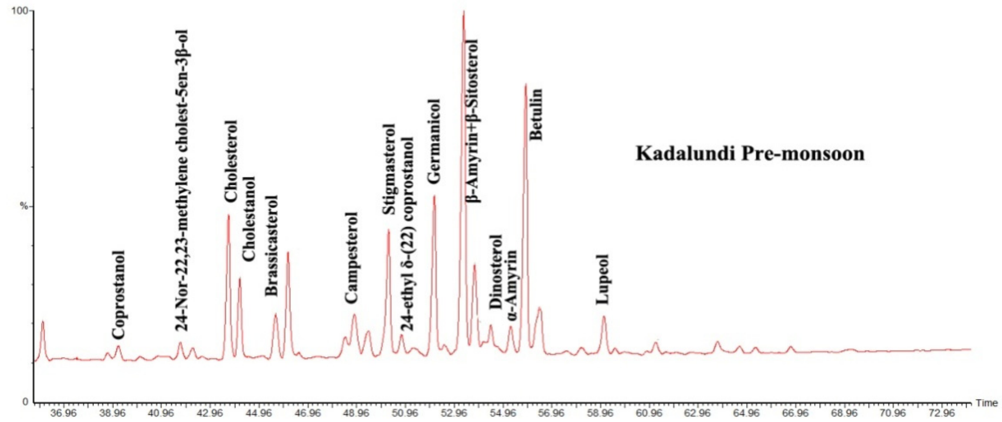

Pappinissery Pre-monsoon
100
%
0
Epi-Coprostanol
Cholesterol
Cholestanol
cholestan-2-one
Brassicasterol
Stigmasterol
Germanicol
β-Amyrin
Stigmastanol
α-Amyrin
Betulin
38.05
40.05
42.05
44.05
46.05
48.05
50.05
52.05
54.05
56.05
58.05
60.05
62.05
64.05
66.05
68.05
70.05
Time

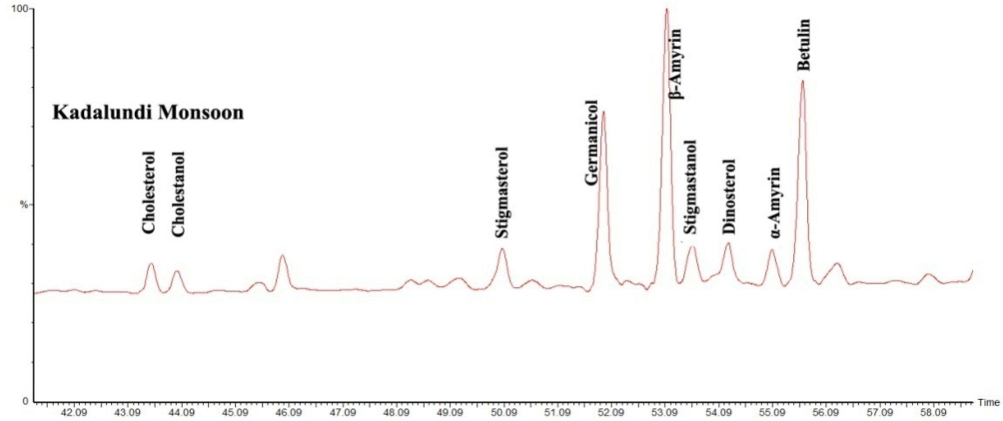

Pappinissery Monsoon
100
%
0
Germanicol
β-Amyrin
α-Amyrin
Betulin
52.07
54.07
56.07
58.07
60.07
62.07
64.07
66.07
68.07
70.07
72.07
74.07
76.07
Time


Thalassery Post-monsoon
100
%
54
Cholesterol
Stigmasterol
β-Sitosterol
Betulin
41.80
42.80
43.80
44.80
45.80
46.80
47.80
48.80
49.80
50.80
51.80
52.80
53.80
54.80
55.80
Time


Thalssery Pre-monsoon
100
%
38
Cholesterol
Stigmasterol
Germanicol
β-Amyrin
α-Amyrin
Betulin
64.54
69.10
40.67
42.67
44.67
46.67
48.67
50.67
52.67
54.67
56.67
58.67
60.67
62.67
64.67
66.67
68.67
70.67
72.67
Time


Thalassery Monsoon
100
%
0
Coprostanol
Cholesterol
Cholestanol
Brassicasterol
Campesterol
Germanicol
Stigmasterol
β-Amyrin+β sitostero
Stigmastanol
α-Amyrin
Betulin
35.05
37.05
39.05
41.05
43.05
45.05
47.05
49.05
51.05
53.05
55.05
57.05
59.05
61.05
63.05
65.05
67.05
69.05
71.05
Time


100
%
0
Coprostanol
24-Nor-22,23-methylene cholest-5en-3β-ol
Cholesterol
Cholestanol
Brassicasterol
Campesterol
Stigmasterol
24-ethyl δ-(22) coprostanol
Germanicol
β-Amyrin+β-Sitosterol
Dinosterol
α-Amyrin
Betulin
Lupeol
Kadalundi Pre-monsoon
36.96
38.96
40.96
42.96
44.96
46.96
48.96
50.96
52.96
54.96
56.96
58.96
60.96
62.96
64.96
66.96
68.96
70.96
72.96
Time


100
%
0
Kadalundi Monsoon
Cholesterol
Cholestanol
Stigmasterol
Germanicol
β-Amyrin
Stigmastanol
Dinosterol
α-Amyrin
Betulin
42.09
43.09
44.09
45.09
46.09
47.09
48.09
49.09
50.09
51.09
52.09
53.09
54.09
55.09
56.09
57.09
58.09
Time

……

List of Publication

[1]. Manju, M. N., **Resmi, P**., Gireesh Kumar T.R., Ratheesh Kumar, C.S., Rahul, R., Joseph, M. M., and Chandramohanakumar, N. Assessment of Water Quality Parameters in Mangrove Ecosystems Along Kerala Coast: A Statistical Approach Int. J. Environ. Res., 6(4):893-902.

Seminars accepted

[1]. **Resmi, P**., Manju, M. N., Gireesh Kumar T.R., Ratheesh Kumar, C.S., Rahul, R., Joseph, M. M., and Chandramohanakumar, N. Monitoring Water Quality in Mangrove ecosystems Along Kerala Coast. Paper accepted in Aquasem '13 Cochin University of Science and Technology.

[2]. **Resmi, P**., Manju, M. N., Gireesh Kumar T.R., Ratheesh Kumar, C.S., Joseph, M. M., and Chandramohanakumar, N. Spatial and seasonal variation of biochemical composition of sedimentary organic matter in Mangrove Ecosystems Along Kerala Coast: A baseline study. Paper accepted in Aquasem' 13 Cochin University of Science and Technology.

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