PROVENANCE, ISOLATION AND CHARACTERISATION OF ORGANIC MATTER IN THE COCHIN ESTUARINE SEDIMENT- "A DIAGENETIC AMINO ACID MARKER SCENARIO"

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By

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Provenance, Isolation and Characterisation of Organic Matter in the Cochin Estuarine Sediment-"a Diagenetic Amino Acid Marker Scenario"

Ph.D. Thesis under the Faculty of Marine Sciences

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Dedicated to my dear Parents .....

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Certificate

This is to certify that the thesis entitled ***PROVENANCE, ISOLATION AND CHARACTERISATION OF ORGANIC MATTER IN THE COCHIN ESTUARINE SEDIMENT-"A DIAGENETIC AMINO ACID MARKER SCENARIO"*** *is an authentic record of the research work carried out by Mr. Salas P.M, 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 fulfilment of the requirements for Ph.D degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any other University. I further certify that all the relevant corrections and modifications suggested by the audience during the pre-synopsis Seminar and recommended by the Doctoral Committee of Mr. Salas P M has been incorporated in the thesis.*

Dr. C. H. Sujatha
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Declaration

I hereby declare that the thesis entitled ***PROVENANCE, ISOLATION AND CHARACTERISATION OF ORGANIC MATTER IN THE COCHIN ESTUARINE SEDIMENT-"A DIAGENETIC AMINO ACID MARKER SCENARIO"*** *is an authentic record of the research work carried out by me under the guidance and supervision of Dr. C. H. Sujatha, Associate 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 from any University/Institution.*

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Preface

Estuaries are among the most productive areas of world's coastal zones which connect the carbon cycle of the oceans to the continents. These ecosystems play a pivotal role in the cycling of carbon and associated elements in tropical environments. Organic matter delivered by rivers can undergo prominent biogeochemical alterations and therefore estuaries are recognised as highly active areas in terms of organic matter and nutrient processing. The relative contribution of allochthonous and autochthonous input results in alterations in the biogeochemical cycling of organic matter. The carbon fixed in estuaries is highly important in the coastal food webs and exerts profound effect on promoting biodiversity richness. Large amounts of organic matter and nutrients, which support not only the fauna and flora of the system but also adjacent coastal habitats. Defining the sources and composition of organic matter within the estuaries is therefore essential to the understanding of the carbon cycle as well as to implement sustainable management practices for their conservation. A better knowledge of the geochemical characteristics is required for the evaluation of nature, source and degradation state of sedimentary organic matter.

Primary production creates large quantities of organic matter in these transitional ecosystems, of which a major fraction sinks through the water column and ultimately preserved in sediments. The quantity and quality of organic matter preserved in sediments varies greatly depending on the nature of material delivered to the sediment and on the depositional environment. Information on the processes controlling the input of organic matter to coastal environments is important for the

understanding of global biogeochemical cycles. Cochin estuary is a highly productive ecosystem, and its complex nature is attributed to permanent connection with the Arabian Sea and the input of significant quantities of organic matter and nutrients via river run off. Eventhough detailed information on organic matter dynamics is available in Cochin estuary, studies based on molecular level distribution characteristics of amino acids and free sugars has not been carried out yet.

The thesis entitled **Provenance, Isolation and Characterisation of Organic Matter in the Cochin Estuarine Sediment-" a Diagenetic Amino Acid Marker Scenario"**is an attempt to evaluate the quantity, quality and degradation state of the organic matter in the surface sediments of Cochin estuarine system with the application of bulk and molecular level approaches. Bulk parameters utilized for source characterisation include: biochemical composition, elemental ratios and stable carbon isotope ratio. The applications of organic molecules to studies of natural systems are their use as source and process indicators. In this study, free sugars and amino acids were selected as the molecular level tools to evaluate the productive nature, source and degradation state of sedimentary organic matter.

The thesis is divided into six chapters, grouping to different facts of the research objectives.

Chapter 1, **General Introduction** and it deals with the aim and scope of the present study. The general geographical location of the sampling sites and salient features of the study area are described in Chapter 2, **Materials and Methods**. It also describes the sampling and analytical methodology. Nine sampling stations spread

across the Cochin estuarine system, Southwest India were selected for the present study. The results of general hydrographic parameters and nutrients are also included here. Chapter 3 entitled "**Geochemistry of Phosphorous and Nitrogen fractions in Sediments"** includes the seasonal and spatial variations of the fundamental geochemical variables in the surface sediments. It also deals with the general sedimentary characteristics, elemental composition, phosphorous and nitrogen fractionation. Chapter 4- "**Quality Assessment of Organic Matter - Bulk Parameter Approach"**, deals with the biochemical composition of organic matter in the surface sediments to examine the quality and quantity of organic matter. Bulk sedimentary parameters such as elemental ratios and stable isotope ratios are also employed for source characterisation of organic matter. Chapter 5, "**Spatio-Temporal Variability of Free Sugar Distribution: Implications on Primary Productivity"**, explain the seasonal and spatial variation of free sugars in the sediments of the estuary and its implications on the primary productivity of the ecosystem. Chapter 6, entitled "**Distribution and Degradation Status of Amino Acids in Estuarine Sediments",** deals with the occurrence, spatio-temporal distributional characteristics of amino acids to evaluate the nature and degradation state of sedimentary organic matter. The salient findings and interpretations derived by detailed analysis of the data in each chapter is briefly outlined in **Summary** section. References are provided at the end of each Chapter. The results of biochemical composition, chromatograms of free sugars and amino acids are given in the **Appendix** provided at the end of the thesis.

Abbreviations

ANOVA	-	Analysis of variance
AAs	-	amino acids
AAs-N	-	amino acid nitrogen
AAs-C	-	amino acids carbon
chl-a	-	chlorophyll-a
chl-b	-	chlorophyll-b
chl-c	-	chlorophyll-c
C/g	-	carbon per gram
cm	-	centi meter
E	-	East
e.g	-	exempli gratia
h	-	hour
kg	-	kilogram
km^2	-	kilometre square
ml	-	millilitre
m/kg	-	milligram per kilogram
mg/g	-	milligram per gram
m^2	-	meter square
m^3	-	meter cube
mg	-	milligram
mgC/g	-	milligram carbon per gram
mg/l	-	milligram per litre
ml/l	-	millilitre per litre
MON09	-	monsoon 2009
MON12	-	monsoon 2012
N	-	north

Nd	-	not detected
nm	-	nano meter
^{o}C	-	degree Celsius
POM09	-	post monsoon 2009
ppm	-	parts per million
PRM09	-	pre monsoon 2009
PRM10	-	pre monsoon 2010
rpm	-	revolutions per minute
S	-	south
W	-	west
wt.	-	weight
µg/g	-	micro gram per gram
µg/l	-	micro gram per litre
µg/kg	-	micro gram per kilogram
µl	-	micro litre
µm	-	micro meter
µmol/g	-	micro mole per gram
µmol/l	-	micro mole per litre
β	-	beta
ϒ	-	gamma
‰	-	per mil

Contents

Chapter 1

INTRODUCTION

Contents

Estuaries represent a biogeochemically active zone, significantly transports both terrestrial and riverine inputs to the coastal zone. These unique, dynamic and complex environments are among the most productive ecosystems in the world (Chapman and Wang, 2001). Estuaries are commonly entitled as semi-enclosed bodies of water, situated at the interface between land and ocean, where seawater is measurably diluted by the inflow of freshwater (Hobbie, 2000). Another definition of the estuary according to Perillo (1995) is "a semi-enclosed coastal body of water that extends to the effective limit of tidal influence, within which sea water entering from one or more free connections with the open sea, or any other saline coastal body of water, is significantly diluted with fresh water derived from land drainage, and can sustain euryhaline biological species from either, part or the whole of their life cycle". These ecosystems perform important role in various ecological and biological functions (Dolbeth et al., 2007), provide a direct resource for commercially important species of fishes, shelter and food resources for commercially important shell species. They are significant for human welfare through their role in trade, transportation, production of food and various recreational pursuits.

1.1 Estuarine biogeochemistry

Estuaries are characterised by high rate of primary production and shallow water depth, both of which allow the accumulation of relatively large fraction of autochthonous and allochthonous organic matter to the sea floor and ultimately preserved in anoxic sediments (Hedges and Keil, 1995). Processing of organic matter along the estuarine mixing zone has the potential to modify the quantity, sources, and composition of organic carbon before its export to the coastal zone. The diversity in the origin of estuarine organic matter depends not only on the biological and geographical factors but also on the socio-economic environment through the effects of urbanisation, industrialisation and regional development (Galois et al., 2000). The sources of organic matter within the estuarine sediments is of utmost important in understanding the roles of terrestrial or estuarine derived organic matter as sources of energy and nutrients to coastal ecosystems (Yamamuro, 2000; Goni et al., 2003) and the potential enhancement of nutrient loads that contribute to eutrophication processes (Yamamuro, 2000). The preservation of organic matter in estuarine sediments is principally controlled by productivity, sedimentation rate, redox potential, adsorption and desorption (Hedges and Keil, 1995).

The diversity in the origin of estuarine organic matter depends on the natural biological and geographical factors, as well as on the anthropogenic interventions like urbanisation, industrialisation and regional development (Galois et al., 2000). The origin of organic matter in estuarine systems is often diverse, due to autochthonous and allochthonous inputs, including: phytoplankton (Meyers, 1997), algae (Meziane and Tsuchiya, 2000), bacteria (Dale, 1974) and terrestrial vegetation (Mfilinge et al., 2005). A greater knowledge of biogeochemical cycling in estuaries, which involves the

transformation, fate and transport of chemical substance, is critical in understanding the effects of these environmental alterations from regional to global context (Bianchi, 2007). Hence on a global basis, estuarine sediments are important sites for evaluating fluxes, cycling and storage of the chemical elements.

1.2 Primary production in estuaries

The photosynthetic fixation of inorganic carbon and nutrients into plant biomass is the primary source of organic matter existing in the estuaries. Primary production is simply defined as the photosynthetic formation of organic matter. Phytoplankton represents an important source of organic matter in most estuaries. Microphytobenthos consist of an assemblage of benthic diatoms that typically migrate vertically in the sediments over a diurnal period (Serodio et al., 1997). Enhanced turbidity in shallow regions from resuspension events can decline light penetration; hence, the most effective time for primary production occurs in intertidal sand and mud flats during daytime exposure periods (Guarini et al., 2000; 2002). Stumm and Morgan (1996) modified the stoichiometry of the chemical reaction of photosynthesis (primary production) and oxidation (degradation) of organic matter by the following equation:

$$106\,CO_2 + 16\,HNO_3 + H_3PO_4 + 122\,H_2O \underset{\text{photosynthesis} \rightarrow}{\overset{\leftarrow \text{respiration}}{\leftrightarrow}} (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138\,O_2$$

This equation offers a different perspective on how photosynthesis and degradation processes are linked to redox chemistry and the stoichiometric constraints on the availability of key elements in many biogeochemical cycles in aquatic ecosystems. Primary production related to cell abundance, diversity of phytoplankton that varies seasonally, concentration of various pigments and

primary productivity. Physico-chemical parameters like nutrient concentrations, chlorophyll, water transparency (Carlson, 1977; Kratzer and Brezonik, 1981) and primary production measurements (Nixon, 1995) have often been employed to assess trophic status. In the pelagic waters, concentration of inorganic nutrients such as nitrate, phosphate and silicate in the water determines the population growth of planktonic primary producers. The nitrogen as nitrate and phosphorus as phosphate greatly augment the primary productivity and both are essential for the survival of primary producers. Primary production can also be limited by Fe availability in coastal environments (Kirchman et al., 2000; Bruland et al., 2001).

Benthic macroalgae and microphytobenthos are important sources of primary production in estuaries and have significant effects on the seagrass, tidal flat and intertidal marsh habitats (Bianchi, 1988; Pinckney and Zingmark, 1993; de Jonge and Colijn, 1994). Common benthic macroalgae found in estuaries include: Chlorophyta (*Ulva latuca*, *Entermorpha intestinales*), Phaeophyta (*Fucus vesciculousus*), and Rhodophyta (*Gracilaria folifera*). The increase in anthropogenic loading of nutrients has resulted in numerous macroalgal blooms consisting primarily of the genera *Ulva*, *Enteromorpha* and *Gracilaria spp*. (Rosenberg and Ramus, 1984; Duarte, 1995; Kamer et al., 2001).

1.3 Nutrients in aquatic sediments

Sources of nitrogen and phosphorus in aquatic environments include: land run off, where synthetic fertilizers and detergents are the major contributors. Concentration of nutrient elements in aquatic systems are governed by the biological uptake, regeneration and other geochemical processes. The study of nutrients in the dissolved and particulate forms would help in understanding the potential availability of life supporting elements in

any particular aquatic region (Klump and Martens, 1981). Phosphate can be present in association with metals like Fe, Al and Ca oxides or adsorbed on the surface of minerals and organic materials. The range of variables like salinity, pH and redox potential in estuarine systems determines the relative importance of each phosphorous fractions (Lebo, 1991; Paludan and Morris, 1999).

Growth rate and reproduction of organisms depends not only upon the availability of carbon, water and energy but also a variety of essential mineral nutrients. The important factors controlling the productivity of estuaries are nitrogen in chlorophyll and amino acids, phosphorous in adenosine triphosphate (ATP) and phospholipids. Some of these essential elements (N, P, Ca and Si) are generally abundant, and so can be termed macronutrients, whereas others (Fe and Mg) are required by organisms in only trace amounts and are called micronutrients. Nitrogen and phosphorous availability can also limit primary production in the aquatic environments, therefore, termed bio limiting elements.

Nitrogen (N) and phosphorous (P) are the primary nutrients that affect sediment and water quality in rivers (Nair et al., 1983; Babu, 1999). Surface runoff can contribute nutrients, particularly P in connection with the application of fertilizers in agricultural fields. Sewage effluent is also another important source of N and P in rivers. Nitrogen and phosphorous are the two elements that react completely different when once emitted to the terrestrial and aquatic environment. The fate of P in the soil is dominated by chemical processes like adsorption-desorption and dissolution-precipitation, whereas the fate of N is dominated mainly by biological processes such as mineralisation, nitrification, and denitrification (Edwards and Withers, 1998). Nutrients limiting the primary production in aquatic ecosystems may vary locally. Nitrogen is the most common limiting element of primary production in most marine ecosystems (Mortimer et al., 1999). Phosphorous can be a limiting

nutrient in coastal systems (Thingstad et al., 1998) due to eutrophication resulting from increased population, urbanisation and industrialisation. Nitrogen and phosphorous exchange at the water-sediment interface is controlled by many complex physicochemical factors, as well as by biological processes. Zoo benthos can influence nutrient dynamics at the water-sediment interface through excretion of nutrient compounds, and through continuous release of nutrients from sediments along the channels created by bioturbation activity (Risnoveanu et al., 2004). Sediment bound organic matter content can function as a limiting factor and act as an important indicator for nutrients and production in both aerobic and anaerobic sediments.

The significant inputs of nitrogen containing compounds to estuaries have been linked to freshwater inputs from rivers (Nixon et al., 1995; Seitzinger et al., 2002; Bouwman et al., 2005). Many of these nitrogen inputs have increased in estuaries around the world as a direct result of human population expansion (Howarth et al., 1996; de Jonge et al., 2002; Bouwman et al., 2005). Export of dissolved inorganic nitrogen to coastal environment, frequently leads to enhanced primary production, since many estuaries are N limited (Nixon, 1995; D'Elia et al., 1992; Howarth et al., 2000). This can result in the formation of harmful algal blooms as well as hypoxia, and ultimately creates anoxic water columns (Valiela et al., 1990; Boynton et al., 1995; Richardson, 1997). Hence in the present investigation, the fractionation of nitrogen was carried out to assess its bioavailability as well as its role on the fertility of the Cochin estuarine system.

Internal loading of sediment bound P can be a significant term in the annual phosphorous budget of an aquatic ecosystem. Moreover release of sediment bound nutrients represent a more ecologically important process than inputs from external nutrient sources because P released from sediments often

contains a larger quantities of bio available portion (Pardo et al., 2003). Therefore, it is critical to characterise sources of P, both external and internal, to rivers and reservoirs in order to manage nutrient inputs to aquatic systems. Several methods can be used to investigate the bioavailability/mobility of sediment bound phosphorus; among these, chemical fractionation involving extraction procedures has widely been used. The fractionation of P can be used as an effective tool for unravelling the redox processes acting along the salinity gradients of the Cochin estuary. Since an important fraction of the chemicals present in the aquatic environment is reversibly associated with sediments, the study of nutrient dynamics was performed in this research work as a prerequisite to understand their behaviour as well as the general geochemical settings of the sedimentary environment.

1.4 Characterisation of bulk sedimentary organic matter

Source characterisation of organic matter associated with estuarine sediments is an essential criteria in unravelling the autochthonous and allochthonous input as well as to assess global biogeochemical cycles (Yamamuro, 2000; Goni et al., 2003).In situ biological production and accumulation of marine particles and terrestrial origin have been recognised as the significant sources of organic matter to estuarine sediments (Mayer et al., 1988; Cifuentes, 1991). However in the case of anthropogenically affected estuaries, urban sewage inputs strongly influence the quantity and quality of incoming materials at sediments and therefore, the nature of organic materials depend on complex physicochemical processes (Cotano and Villate, 2006).

1.4.1 Biochemical composition

Indications on the origin and the processes involved in the transformation of organic matter in sediments can be achieved by several methodological approaches. Biochemical composition (total proteins, total lipids and total carbohydrates) serves not only as a valid methodology to quantify the organic matter (Colombo et al., 1996), but also a useful tool to evaluate its nutritional quality - as available food source for benthic consumers (Dell Anno et al., 2000; Cividanes et al., 2002; Joseph et al., 2008). The portions of sedimentary organic matter which are more readily available to benthic consumers (labile fraction), have usually been evaluated by estimation of the main biochemical classes of organic compounds (Danovaro et al., 1993; Fabiano et al., 1995; Dell'Anno et al., 2002). Composition of organic matter in sediments has been established as the major factor affecting metabolism, distribution and dynamics of benthic organisms (Grant and Hargrave, 1987; Graf, 1989; Duineveld et al., 1997) and has been widely employed to evaluate the trophic state of marine ecosystems (Cloern, 2001; Dell' Anno et al., 2002; Renjith et al., 2012). Moreover protein to carbohydrate ratio and the lipid to carbohydrate ratio have been used as valuable indicators to investigate the status of biochemical degradation processes (Galois et al., 2000).

1.4.2 Elemental composition

Elemental analysis can meaningfully constrain the structural characteristics and biochemical compositions of individual organic materials (Mitchell et al., 1997; Andrews et al., 1998; Graham et al., 2001). The major elements occurring in pure organic substances viz. C, H, N, O, and S can be routinely analysed by a combination of methods involving combustion and pyrolysis. C, H, N, and S are measured simultaneously by high temperature

(>1000 ^{o}C) combustion to CO_2, H_2O, N_2 and SO_2 gases, which are separated and quantified (Hedges and Stern, 1984; Verardo et al., 1990). Among the bulk parameters, TOC/TN ratio has been used as best descriptor for the quality of organic matter in sediments (Huston and Deming, 2002). Besides TOC/TN ratios have been widely used to differentiate the sources of organic matter based on the assumption that algal derived organic matter exhibit TOC/TN ratios between 4 and 10, whereas organic matter delivered from terrestrial vascular plants record TOC/TN ratios of 20 and greater (Redfield et al., 1963; Ishiwatari and Uzaki,1987; Ruttenberg and Goni, 1997; Lehmann et al., 2002). The distinction in organic matter sources arises on account ofthe absence of cellulose in algae and its greater abundance in terrestrial vascular plants. The characteristic lowering of TOC/TN ratios in sedimentary phase might be attributed to microbial immobilisation of nitrogenous material accompanied by the remineralisation of carbon (Sollins et al., 1984). According to the previous investigation, the selective degradation of organic matter during early diagenesis, results in the variation of TOC/TN/P ratios in sediments (Meyers, 1997).

1.4.3 Stable isotope composition

$\delta^{13}C$ values are most commonly employed as indicators of ultimate plant or geographic sources (Dittmar et al., 2001; Bouillon et al., 2003). In order to distinguish between the relative contributions of terrestrial versus marine organic matter in sediments, comparison of the more negative $\delta^{13}C$ value of common C3 or C4 land plants with marine plankton is a widely accepted methodology (Smith and Epstein, 1971; Rau, 1978; Forsberg et al., 1993). The principle involved in the application of stable isotopes in natural ecosystems is the alterations in the relative abundance of lighter isotopes arising due to chemical processes (Hoefs, 1980). The faster reaction kinetics of the lighter isotope of an element, results in a situation where the reaction products in nature can be enriched with lighter isotopes (Killops and Killops,

2005). The process is known as isotopic fractionation, which have proven to be useful in determining source of organic matter in biogeochemical studies.

1.5 Molecular level characterisation

Molecular characterisation can be defined as the analysis and quantification of an essentially pure type of organic compound or classes of compounds. Natural organic materials usually contain many molecular components, their characterisation typically involves several preparation and isolation steps prior to analysis. For biopolymers, a common preparation step is necessary to break the parent biochemical into its structural units that can be chromatographically separated and quantified. The analysis step may involve simple detection of individual compounds as they elute through a liquid chromatograph or other separation system, coupled with characterisation methods (often spectral or mass based) that have sufficiently high sensitivities and fast enough response times to operate in a continuous flow mode.

A number of methodologies have been organised and utilised for the characterisation of organic matter in aquatic sediments. Among these tools (bulk and molecular level approaches), lipid class of organic compounds are of particularly advantageous since they can reveal valuable information on sources of organic matter at the molecular level (Meyers, 2003). Even though bulk organic matter source indicators play vital role in identifying the origin of organic matter, molecular constituents provide details of production, delivery and preservation of sedimentary organic matter (Meyers, 1997). Previous investigations have successfully used molecular level approach to explore the origin of organic matter in coastal areas, estuaries, rivers and lakes (Jaffe et al., 2001; Bianchi et al., 2002; Mead et al., 2005). Analysis of lipids in sediments has been successfully employed to unravel the environmental changes that have brought about alterations in the sources of organic matter (Zimmerman

and Canuel, 2000). Amino acids and carbohydrates (free sugars) were the organic compounds selected for the molecular level studies in the present investigation.

1.6 Carbohydrates in sedimentary organic matter

The cycling of carbohydrates is a key process in the marine carbon cycle due to its abundance and omnipresent nature in the marine ecosystem. Carbohydrates form an important fraction of the organic carbon produced by phytoplankton (Biddanda and Benner, 1997; Biersmith and Benner, 1998) as well as major constituents of dissolved, particulate and sedimentary organic matter (Cowie and Hedges, 1984; Benner et al., 1992). Different types of carbohydrates, engaged with various functions are synthesised by phytoplankton. In addition to this phytoplankton releases a portion of the organic compounds formed by primary production directly as dissolved organic carbon (Nagata, 2000; Teira et al., 2001), a significant part of which typically consists of carbohydrates (Biersmith and Benner, 1998; Aluwihare and Repeta, 1999). Carbohydrates represent important structural and storage materials in both terrestrial and aquatic organisms and denote the most abundant class of organic compounds in the environment. Algae and other chlorophyll bearing organisms, in presence of sunlight transform CO_2 and water to polymers such as cellulose, starch and related compounds (Rawn, 1990).

Carbohydrate constitutes a major resource of reduced carbon, which is predominantly recycled in the water column, where it is used as energy and a carbon source for the food web. In general, at the basis of the food web, particulate organic matter is taken up by grazers, while the dissolved organic matter is utilised by bacteria. A large fraction of the dissolved and particulate marine organic matter comprised of carbohydrates released from

polysaccharides and other biopolymers during hydrolysis (Benner, 2002). Biogeochemical studies on sedimentary organic matter, have achieved greater attention during the past few decades, with numerous advances in understanding the diagenetic fate and versatile of applications (Benner and Opsahl, 2001; Amon and Benner, 2003; Jia et al., 2008). Sediments occurring in estuaries are vital reservoirs of carbohydrates, originated via photosynthesis. Carbohydrates comprise 75% of weight of terrestrial plant tissue, present in structural polysaccharides such as cellulose, hemicellulose and pectin (Aspinall, 1970; Sjostrom, 1981); whereas constitutes only 20 and 40% of weight in plankton and bacteria respectively (Parsons et al., 1984; Moers et al., 1993).

The individual neutral sugars in aquatic ecosystems may have different origins (phytoplankton, zooplankton, bacteria, debris from local vegetation and soil organic matter) (Cowie and Hedges, 1984; Guggenberger et al., 1994b). Production of carbohydrate by microorganisms depends on several factors such as phytoplankton biomass, phytoplankton species, phase of growth, nutrient status and bacterial activities (Morris, 1981; Sakugawa and Handa, 1985). Most photosynthesizing organisms are aerobes: vascular plants, macroscopic algae (seaweeds), unicellular algae (phytoplankton), cyanobacteria and prochlorophytes.

1.7 Amino acids in sedimentary organic matter

General structure of amino acids (AAs) is $NH_2CH(R)$-COOH, where the side chain (R group; see Figure 1.1) may vary in size, shape, charge and hydrophobicity. There are hundreds of amino acids in nature, among them only 20 are commonly found in proteins, which are reported to have significant concentration in the aquatic ecosystems. It can be placed in the category of either essential or non-essential amino acids. Essential amino acids are those that are

"essential" in the diet; the living organisms cannot create them through their own metabolism. Therefore, organisms need to obtain them through foods containing them. The non-essential amino acids are those which can be produced from other amino acids and substances in the diet and metabolism. Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryphtophan and valine are grouped as essential AAs. Meanwhile, arginine, alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine and tyrosine are nonessential AAs.

Amino acids are the major N forms and important components of organic carbon in most of the marine organisms (Parsons et al., 1977). They are typically labile relative to bulk carbon and N and account for a considerable portion of the particulate organic carbon and N recycled in both water column and sediments (Henrichs and Farrington, 1987; Burdige and Martens, 1988) and therefore are important nutrients for secondary producers. Amino acids are structural components of proteins and are the most important nitrogen bearing compound in most organisms. Their analysis in sediments provides useful tool to evaluate the reactivity of particulate organic matter (Jennerjahn and Ittekkot, 1999; Kerherve et al., 2002; Pantoja and Lee, 2003). Plant remains and other debris contribute nitrogen in the form of amino acids. Amino acids exist in soil in several different forms, like free amino acids in the sediment micropores; as amino acids, peptides or proteins bound to clay minerals; as amino acids, peptides or proteins bound to humic colloids. Proteins most likely form the principal source of nitrogen for benthic heterotrophs (Cowie and Hedges, 1994; Wakeham et al., 1997; Dauwe and Middleburg, 1998). Despite the ubiquitous distribution among living organisms, the molar composition of amino acids provide clues to the sources of organic matter (da Cunha et al., 2002; Jennerjahn, 2004).

R group	name	R group	name
—H	glycine	—CH_2CNH_2 (O)	asparagine
—CH_3	alanine		
—$CH(CH_3)_2$	valine	—$CH_2CH_2CNH_2$ (O)	glutamine
—$CH_2CH(CH_3)_2$	leucine	—$(CH_2)_4NH_2$	lysine
—$CHCH_2CH_3$ (CH_3)	isoleucine	—$(CH_2)_3NHCNH_2$ (NH)	arginine
—CH_2—(benzene)	phenylalanine	—CH_2—(indole)	tryptophane
—CH_2OH	serine		
—$CHOH$ (CH_3)	threonine		
—CH_2—(benzene)—OH	tyrosine	—CH_2—(imidazole)	histidine
—CH_2OOH	aspartic acid	—CH_2SH	cysteine
—CH_2CH_2OOH	glutamic acid	—$CH_2CH_2SCH_3$	methionine
(H_2N—C—H, COO^-)	proline		

Figure 1.1 Structure of the side chains (R groups) in various amino acids; source: Killops and Killops, 2005

1.8 Aim and scope of the study

The peculiar geographic location of estuaries make them to act as receptors of organic matter - both natural and anthropogenic; originate from terrestrial run off, riverine and aeolian input. Estuarine ecosystems are proper environments for studying the source, pathway and fate of organic matter due to the rapid accumulation of fine sediments and enhanced preservation potential (Hedges and Keil, 1999). It is therefore, crucial to distinguish the relative contribution of different sources of organic carbon to the biogeochemical cycles in estuarine environments to substantiate their ecological importance.

Eventhough a number investigations established their ecological, economical and societal value, estuarine ecosystems are under the threat of destruction due to urbanisation, industrialisation, coastal development, altered hydrology and sea level rise. The ecological functioning of these ecosystems has been varied without public notice. On account of ecological and economic importance as a coastal resource, estuaries requires more attention to protect these fragile ecosystems. Ever increasing demographic pressure and indiscriminate anthropogenic interventions during the past decades have delivered bulk quantities of nutrients, heavy metals and organic constituents to these vulnerable ecosystems. The evidence has been reported for nutrient enrichment, heavy metal accumulation and decline in productivity associated with the reduction in phytoplankton density in the Cochin estuarine System (Selvaraj et al., 2003; Jyothybabu et al., 2006; Ratheesh Kumar et al., 2010; Renjith et al., 2012; Selvam et al., 2012). The investigations on biogeochemical aspects of sedimentary organic matter have been carried out as an essential requirement to evaluate the quality, source and carbon budget. Since the organic matter deposited in aquatic sediments is directly related with the primary productivity, biogeochemical characterisation has to be adopted as a tool for the conservation and sustainable management of these vulnerable ecosystems.

The biogeochemical functioning of estuarine environments has been assessed in a number of regions around the world (Galois et al., 2000; Bianchi, 2007; Gireeshkumar et al., 2015), but the ability to elucidate carbon and nutrient budgets of these ecosystems is still incomplete. Long term monitoring of the geochemical parameters is required since the biogeochemistry of estuaries are complex due to the tidal influx of allochthonous organic matter via terrestrial run off. In order to understand the relative importance of biogeochemical processes, it is necessary to characterise the organic matter as well as to identify

its origin. Common chemical parameters are insufficient to explain the biogeochemical character of these fragile ecosystems effectively. Bulk geochemical parameters such as biochemical composition, elemental composition and stable carbon isotope ratio are relatively reliable proxies of organic matter origin. More specific information on organic carbon dynamics can be assessed through effective tools like molecular level characterisation.

Cochin estuarine system, a highly productive tropical wetland (Ramsar site No. 1214) play an important role as a nursery ground for a number of species of fishes, molluscs and crustaceans. Patches of mangroves distributed around the estuary create shelter to juveniles of many important species. But the developmental activities around Cochin estuarine system have added to the complexities and environmental alterations. The estuary is under the influence of severe contamination in connection with the release of untreated effluents from industries and domestic sectors. Moreover, indiscriminate reclamation of land has declined the area of this unique tropical wetland from 365 to 265 km^2 (Gopalan et al., 1983). Extensive research on the physical, chemical and biological characteristics of Cochin estuary have been attempted by scientific community during the past decades, on account of its economical as well as environmental relevance. A number of studies have focussed on various physical, chemical and biological characteristics of this tropical wetland which have clearly evaluated the productivity, nutrient enrichment and heavy metal contamination (Balachandran et al., 2005; Deepulal et al., 2011, Selvam et al., 2012). Severe encroachment, developmental and demographic pressure have exerted marked fluctuations in ecological functions.

Studies on hydrodynamic conditions of Cochin estuary carried out in the last four decades have revealed drastic alterations in hydrological, biological and geological conditions (Qasim, 1980; Lakshmanan et al., 1982;

Joseph and Kurup, 1990; Menon et al., 2000; Balachandran, 2001; Joseph and Ouseph, 2009; Revichandarn et al., 2012). Total organic content in sediments play a significant role in the biogeochemical cycles and its degradation causes the production CO_2 and other green house gases, which can trigger climatic changes. Therefore, degradation state of OM in sediments constitute an integral processes of estuarine ecosystem dynamics. Review of literature reveals that the characterisation of organic matter and the associated biogeochemical processes using amino acids and carbohydrates is poorly studied in this highly dynamic ecosystem. In this context, the present investigation intend to unravel the nature, preservation and degradation state of sedimentary organic matter in the surface sediments of Cochin estuary.

Organic matter content in sediments is the most significant variable in biogeochemistry which regulates the distribution of other significant variables in the aquatic environment. Therefore, the quality and quantity of organic matter has to be assessed to have a better understanding of the biogeochemical cycling of carbon and other associated elements. Organic matter content in estuarine sediments are either originated from in situ primary production or by allochthonous inputs, resulting in a highly complex nature. The origin, distribution and degradation state of organic matter in sediments can be evaluated either by bulk parameters and molecular level approaches. The present study employed the bulk organic matter parameters like elemental ratios, stable carbon isotope and biochemical composition for source characterisation. The productive nature of the estuary was unravelled by biopolymeric carbon which categorised the stations as oligotrophic (unproductive), mesotrophic (intermediate productivity) and eutrophic (highly productive) states.

Since nutrient content is directly linked with primary productivity, nutrient fractionation studies was carried out to assess concentration of

bioavailable N and P in the sedimentary environment as well as nutrient enrichment. Although, there have been numerous works that attempted to trace the source of organic matter in estuarine systems (Akhil et al., 2013; Gireeshkumar et al., 2015), the degradation status of organic matter has not been investigated yet now. Molecular level detection and concentration of amino acids was utilised to describe the distribution, quality and degradation status of sedimentray organic matter in the estuarine system under investigation. Degradation status of the sediments is a useful criteria while evaluating the carbon budget of aquatic environments. Since carbohydrates form an integral part of estuarine organic matter, distribution and content of free sugars in sediments was also analysed to establish the productive nature of the estuary. Understanding the biogeochemical processes is a fundamental aspect of scientific investigations to put forward strategies to conserve vulnerable ecosystems and to implement proper management practices and therefore the study implies the social relevance to the public.

Objectives of the present study

- To find the nutrient enrichment in the estuarine sediments using phosphorous and nitrogen fractionation.
- To assess the spatio-temporal variations, nature and quality of bulk sedimentary organic matter as well as the benthic trophic status of the estuary.
- Extraction, quantification and distribution of free sugars in sedimentary organic matter and its implications on productivity.
- Distribution pattern and diagenetic process of amino acids in order to unravel the quality of estuarine sedimentary organic matter.

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Chapter 2

MATERIALS AND METHODS

Contents

2.1 Description of the study region

Cochin estuarine system (Latitude: 9° 40' & 10° 12' N and Longitude: 76° 10' & 76° 30' E) forms a complex, network of shallow brackish water environment (250 km^2) running parallel to the Kerala coast, with two permanent openings to the Arabian Sea. It is one of the most productive estuarine ecosystems (Qasim, 2003) and has been designated as a 'Ramsar site' (No. 1214). This tropical aquatic system is under the profound influence of monsoon, which contributes to about 71% of the annual rainfall (Jayaprakash, 2002) and accordingly there are three seasonal conditions prevailing viz. monsoon (June-September), post-monsoon (October-January) and pre-monsoon (February-May). Tides occurring in this estuary are of a mixed semi-diurnal type, exhibiting a maximum spring tide range of approximately1m (Srinivas et al., 2003). Constant mixing with seawater through tidal exchanges has provided the characteristics of a tropical estuary (Balchand and Nair, 1994; Ajith and Balchand, 1996). Investigations on hydrobiological aspects of the estuary pointed out the fact that high flushing

process during monsoon completely transforms the estuary into a freshwater habitat (Menon et al., 2000). Six rivers (namely: Periyar, Muvattupuzha, Pamba, Manimala, Meenachil and Achencovil) discharge about 20000 x 10^6 m^3 of fresh water into the estuary annually (Srinivas, 1999) and variation in the river discharge induces a salinity gradient, which in turn causes remarkable diversity in plankton population. Moreover the circulation patterns in the northern and southern arms of the Cochin estuary are distinctly different, ascribed to the peculiar topography. The north-western part frequently develops flow restrictions due to converging tides entering from two adjacent inlets, whereas the southern arm experiences tidal amplification (Balachandran et al., 2008).

Cochin estuarine system, the well-known biodiverse wetland is under the threat of severe ecological degradation due to massive reclamation (Gopalan et al., 1983), increased industrialisation and urbanisation (Menon et al., 2000; Qasim 2003). The major industries located around the study region include: Fertilizers and Chemicals Travancore Ltd. (FACT), oil refinery (Bharath Petroleum Corporation Limited- Kochi Refinery), rare earth processing plant (Indian Rare Earth limited), mineral sand rutile plant, zinc smelter plant (Binani Zinc), insecticide manufacturing unit (Hindustan Insecticides Limited) and organic chemical plant (Hindustan Organic Chemicals Limited). The hydraulic barriers constructed on the southern limb of the estuary at Thanneermukkam region to prevent saline ingression into the upstream agricultural fields has imposed severe flow restrictions and increased sedimentation in the estuary (Menon et al., 2000).

2.2 Sampling, storage and analytical methods

The water and sediment samples from nine stations situated along the Cochin estuary were collected in five sampling campaigns viz., April 2009 (pre monsoon 2009: PRM09), August 2009 (monsoon 2009: MON09), January 2010 (post monsoon 2009: POM09), April 2010 (pre monsoon 2010: PRM10) and September 2012 (monsoon 2012: MON12). As the estuary has been continuously subjected to severe deterioration, on account of urbanisation and industrialisation, a regular monitoring of the physicochemical variables is an essential requirement to assess the variability in parameters and ecological health. Monsoon bring about heavy rainfall and the terrestrial run off delivers huge loads of allochthonous materials to the estuary, causing wide fluctuations in physicochemical variables. Hence to understand the recent state of the system and to gather an updated information on the biogeochemical status of the estuarine system, sampling during the monsoon 2012 (MON12) was also carried out.

The exact geographical location of sampling points and their characteristic features are depicted in Figure 2.1 and Table 2.1. Among the stations, Karippadam (S1), Murinjapuzha (S2), Perumbalam (S3) are river influenced with thickly populated banks and characterised by input of domestic wastes. Meanwhile fishing activities are prevailing at stations Thevara (S4), Marine Science Jetty (S5), Bolghatty (S6), Mulavukad (S7), and Cheranellur (S8) and are contaminated with domestic sewage. Inland navigation and tourism activities are prevalent at S8 and S9, while industrial belt (Northern part of the study area) include the station Eloor (S9), severely contaminated with untreated industrial effluents.

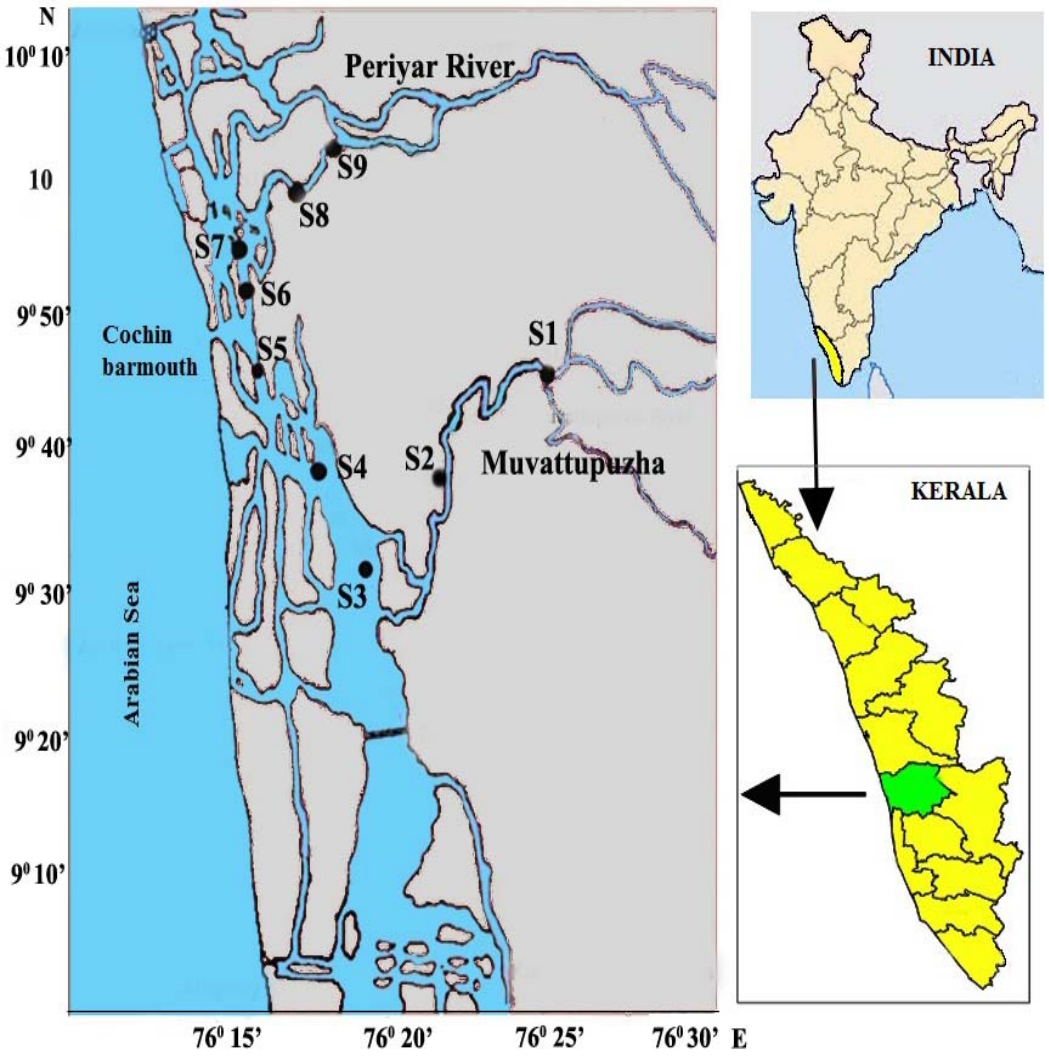

Figure 2.1 Location map of the sampling stations

Table 2.1 Geographical locations and characteristic features of sampling points

Station Code	Sampling site	Depth (m)	Latitude	Longitude	Description
S1	Karippadam	4.3	9^0 47'N	76^0 25'E	Thickly populated area with outflow of domestic wastes.
S2	Murnijapuzha	4.6	9^0 49'N	76^0 21'E	Disposal of domestic wastes
S3	Perumbalam	3.2	9^0 33'N	76^0 19'E	Disposal of Domestic and fish processing wastes
S4	Thevara Bridge	2.7	9^0 39'N	76^0 17'E	Sewage Outfall
S5	Marine Science Jetty	3.3	9^0 44'	76^0 16'E	Sulphur Jetty input and sewage. Industrial pollution
S6	Bolghatty	2.5	9^0 54'N	76^0 16'E	Inland navigation and other tourism operations-waste disposal
S7	Mulavukad	2.2	9^0 57'N	76^0 15'E	Disposal of domestic sewages and fish wastes.
S8	Cheranellur	2.1	10^0 04'N	76^0 17'E	Disposal of domestic sewage and wastes.
S9	Eloor	4.4	10^0 55'N	76^0 18'E	Industrial region

2.2.1 Analysis of general hydrographical parameters

Water samples (both surface and bottom) were collected using a Niskin Sampler (GO-FLO, USA). Sub sampling for determination of pH and DO was done in situ. The remaining portion of the water samples from Niskin Sampler were transferred carefully to pre-cleaned polythene bottles for the analysis of nutrients and other water quality variables. The water samples were kept in ice boxes and carried to the laboratory carefully without contamination. The analyses of nutrients were performed in the laboratory on the same day of sampling without delay. General hydrographical parameters and nutrients of the surface waters were analysed using standard methods. pH of the surface and bottom water samples was measured in situ using portable pH meter (Eutech, pH Tester 10). Salinity of the water samples was estimated by Mohr- Knudsen method (Muller, 1999). Modified Winkler method was used for the estimation of dissolved oxygen (Hansen, 1999). Alkalinity of the water samples was estimated by the method of Koroleff (Anderson et al., 1999). The concentration of nutrients (ammonia, nitrite, nitrate, phosphate and silicate) was estimated using spectrophotometric methods (Grasshoff et al., 1983). Ammonia reacts in moderately alkaline solution with hypochlorite to give monochloramine which, in the presence of phenol, catalytic amounts of nitroprusside ions and excess of hypochlorite, gives indophenol blue; its absorbance is measured using UV visible spectrophotometer (Genesys 10 UV Thermospectronic) at wavelength of 630 nm. Nitrite was converted to an azo dye with sulphanilamide and N- (1-naphthyl) ethylene diamine (Grasshoff et al., 1999). Nitrate was reduced to nitrite using copper-coated Cd granules and estimated as nitrite (Grasshoff et al., 1999). Determination of inorganic phosphate was based on the reaction of ortho phosphate ions with acidified molybdate reagent to yield a phosphor molybdate heteropoly acid, which is then reduced to a blue coloured compound (Grasshoff et al., 1999). Silicate was

analysed by converting it into silicomolybdate complex, which is reduced, using ascorbic acid and oxalic acid, to produce a blue solution (Grasshoff et al., 1999). Light and dark bottle method (APHA, 1995) was used for the estimation of primary productivity. The "Winkler" method for determining dissolved oxygen is normally used in the 'light and dark bottle' technique for the measurement of primary productivity.

Quantitative determination of chlorophyll pigments (chlorophyll-a.b,c) and phaeophytin in water samples was done by spectrophotometric analysis (APHA, 1995). For the estimation of chlorophyll, the water sample was filtered through GF/C glass fibre filter paper and a thin bed of magnesium carbonate was applied to the filter paper. The filter paper containing the pigments were transferred to a clean beaker and added 5 ml of 90 % acetone, and the beaker was wrapped with aluminium foil, kept overnight at $4^{o}C$ in a refrigerator. The contents were macerated and made up the extract solution to 10ml .The absorbance at wave lengths of 750 nm, 665 nm, 645 nm, 630 nm and 450 nm of the resulting acetone. Concentration of phaeophytin was determined by adding 2 drops of 0.5 N HCl to the same sample and measurement of absorbance were performed at wavelengths 750 nm and 665 nm.

2.2.2 Analysis of sedimentary parameters

Sediment samples were collected using a stainless steel van Veen Grab (0.042 m^2) and stored in polythene bags and kept deep frozen till analyses. pH of the sediments was determined in situ using a portable pH meter (Eutech, pH Tester 10). Redox potential of the fresh wet sediments was measured in situ by portable Eh meter (Eutech, ORP Tester 10) which was calibrated with Zobell solution (Brassard, 1997). The sediment texture (contents of sand, silt and clay) was determined by pipette analysis (Krumbein and Pettijohn, 1938), based on

Stoke's law after removing the inorganic carbonates using 10% HCl and organic matter using 15% H_2O_2. Sediment was dispersed in sodium hexametaphoshate overnight and then wet sieved through a 63 μm sieve to collect the sand fraction. The mud fraction was divided into silt and clay fractions by the timed gravimetric extraction of dispersed sediments (Folk, 1974). Sediment samples were freeze-dried and finely powdered using agate mortar and pestle for further analyses. Total carbon, total nitrogen (TN) and total sulphur were determined using CHNS analyser (Vario EL III). Total organic carbon (TOC) was estimated by TOC analyzer (VARIO TOC SELECT- Elementar), after removing inorganic carbon using 2M HCl. The amount of total organic matter (TOM) was obtained by multiplying the organic carbon values with 1.724 (Nelson and Sommers, 1996). Stable carbon isotope analysis of Total Organic Matter ($\delta^{13}C_{TOM}$) was carried out using Flash EA interfaced with IRMS (FINNIGAN DELTA PLUS XP, Thermo Electron Corporation). Stable carbon isotope abundance are presented as $\delta^{13}C$ values and are expressed relative to the PDB (Pee Dee Belemnite) standard:

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

2.2.3 Fractionation of phosphorous in sediments

Method of sequential extraction proposed by Golterman (1996) employs chelating agents for the determination of different phosphorus fractions (Figure 2.2). Iron bound phosphorous (Fe(OOH)-IP) was leached with buffered Ca-EDTA/dithionite and calcium bound fraction ($Ca\ CO_3$-IP) subsequently with Na-EDTA. In the next step, acid soluble organic phosphorous (ASOP) was eluted with H_2SO_4 and then alkali soluble organic phosphorous (Alkali-OP) with 2M NaOH at 90°C for 2 hours. Residual organic phosphorous (R-OP) was measured after 1 hour $K_2S_2O_8$ digestion in acid medium.

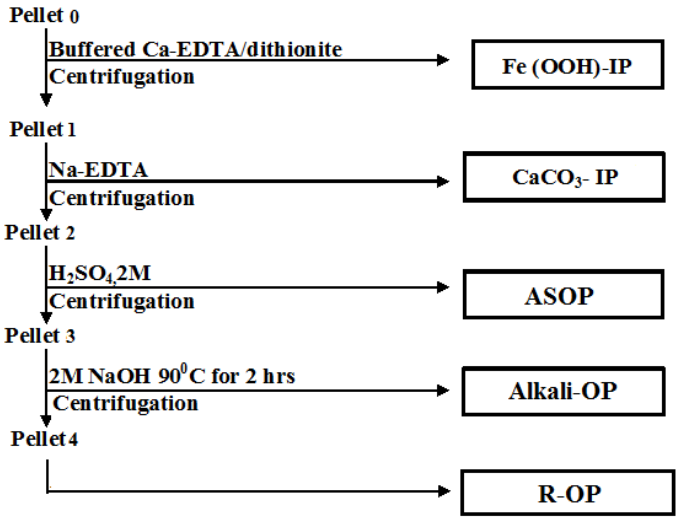

Figure 2.2 Sequential extraction scheme for phosphorus fractionation

2.2.4 Fractionation of nitrogen in sediments

The scheme for nitrogen fractionation in sediments is shown in Figure 2.3. The extraction and estimation of nitrite, nitrate, ammonia and urea were carried out by the KCl equilibrium extraction method (Agemian, 1997). Sediment samples were shaken with a solution of KCl (2N) at room temperature for a period of one hour followed by filtration through Whatmann 42 filter paper. The filtrate containing the dissolved nitrogen was stored at 4^{o}C until analysis. From the filtrate the nitrite, nitrate, ammonia and urea were quantitatively estimated by spectrophotometric analyses (Grasshoff et al., 1999). For the estimation of total nitrogen, sediment samples were digested in H_2SO_4 in the presence of potassium sulphate and copper catalyst (Selenium Reagent mixture-MERCK) (Agemian, 1997). Organic compounds of nitrogen constituent as well as free inorganic forms were thus converted to ammonium ions which is determined spectrophotometrically using indophenol blue method (Grasshoff et al., 1999). Kjeldahl extraction technique determines the concentrations of nitrogen apart from nitrate-N and nitrite-N. The sum of the

concentration of nitrate-N, nitrite-N and Kjeldahl-N gives an estimate of the total nitrogen. The difference between the total nitrogen and the sum of nitrate-N, nitrite-N, ammonia-N and urea-N in sediments is expressed as residual N.

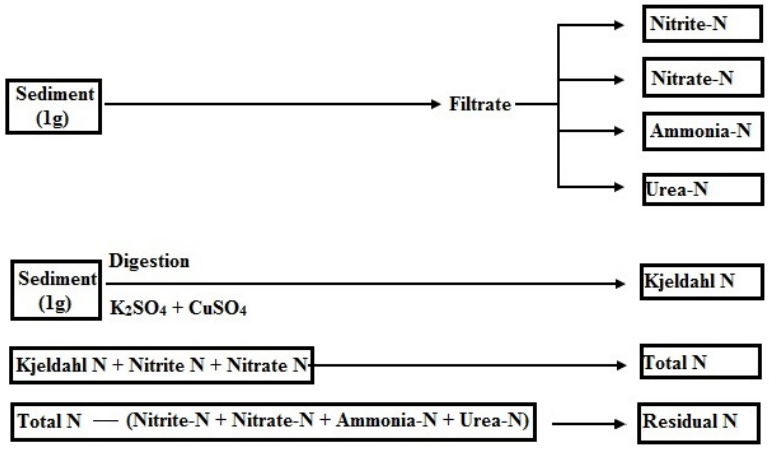

Figure 2.3 Sequential extraction scheme for nitrogen fractionation

2.2.5 Analysis of biochemical composition in sediments

Spectrophotometric methods were employed for the determination of biochemical constituents in sediments. Extraction and estimation of total protein (PRT) in sediments were carried out as per the standard methods (Lowry et al., 1951; Rice, 1982), with albumin as the calibration standard. The quantity of protein nitrogen was obtained by multiplying protein with a factor of 0.16 (Mayer et al., 1986). Total carbohydrates (CHO) were analysed according to Dubois et al (1956), using glucose as the calibration standard. Analysis of total lipids (LPD) was carried out spectrophotometrically using cholesterol as the calibration standard (Bligh and Dyer, 1959; Barnes and Blackstock, 1973). The sum of all PRT, CHO and LPD was defined as the labile or easily assimilable organic fraction (Danovaro et al., 1993; Cividanes et al., 2002). PRT, CHO and LPD concentrations were converted to carbon equivalents by using the following conversion factors: 0.49, 0.40 and 0.75 g of C/g, respectively (Fabiano and

Danovaro, 1994). The sum of PRT, CHO and LPD carbon is referred to as biopolymeric carbon (BPC) (Fichez, 1991; Fabiano et al., 1995).

Tannin and lignin in sediments were extracted using 0.05M NaOH at 60^{o} for 90 minutes and estimated spectrophotometrically by the sodium tungstate-phosphomolybdic acid method (Nair et al., 1989; APHA, 1995), using tannic acid as the calibration standard. The principle involved is the development of a blue colour on reduction of Folin phenol reagent by the aromatic hydroxyl groups present in tannin and lignin. The effects of Mg and Ca hydroxides and/or bicarbonates present in the water samples were suppressed by the addition of trisodium citrate solution (Nair et al., 1989).

Analysis of chlorophyll and phaeopigments in sediments of the study area was carried out according to standard procedures (Lorenzen and Jeffrey, 1980; APHA, 1995). Pigments in sediments were extracted with 90 % acetone (24 hrs in the dark at 4 °C). After centrifugation, the supernatant was used to determine chlorophyll pigments (chl-a, chl-b and chl-c) and there after acidified with 0.1N HCl to estimate the concentration of phaeophytin. Details of the spectrophotometric measurements are provided at section 2.2.1.

2.2.6 Analysis of free sugars in sediments

Finely powdered sediments (10g) were suspended in aqueous ethanol (70%, 2 h, thrice) and stirred continuously at room temperature. It was then centrifuged (KUBOTA 6500, Japan) and the pooled extracts (free sugars) were deionized by passage through Dowex-50 (H+) and Dowex-1 (OH−) resins to remove cationic as well as anionic contaminants. The purified sugars were concentrated by rotary evaporator (Heidolph, Germany). The deionised samples dissolved in known volume of distilled water were analysed by high-

performance liquid chromatography (HPLC) method (Vallentyne and Bidwell, 1956; Suhasini, et al., 1997; Revanappa, 2009) [Shimadzu LC 2020 equipped with RID 10A detector and SUPELCOSIL LC-NH_2 column (purchased from Sigma Aldrich)]. The separation was done at 30ºC with the mobile phase acetonitrile: water (8:2) with a flow rate of 0.8 ml per minute.

The identification of individual compounds was done by comparison of retention time with those of standard compounds. The calibration curve was plotted using varying concentrations of the standards starting from 5 ppm to 100 ppm. The concentration of the free sugars in the sample was determined from the peak area of the detected sugar.

2.2.7 Analysis of amino acid in sediments

Total hydrolysable amino acid (THAA) were extracted by adding 5ml HCl (6M) to freeze dried homogenized sediment (100 mg) in pre-cleaned and muffled (450^{o}C for 3 hrs) glass vials, and purging the headspace with N_2. The vials were kept in an oven at 110^{o}C for 24h. The extracts were then centrifuged at a speed of 5000 rpm for 10 min (KUBOTA 6500, Japan) and for neutralisation, it was washed with distilled water, HCl content in extract was removed (Stevenson and Cheng, 1970; Cheng, 1975) using rotary evaporator (Heidolph, Germany). For the detection and quantification of the extracted amino acids, pre-column derivatisation with phenyl isothiocyanate (PITC) (Bidlingmeyer et al., 1984) was used. In this technique, dried samples (HCl removed) were dissolved in 20 µmol/L of ethanol : water : triethylamine (TEA) (2:2:1) and dried again under vacuum. Then to the dried sample, 20 µl freshly prepared reagent consisted of ethanol : water : TEA : PITC (7:1:1:1) were added under nitrogen atmosphere and sealing them for 30 minutes at room temperature. The reagents were then

removed under vacuum at 45° C to reduce the evaporation time without any significant sample difference in comparison to drying at lower temperatures (the dried derivatives could be kept dried and frozen for several weeks without significant degradation). The individual amino acids (AAs) - aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), cysteine (Cys), isoleucine (Ile), leucine (Leu), phenyl alanine (Phen) and lysine (Lys) were quantified according to Lindorth and Mopper (1979) by HPLC (Shimadzu LC 2020).

The proposed solvent system consisted of two eluents. Solvent A, an aqueous buffer, was a solution of 50 mM sodium acetate containing TEA as modifier. The solution was degassed before the addition of TEA. The pH was adjusted to the desired value (6.8) using glacial acetic acid, and the solution was filtered through a 0.2 µm membrane filter. Solvent B consisted of water, acetonitrile, and methanol. Aqueous solutions containing acetonitrile and methanol are the most common solvents used in amino acid analysis in HPLC (Bidlingmeyer et al., 1984; Hariharan et al., 1993). The pH and TEA content of solvent A, the composition of solvent B, the temperature, and the mobile phase flow rate are specified as required for the experimental design. Ultraviolet spectrophotometric detection was carried out at 254 nm. Before starting the gradient for a certain run, the column was equilibrated for 30 minutes as required with the associated experimental design.

Prior to HPLC, the derivatised forms of the standard amino acid mixture and the individual amino acids were first dissolved separately in 12 µl of a 60% solution of acetonitrile and mixed thoroughly (ROTEK CYCLO-

vortex mixer). Then, 113 µl of the corresponding solvent A was added to each sample and mixed well using vortex mixer.

Instrument: High pressure Liquid chromatograph (Shimadzu Ultrafast LC 2020) equipped with UV&RI detector. Column C18 (octadecayl Cilane) 25cm*2.1mm* (particle size: 5µm diameter). Temperature: room temperature, flow rate 1 ml/minute. Calibration range: 0.0087 µmol/ml to 0.14 µmol/ml.

The computation of amino acid carbon to TOC (THAA-C %) and amino acid nitrogen to TN (THAA-N %):-

First calculate the C, N and Molecular weight (Mol.Wt.) for each individual amino acid (AAs) of standard used as given below:

AAs	C	N	Mol.Wt.
Lysine	72	28	146.20
Tyrosine	108	14	181.20
Average	?	?	?

Then take the average of C, N and Mol. Wt. for all AAs

Now Average C value divided by Average Mol. Wt. that will give carbon content in AAs. The nitrogen content in AAs was calculated in the similar way.

Now to calculate THAA-C% in terms of TOC THAA concentration, THAA-C and TOC

1) THAA-C= THAA conc. x Carbon content in AA
2) THAA-C%= THAA-C/TOC X 100

Similarly, THAA-N% in terms of TN can be computed as follows.

1) THAA-N= THAA conc. x Nitrogen content in AA
2) THAA-N% = THAA-N/TN X 100

2.3 Statistical analysis

All the estimations were carried out in triplicates and the average value are reported. Relevant data were subjected to statistical analysis wherever necessary. Pearson correlations were determined to find out the inter relations between different parameters. Statistical significance of the observed spatial and temporal variations in sediments was checked using two way ANOVA (stations x seasons). Principal component analysis was carried out using the software (SPSS 15.0) to find out the biogeochemical processes governing the distribution of estimated variables in the study area.

2.4 Quality control

All the bottles and collecting containers were acid washed and thoroughly rinsed with Milli-Q water before use. Chemicals/solvents used for the analysis of various parameters were purchased from Merck (India/Germany). Caliberation standards for amino acids and carbohydrates were purchased from Sigma-Aldrich (USA). All the glass wares were cleaned by ultra sonic bath followed by heating at high temperature in oven.

2.5 Results of general hydrographic parameters

The main factors which influence the hydrographic conditions of an estuary are the saline water ingression from ocean associated with tides and influx of fresh water brought in by the rivers. The bottom topography and geographical shape also play an important role in controlling the hydrographic regime of an estuary. The variability in physical, chemical and biological processes in estuarine salinity gradient has considerable impact on the

composition and distribution of sedimentary organic matter (Carreira et al., 2011; Costa et al., 2011). The general hydrographic parameters and nutrients in water column have a direct control over the in situ primary production, distribution and fate of sedimentary organic matter. Therefore, a brief description of the spatio-temporal variation and concentration of water quality variables is presented in this section.

The spatial and seasonal variation of different hydrographical parameters of surface and bottom waters are represented in Figures 2.4, 2.5 and 2.6. Terrestrial run off associated with monsoon rainfall creates remarkable seasonal variations in the hydrographical parameters in Cochin estuarine system. Present investigation recorded a variation in pH from 6.70±0.19 (S2; MON12) to 8.10±0.14 (S6; POM09) in surface and 6.50±0.17 (S8; MON12) to 8.40±0.12 (S5; PRM09) in bottom. Observed salinity ranged from 0.04±0.01 to 29.93±2.60 psu and 0.04±0.02 to 33.50± 3.10 psu for surface and bottom waters respectively. The maximum salinity was recorded during POM09 and PRM10 at stations S6 (surface) and S5 (bottom) respectively, which are located at the confluence of estuarine mouth. Present study revealed an alkalinity ranging from 6.40±0.67 mg $CaCO_3$/l (S5; POM09) to 112.70± 12.67 mg $CaCO_3$/l (S5; PRM09) for surface and 6.40±0.56 mg $CaCO_3$/l (S5; POM09) to 105.80± 11.56 mg $CaCO_3$/l (S5; PRM09) (mg $CaCO_3$/l) for bottom waters (Figure 2.4).

The maximum concentration for inorganic nitrite was observed at S9 (PRM09; surface) and S4 (PRM10; bottom). The observed variation from 0.02 to 1.01 µmol/l (surface) and 0.01 to 1.10 µmol/l (bottom); with an estimated average of 0.31±0.22 µmol/l. The observed nitrate content varied from

0.93±0.04 µmol/l (S3; PRM10) to 32.51±0.70 µmol/l (S1, MON09) in surface waters and 0.79±0.03 µmol/l (S6, PRM10) to 41.80±0.56 µmol/l (S2; MON09) in bottom waters. The ammonia recorded its maximum concentration at S4 (410.50±0.70 µmol/l) during PRM10 and minimum recorded at S1 (0.02±0.01 µmol/l) during MON12 for surface water. However in the case of bottom water, maximum concentration was displayed at S7 (132.80±0.70 µmol/l, PRM10) and minimum at S1 (0.03±0.01 µmol/l, MON12) (Figure 2.5).

During the pre-monsoon season, when river run off weakens, no vertical stratification was observed and fresh water conditions were prevalent during the monsoon season. However, stratification was seen during the post-monsoon season. Wide fluctuation was exhibited by dissolved oxygen (DO) and its concentrations were in the range: 3.32±0.33 to 7.20±0.11 mg/l (surface) and 2.24±0.03 to 7.92± 0.04 mg/l (bottom). The observed maximum DO was noted at stations S1 (surface) and S9 (bottom) during MON12. The estimated inorganic phosphate content ranged from 0.01±0.03 µmol/l (S2; PRM10) to 3.06±0.05 µmol/l (S6; PRM09) (surface) and 0.03±0.04 µmol/l (S3; POM09) to 4.16±0.05 µmol/l (S4; PRM09) (bottom). The concentration of silicate in surface water samples varied from 8.53±0.05 to 121.10±0.06 µmol/l, while in bottom samples it ranged between 10.00±0.45 and 118.90±0.55 µmol/l. The maximum content for silicate (Figure 2.6) was recorded for surface water at S6 (POM09) and bottom water at S9 (POM09).

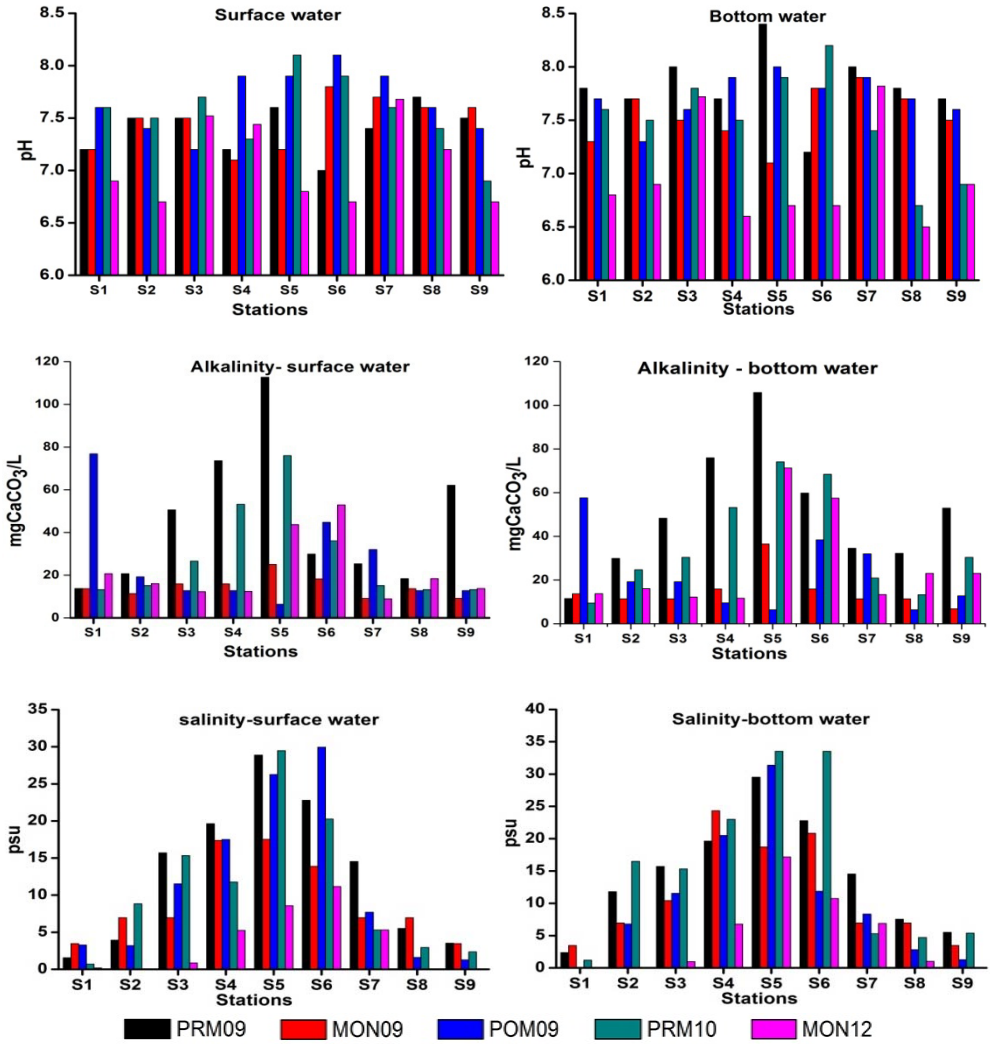

Figure 2.4 Distribution of pH, alkalinity and salinity in the water samples

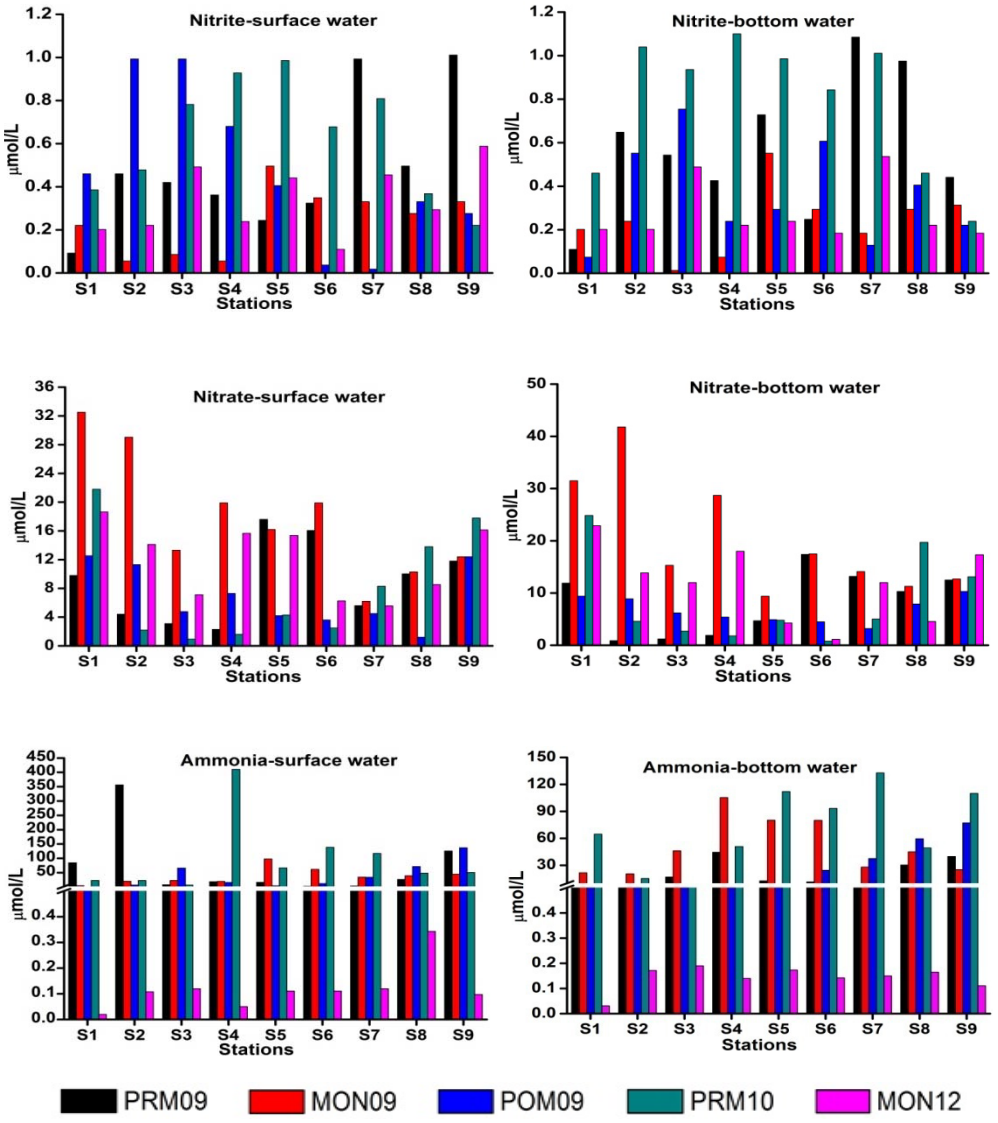

Figure 2.5 Spatio-temporal variations of inorganic nitrite, nitrate and ammonia in water samples

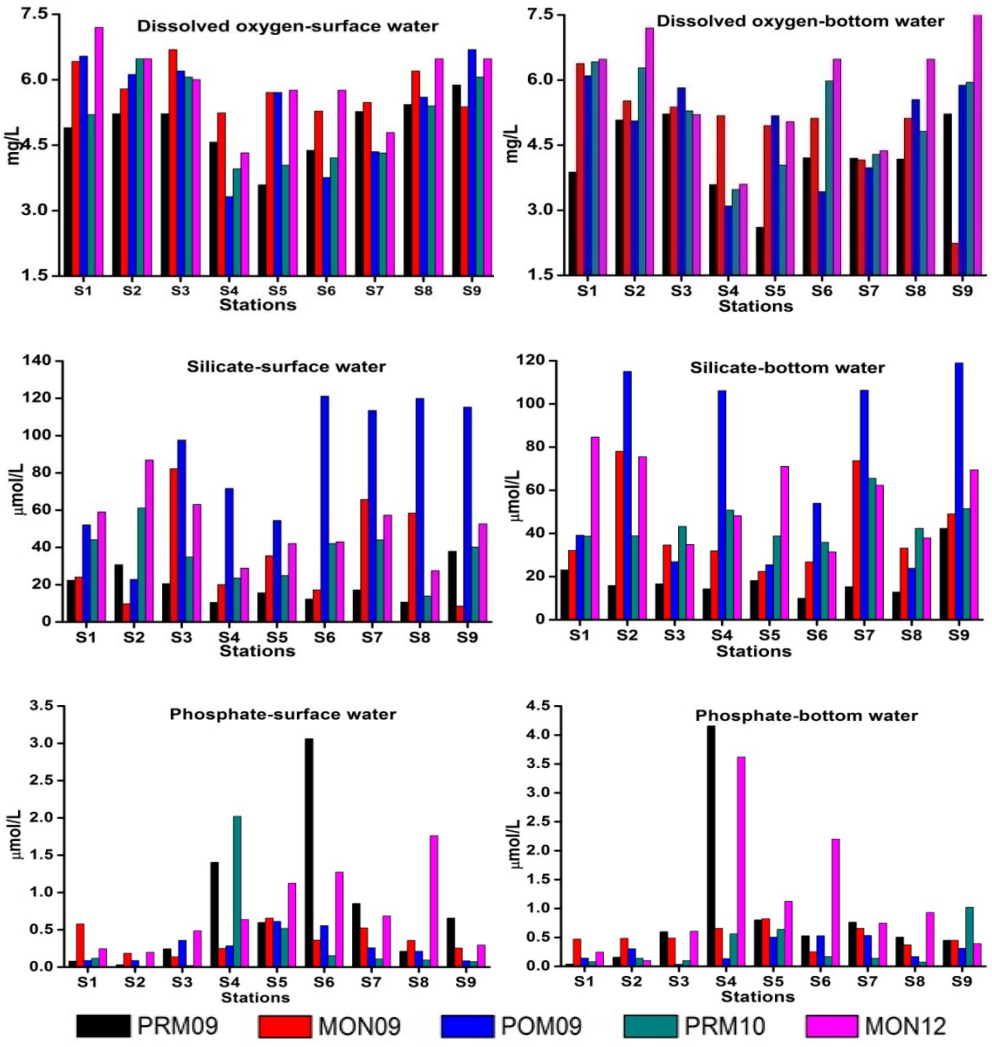

Figure 2.6 Concentration of dissolved oxygen, silicate and inorganic phosphate in water samples

Anthropogenic activities have remarkable influences on the water quality of aquatic ecosystems (Siddiqui, 2011; Clemente et al., 2012; Kiteresi et al., 2012). The stability of the ecosystem is influenced by salinity, grain size, nutrient content and dynamics, physiological tolerance, predation and competition at local level (Smith et al., 2003). Despite the fact that nutrients in the tropical marine ecosystems are generally low (Qasim and Wafar, 1990). Salinity showed minimum value during monsoon season (Figure 2.4) due to fresh water runoff. The maximum was reported from station S6, due to the peculiar geographical location in the vicinity of Arabian Sea and the tidal activity altered the salinity of the other estuarine stations significantly (Manju et al., 2012). In aquatic systems, oxygenation is the result of an imbalance between the process of photosynthesis, degradation of organic matter, reaeration (Granier et al., 2000), and physicochemical properties of water (Aston, 1980). The anoxic conditions observed at various stations were attributed to the higher concentration of organic matter and salinity variations.

In the present study nitrate exhibited higher concentration during monsoon in both surface and bottom water and lower content was found during premonsoon (Figure 2.5). Distribution of nitrogen content in water column is controlled by the balance between assimilation, mineralisation, nitrification, denitrification and nitrogen fixation (Solanki et al., 2010). Stations S1, S2, S8 and S9 received large quantities of sewage and industrial wastes resulting in the creation of reducing environment and recorded elevated levels of nutrients.

Comparatively higher content of phosphate was recorded during PRM09. During the study period (in MON09 and PRM09 season) nitrate and phosphate in water body considerably increases due to land drainage and anthropogenic activities. The lower content of these nutrients observed in the

study period (Figure 2.5 and 2.6) might be due to the decreased runoff, adsorption on to sediments, and utilisation by phytoplankton (Ramakrishnan et al., 1999). Higher concentration of silicate in surface and bottom water was observed at stations S6 and S9 respectively during POM09 (Figure 2.6) attributed to the riverine input. Meanwhile, lower content was observed at stations S9 (surface water-MON09) and S6 (bottom water-PRM09). Weathering process and land run-off largely contribute to silicate concentration of these estuarine ecosystems (Manju et al., 2012).

Various chemical processes affect diurnal variation of nitrogenous nutrients in the system is depicted in Table 2.2. Nutrient distribution and variation in estuarine systems are generally controlled by a variety of physical, chemical and biological processes (Pritchard and Schubel, 1981). The negative correlation (Table 2.2) between salinity and nutrients (nitrate and silicate) within the estuary indicated that the nutrient levels are controlled by the discharge inputs. From the interrelationships between nutrients (Table 2.2), it could be inferred that they were originated from the same source. Comparing the hydrographical data of the present study with those of previous investigations (Table 2.3), revealed that the system maintains its unique character.

Table 2.2 Correlation matrix of the various hydrographical parameters in water column (n=45)

	pH	DO	Salinity	Alkalinity	Phosphate	Silicate	Nitrate	Nitrite	Ammonia
pH	1								
DO	-0.52(**)	1							
Salinity	-0.49(**)	0.63(**)	1						
Alkalinity	0.52(**)	-0.28(**)	-0.20	1					
Phosphate	-0.59(**)	0.12	0.23(*)	-0.48(**)	1				
Silicate	0.53(**)	-0.31(**)	-0.41(**)	0.26(*)	-0.44(**)	1			
Nitrate	0.53(**)	-0.26(*)	-0.41(**)	0.36(**)	-0.54(**)	0.26(*)	1		
Nitrite	-0.45(**)	0.41(**)	0.31(**)	-0.34(**)	0.39(**)	-0.31(**)	-0.37(**)	1	
Ammonia	0.09	-0.23	-0.04	0.08	0.07	-0.04	-0.26	0.31(*)	1

Table 2.3 Earlier studies of important water quality parameters in Cochin estuary

Parameters	Concentration	Reference
Salinity	0.5-34	Ramamirtham and Jayaraman, 1963
Phosphate	0.22-2.90 µg/L	Ansari and Rajagopal, 1974
Salinity	~33 (maximum)	Kunjukrishnan Pillai, et al., 1975
DO	5.9 µg/L	
Phosphate	32 µg/L	
Nitrite	~2.6 µg/L (maximum)	
Nitrite	~2.6 µg/L (maximum)	
Salinity	0.30-35.50	MadhuPratap, 1978
DO	0.5-5 mg/L	
Salinity	0.21-34.30	Remani et al., 1983
DO	0.05-4. 4 mg/L	
pH	6.08-8.15 (surface) 5.95-8.77 (bottom)	Ratheeshkumar et al., 2010
Salinity	6.08-8.15 (surface) 5.95-8.77 (bottom)	
DO	4.57-7.68 mg/L (surface) 3.04-8 mg/L (bottom)	
Alaklinity	12-314 mg/L (surface) 16-208 mg/L (bottom)	
Phosphate	5.29-49.73 µmol/l	
Silicate	3.55-63 µmol/l	
Slicate	3.2-123.50µM	Selvam et al., 2011
pH	6.3-8.3	
Salinity	5.4-28.40	
DO	4.3-8.6 mg/l	
Salinty	0.01-32.95	Renjith et al., 2012
DO	1.96-10.16 mg/l	
Nitrite	0.11-4.65 µM	
Nitrate	0.97-49.96 µM	
Ammonia	BDL-253.70µM	
Phosphate	BDL-13.70µM	
Salinity	0.04±0.01 to 29.93±2.60 (surface) 0.04±0.02 to 33.50± 3.1 (bottom)	Present study
pH	6.70±0.19-8.10±0.14 (surface) 6.50±0.17-8.40±0.12 (bottom)	
DO	3.32±0.33 to 7.20±0.11 mg/l (surface) 2.24±0.03 to 7.92± 0.04 mg/l (bottom)	
Alaklinity	6.40±0.67-112.70± 12.67 mg$CaCO_3$/l (surface) 6.40±0.56 - 105.80± 11.56 mg$CaCO_3$/l (bottom)	
Nitrite	0.02 to 1.01µmol/l (surface) 0.01 to 1.10 µmol/l (bottom)	
Nitrate	0.93±0.04- 32.51±0.70 µmol/l (surface) 0.79±0.03 - 41.80±0.56 µmol/l (bottom)	
Ammonia	0.02± 0.01-410.50±0.70 µmol (surface) 0.03±0.0-132.80±0.70 µmol /l(bottom)	
Phosphate	0.01±0.03 - 3.06±0.05 µmol/l(surface) 0.03±0.04 - 4.16±0.05 µmol/l (bottom)	
Silicate	8.53±0.05 to 121.10±0.06 µmol/l (surface) 10.00±0.45-118.90±0.55 µmol/l (bottom)	

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Chapter 3

GEOCHEMISTRY OF PHOSPHOROUS AND NITROGEN FRACTIONS IN SEDIMENTS

Contents

3.1 Introduction

Biologically available nitrogen (N) and phosphorous (P) play a key role in determining the ecological status of aquatic systems (Jarvie et al., 1998). Both N and P can act as limiting element for primary production in most marine and coastal ecosystems (Thingstad et al., 1998; Mortimer et al., 1999). The benthic release of N could play a prominent role in sustaining the productivity of estuarine ecosystems (Renjith et al., 2011). In aquatic environments, N and P exchange at the water-sediment interface is controlled by many complex physicochemical and biological processes. Nutrient sources to estuaries vary with inputs associated with freshwater flow (Peierls et al., 1991), atmospheric deposition (Paerl et al., 2002), submarine groundwater discharge (Santos et al., 2008), and nitrogen fixation (Gardner et al., 2006; Fulweiler et al., 2007). Anthropogenic impacts including rising nutrient loads in rivers and estuaries from fertiliser application, rapid development of aquaculture facilities and urbanisation (Zhang etal., 1996; Li et al., 2002; Liu

et al., 2009) can alter the nutrient content in sediments. The impacts of these anthropogenic activities on aquatic systems include: shifts in the composition of plankton species (Sanders et al., 1987; Oviatt et al., 1989) and the enhancement of primary production, which results in increased respiration, leading to the formation of oxygen deficient zones (Diaz and Rosenberg, 2008; Zhang et al., 2010). A major fraction of the growth limiting elements in the aquatic environment is reversibly associated with surficial sediment, hence the evaluation of nutrient dynamics in sediments can be used as a tool to describe the general geochemical setting and the health of the estuarine system. This Chapter, focussed on the distribution of the different fractions of N (Nitrite-N, Nitrate-N, Urea-N, ammonia-N, Kjeldahl-N and Residual-N) and P (Iron bound inorganic-P (Fe(OOH)–IP), Calcium bound Inorganic-P ($CaCO_3$-IP), Acid soluble organic-P, Alkali soluble organic-P, Residual organic-P), in the sediments of Cochin estuary to assess their geochemical implications.

3.2 Results

3.2.1 General sediment characteristics

Wide fluctuations intexture (sand, clay and silt) were observed in the sediments of the study region (Figure 3.1). The content of sand ranged from 0.38±0.01% (S8; MON12) to 99.14±0.06% (S9; MON09) and exhibited significant seasonal variations. Similarly clay content also displayed seasonal variation ($p<<0.01$) and ranged from 0.10±0.01% (S9; MON09) to 47.59±1.21% (S8; MON12). Meanwhile, in the case of silt, the observed variation was from 0.24±0.02 % (S9; MON09) to 86.60±1.45% (S4; POM09).

Sediment pH (Table 3.1) showed slightly alkaline nature during the investigation with its minimum and maximum was recorded at S8 (6.65±0.12; PRM10) and S5 (8.30±0.11; PRM09) respectively. Characteristic reducing conditions was indicated by Eh values (Table 3.1); which displayed a range of -345±1.5 mV (S6; PRM09) to -29.78±1.7 mV (S9; MON12).

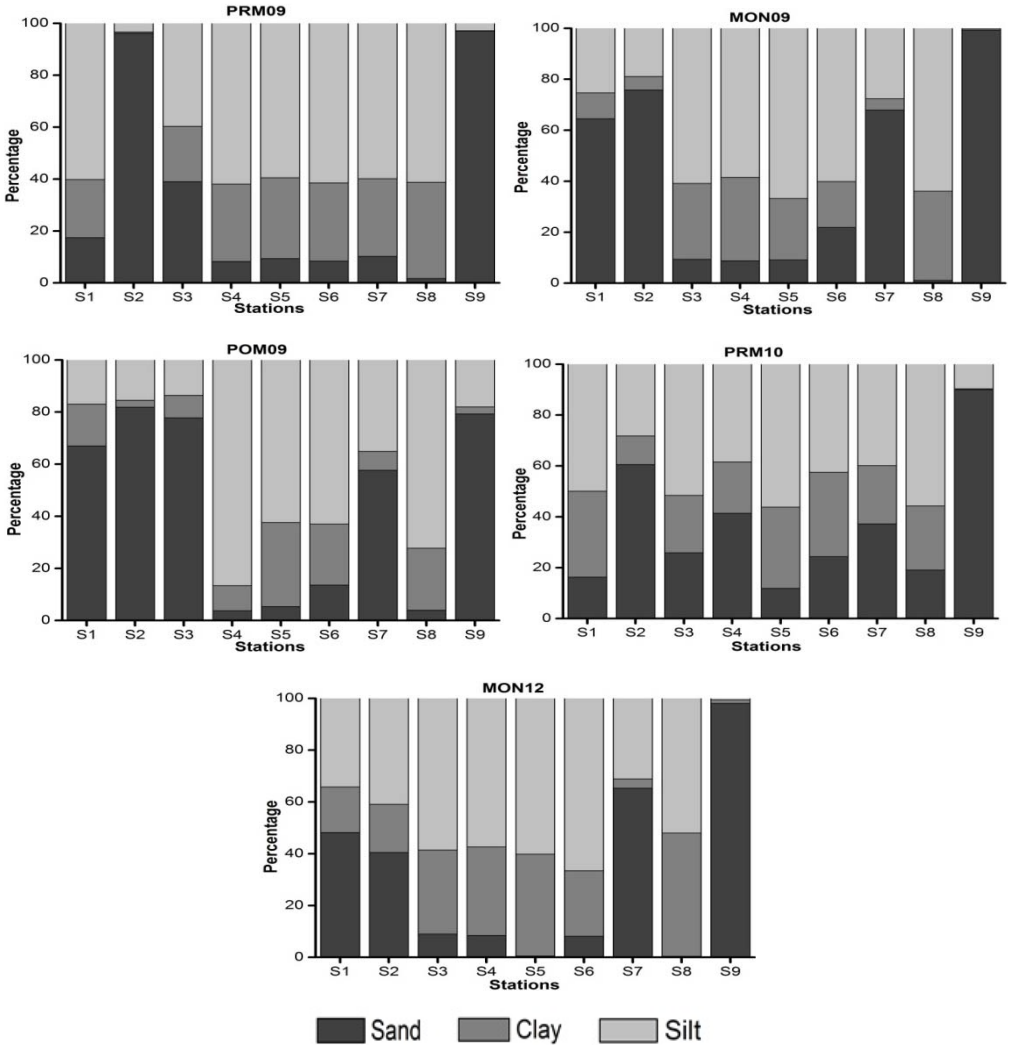

Figure 3.1 Grain size variations in surface sediments of the study area

Table 3.1 Values of pH and Eh (mV) in sediments of the estuary

Stations	PRM09		MON09		POM09		PRM10		MON12	
	pH	Eh	pH	Eh	pH	Eh	pH	Eh	pH	Eh
S1	7.67±0.12	-84±1.54	7.22±0.15	-42±1.25	7.65±0.15	-67.2±1.23	7.56±0.13	-103±1.78	7.33±0.15	-38.03±1.22
S2	7.56±0.08	-114±1.25	7.56±0.12	-57±1.19	7.2±0.22	-91.2±2.35	7.32±0.14	-80±1.17	7.67±0.13	-53.03±1.25
S3	7.7±0.06	-127.5±1.32	7.3±0.14	-63.75±1.25	7.57±0.11	-102±2.56	7.66±0.13	-105±1.32	7.41±0.11	-59.78±2.23
S4	7.6±0.04	-208.5±1.11	7.3±0.11	-104.3±2.1	7.88±0.08	-166.8±2.18	7.34±0.11	-89±2.23	7.41±0.08	-100.3±3.13
S5	8.3±0.11	-270±1.98	6.97±0.13	-135±3.5	7.87±0.92	-216±1.95	7.86±0.08	-73±1.5	7.08±0.13	-131±2.28
S6	7.1±0.02	-345±1.5	7.67±0.16	-172.5±2.8	7.67±0.27	-276±2.35	8.1±0.03	-79±2.36	7.78±0.15	-168.5±1.7
S7	7.89±0.13	-337.5±1.76	7.87±0.08	-168.8±2.6	7.88±0.17	-270±3.25	7.34±0.15	-107±2.25	7.98±0.23	-164.8±2.3
S8	7.67±0.07	-165±1.89	7.69±0.17	-82.5±2.17	7.65±0.12	-132±2.27	6.65±0.12	-109±3.36	7.8±0.07	-78.53±1.8
S9	7.87±0.05	-67.5±1.56	7.43±0.13	-33.75±1.29	7.54±0.15	-54±1.17	6.87±0.03	-81±1.89	7.54±0.04	-29.78±1.7

3.2.2 Phosphorous fractions in sediments

The various fractions ofphosphorous in the surface sediments of the study area are depicted in Table 3.2. Concentration of total phosphorous-TP (sum of all P fractions) in the estuarine sediments ranged from 222.92±3.89µg/g (S9; POM09) to 4348.66±15.35µg/g (S8; PRM10). Fe (OOH) -IP recorded its maximum, during POM09 at S8 (2724.66± 2.86 µg/g) while the minimum was noticed during MON12 at S9 (115.27±1.27 µg/g). The content of $CaCO_3$-IP recorded its maximum at S8 (2726.76±12.23 µg/g; PRM10) and minimum at S9 (20.39±0.54 µg/g; MON12) during the study period. Acid soluble organic phosphorous (ASOP) found to vary from 10.29±0.17µg/g(S9; MON12) to 555.42±2.87µg/g(S8; PRM10). Meanwhile, alkali soluble organic-P (Alkali-OP) in the sediments ranged between 0.96±0.12µg/g(S3; POM09) and 3242.40±11.96µg/g(S9; MON09). Residual organic phosphorous (R-OP) in the study region varied from 0.39 ± 0.02µg/g(S2; POM09) to 148±2.23µg/g(S8; PRM09).

Table 3.2. Various phosphorous fractions (µg/g) in the sediments of the study area

P- fraction	Stations	PRM09	MON09	POM09	PRM10	MON12
Fe(OOH)-IP	S1	513.36±5.15	666.37± 6.22	731.69±7.82	622.29±8.78	661.34±6.11
	S2	187.79±2.38	378.42±6.78	286.34±8.32	880.16±8.43	369.04±3.21
	S3	1024.83±7.89	348.14±7.48	282.93±7.63	1024.03±9.67	336.83±2.25
	S4	523.17±4.78	333.60±3.56	1320.17±6. 29	1017.76±11.42	317.41±2.18
	S5	1088.73±8. 56	1095.86±4.42	1142.62± 7.26	1358.70±10.23	1081.61±8. 43
	S6	1192.74±4.79	843.65±5.22	1128.19±5.48	1144.88±8.94	827.86±4.39
	S7	858.83±5.78	526.26±6.73	538.12± 5.97	1024.94±9.69	514.16±1.55
	S8	1237.93±6.74	916.01±5.65	2724.66±2.86	738.22±11.21	916.00±1.33
	S9	754.68±3.54	123.06±4.32	136.93±3.76	148.98± 1.12	115.27±1.27
$CaCO_3$-IP	S1	435.75±7.66	133.67±5.73	143.07±7.27	217.39±2.48	127.67±1.06
	S2	363.19±6.98	76.02±3.78	43.16±4.56	129.08±1.22	70.02±1.18
	S3	410.10±5.87	88.68±2.56	40.45±3.21	159.13±1.31	82.68±1.02
	S4	183.17±3.86	229.04±3.28	226.42±7.67	187.36±1.13	223.04±1.33
	S5	652.13±4.62	537.07±3.19	180.13±6.59	194.34±2.21	531.07±1.12
	S6	439.55±6.28	519.31±4.54	290.87±4.89	242.08±2.15	513.31±0.94
	S7	238.37±4.72	265.68±2.43	222.72±3.65	164.00±1.73	259.68±0.28
	S8	996.84±3.95	1359.93±12.34	997.34±5.89	2726.76±12.23	1353.93±1.32
	S9	109.12±2.43	26.39±1.87	58.71±3.33	161.83±1.43	20.39±0.54

ASOP	S1	118.61±6.39	103.98±2.11	152.15±4.53	153.38±1.25	97.98±0.28
	S2	77.29±2.31	79.29±1.13	67.95± 4.26	112.77±1.11	73.29±1.01
	S3	149.26±3.29	26.15±0.56	29.51±1.78	101.38±1.82	20.15±0.79
	S4	182.63±3.45	119.81±0.78	156.40±2.34	121.32±1.43	113.81±0.77
	S5	255.99±3.67	231.09±2.19	146.40 ±3.57	134.12±1.58	225.09±1.28
	S6	216.19±4.41	296.65±1.87	209.06±6.88	162.21±2.12	290.65±0.97
	S7	148.09±3.97	241.89±1.59	189.57±5.68	204.50±4.19	235.89±0.89
	S8	418.45±4.25	287.92±1.94	346.71±6.97	555.42±2.87	281.92±0.27
	S9	220.42±3.47	16.29±1.19	25.42±3.94	54.16±1.13	10.29±0.17
Alkali-OP	S1	341.37±2.36	94.40±1.11	180.43±8.93	350.24±1.28	88.40±0.08
	S2	870.23±3.14	128.03±1.18	2.20±0.89	81.52±1.56	122.03±1.17
	S3	922.17±4.11	24.77±1.32	0.96±0.12	92.17±0.96	18.77±1.01
	S4	306.06±2.24	821.96±1.78	10.55±0.77	90.50±1.21	815.96±0.88
	S5	616.77±1.15	268.45±3.22	12.45±0.58	106.76±0.95	262.45±1.32
	S6	675.22±1.27	341.11±2.75	13.34±0.48	135.69±0.91	335.11±1.07
	S7	1184.75±3.47	200.38±3.18	11.05±0.85	127.48±0.50	194.38±1.03
	S8	613.44±3.65	412.06±2.43	26.84±1.01	313.12±1.26	406.06±0.85
	S9	203.69±1.74	3242.40±11.96	1.45±0.06	53.51±2.27	3236.40±10.06
R-OP	S1	46.29±1.27	131.76±1.94	13.79±0.18	40.34±0.78	125.76±1.21
	S2	45.80±2.23	69.84±1.21	0.39±0.02	3.06±0.82	63.84±0.88
	S3	130.05±1.71	47.71±1.98	0.75±0.03	3.34±0.18	41.71±0.95
	S4	77.00±1.15	57.37±1.45	2.10±0.01	5.37±0.21	51.37±0.67
	S5	131.74±1.94	75.65±2.23	1.47±0.05	0.73±0.05	69.65±0.59
	S6	90.82±2.11	62.20±1.87	5.33±0.28	20.35±1.15	56.20±0.62
	S7	78.20±1.35	49.62±1.65	1.03±0.08	2.02±0.08	43.62±0.48
	S8	148.00±2.23	114.62±2.27	1.16±0.03	15.13±0.25	108.62±0.54
	S9	81.47±2.36	24.33±2.19	0.41±0.02	0.77±0.07	18.33±0.95
TP	S1	1455.38±2.34	1130.17±2.36	1221.13±6.98	1383.65±11	1099.19±6.93
	S2	1544.30±4.41	731.60±1.86	400.04±5.96	1206.59±8.12	700.62±5.19
	S3	2636.40±23.42	535.44±2.43	354.61±4.87	1380.05±9.45	504.46±5.28
	S4	1272.03±12.48	1561.78±3.18	1715.64±11.02	1422.31±8.44	1530.80±6.74
	S5	2745.35±10.05	2208.11±2.87	1483.06±10.22	1794.65±6.87	2177.13±10.05
	S6	2614.52±8.74	2062.91±3.82	1646.79±8.93	1705.23±9.58	2031.93±8.94
	S7	2508.25±6.83	1283.83±4.18	962.50±7.45	1522.93±10.34	1252.85±9.48
	S8	3414.66±9.56	3090.54±3.85	4096.71±6.89	4348.66±15.35	3059.56±11.21
	S9	1369.38±7.93	3432.46±4.46	222.92±3.89	419.24±5.88	3401.48±18.32

3.2.3 Nitrogen fractions and total sulphur in sediments

The different fractions of nitrogen estimated in the sediments of the estuary were: nitrite-N, nitrate-N, ammonia-N, Kjeldahl-N, urea-N, total-N and residual-N. The spatial and temporal distribution of various N fractions in sediments is presented in Table 3.3. Concentration of sedimentary nitrite-N recorded its maximum (13.88± 0.19 μg/g) at S9 during POM09, while minimum (0.71± 0.15 μg/g) was noted at S8 during POM09, and also exhibited strong spatial variation ($p<0.05$). While the maximum content of nitrate-N was observed at S7 (2700.50±8.43 μg/g) and the minimum was recorded at S4 (28.32±1.83 μg/g) during the period of PRM10 and MON09 respectively with seasonal variation ($p<0.01$). It was observed that ammonia-N recorded its maximum at S2 (3.60± 0.21 μg/g) and the minimum was observed at S8 (0.06±0.02μg/g) during POM09 and MON12 respectively, with significant spatial and seasonal variation ($p<<0.01$). Meanwhile the Kjeldahl-N (Kj-N) during the study period ranged between 35.30 ± 1.58 μg/g (S9; MON09) and 12886.44 ± 9.65 μg/g(S7; PRM10) and revealed significant seasonal variation ($p<0.01$). The urea-N fluctuated between 0.45±0.03μg/g (S1; PRM09) and 6.22±0.07 μg/g (S5; MON12), with the spatial and seasonal variations ($p<0.01$). In the case of residual-N (subtracting sum of nitrite, nitrate, ammonia, and urea nitrogen from total-N), the maximum was observed at S4 (8898.66±12.32μg/g; MON12) and minimum at S9 (33.40±1.43 μg/g; MON09) with seasonal variation ($p<0.01$). The observed variation in total-N (TN = Kj-N + Nitrate-N + Nitrite-N) was from 294.75±1.18 μg/g(S9; MON09) to 15594.43±13.22 μg/g(S7; PRM10) and exhibited spatial and seasonal variations ($p<0.01$). The total sulphur (TS) in the sediments of the study area varied from 0.06 % (S4; MON12) to 3.10 % (S4; PRM10) with significant spatial variation ($p<<0.01$).

Table 3.3 Various nitrogen fractions (µg/g) and total sulphur (%) in surface sediments of the study area

Nitrogen fraction	Stations	PRM09	MON09	POM09	PRM10	MON12
Nitrite-N	S1	10.08±1.12	7.65±0.19	13.33±1.19	7.14±0.21	5.21±0.32
	S2	8.72±0.78	5.51±1.12	5.71±0.84	7.26±0.34	6.25±0.52
	S3	4.96±0.29	1.22±0.76	2.62±0.05	6.78±0.56	3.45±0.05
	S4	6.64±0.18	4.08±0.28	3.09±0.08	2.62±0.07	2.95±0.04
	S5	5.76±0.22	2.14±0.41	2.38±0.03	7.74±0.17	6.99±0.21
	S6	6.20±0.24	2.86±0.09	2.14±0.03	3.81±0.26	8.03±0.34
	S7	4.32±0.17	3.47±0.04	9.40±0.17	7.50±0.28	4.88±0.28
	S8	11.60±0.78	3.16±0.11	0.71±0.15	5.36±0.31	7.48±0.21
	S9	5.33±0.05	10.47±0.28	13.88±0.19	7.67±0.27	6.74±0.31
Nitrate-N	S1	511.00±3.72	475.64±3.18	2379.49±10.16	2593.88±6.74	784.80±1.11
	S2	409.90±2.11	235.25±3.29	1074.50±4.93	753.57±1.23	571.33±1.29
	S3	308.29±2.19	138.78±2.19	810.40±2.73	363.24±1.31	176.61±0.91
	S4	83.12±1.78	28.32±1.83	1258.03± 8.87	1572.27±7.56	397.05±2.73
	S5	226.25±2.25	291.14±1.58	873.16±2.11	697.60±6.21	999.82±8.11
	S6	429.41 ±1.83	147.87±1.93	246.53±1.18	558.27±2.92	1700.93±8.96
	S7	344.76±1.74	234.24±2.18	186.59 ± 2.17	2700.50±8.43	1644.89±7.48
	S8	191.56±1.28	664.85±3.19	870.56±1.92	805.34±5.81	982.83±8.14
	S9	246.53±1.83	248.98±3.34	296.43±6.68	600.84±4.97	201.17±3.18
Ammonia-N	S1	0.25±0.04	1.13±0.14	2.13±0.18	0.25±0.03	0.57±0.02
	S2	1.38±0.03	3.38±0.11	3.60±0.21	1.63±0.02	2.82±0.04
	S3	1.25±0.04	2.50±0.06	2.70±0.12	1.38±0.03	1.94±0.03
	S4	0.25±0.12	0.88±0.03	0.90±0.07	0.25±0.02	0.32±0.04
	S5	0.50±0.08	1.13±0.05	1.63±0.13	0.25±0.02	0.65±0.02
	S6	0.50±0.04	1.13±0.06	1.40±0.07	0.50±0.01	0.46±0.03
	S7	0.38±0.03	0.90±0.02	1.00±0.09	0.38±0.02	0.34±0.01
	S8	0.50±0.04	0.63±0.04	0.90±0.03	0.50±0.03	0.06±0.02
	S9	0.38±0.05	1.13±0.06	2.40±0.12	0.50±0.01	0.23±0.02
Kjeldahl-N	S1	997.83±1.28	1880.67±4.76	2971.31±8.39	1964.87±8.93	712.61±3.43
	S2	327.76±1.76	995.08±2.19	826.05±4.19	1725.66±7.95	1083.21±6.83
	S3	2267.38±1.47	945.01±3.18	867.71±3.35	1665.47±9.92	1340.00±6.97
	S4	756.38±1.82	910.46±3.11	2260.40±14.18	1295.65±10.17	8900.00±9.93
	S5	2051.87±9.94	1839.93±7.29	1665.19±8.73	1217.60±13.14	2461.60±11.31
	S6	1568.78±11.19	2061.83±8.38	1938.55±7.49	1585.00±10.23	1749.12±12.26
	S7	354.84±2.85	859.10±2.84	797.72±3.92	12886.44±9.65	960.00±6.79
	S8	4604.69±8.93	4006.00±8.83	3529.60±10.52	2748.04±12.86	4457.46±10.93
	S9	252.11±1.17	35.30±1.58	281.75±1.12	100.85±7.67	113.20±4.50

Urea- N	S1	0.45±0.03	1.29±0.45	2.87±0.18	2.43±0.24	1.87±0.18
	S2	1.89±0.05	0.69±0.05	4.20±0.11	1.55±0.11	1.52±0.13
	S3	1.77±0.18	2.97±0.11	3.60±0.25	1.26±0.08	1.68±0.05
	S4	0.87±0.07	1.45±0.18	0.87± 0.03	0.50±0.01	1.03±0.03
	S5	0.65±0.08	1.66±0.29	1.88±0.01	2.74±0.88	6.22±0.07
	S6	0.69±0.07	0.61±0.08	1.67±0.02	1.03±0.05	1.77±0.17
	S7	0.89±0.09	1.18±0.02	1.37±0.03	2.02±0.18	1.89±0.04
	S8	0.95±0.03	1.14±0.04	1.30±0.01	1.83±0.04	1.57±0.05
	S9	0.56±0.03	0.78±0.05	2.80±0.08	0.80±0.02	0.49±0.02
Residual-N	S1	997.13±2.18	1878.26±11.23	2966.32±10.81	1962.19±8.94	710.17±3.76
	S2	324.50±3.74	991.02±3.48	818.25±5.19	1722.49±10.23	1078.87±8.76
	S3	2264.36±8.81	939.54±3.82	861.41± 6.11	1662.84±7.49	1336.38±8.37
	S4	755.26±4.19	908.13±2.84	2258.63±8.93	1294.90±10.11	8898.66±12.32
	S5	2050.72±9.65	1837.15±3.88	1661.68±10.21	1214.62±9.34	2454.73±8.93
	S6	1567.60±4.79	2060.10±8.72	1935.48±8.72	1583.48±7.94	1746.89±8.38
	S7	353.58±5.82	857.02±4.83	795.36±3.30	12884.05±8.87	957.77±6.92
	S8	4603.24±8.25	4004.24 ± 10.82	3527.40±8.36	2745.72±3.85	4455.82±4.18
	S9	251.17±2.19	33.40±1.43	276.55±7.49	99.55±1.16	112.48±2.18
TN	S1	1518.92±13.18	2363.97±10.94	5364.13±13.51	4565.89±9.77	1502.61±7.88
	S2	746.38±6.12	1235.84±8.79	1906.27±10.81	2486.50±8.92	1660.78±9.48
	S3	2580.64±14.12	1085.01±7.43	1680.73±8.92	2035.49±6.32	1520.06±4.57
	S4	846.14±4.82	942.86±3.68	3521.52±7.96	2870.53±7.38	9300.00±6.94
	S5	2283.88±7.85	2133.22±3.84	2540.73±5.82	1922.94±12.11	3468.41±10.37
	S6	2004.39±8.50	2212.56±3.81	2187.22±8.76	2147.08±10.34	3458.09±10.18
	S7	703.92±3.91	1096.80±3.92	993.71±5.92	15594.43±13. 22	2609.77±11.02
	S8	4807.85±15.82	4674.02±4.13	4400.87±10.85	3558.73±10.94	5447.77±8.99
	S9	503.97±4.92	294.75±1.18	592.07±7.28	709.35±9.32	321.11±4.89
TS	S1	0.72±0.01	1.51±0.04	2.24±0.03	2.51±0.04	0.46±0.07
	S2	0.26±0.04	0.52±0.01	0.55±0.02	0.41±	0.36±0.03
	S3	1.41±0.02	0.46±0.02	0.40±0.01	0.51±0.08	0.16±0.03
	S4	0.61±0.03	0.36±0.03	2.52±0.05	3.10±0.04	0.06±0.01
	S5	1.78±0.05	1.64±0.05	1.66±0.08	1.45±0.05	1.11±0.01
	S6	1.34±0.09	1.38±0.01	1.46±0.08	1.81±0.03	1.12±
	S7	0.30±0.07	0.77±0.02	0.80±0.05	0.92±0.1	0.47±0.02
	S8	2.06±0.03	2.15±0.04	2.07±0.03	1.91±0.02	0.80±0.03
	S9	0.50±0.1	0.16±0.1	0.19±0.02	0.31±0.03	0.10±0.01

3.3 Discussion

The texture of the sediment displayed distinct spatial as well as seasonal variation ($p<<0.01$). Increased river runoff associated with high precipitation during monsoon season (Menon et al., 2000), resulted in higher sand content, which was more pronounced at Eloor (S9; MON09, Figure 3.1). Fine grained fractions of sediments viz., silt and clay displayed positive correlations with most of the sedimentary variables, while sand exhibited strong negative correlations (Table 3.4). This observation revealed the fact that the grain size of the sediments have a profound role in the distribution pattern of geochemical parameters of the sediments in the study area (Prasad and Ramanathan, 2008; Wen et al., 2008; Renjith et al., 2011). Sediments from the majority of the stations exhibited significant fluctuation in textural characteristics indicating the discharge dependency in riverine and estuarine zones (Saraladevi et al., 1992; Muraleedharan Nair and Ramachandran, 2002). A gradual downstream decrease in sand content denoted textural maturity of the sediments. Northern side of the study region was characterised by sandy sediments. The complex current pattern prevalent in the area and dredging also influence the varying textural characteristics of the sediments.The physical processes of transportation and deposition can alter the grain size of sediments. Depending on the competency of flow, finer material gets entrained in the runoff, thus leaving behind the coarser sediments, resulting in the predominance of sand in the upper reaches of the study area.On account of the slackening of river flow while nearing the confluence results in incompetence to carry coarser material leading to the fining of sediment grain size. Another important factor that influences sediment texture in the estuarine region is the fine material transfer from seaside associated with the flood tide. In addition to

this, random water movements arising from tidal cycles also control the sediment distribution pattern.

Elemental ratios: Stoichiometric nutrients ratios are utilised to determine the origin and transformation of organic matter in sediments (Yamamuro, 2000). Redfield (1958) proposed a TOC/TP ratio of 7 for algal input. Wide range of TOC/TP (28 - 56) and TN/TP (4 -9) ratios in aquatic sediments can still obey Redfield ratio (Hecky et al., 1993). The TOC/TP ratios (Figure 3.2) displayed large variations in the study region which varied from 0.47 (S9; MON09) to 47.27 (S1; POM09). The TN/TP ratios were very low and did not exhibit much variation in the study region, exhibiting a range of 0.09 (S9; MON12) to 10.24 (S7; PRM10). Meanwhile depleted TN/TP ratio reflected enrichment of phosphorus as well as higher benthic nitrogen recycling. Denitrification and the benthic release of nitrogen also play a significant role in sustaining the productivity of the system (Renjith et al., 2011). The TOC/TP and TN/TP ratios were lower than the Redfield ratio (Hecky et al., 1993), pointed out the fact that the organic matter was enriched with P and tends to accumulate in sediments.

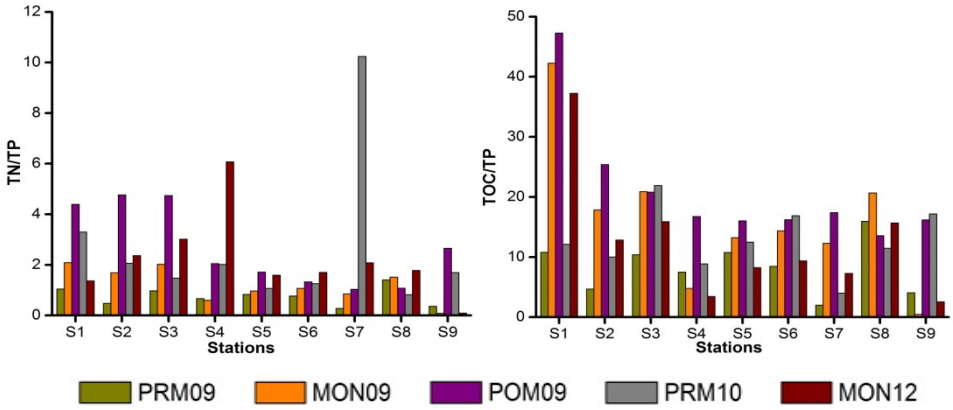

Figure 3.2 Bulkelemental ratios (TN/TP and TOC/TP) in the sediments of the study area

3.3.1 Biogeochemistry of phosphorous fractions in the sediments

Chemistry of phosphorous in sediments is largely governed by redox conditions, and the redox cycle of Fe greatly affects P geochemistry after burial (Slomp et al., 1996; Cha et al., 2005). Fractions of phosphorous undergo a wide range of chemical and biological transformations along the salinity gradient of estuaries. A substantial portion of released P contributes to the formation of authigenic P minerals and thereby immobilised in the sediments (Ruttenburg and Berner, 1993; Schuffert et al., 1994; Kim et al., 1999; Cha et al., 2005).

The involvement of Fe in the dynamic equilibrium between the sediment bound and dissolved PO_4^{3-} levels implied that the Fe dependent threshold limit exists for the sediment to bind phosphate (Sondergaard et al., 2003). Present study recorded a Fe/TP ratio ranging from 1.61 (S9; MON09) to 56.41 (S2; MON12). The Fe/TP ratios were considered as a measure of free sorption sites for PO_4^{3-} ion on iron hydroxides (Jensen and Thamdrup, 1993; Coelho et al., 2004). In order to regulate P release in sediments, Fe/TP ratio should exceed 10 (Caraco et al., 1990). Fe/TP ratios lacked seasonal variations in the study region, but displayed significant spatial variation (Figure 3.3). The river influenced areas showed higher Fe/TP indicating the presence of enough Fe in surface sediments to bind with P, while lower ratios obtained towards the seaward sites suggest the saturation of sorption sites or less capacity to bind with PO_4^{3-} which leads to the transfer of P from the sediment to the water column.Fe(OOH)-IP exhibited positive correlations with silt (r=0.59), clay (r=0.43), TOC (r=0.53), TN (r=0.40), Fe (r=0.57), TS (r=0.65) and TP (r=0.50), which reflected granulometric dependence on the distribution pattern (Table 3.4). The main inorganic forms of P are the fraction associated with Al, Fe and Mn oxides and hydroxides. Phosphorus and iron are usually strongly associated with sediments; P being adsorbed onto iron

compounds with the formation of iron phosphate complexes. The content of FeOOH is therefore one of the major factor controlling P release from sediment.Fe(OOH)-IP is more important than $CaCO_3$-IP in terms of the potential availability of phosphorous under the redox variations observed in the sediments (Caraco et al., 1989; Silva and Mozeto, 1997). Significant positive correlation of $CaCO_3$-IP with sulphur (Table 3.4), the redox indicator pointed out that there is preferential accumulation of $CaCO_3$-IP under reducing conditions. Generally, the release of this phosphate fraction from the sediment is controlled by sulphate reduction (Caraco et al., 1989) and is considered more bioavailable under the redox variations (Caraco et al., 1989; Silva and Mozeto, 1997). Sulphide produced from sulphate reduction may reduce the iron-oxides and thus promote the release of iron-bound phosphorous (Jensen et al., 1995; Howarth et al., 1995).

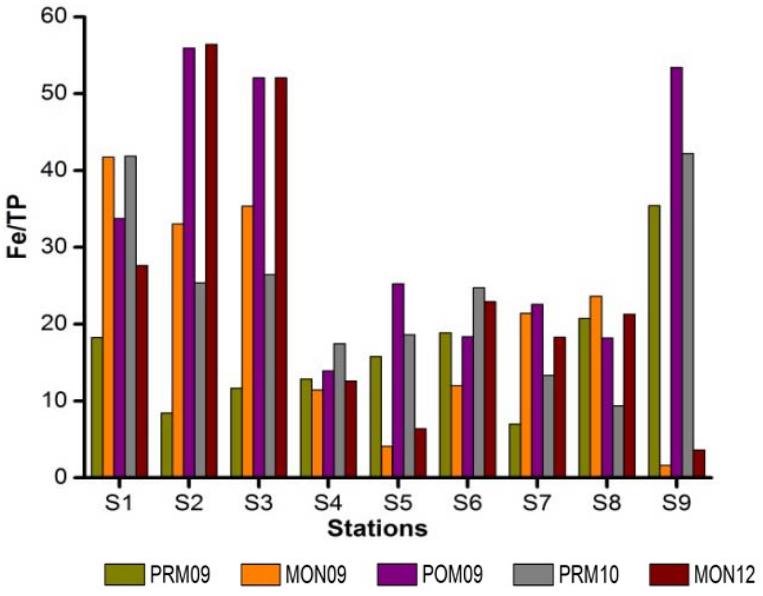

Figure 3.3 Variation of Fe/TP ratio in the sediments of the study area

Table 3.4 Correlation matrix for phosphorous fractions with other sedimentary variables (n=45)

	pH	Eh	sand	Silt	clay	Fe (OOH) -IP	$CaCO_3$-IP	ASOP	Alkali-OP	ROP	Salinity	TOC	TN	Fe	TS	TP
pH	1															
Eh	-0.27	1														
sand	-0.09	0.40 (**)	1													
Silt	0.10	-0.46 (**)	-0.96 (**)	1												
clay	0.05	-0.24	-0.90 (**)	0.76 (**)	1											
Fe (OOH) -IP	0.21	-0.33 (*)	-0.56 (**)	0.59 (**)	0.43 (**)	1										
$CaCO_3$-IP	-0.20	-0.10	-0.40 (**)	0.37 (*)	0.39 (**)	0.33 (*)	1									
ASOP	0.03	-0.37 (*)	-0.47 (**)	0.47 (**)	0.38 (**)	0.56 (**)	0.82 (**)	1								
Alkali-OP	-0.01	0.08	0.27	-0.31 (*)	-0.16	-0.28	-0.05	-0.18	1							
ROP	0.09	-0.11	-0.25	0.17	0.34 (*)	0.04	0.22	0.27	0.10	1						
Saln	0.25	-0.42 (**)	-0.37 (*)	0.37 (*)	0.32 (*)	0.37 (*)	-0.08	0.08	-0.14	-0.07	1					
TOC	0.04	0.02	-0.43 (**)	0.38 (**)	0.44 (**)	0.53 (**)	0.63 (**)	0.61 (**)	-0.22	0.40 (**)	-0.06	1				
TN	-0.02	0.07	-0.28	0.22	0.35 (*)	0.40 (**)	0.30 (*)	0.40 (**)	-0.26	-0.06	-0.06	0.46 (**)	1			
Fe	0.25	-0.05	-0.38 (**)	0.32 (*)	0.43 (**)	0.57 (**)	0.49 (**)	0.56 (**)	-0.24	0.35 (*)	-0.08	0.72 (**)	0.43 (**)	1		
TS	0.09	-0.14	-0.46 (**)	0.44 (**)	0.41 (**)	0.65 (**)	0.43 (**)	0.53 (**)	-0.26	0.13	0.31 (*)	0.64 (**)	0.58 (**)	0.57 (**)	1	
TP	0.01	-0.20	-0.33 (*)	0.30 (*)	0.34 (*)	0.50 (**)	0.70 (**)	0.65 (**)	0.50 (**)	0.27	0.04	0.48 (**)	0.19	0.41 (**)	0.39 (**)	1

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

Total phosphorousin sediments displayed both spatial and seasonal variations in the study area ($p<0.01$). The distribution of various P fractions and other geochemical parameters in the surface sediments of CES displayed significant relationship with texture. Content of TP displayed an increasing trend from fresh water region to saline area (Figure 3.4). Correlation analysis (Table 3.4) revealed that silt and clay fractions possess remarkable positive correlations with most of the sedimentary parameters, which indicate the fact that the main factor influencing the geochemistry of sediments in the study region could be sediment texture. These high correlations of P-fractions with fine grained sediments (silt+clay) may have resulted from the greater surface area of fine particles, providing more adsorption sites for phosphate ions (Liu et al., 2002; Zhou et al., 2005). It is well documented that organic carbon and nutrients are enriched in silt and clay fractions compared to sand (Krishna Prasad and Ramanathan, 2008; Wen et al., 2008; Renjith et al., 2011).

ANOVA indicated that Fe(OOH)-IP possess significant spatial variation ($p<0.01$) but devoid of seasonal variations. During the monsoon seasons (both MON09 and MON12), the estuary is virtually transformed to a freshwater basin (Menon et al., 2000; Renjith et al., 2011). During post monsoon, river discharge gradually declines and tidal activity attains momentum as the estuarine conditions changes to a partially mixed type, which weakens stratification. In PRM09 and PRM10, the river discharge is minimised and seawater influence was maximum in the study area and the estuary was well-mixed, similar to the previous observation (Menon et al., 2000). Oxidative decomposition of organic matter and reductive dissolution of Fe(OOH)-IP releases the phosphorous to the sediment interstitial water and a portion of released P may return to the water column. The seasonal distribution of Fe(OOH)-IP followed the pattern: POM09>PRM10> PRM09> MON09>MON12. From the Figure 3.4, it is clear thatfrom station S5 to

S8higher concentration of Fe(OOH)-IP was noticed, but at the sanddominated station (S9) lower content was found. The increased pH and salinity in those stations may inhibit phosphate adsorption onto Fe oxides/hydroxides and by altering the surface charge on the Fe oxides and hydroxides (Lebo, 1991; Zwolsman, 1994). Moreover, the concentration of Fe oxides and hydroxides reduced in sulphide rich environments by the formation of solid Fe sulphides and the sulphate reduction rate may be more pronounced at stations with higher salinity (Paludan and Morris, 1999; Hou et al., 2009).

Carbonate-adsorbed P has been regarded as most dominant phase of P in carbonate-rich sediments, owing to the greater adsorption capacity of carbonates (Short et al., 1990; Millero et al., 2001). $CaCO_3$-IP exhibited significant spatial difference in the study region recording higher concentrations at S8 (PRM10) and did not display any seasonal variation. $CaCO_3$-IP displayed strong positive correlation with salinity (Table 3.4) and its concentration increased towards the zones with high salinity, ie; from southern to northern side (Figure 3.4).The maximum content of calcium-bound P in sediments of the regionswith higher salinity, might be resulted from the favourable accumulation of $CaCO_3$under alkaline pH providing adsorption sites for phosphorous ions (Huanxin et al., 1994; Zwolsman, 1994; Silva and Mozeto, 1997). A similar trend is reported in marine sediments presumably by the accumulation of calcium under high salinity, which favours apatite formation (Ryden et al., 1997). The increased concentration of $CaCO_3$-IP (S5 to S8; Figure 3.4) caused by the interactions between PO_4^{3-} and $CaCO_3$ with increasing salinity (Coelho et al., 2004; Anshumali and Ramanathan, 2007; Hou et al., 2009; Hartzell and Jordan,2010). Calcite in estuarine environment is produced at higher salinities through precipitation reactions and biological activity forming an adsorption substrate for dissolved phosphate (Coelho et al., 2004). $CaCO_3$-IP also exhibited strong positive correlation with ASOP which may have resulted from the mineralisation of organic-P. During

microbial decomposition, organic P may have transformed into authigenicfluroapatite (Anshumali and Ramanathan, 2007; Katsaounos et al., 2007; Hou et al., 2009). The southern part of the study region is well known for the black Clam fishery (Lakshmilatha and Appukuttan, 2002) and the shell of the black Clams is thick as well as rich (93.3 to 95.8%) in calcium carbonate (Kripa et al., 2004). Apart from live Clam beds, the estuary has extensive sub fossil deposits (Renjith et al., 2011). The periodical tidal ingression in the estuary favours the formation of calcite which can bind with P results in the enhanced levels of this fraction during non monsoon seasons.$CaCO_3$-IP positively correlate with silt, clay and Fe(OOH)-IP, while negatively correlated with sand ($r=-0.40$) (Table 3.4) revealed the dependence of texture.

ASOP includes apatite-bound phosphate and biochemical components such as nucleic acids, lipids, and sugars that are bound to phosphate (De Groot, 1990). ASOP exhibited spatial ($p<0.01$) variation with maximum concentrations in the PRM10, and displayed an increasing trend towards the regions with higher salinity (Figure 3.4). The degradation of ASOP compounds release phosphate which become readily bioavailable to phytoplankton. ASOP exhibited a highly significant positive correlation with total sulphur(Table 3.4) indicated that reducing environment favour the retention of ASOP in sediments.

The main component of Alkali-OP has been reported ashumic substances (Golterman, 2001). Phosphorous associated with humic acids has been considered either to be an integral part of humic acids or as a phosphate/organic matter complex (Stevens and Stewart, 1982). This fraction also contains phytate, an organic phosphate commonly occuring in plants and sediments (De Groot and Golterman, 1993; Dvorakova, 1998). Alkali-OP does not display any seasonal or spatial variation during the study period and

recorded differences inconcentration with maximum at S9 (3242.40±11.96µg/g; MON09) and minimum at S3 (0.96±0.12µg/g; POM09). Higher Alkali-OP concentrations were observed in some stations (Figure 3.4) of the study region, may be due to the flocculation and precipitation processes involving humic acids, Fe/Al oxides and dissolved reactive P complexes by advection (Coelho et al., 2004).

Generally, organic bound phosphorus accounted for 6 to 19% of the total-P in coastal sediments (Hirata, 1985). The minimum content of residual organic phosphorous recorded was 0.39±0.02µg/g(S2; POM09) and a maximum of 148±2.23 µg/g(S8; PRM09) during the study period. Organic phosphorus is a complex fraction, the exact nature of which is not clearly described yet (Reitzel et al., 2007). As a result of diageneticreorganisation of phosphorus within sediments, organic-P concentrations gradually decreases and it is ultimately transformed to authigenic-P during diagenesis (Ruttenberg and Berner, 1993; Andersen et al., 2001). Residual organic-P (R-OP) was the smallest fraction estimated in sediments from estuarine stations and the lower content of this fraction might be attributed to diagenesis. Degradation of organic P compounds also releases phosphate, making it available to bacteria and algae. Bacteria are generally considered to be the catalysts that accelerate the solubilization of P (Gachter and Meyer, 1993) and the processes of anoxic mineralization of phytate (Golterman et al., 1998) could release organic P buried during monsoon season. The higher concentration of organic bound phosphorus (R-OP) recorded during PRM09 (S3, S5 and S8), MON09 (S1 and S8) and MON12 (S1 and S8), indicated that mineralisation of P was less. It has been well documented that mineralisation of organic P and C/P ratio is stronglycorrelated under anaerobic conditions compared to aerobic conditions (Bridgam et al., 1998; Reddy and Delaune, 2008). Highly depleted redox potential at certain stations (S4, S5, S6 and S7-PRM09; S5, S6 and S7-POM09) results in higher mineralisation and

subsequentlylower the concentration of organic phosphorus. The higher content of inorganic phosphorus in the estuary seems to be a signal of higher levels of diagenetic activity. In the case of organic-P in sediment, very low concentration was noted compared to other P fractions (Figure 3.4). Degradation of organic P compounds also releases phosphate, making it available to bacteria and algae. Some worldwide reference values of different P fractions in sediments arefurnished in Table 3.5.

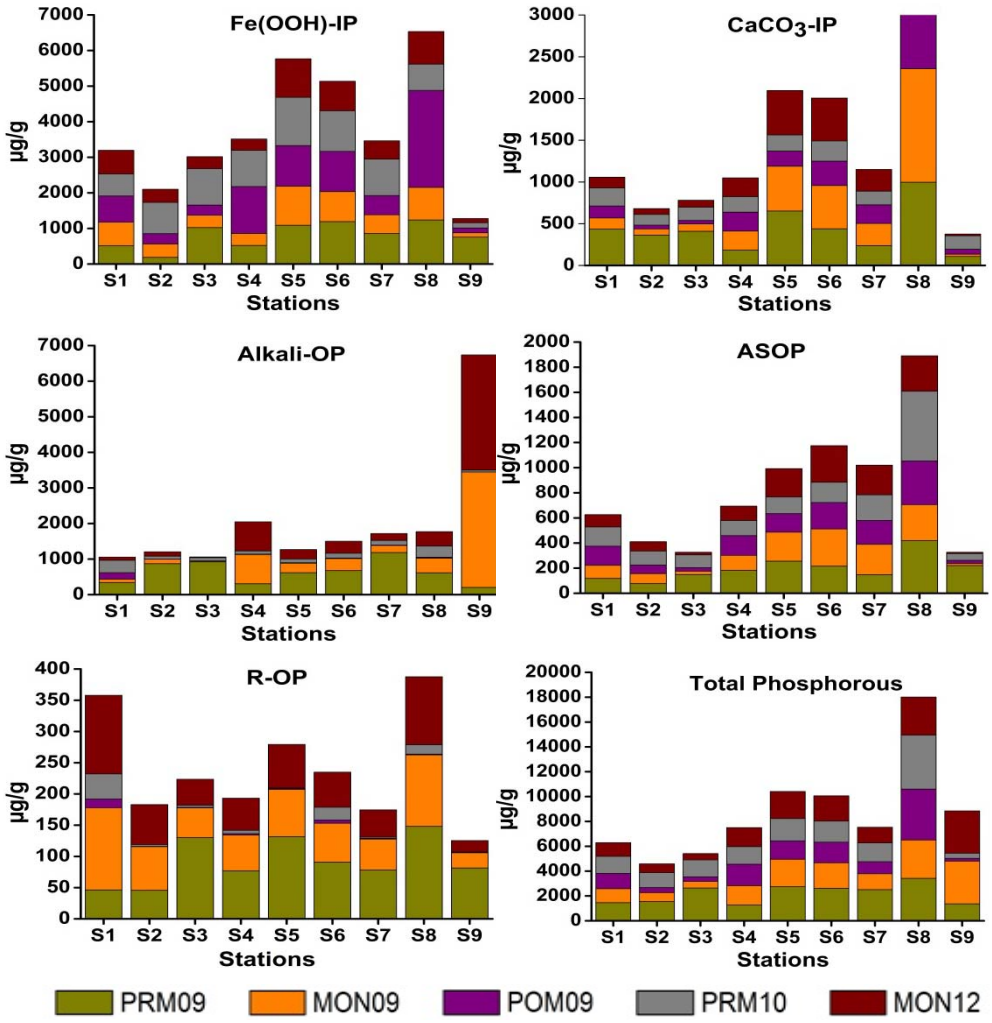

Figure 3.4. Various phosphorous fractions in the sediments of the study area

Table 3.5 Worldwide reference values of different phosphorous fractions in sediments

Aquatic system	Concentration of various phosphorous fractions			References
Southwest coast of India (different extraction techniques)	Ca bound P Exchangeable-P Fe/Al bound P	2.0—44.3 11.8—59.4 12.4—34.6	µg/g µg/g µg/g	Nair et al., 1993
Bay of Seine, France	Fe/Al bound P Ca bound P Exchangable-P Organic-P TP	0.04 — 4.25 0.1- 9.2 0.03- 2.16 0.27- 4.37 0.3- 18.60	µmol/g µmol/g µmol/g µmol/g µmol/g	Andrieux and Aminot, 1997
Morales Stream, Argentina	Fe bound P Ca bound P ASOP Alkali-P TP	~440 ~400 ~170 ~210 ~600	mg/kg mg/kg mg/kg mg/kg mg/kg	Garcia and de Iorio, 2003
Cochin estuary, India	Fe bound P Ca bound P ASOP Alkali-OP R-OP TP	5.04-474.24 11.16-826.09 22.22-365.86 51.92-1002.45 29.27-279.83 319.54-2,938.83	µg/g µg/g µg/g µg/g µg/g µg/g	Renjith et al., 2011
Dianchi Lake, China	Fe bound P Ca bound P Residual-P TP	210.19-441.45 188.38 -899.71 359.12-1304.15 1465.27-2544.73	mg/kg mg/kg mg/kg mg/kg	Li et al., 2012
Cochin estuary, India	Fe bound P Ca bound P ASOP Alkali-OP R-OP TP	33-963 17-1355 17-207 72-1492 2-142 313-2383	mg/kg mg/kg mg/kg mg/kg mg/kg mg/kg	Gireeshkumar et al., 2013
Zhujiang (Pearl) River Estuary, China.	Fe/Al bound P Ca bound P Inorganic-P Organic-P TP	~100- 480 ~100- 300 ~450-650 ~120-320 ~650-1075	mg/kg mg/kg mg/kg mg/kg mg/kg	Wang Lili et al., 2013
Changjiang estuary (China)	Detrital-P Refractory-P Organic-P TP	6.6-13.2 1.72-3.93 0.82-4.27 15-21.40	µmol/g µmol/g µmol/g µmol/g	JiaMeng et al., 2014
Santos—Sao Vicente Estuary, Brazil	Fe bound P TP	1.29-34.17 3.57 - 74.11	µmol/g µmol/g	Berbel et al., 2015
Cochin Estuary	Fe(OOH)—IP $CaCO_3$-IP ASOP Alkali-OP R-OP TP	115.27±1.27-2724.66± 2.86 20.39±0.54-2726.76±12.23 10.29±0.17- 555.42±2.87 0.96±0.12- 3242.40±11.96 0.39 ± 0.02 - 148±2.23 222.92±3.89-4348.66±15.35	µg/g µg/g µg/g µg/g µg/g µg/g	Present study

Bioavailable phosphorous (BAP) in the sediment can be defined as the sum of immediately available phosphorus and potential phosphorus that can be transformed into an available form by naturally occurring physical, chemical and biological processes (Wang et al., 2009). Fe(OOH)-IP, $CaCO_3$-IP and ASOP were considered as the source of BAP for phytoplankton (Diaz-Espejo et al., 1999). Knowledge of P fractions is of utmost importance in determining the upper limit of the potentially BAP in aquatic ecosystems (Hou et al., 2009). The bioavailability of Fe(OOH)-IP depends primarily on redox potential of the sediment (Andrieux and Aminot, 1997; Rozan et al., 2002; Alvarez-Rogel et al., 2007). In the areas characterised by frequent change of sediment redox potential, Fe(OOH)-IP can occasionally be reduced and released from sediments to the water column (Jensen and Thamdrup, 1993; Coelho et al., 2004). Organic P could become bioavailable by microbial remineralisation (Andrieux and Aminot, 2001; Hou et al., 2009). The spatial and seasonal variation of BAP is depicted in Figure 3.5. The concentration of BAP ranged from 145.94 µg/g(S9; MON12) to 4068.71 µg/g(S8; POM09). Two way ANOVA revealed that BAP in surface sediments displayed significant seasonal ($p<0.05$) and spatial ($p<0.01$) variations. The Figure 3.5, displayed that the BAP exhibit an increasing trend from southern side (S1) to the northern side (S9). BAP was relatively higher in the Cochin estuary, which also revealed that the sediment can act as an important internal source of P. Fe(OOH)-IP displayed highly significant positive correlations with other two bioavailable fractions ($CaCO_3$-IP and ASOP). The P-fractions available for biological uptake are all well correlated (Table 3.4) indicating a common biogeochemical balance.

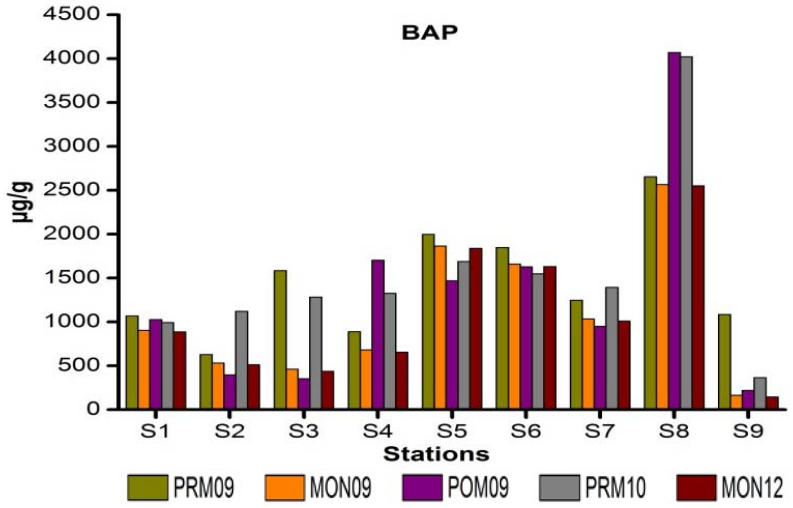

Figure 3.5 Concentration of bioavailable phosphorous (BAP) in the sediments of the study area

Principal component analysis (PCA) of geochemical parameters was carried out to discover and interpret various geochemical variables and thereby identifying the major geochemical processes acting in the estuary (Table 3.6). Varimax rotation was applied in order to identify the variables that are more significant to each factor based on the significance of their correlations that are expressed as factor loading (Buckley et al., 1995; Davis, 2002). The various biogeochemical processes acting along the salinity gradients strongly depend on the hydrodynamic conditions prevailing in the water column.

Factor analysis provided three components for the monsoon season with a total cumulative variance of 71.50 %. The component1 accounted for a total variance of 42.15% and exhibited strong positive loadings on silt, clay, Fe(OOH)-IP, Fe and TP. The close association of clay particles with organic matter and P fractions indicate the granulometric factor. The physical adsorption on organic matter and subsequent sinking to the surface sediments are significant geochemical processes governing the concentration of various P fractions in sediments.

Component 2 consists of $CaCO_3$-IP, ASOP, salinity, TOC, TS and TP contributing 29.35% of total variance, indicated that salinity has a direct control on the concentration of $CaCO_3$-IP in sedimentary phase of the study area. The major role of salinity is the creation of alkaline conditions favouring the formation of $CaCO_3$ which provide adsorption sites for phosphorous. The enhanced flocculation and sedimentation of organic matter is also resulting from the salinity gradient. The complete flushing of the estuary takes place during the peak monsoon period (Revichandran et al., 2012) and the salinity variation can cause strong hydrodynamic forcing. The positive loading of sulphur also gives an indication of the diagenetic processes acting on the surface sediments. Diagenesis, a redox process, largely mediated by microorganisms and the in turn suitable indicators of this process are TOC, TN and TS.

Grouping of salinity and phosphate with moderate negative loading of Fe(OOH)-IP suggests the desorption and reductive dissolution of Fe(OOH) in the stations with high salinity and release of Fe(OOH)-IP to the water column. Reducing condition prevails in the surface sediments during the pre monsoon season and the P fractionation indicated lowest concentrations of Fe(OOH)-IP in surface sediments during this period. The high temperature induces greater bacterial activity in sediments and together with the high salinity initiate sulphate reduction, denitrification and iron involved redox reactions (iron cycling) in sediments. These processes results in the formation of sulphide minerals such as greigite (Fe_3S_4) and mackinawite (FeS) in sediments, which grows as a precursor to pyrite during early diagenetic sedimentary sulphate reduction (Sobrinho et al., 2011).

Table 3.6 Results of Principal Component Analysis

Factor loadings for N fraction				Factor loadings for P Fraction		
Parameters	PC1	PC2	PC3	Parameters	PC1	PC2
Sand	-0.665	-0.413	0.22	Sand	-0.436	-0.092
Silt	0.753	0.504	0.02	Silt	0.384	0.087
Clay	0.776	0.445	-0.048	Clay	0.401	0.053
Salinity	0.125	0.665	-0.051	Fe(OOH)-IP	0.207	0.191
TOC	0.78	-0.048	-0.097	$CaCO_3$-IP	0.275	0.243
Residual-N	0.915	0.166	0.141	ASOP	0.203	0.247
Nitrite-N	-0.171	-0.628	-0.137	Alkali-OP	-0.098	0.083
Nitrate-N	0.516	-0.647	0.205	R-OP	0.203	-0.206
Ammonia-N	-0.279	0.152	0.825	Salinity	0.046	0.28
Urea-N	0.225	-0.151	0.79	TOC	0.153	0.055
Kjeldahl-N	0.916	0.165	0.142	TN	0.027	0.023
TN	0.929	-0.084	0.146	Fe	0.143	0.055
TS	0.754	-0.033	-0.224	TS	0.223	0.251
-	-	-	-	TP	0.053	0.159
% of Variance	44.40	15.30	11.85	-	42.15	29.35

3.3.2 Biogeochemistry of nitrogen fractions in sediments

Nitrogen is a key nutrient element which governs the functions of estuarine ecosystems by limiting the biological growth and is capable of driving eutrophication (Montagna et al., 2002;Gruber and Galloway, 2008). The relative importance of sediments as nitrogen source is greatly understood by its fractionation. Various fractions of nitrogen in sediments of the study area include: nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3), and urea ($CO(NH_2)_2$). The first three are the most significant inorganic forms and urea is a significant organic form of the element. The content of various nitrogen fractions estimated are given in Table 3.3.

The concentrations of nitrite-N in the sediment ranged from 0.71± 0.15 to 13.88± 0.19 mg/kg with significant spatial variation ($p<0.05$). During the study period its seasonal concentration followed the trend: POM09>PRM09>MON09>MON12>PRM10 (Figure 3.6). Enhanced levels of nitrite-N were found at region with lower salinity and their significant concentration was recorded in the sediments of the riverine region. The depleted concentrations of nitrite-N in the sediments, (S3; MON09) and (S8; POM09) revealed the fact that the nitrite-N is intermediate in oxidation state between ammonia and nitrate, and as such it can appear as a transient species in both the oxidation of ammonia and the reduction of nitrate. In certain stations, the concentration of nitrite-N was slightly lower during monsoon period (MON09: S3 to S8 as well as MON12: S1, S3, S4 and S7). Several aerobic and anaerobic microbial processes contribute to the production of nitrogen compound especially NO_2^-, including denitrification and nitrifier-denitrification (Wrage et al., 2001), nitrification and dissimilatory nitrate reduction to ammonium (Smith and Zimmerman, 1981).

Figure 3.6, clearly demonstrates that the distribution of nitrite-N and nitrate-N in surface sediments of the estuary was not uniform. ANOVA revealed that the nitrate-N during the study period varied significantly ($p<<0.01$) without any spatial variation. The maximum content for nitrate-N was found during the period of PRM10 and followed the trend POM09> MON12>MON09>PRM09. The concentration of nitrate-N in sediments were comparatively higher than nitrite-N at all stations. The higher levels of nitrate-N are related to more intense mixing of the upper layers of the water column due to rain, land run off and increased rate of river discharges. Nitrification, the conversion of ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-), is an essential part of the

nitrogencycle in aquatic environments (Urakawa et al., 2006). According to Al Bakri and Chowdhury (2006) bottom sediments act as a source of ammonia nitrogen and function as a sink for nitrate-N. The higher content of sedimentary nitrate-N recorded in the present study indicates a higher rate of nitrification and less denitrification owing to the periodically fluctuating oxic/anoxic conditions. When the dissolved oxygen in water column gets used up for the degradation of organic matter, substitute source for oxygen are sulphate and nitrate. On the other hand, during the monsoon period, the concentrations of nitrate-N was comparatively lower than non-monsoon period. This may be due to the anoxic condition created by the oxidation of large quantities of allochthonous organic matter deposited in sediments via land runoff. The elevated levels of nitrate-Nin the sediments of thestudy area (Figure 3.6) might be due to the influence of agriculture, industries and waste water and also the physical degradation of the land area.

Biological nitrogen fixation, the conversion of atmospheric N_2 to NH_4^+, is an important source of new nitrogen in the sedimentary environment. Organic nitrogen mineralisation in sediments can be a significant source of ammonia-N to the overlying water. Nitrification and denitrification are often tightly coupled near the oxic-anoxic boundary of the sediment with little loss of fixed nitrogen (NO_x and NH_4^+) to the overlying water (Thamdrup and Dalsgaard, 2008). It has been proposed that NH_4^+ can be anaerobically oxidized to N_2, NO_2^- or NO_3^- by Mn (hydr) oxides or organic complexes of Fe(III) and Mn (III/IV), which are ubiquitous and abundant in sediments (Luther et al., 1997; Hulth et al., 1999; Madison et al., 2011). Compared to other seasons, concentrations of ammonia-N in sediments were found to be higher at all locations during POM09 (Figure 3.6). Meanwhile, at S2 and S3 (region with lower salinity), the higher

concentration of ammonia-N was observed throughout the study period. The higher concentration of ammonia-N was observed in POM09 and its content followed the trend of MON09>MON12>PRM10>PRM09 during the study period. It could be seen that during the study period, the ammonia-N varied spatially and seasonally ($p<<0.01$). From the Figure 3.6, it is clear that stations with higher salinity had a lower content of ammonia-N in all seasons. The lower concentrations of ammonia in sediments provide a site for denitrification, which along with nitrogen fixation and other processes determines available nutrient ratios (McCarthy et al., 2007). During periods of hypoxia and anoxia, the ammonium from sediments usually gets released (Berman et al., 1999). The Figure 3.6 indicates that, during monsoon and non-monsoon seasons, the stations from S4 to S8 exhibitedsimilar trendin the concentration of ammonia-N. Dissimilatory nitrate reduction to ammonium was the dominant process compared to denitrification. The anoxic conditions were more favourable in maintaining higher NH_3/NH_4^+levels as the conversion of $NH_4^+$to nitrate by nitrification is less due to the absence of dissolved oxygen under these conditions. Ammonium is the end product of organic nitrogen decomposition in anaerobic condition and a portion of ammonium also gets assimilated in bacterial cells (Blackburn, 1980). The rate of sedimentary nitrification (oxidation of ammonium into nitrate) is generally regulated by the availability of oxygen and ammonium for nitrifying bacteria, and the supply of both components is commonly highest in the top layer of sediment (Henriksen and Kemp, 1988).Positive correlations of ammonia-N with sand and negative correlation with silt and clay (Table 3.7) suggest the desorption process occurring in sediments.

Urea-N in the sediments of the study area exhibited elevatedconcentrations at four stations (S1, S2, S3 and S9), during POM09. The maximum concentration was noted at S5 during MON12 (Figure 3.6), suggesting the influence of river discharges in transporting urea from land during rain and getting engulfed in sediments. The levels of urea-N varied remarkably at the majority of the stations; while a similar pattern of distribution was observed at S7 and S8 in each season (Figure 3.6). The sand dominated station S9 recorded the minimum concentration of urea-N during all the seasons. The urea usually gets released from sediments by similar mechanisms as for ammonium (Berman et al., 1999). The fluctuating urea-N levels in sediments will increase the potential for sediment associated contaminant fluxes and have a direct effect on the distribution of organisms which in turn affect the water quality. Kjeldahl-N positively correlated with clay, residual-N, nitrate-N, but negatively correlated with sand, indicating the adsorption on fine grained sediment fraction.

The estimated residual nitrogen mainly consists of organic forms (other than urea) such as proteins, lipids, etc. During the study period S2, S3 and S9 recorded lower level of residual-N in most of the season and the maximum was noted at S7 (Figure 3.6). With the onset of monsoon season (MON09 and MON12), in most of the stations (except S8-MON09 andS4, S5, S8-MON12) depleted levels of residual-N was observed. Residual-N dynamics in sediments can strongly affect the total N pool because small changes in concentration can impart measurable changes in the biogeochemical environment. From the Table 3.7, it is clear that residual-N correlated negatively with sand and positively with clay, indicating the influence of texture on its distribution pattern.

Table 3.7 Correlation matrix of various nitrogen fractions with other variables in the sediments of the CES (n=45)

	pH	Eh	sand	silt	clay	Residual-N	Nitrite-N	Nitrate-N	Ammonia-N	Urea-N	Kj-N	TN	TS	TP
pH	1													
Eh	-0.27	1												
sand	-0.09	0.40 (**)	1											
silt	0.10	-0.46 (**)	-0.96 (**)	1										
clay	0.05	-0.24	-0.90 (**)	0.76 (**)	1									
Residual-N	-0.07	0.01	-0.34 (*)	0.28	0.39 (**)	1								
Nitrite-N	-0.01	0.18	0.35 (*)	-0.40 (**)	-0.21	0-.03	1							
Nitrate-N	-0.02	0.12	-0.02	0.01	0.06	0.42 (**)	0.16	1						
Ammonia-N	-0.12	0.23	0.33 (*)	-0.29 (*)	-0.35 (*)	-0.23	-0.01	-0.12	1					
Urea-N	-0.26	0.15	-0.01	-0.04	0.10	0.03	0.09	0.31 (*)	0.37 (*)	1				
Kj-N	-0.07	0.01	-0.34 (*)	0.28	0.39 (**)	1.00 (**)	-0.03	0.42(**)	-0.23	0.03	1			
TN	-0.07	0.03	-0.30 (*)	0.24	0.36 (*)	0.97 (**)	0.01	0.62 (**)	-0.23	0.10	0.97 (**)	1		
TS	0.09	-0.14	-0.46 (**)	0.44 (**)	0.41 (**)	0.21	-0.13	0.43 (**)	-0.31 (*)	-0.02	0.21	0.29	1	
TP	0.01	-0.20	-0.33 (*)	0.30 (*)	0.34 (*)	0.21	-0.05	-0.06	-0.51 (**)	-0.25	0.21	0.17	0.39 (**)	1

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

In the case of total-N in sediments,an inconsistent trend was observed, where its maximum content was recorded at S7 (Figure 3.6). As can be seen from Figure 3.6, in most of the stations, the higher abundance of TN was observed during non-monsoon period (POM09 and PRM10), while at stations S4, S5, S6 and S8 during MON12, the higher abundance of TN was recorded.The loss of total nitrogen from the sediments, induced by benthic macrofauna has already been reported in estuarine environments (Clavero et al., 1994). Station S8 recorded a consistent trend in concentration of TN during the study period. The observed deviations at other stations could be because of the nature of local input from land, and also due to the influence of suspended particulate which are rapidly removed from the water column into the sediments associated with salinity gradient. In addition to this, during all seasons, the lowest mean concentration of TN was found at S9, where the sandy nature of the sediments results in very poor retention of N contents. Station S7 recorded the maximum during PRM10, where the fine grained sediments accumulatesthe N compoundstransported through rivers. Moreover, the observed wide fluctuations in the distribution of total N may be due to the variations in magnitude of discharges from land. With the increase of industrialisation and human interventions, inflow water can carry high concentrations of nutrients that arise from sewage disposal. The fertiliser industries located on the northern arm of the estuary acts as a point source of nutrients to the estuary. Data onfractions of nitrogen in sediments from the different parts of the world are furnished in Table 3.8.

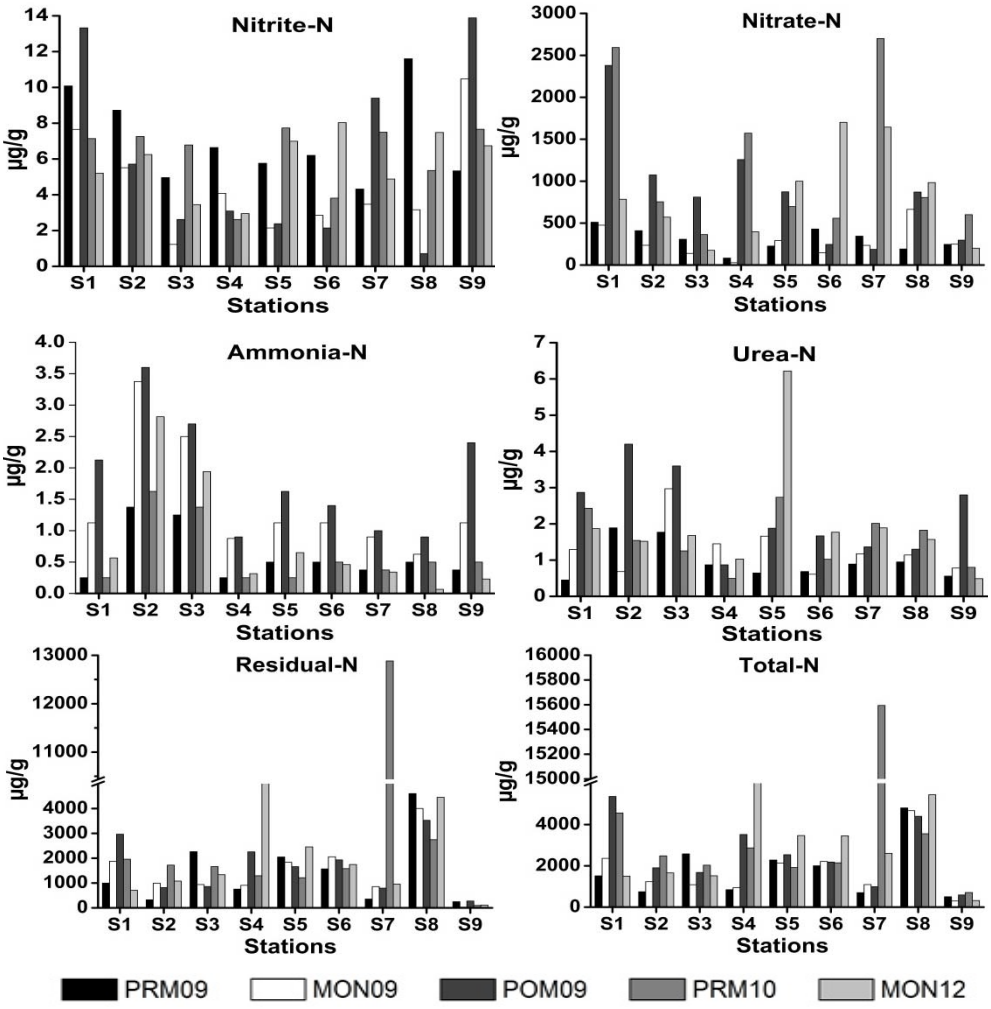

Figure 3.6 Various nitrogen fractions in surface sediments of the CES

Table 3.8 Some worldwide reference values of nitrogen fractions in sediments

Aquatic system	Concentration of various Nitrogen compounds			References
Mississippi estuaries, Gulf of Mexico	TN	1.25	mg/g	Ruttenberg and Goni,1997
Cochin Estuarine system, India	TN	2.24-7.84	mg/g	Lizen Mathews and Chandramohanakumar N, 2003
Mangrove Ecosystems, Kochi, India	Nitrite-N	3.29-30.71	µmol/g	Shaly John, 2003
	Nitrate-N	3.29- 64.31	µmol/g	
	Ammonia-N	0.08- 0.84	µmol/g	
	TN	0.97-30.71	mg/kg	
Anavissos, Greece (surface layer)	TN	1.31-4.89	mg/g	Manos Ladakis et al., 2006
Tigris River, Turkey	TN	507.50-14499.20	mg/kg	Memet Varol, 2012
Cochin estuary	Nitrite-N	0.71± 0.15-13.88± 0.19	µg/g	Present study
	Nitrate-N	28.32±1.83-2700.50±8.43	µg/g	
	Ammonia-N	0.06±0.02-3.60± 0.21	µg/g	
	Kj-N	35.30 ± 1.58-12886.44 ± 9.65	µg/g	
	Urea-N	0.45±0.03-6.22±0.07	µg/g	
	Residual-N	33.40 ± 1.43-8898.66±12.32	µg/g	
	TN	294.75 ± 1.18-15594.43±13.22	µg/g	

Nitrification coupled with denitrification converts biologically available N forms (NH_4^+ and NO_3^- respectively) to N_2 gas and may reduce the effects of excessive N inputs and eutrophication. Nitrate for coupled denitrification derive from organic matter mineralization to NH_4^+ followed by nitrification. Under oxic conditions, nitrifying bacteria convert ammonium (NH_4^+) to nitrite (NO_2^-) and subsequently to NO_3^-, while in anoxic zones, denitrifying bacteria convert NO_3^-or NO_2^- into gaseous forms, either dinitrogen (N_2) or nitrous oxide (N_2O).Benthic nitrification and denitrification influence the inorganic nitrogen budget of estuaries. Microbial processes regulate the availability of nutrients in the estuarine water column by mediating the balance among inputs, recycling and removal to sediments.

In estuarine systems, nitrogen often limits primary production (Capone et al., 2008), and coastal eutrophication, resulting from nitrogen loading to rivers and estuaries and hence is a growing global concern (Cloern, 2001; Capone et al., 2008; Breitburget al., 2009). Distribution of nitrogen fractions in estuaine sediments results from interacting physical processes (advection, diffusion), biological phenomena (uptake, recycling) and reactions with the solid phase (adsorption-desorption) (Treguer and Queguiner, 1989). Due to the wide variations in input and frequent interconversions, principal component analysis (PCA) was carried out to assess the factors controlling the distribution pattern of various nitrogen fractions (Table 3.6). PCA revealed three components which accounted for 71.55% of total variance. Component 1 consisted of negative loadings of sand, positive loadings of silt, clay, TOC, residual-N, nitrate-N, Kjeldahl-N, TN and TS indicating granulometric dependence. Inorganic nutrients (primarily NH_4^+, NO_3^-, and NO_2^-) in pore waters, generated from diagenetic transformation of organic matter, have been important sources of nitrogen for phytoplankton and benthic macroalgae/microphytobenthos in

estuarine environments (Christensen et al., 1987; Cerco and Seitzinger, 1997; Risgaard-Petersen, 2003). The distribution and cycling of N-fractions in sediments has been primarily attributed to various bacterial metabolic processes (Fenchel and Blackburn, 1979). Parameters included in component 2 consisted of silt, salinity and negative loadings of nitrite-N and nitrate-N. This factor denotes a periodically varying redox condition generated by tidal ingression from the Arabian Sea as well as riverine input, which controls the interconversion of nitrite and nitrate. Important factors controlling the content of nitrogen fractions in sediments include seasonality of freshwater flows, nutrient loading, organic matter deposition from phytoplankton blooms and DO content.

3.4 Conclusion

Texture analysis revealed the dominance of sand at the riverine regions of the study area; while the silt and clay content was more pronounced at the estuarine stations. Sand and clay displayed marked seasonal variations ($p<<0.01$). Sediment pH was slightly alkaline during the investigation with its minimum and maximum value was recorded at S8 and S5 respectively. The surface sediments in the entire estuarine region remain oxic during the monsoon season and gradually become reducing during the post monsoon season, which in turn shifts to strongly reducing conditions during the premonsoon season. Characteristic reducing conditions were indicated by Eh values.Sequential chemical extraction of P and N were used for a better understanding of the nutrient enrichment of the estuary. An abrupt increase in the concentration of TP with an increase in salinity was observed in the study region.

Among the various fractions of phosphorous, Fe(OOH)-IP was the most dominant component observed in the sediment. The processes of reductive dissolution of iron hydroxides and biogenic or geochemical formation of

calcium carbonate minerals in the saline areas can be inferred from the distribution dynamics of Fe(OOH)-IP and $CaCO_3$-IP in the estuary. The input of organic matter enriched with P from rivers and from different industrial, agricultural and aquaculture activities lead to a large scale accumulation of refractory organic P in the surface sediments of the estuary**.**During the study period, nitrogen compounds followed the trend: residual-N> nitrate-N> nitrite-N> urea-N> ammonia-N. Among the P fractions, Fe(OOH)-IP exhibited a distinct seasonal distribution pattern with maximum content displayed during the monsoon, when estuary act as a fresh water environment. Fractionation of P in sediments of the study area,resulted in a mixed or metastable digenetic character with strong seasonal signatures. The PCA results generated by considering various P fractions and N fractions support the periodic interchange of oxic/anoxic character of the surface sediments. Intense land use change, unscientific agriculture practices and population growth have significantly altered river fluxes of nutrients. Hence it can be deduced that the major factors controlling the spatio-temporal distribution of N and P fractions in sediments was the salinity, granulometry,OM content, adsorption, desorption, redox status and microbial process.

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Chapter 4

QUALITY ASSESSMENT OF ORGANIC MATTER - BULK PARAMETER APPROACH

Contents

4.1 Introduction

Biogeochemical processes associated with organic matter remineralisation in sediments depend greatly on its quality. Redox conditions prevailing in aquatic environment is closely linked with organic matter dynamics. The components of organic detritus comprise of planktonic materials, faecal pellets of animals and vascular plant debris which display diverse reactivity to leaching and remineralisation processes. Quantity and the composition of organic matter in sediments is strongly influenced by heterotrophic microorganisms (Findlay et al., 1992; Tremblay and Benner, 2006). Bulk sediment parameters are available for the evaluation of sources of organic matter and its fate within marine sediments; which include biochemical composition, elemental and stable isotope ratios. Among the bulk parameters, biochemical composition has been commonly utilised to achieve vital informations on the origin and parameters governing the diagenetic fate of sedimentary organic matter. The major applications of organic molecules to studies of natural systems are their use as source and process indicators. The total concentrations of proteins, carbohydrates and lipids in sediments are generally referred to as biochemical composition and has already

been established as a reliable methodology for the assessment of nature and quality of sedimentary organic matter (Colombo et al., 1996; Dell' Anno et al., 2002; Pusceddu et al., 2009; 2011; Venturni et al., 2012). Sources of the organic matter and the significant processes involved in the transformation of organic matter has been reported in terms of bulk parameters such as elemental ratios (Tan et al., 1991; Thornton and Mc-Manus, 1994; Mitchell et al., 1997; Andrews et al., 1998; Graham et al., 2001). Stable carbon isotope ratios ($\delta^{13}C$) of the various carbon inputs are usually different, making them powerful tracers to differentiate between allochthonous as well as autochthonous organic carbon inputs (Middelburg et al., 1997; Bianchi et al., 2002).

Detailed information on the nature, quality and the relative contribution of different sources (in situ production versus terrestrial input) is of immense importance in understanding the organic carbon dynamics in estuaries. Literature review suggested that huge quantities of sewage and other untreated pollutants have been discharged into Cochin estuarine system, which results in significant impact on the aquatic environment (Balachandran et al., 2005; 2008). Besides detailed investigations on organic geochemical aspects of sediments in Cochin estuary are limited (Aneeshkumar and Sujatha, 2012; Renjith et al., 2012; Gireeshkumar et al., 2012), which prompted to carry out a long term assessment of bulk organic matter parameters, to unravel the nature of organic matter and its origin. This Chapter therefore, intends to focus on the nature and quality of organic matter in the surface sediments of CES and attempts to unfold the sources of sedimentary organic matter by the application of bulk organic matter techniques.

4.2 Results

Spatial and seasonal variation in biochemical components in the surface sediments of the study area is depicted in Figure 4.1 and Appendix 1.1. Total

organic carbon (TOC) in the estuarine sediment during the study period varied from 0.16±0.02 % (S9) to 6.89 ±0.21 % (S8) during MON09 and MON12 respectively. Figure 4.1, revealed that at estuarine stations S1 and S8, higher content of TOC, while at S9 (sand dominated station) lower concentration of TOC was observed. Total organic matter (TOM) content in the sediments varied from 0.29±0.01 % to 12.27±0.18 % during MON09 and MON12 at S9 and S8 respectively. Estimated protein (PRT) concentrations ranged from 110.67±7.77 µg/g to 15250±35.32 µg/g at S4 (PRM09) and S1 (POM09) respectively. Stations with more salinity and also sand dominated station (S9) recorded lower concentration of PRT. While higher concentration of PRT was observed during POM09 (S1), PRM10 (S8) and MON12 (S8) seasons compared to MON09 and PRM09. Carbohydrate (CHO) level in the sediments ranged from 434.19±8.74 µg/g (S9; POM09) to 13285±10.33 µg/g (S8; MON12). CHO exhibited decreasing trend from riverine region to estuarine region (Figure 4.1). While at S9, predominance of sand and an associated lowering of CHO content was noticed. Concentration of total lipids (LPD) in the sediments ranged from 115.29±4.25 µg/g (S9) to 5795.62±10.58 µg/g (S8) during POM10 and MON09 respectively. LPD revealed its maximum content at S1 and S8 during PRM10 and MON09 respectively. Biopolymeric carbon (BPC) content in the sediments varied from 0.02±0.01 % to 1.13±0.03 % at S9 (POM09) and S8 (MON12). Meanwhile, tannin and lignin (TL) in the study area ranged from 97.42±1.25 µg/g (MON12) to 4207±5.48 µg/g (PRM10) at stations S9 and S1 respectively. ANOVA for tannin and lignin revealed highly significant spatial and seasonal variations ($p<<0.01$).

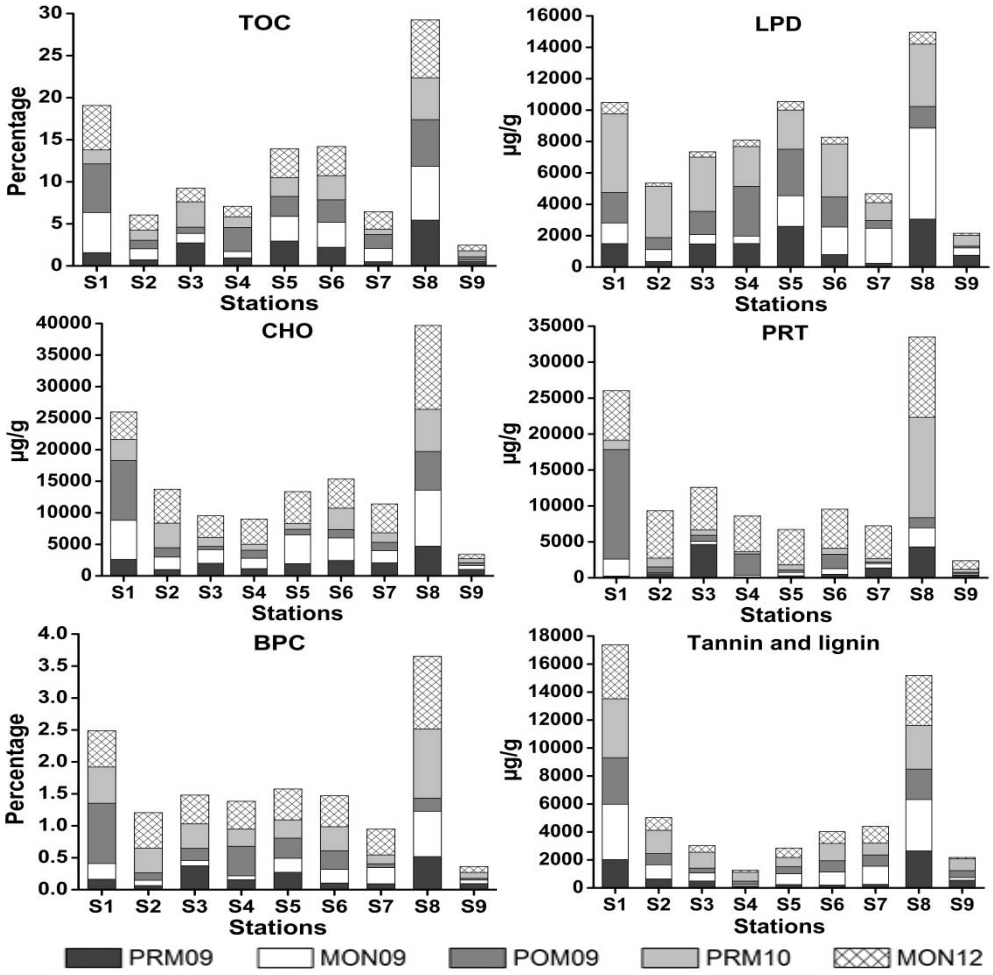

Figure 4.1. Spatial and seasonal variation of biochemical components in the sediments of the study area

The spatial and seasonal variations in chlorophyll-a (chl-a), chlorophyll-b (chl-b), chlorophyll-c (chl-c) and phaeophytin (Phe) are presented in Figure 4.2 and Appendix 1.2. Concentrations of chl-a in the sediments ranged from 0.37±0.03 µg/kg (S9; MON09) to 16.89±1.34 µg/kg (S4; POM09) and revealed highly significant spatial variation ($p<<0.01$). Higher content of chl-a was noticed at S3 (PRM10), S4 (POM09) and S8 (MON09), while lower concentration of chl-a was observed at sand dominated station-S9. Chlorophyll-b content ranged between 0.23±0.01 µg/kg (S9) and 7.34±0.43 µg/kg (S4) during MON12 and POM09 respectively. In the case of chlorophyll-b, the

higher content was recorded at S4 and S5 during POM09 and MON09 seasons respectively. From the results (Figure 4.2), it was noted that chl-b displayed highly significant spatial variations ($p<0.001$) in the study region but devoid of seasonal variation. Maximum content for chl-c recorded in the sediments during the present investigation was 7.10±0.61 µg/kg (S4; POM09); while minimum was 0.22±0.05 µg/kg (S9; MON12). The lower concentration of chl-c was observed at S9 throughout the study period. However in the case of chl-c remarkable differences among sampling sites ($p<0.05$) was noticed. Concentration of phaeophytin in the sediments ranged from 0.55 ±0.02 µg/kg (S9; MON12) to 28.98±2.18 µg/kg (S3; POM09). Phaeophytin recorded its higher concentration at S3 (PRM09, POM09 and PRM10), S4 (POM09), S5 (MON09, MON12) and S8 (MON09, MON12), while lower content was observed at S9 (during all seasons). However, ANOVA displayed highly significant spatial ($p<<0.01$) variation but seasonal variation was absent.

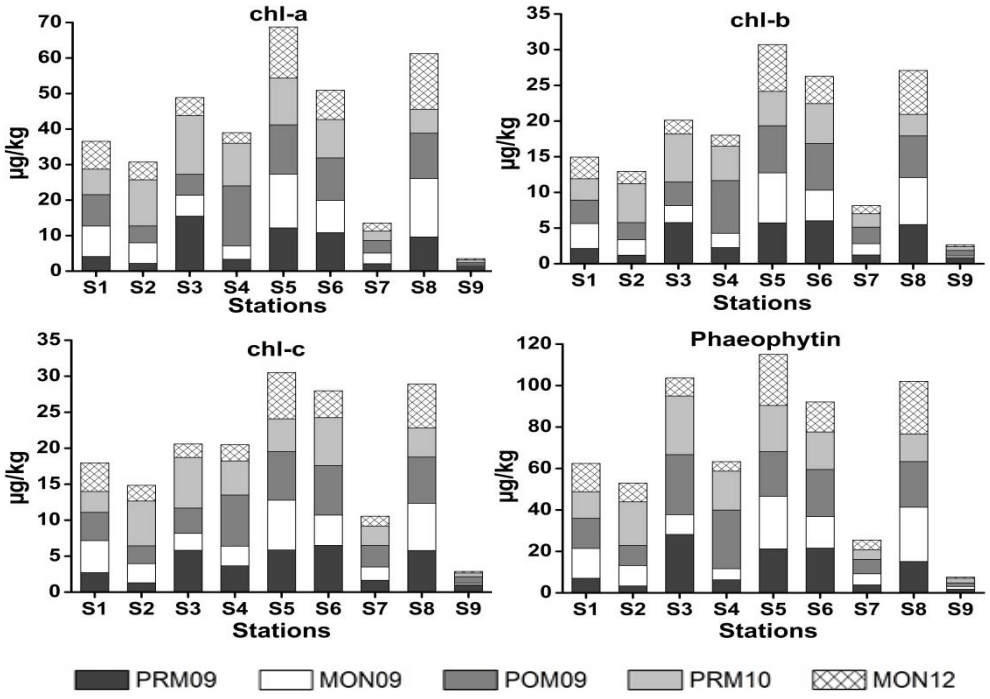

Figure 4.2 Distribution of pigments in the sediments of the study region

4.3 Discussion

4.3.1 Composition of sedimentary organic matter

Organic carbon exhibited higher concentrations in the river dominated regions (S1 and S8) on account for the transport of allochthonous material from the catchment area via terrestrial run-off (Martin et al., 2010; Renjith et al., 2012; Gireeshkumar et al., 2012), while at sand dominated station (S9) lower TOC content was observed. Organic matter, the integral part of aquatic sediments, exhibited a strong spatial variability ($p<<0.01$). Surface sediments collected from CES exhibited a moderate level of TOC (Figure 4.1), which was in good agreement with earlier investigations (Balachandran et al., 2005; Joseph et al., 2008; Martin et al., 2010; Deepulal et al., 2012; Gireeshkumar et al., 2012; Renjith et al., 2012; Akhil et al., 2013). TOC content in the study region was noted to be controlled by the in situ primary production, addition of terrestrial materials, deposition rate and texture of the sediments. Textural control over TOC was suggested by the correlation of TOC with sand, silt and clay. In the estuarine sediments, TOC exhibited significant positive correlation (Table 4.3) with both silt ($r = 0.32$) and clay ($r = 0.25$) and an inverse relationship with that of sand ($r = -0.33$). According to previous study (Table 4.3), positive relationship of TOC with clay and silt implies its size dependent scavenging nature (Muraleedharan Nair and Ramachandran, 2002). Furthermore, organic matter adsorbed onto clay minerals prominently influence the size distribution and sedimentation (Cotano and Villate, 2006; Ramaswamy et al., 2008). The observed concentration of TOC in the sediments of CES during the present study was also comparable with the estuarine data from other regions of the world (Table 4.1).

Table 4.1 Comparison of present study with bulk sedimentary parameters from different estuaries in the world

Study region	LPD, µg/g	PRT, µg/g	CHO, µg/g	$\delta^{13}C$ ‰	TOC/TN	Reference
South eastern Arabian sea	900-5100	100- 7300	400-3500	-	-	Ramya et al., 2013
East coast of India	-	-	2800 – 4710	-23.2 to -19.6	15-23	Kumar et al., 2013
Cochin estuary	41.50-3160	24.48-2600	166-6339	-28.25 to -24.73	0.73-33.62	Renjith et al., 2012
Rio de la Plata estuary	500-8350	1080-16370	290-8860	-	-	Venturini et al., 2012
Cochin estuary	-	-	-	-27.5 to -21.7	7-21	Gireeshkumar et al., 2012
Pearl river estuary	-	-	-	-25.1 to -21.3	6.50 -13.30	He et al., 2010
Pearl river delta and estuary	-	-	-	-25 to -21	6.8 -15.2	Fengling Yu et al., 2010
Pichavaram estuary	-	-	-	-28.92 to -25.34	-	Prasad and Ramanathan, 2008
Cochin estuary	312 – 2815	205 -1924	250 –1229	-	4.80 -10.62	Joseph et al., 2008
Mundaka estuary	300 – 5000	*BDL -16700	200 -5700	-	-	Cotano and Villate, 2006
Yangtze estuary, China	-	-	-	-29.8 to -26.0	1.6 –5.5	Liu et al., 2006
Chunnambar, India	-	-	-	-23.3to -24.5	-	Bouillon et al., 2004
Winyah Bay, SC, USA	-	-	-	-28 to -23	5 - 45	Goni et al., 2003
Harney river estuary Florida	-	-	-	-28.9 to -19.0	-	Hernandez et al., 2001
Lower St.Lawrence estuary	820 – 1470	110- 400	7580-10700	-	-	Colombo et al., 1996
River estuary (Tyrrhenian Sea)	2.9-12.6	250-1670	350-1890	-	-	Fabiano and Danovaro, 1994
Cochin estuary	115.29±4.25-5795.62±10.58	110.67±4.32-15250±11.25	434.19±8.74-13285±10.33	-32.34±1.25 to -25.07±1.02	0.39-35.11	Present study

BDL: below detectable limit

Organic detritus in aquatic sediments has long been documented for its influence on the biogeochemical cycles and its importance as a benthic food resource (Mann and Lazier, 1991; Bianchi and Bauer, 2011). Predominance of carbohydrates in the study region and the estimated contents were comparable with data from other estuaries in the world (Table 4.1). Decay of floating plants in the estuary greatly contributes to the comparatively higher concentration of carbohydrates. Increased levels of CHO in the sediments of the study region could have also been attributed to the accumulation of aged organic detritus owing to faster utilisation of proteins compared to carbohydrates, by microbial processes (Joseph et al., 2008; Venturini et al., 2012). The enhanced CHO content was recorded at stations S1 and S8 (Figure 4.1), which are located in the upstream locations of the study area, implying greater contribution of vascular plant debris to sedimentary OM (Cowie and Hedges, 1984). The season wise variation in the content of biochemical components in sediments of CES was in the order: PRM09-CHO>PRT>LPD, MON09-CHO>LPD>PRT, POM09-PRT>CHO>LPD, PRM10 - PRT>CHO>LPD, MON12 - CHO>PRT>LPD. The dominance of CHO over PRT and LPD during PRM09, MON09 and MON12 pointed out the input of terrestrially derived OM and also implied the detrital-heterotrophic nature of CES (Danovaro, 1996; Renjith et al., 2012). The observation is comparable with the previous records of this wetland ecosystem (Joseph et al., 2008; Renjith et al., 2012; Akhil et al., 2013).

Compared to other seasons, the MON12 displayed higher concentration of protein in most of the stations except S1 and S8 (Figure 4.1). The increased levels of total protein (comparable with other studies- see Table 4.1), in sediments of the study area revealed the productive nature of the estuary and the better preservation potential of this class of compounds (Nguyen and Harvey, 2001; Knicker and

Hatcher, 2001). Proteins in sediments constitute a significant portion of labile organic matter, originating from either autochthonous or via allochthonous inputs. Wide fluctuations in PRT content were noticed in the estuary; with strong spatial variability ($p<0.01$) and the maximum was noted at southern arm (S1) of CES. Fish processing industries situated on the banks of CES release bulk quantities of waste materials into the estuary contributing OM enriched with protein, which ultimately adsorbed/settled in the sediment (Vasudevan, 2000; Balasubramanian et al., 2012).

Lipids are produced by living organisms and comprise of a major fraction of dissolved and particulate organic matter in aquatic ecosystems (Borsheim et al., 1999; Burdige et al., 2000). Elevated levels of total lipid recorded in the present study (during PRM10, Figure 4.1) reflected the biological activity together with the highly productive nature of the estuarine environment (Gremare et al., 1997; Akhil et al., 2013). The influx of surplus quantities of allochthonous OM into the Cochin estuary has already been reported (Balachandran et al., 2003; Babu et al., 2006; Thottathil et al., 2008; Martin et al., 2010). Comparatively higher concentrations of dissolved and particulate organic carbon were reported from the central part of CES (Martin et al., 2010) which implied sewage derived OM delivered from various channels of Cochin city. Thus the general distribution pattern of lipid revealed higher content during the investigation period at stations S1, S5, S6 and S8 indicating terrestrial runoff coupled with industrial input. LPD constitutes a significant portion of the labile OM and provide useful informations on meiofauna abundance and biomass (Cartes et al., 2002; Gremare et al., 2002). The southern arm of the estuary receives sewage enriched from aquaculture, agricultural fields and coconut husk retting yards, which ultimately increases

the organic carbon level in sediments (Thomson, 2002; Babu et al., 2006; Martin et al., 2010). Besides the enriched content of lipids in the sediments also pointed towards the greater availability of labile organic matter (Cartes et al., 2002; Gremare et al., 2002).The concentration of total lipids recorded in the present study was comparable with the previous investigations (Table 4.1).

Tannin and lignin, well known aromatic polycyclic phenolic compounds biosynthesised by higher plants (Finar, 1976; Field and Lettinga, 1987; Hernes and Hedges, 2000), which have been delivered to aquatic environment through terrestrial run off. These form a major fraction of refractory OM and its quantitative determination provides valuable information on the input of terrestrially derived organic detritus in to the sediments (Lin et al., 2006). The enhanced levels of tannin and lignin observed in the sediments of CES (Figure 4.1), seemed to be originated from terrestrial vascular plant debris, accumulated in sediments by land runoff. The successful application of these unique class of phenolic compounds as biomarkers of terrestrial organic matter has already been documented in Cochin estuary (Renjith et al., 2012; Akhil et al., 2013).

Evaluation of the nature of organic matter (labile or refractory), is an inevitable part of organic geochemical research, which can be achieved by the use of biochemical composition. Labile organic matter can be defined as the sum of all proteins, carbohydrates and lipids (Danovaro et al., 1993; Cividanes and Souza, 2003). Labile fraction denotes the easily assimilable portion of organic matter that is easily available for the use of aquatic organisms including benthos. The labile organic matter (LOM) content in sediments recorded a remarkable variation from 746.48±4.86 μg/g to 26687.23±9.78 μg/g (Figure 4.3) and its contribution to TOM varied from 7.05±0.22 % to 45.14±3.12 %. The river and industrial area (Figure 4.3) indicated higher contribution of LOM to TOM and

the elevated values establish enhanced productivity, coupled with external supply of terrigenous materials. Previous investigations from the CES recorded contribution of LOM to TOM ranging from 9.43 % to 31.10 % (Joseph et al., 2008), pointed out the fact that a significant fraction of TOM represented refractory material.

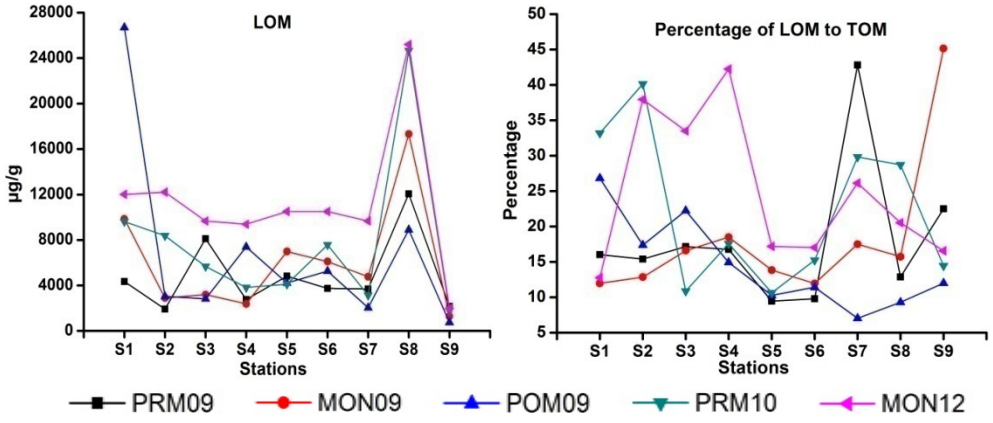

Figure 4.3 Distribution of LOM and percentage contribution of LOM to TOM in surface sediment

4.3.2 Phyto pigments in sediments

Concentration of chlorophyll pigments in the sediments followed the trend: chl-a >chl-b >chl-c. The ratio of chl-a/chl-a+Phe, ranged from 0.17 to 0.45 at S3 and S9 respectively and did not exhibit any spatial and temporal variations (Figure 4.4). The enhanced values of this ratio supported rapid and recent deposition of phytoplankton in sediments (Josefson and Conley, 1997; Hagy et al., 2005). Autochthonous inputis a significant contributor of sedimentary chl-a (Szymczak-Zyła and Kowalewska, 2007) and allochthonous inputs include terrestrial plants.

Previous investigations established the fact that eutrophic systems have a tendency to accumulate organic matter having refractory nature (Pusceddu et al.,

2009; Pusceddu et al., 2011; Venturini et al., 2012) and the results of the present study were in good agreement with these observations. Chlorophyll-a content obtained were comparable with those reported from the other estuaries (Fabiano and Danovaro, 1994; Liu et al., 2006; Venturini et al., 2012). Various biotic and abiotic factors affect the spatio-temporal variations of chlorophyll pigments in the sediments (Moreno and Niell, 2004). Chlorophyll-a content in sediment displayed distinct spatial variations with maximum content observed during POM09 and minimum during MON09 (Figure 4.2). Apart from these, the depleted levels of pigments during monsoon seasons (MON09 and MON12) in most of the stations might also be due to high flushing which results in the faster removal of phytoplankton to the coastal regions (Jyothibabu et al., 2006). Light availability and dissolved oxygen content in the water column have been regarded as the key factors controlling the concentration of phytopigments in sediments (Kowalewska and Szymczak, 2001; Kowalewska et al., 2004). The light attenuation is in turn controlled by the fluctuations in the prevailing hydrodynamic conditions (Moreno and Niell, 2004). Decreased daily total solar insolation resulting from cloud cover during the monsoon is a general phenomenon in the study region (Qasim, 2003). Furthermore, the monsoon season causes the transportation of suspended particulate matter to the estuary via river run off which bring about enhanced water column turbidity and causes poor light penetration. The higher turbidity and reduced solar insolation, limit the primary production in water column as well as in the benthic compartments.

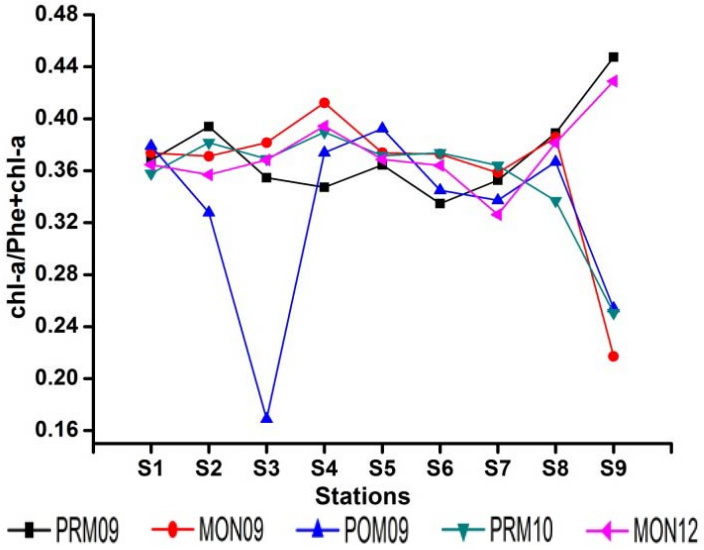

Figure 4.4 Spatio-temporal variation of pigment ratios in sediments of the study area

4.3.3 Nutritional quality

Concentration of lipid and lipid to carbohydrate ratio have been employed as suitable index to unravel the food quality of the sedimentary organic matter (Fabiano and Pusceddu, 1998; Gremare et al., 2002). It has been well documented that lipid content in sediments can function as an effective methodology to describe the benthic trophic state (Danovaro et al., 1999; 2000; Dell' Anno et al., 2002). The higher lipid content estimated in sediments of most of the stations resulted in higher LPD/CHO ratio and the improved nutritional quality of labile organic matter to support benthic fauna of the estuary. The LPD/CHO ratio ranged from 0.04 to 3.34 and displayed highly significant spatio-temporal variability ($p<0.01$) with a maximum value at S5 (POM09), which also indicates the freshness of sedimentary OM (Figure 4.5). These observed values of LPD/CHO ratio were comparable with the previous reports from CES (Joseph et al., 2008; Renjith et al., 2012). Furthermore, significantly lower ratios observed during the monsoon season ($p<0.01$), provided a clear evidence of lower productivity and higher allochthonous organic input associated with land runoff (Jacob et al., 2008;

2009). The contribution of labile organic matter to total organic matter was very high in most stations, arising from input of bulk quantities of sewage (Vasudevan, 2000; Balasubramanian et al., 2012).

PRT/CHO ratio has been used as an index to assess the origin of materials present and to differentiate between the fresh and aged organic matter in sediments (Danovaro et al., 1993; Cividanes et al., 2002). PRT/CHO ratio > 1 indicates recently deposited fresh organic matter, while PRT/CHO ratio <1 suggest the predominance of aged organic matter in sediments (Danovaro et al., 1993). Marked variation in PRT/CHO, from 0.08 (S2; MON09) to 2.34 (S4; POM09), was noticed in the study area (Figure 4.5). It has been well documented that proteins are more readily utilised by bacteria compared to carbohydrates (Williams and Carlucci, 1976; Newell and Field, 1983). The observed increased values of PRT/CHO ratios (Figure 4.5) suggested freshly deposited detritus in sediments (Danovaro, 1996), during the seasons PRM09 (S3), POM09 (S1, S3, S4 and S6) and MON12 (except S5 and S8). The present investigation revealed the dominance of carbohydrates and lower PRT/CHO ratio (<1) during MON09 and PRM10 (see Figure 4.5), pointing out a detrital heterotrophic environment (Danovaro, 1996).

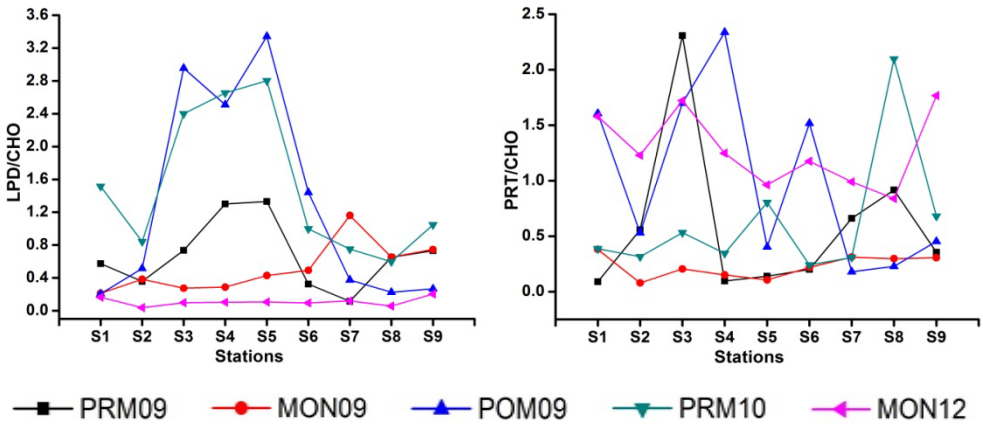

Figure 4.5 Variation of LPD/CHO and PRT/CHO ratios in sediments

4.3.4 Trophic status

Cochin estuarine system possesses a positive net ecosystem production (Qasim, 2003); but as the consequence of terrestrial organic matter input a seasonal shift in net pelagic production to heterotrophic conditions has been treated (Thottathil et al., 2008). Assessment of trophic state in aquatic environments is crucial in understanding food web linkages as well as biogeochemical characteristics (Smith, 2003). Similar to other ecosystems, estuaries have a biotic community that depends on carbon resource to fuel food webs and maintain the organisms that inhabit in them. General classification of trophic status of aquatic environments is as follows: oligotrophic (unproductive), mesotrophic (intermediate productivity) and eutrophic (highly productive). According to Dodds and Cole (2007), the nature of the trophic state can be influenced by light, external carbon source, nutrients, hydrology and food web structure. The indiscriminate and unscientific application of fertilisers, industrial input and domestic sewage had introduced bulk quantities of nutrients into the estuary which have affected the food web structure and alterations in trophic state. In the present investigation, evaluation of trophic state was carried out based on biopolymeric carbon (BPC) and the algal contribution to BPC as per the methods prescribed by Pusceddu et al (2011). The BPC was estimated as the sum of protein, carbohydrate and lipid carbon and has been reported as bioavailable fraction to the benthic consumers (Pusceddu et al., 2009).

In the present investigation, BPC exhibited marked variations, which might be accredited to the changes in organic matter deposition associated with the strong river discharge from upper reaches of the estuary. Algal contribution to BPC was also calculated as the percentage of chlorophyll-a to BPC concentrations, after converting chlorophyll-a concentrations into carbon

equivalents (chlorophyll carbon) using a mean value of 40 (Pusceddu et al., 2009). In general, the ratio of chlorophyll carbon to chlorophyll-a in sediments range from 10 to >100 (De Jonge, 1980) and using this mean value, comparison of the present data with literature information from other marine and coastal areas was possible (Pusceddu et al., 1999; 2009; 2011). Algal contribution to BPC ratio in the study area recorded maximum at S6 (41.73 %) and minimum at S9 (1.76 %) during PRM09 and MON12 respectively; revealed distinct spatio-temporal variability ($p<<0.01$) during the study period. BPC (mgC/g) showed maximum during MON12 (S8) and minimum during POM09 (S9) with highly significant spatial and seasonal variability ($P<<0.001$) (Table 4.2). The trophic classification (Pusceddu et al., 2011) based on BPC concentrations and the algal contribution to BPC was employed in the present study (eutrophic→ BPC>3 mgC/g, algal fraction <12 % of BPC), (mesotrophic→ BPC = 1-3 mgC/g, algal fraction = 12-25 % of BPC), (oligotrophic→ BPC <1 mgC/g, algal fraction >25 % of BPC). Based on these criteria, present investigation categorised the stations under eutrophic, mesotrophic and oligotrophic classes. PRM09 was characterised by eutrophic (S3 and S8), mesotrophic (S5) and oligotrophic states (S6). Meanwhile during MON09, stations revealed mesotrophic condition (S1 and S6), oligotrophic level (S2, S3, S4) and eutrophic state (S8). During POM09, S1 displayed eutrophic level, but S2, S3, S6 and S8 were categorised under mesotrophic classes. Eutrophic state was assigned for S1, S6 and S8 during PRM10, while S4 and S5 were ranked as mesotrophic. While during MON12, all stations except S9 displayed the eutrophic level (Martin et al., 2011).Seasonal and spatial variation was recorded for the benthic trophic state of the estuarine system.

Table 4.2 Algal contribution to BPC% (A) and BPC concentration-mgC/g (B) estimated in the sediments of the study area

Stations	PRM09		MON09		POM09		PRM10		MON12	
	A	B	A	B	A	B	A	B	A	B
S1	10.10	1.63	13.90	2.47	3.76	9.42	5.06	5.67	5.50	5.68
S2	13.61	0.64	27.75	0.84	16.44	1.15	13.41	3.87	3.62	5.55
S3	16.52	3.75	28.86	0.81	12.42	1.90	17.24	3.84	4.51	4.51
S4	8.60	1.56	24.95	0.60	14.62	4.62	17.73	2.70	2.76	4.34
S5	17.83	2.73	27.66	2.19	17.65	3.15	18.48	2.86	11.90	4.83
S6	41.73	1.04	16.90	2.14	16.48	2.90	11.47	3.77	6.82	4.85
S7	9.11	0.91	4.75	2.53	23.12	0.61	7.77	1.38	2.21	4.04
S8	7.45	5.17	9.27	7.09	24.75	2.06	2.49	10.84	5.52	11.35
S9	6.04	0.94	2.56	0.58	9.56	0.25	3.19	0.93	1.76	0.93

4.3.5 Elemental ratios

The total sulphur (TS) in study area varied from 0.06 % (S4; MON12) to 3.10 % (S4; PRM10) with significant spatial variation ($p<<0.01$; Figure 4.6). Origin and transformation of organic matter can be aseesed by the application of bulk parameters such as stoichiometric elemental ratios (Yamamuro, 2000). According to Raiswell et al (1987), qualitative evaluation of the redox status of the sedimentary environment can be achieved using total organic carbon to sulphur ratios. Under normal conditions, TOC/TS ratios > 5 has been categorised as oxic sediment with oxygenated bottom water, TOC/TS = 1.5-5 indicates sediments deposited under periodic anoxia and TOC/TS < 1.5 reflects anoxic sediment with anoxic water (Raiswell et al., 1987). TOC/TS ratio varied from 0.41 (S4; PRM10) to 22.39 (S4; MON12) provides a qualitative indication of the redox status of the environment of deposition, when TS concentrations are higher (Raiswell et al., 1987). The observed average TOC/TS values in the study region

can be included in the second category (periodic anoxia) (except at S3-PRM10 and S4, S8, S9-MON12, Figure 4.6), which implied the fact that the sediments undergo sulphate reduction below an oxygenated water column (Hedges and Keil, 1995; Niffy Benny, 2009; Renjith et al., 2012; Akhil et al., 2013).

Organic carbon to nitrogen ratios have been used as an effective tool to trace the organic matter sources based on the fact that marine and terrestrial derived organic matters have a TOC/TN ratio of 5-8 and >15 respectively (Meyers, 1997). Typically lower TOC/TN ratio (between 4 and 10) is assigned for bacteria and algae; but higher values >20 have been displayed by vascular land plants (Hedges et al., 1988; Hedges and Oades, 1997). During the present investigation, TOC/TN ratio recorded its higher values at S1 (35.11; MON12) and minimum was found at S7 (0.39; PRM10) and was comparable with other studies (Table 4.1). Intermediate values for TOC/TN ratios recorded in the present study (Figure 4.6), signalled a combined input of both autochthonous and terrestrial organic matter to the estuarine sediments (Verma and Subramanian, 2002; Muri et al., 2004; Gireeshkumar et al., 2012).

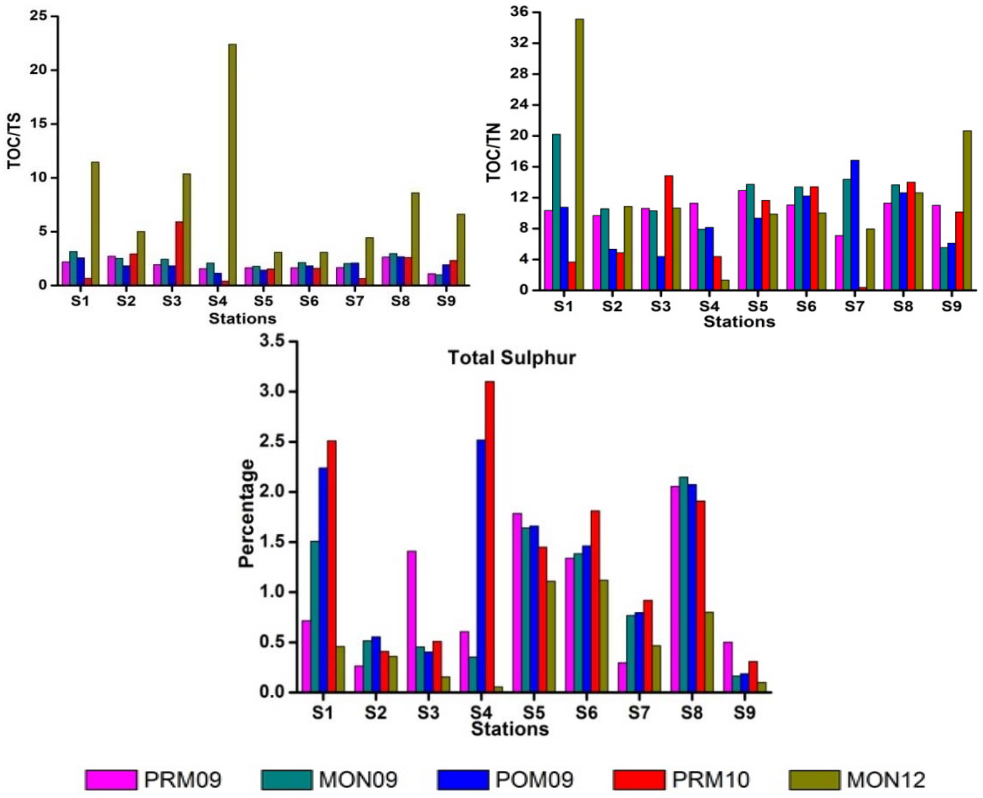

Figure 4.6 Variations of TOC/TS, TOC/TN and TS in surface sediment

4.3.6 Stable carbon isotope ratio

Organic matter of marine origin typically possess $\delta^{13}C$ values which for terrestrial C3 plants (-26‰ to -28‰) and phytoplankton (-19‰ to -22‰), has successfully been used to evaluate the sources of organic matter in estuarine sediments (Gearing et al., 1977; Meyers, 1997; Bianchi et al., 2002). The sediment $\delta^{13}C$ values determined in the present investigation ranged between -32.34±1.25 ‰ (S2; MON09) and -25.07±1.02 ‰ (S4; MON09). River dominated stations (S1, S8 and S9) exhibited more depleted $\delta^{13}C$ values, suggesting a major input of terrestrial higher plant debris to sedimentary organic matter. The stable carbon isotope ratios recorded in the present investigation was comparable with data reviewed from different estuaries in the world (Table 4.1).

Table 4.3 Correlation between sedimentary parameters in the study region[a] (n=45)

Parameters	Sand	Clay	Silt	TOC	TN	TS	PRT	CHO	LPD	chl-a	chl-b	chl-c	Phe	TL
Sand	1													
Clay	**-0.78**	1												
Silt	**-0.93**	**0.49**	1											
TOC	**-0.33**	**0.25**	**0.32**	1										
TN	-0.18	0.2	0.13	**0.41**	1									
TS	**-0.43**	**0.38**	**0.37**	**0.58**	**0.6**	1								
PRT	-0.07	0.03	0.08	**0.52**	**0.42**	**0.4**	1							
CHO	-0.19	*0.25*	0.12	**0.68**	**0.48**	**0.48**	**0.6**	1						
LPD	**-0.41**	**0.46**	**0.3**	**0.62**	**0.47**	**0.56**	**0.37**	**0.54**	1					
chl-a	**-0.41**	**0.39**	**0.34**	**0.41**	*0.27*	**0.54**	0.07	**0.35**	**0.44**	1				
chl- b	*-0.25*	*0.25*	0.2	**0.41**	0.16	**0.39**	0.04	*0.27*	**0.45**	**0.83**	1			
chl-c	-0.13	0.12	0.011	**0.38**	0.11	*0.27*	0.07	*0.24*	**0.43**	**0.66**	**0.95**	1		
Phe	**-0.4**	**0.38**	**0.33**	**0.34**	**0.25**	**0.48**	0.06	*0.3*	**0.37**	**0.94**	**0.72**	**0.52** 1		
TL	-0.05	-0.04	0.1	**0.5**	**0.42**	**0.35**	**0.61**	***0.52***	**0.49**	0.12	0.17	*0.23*	0.05	1

[a]*Correlation significant at 0.01 level are shown in bold and correlation significant at 0.05 level are shown in italic*

4.3.7 Pearson correlation

Grain size of sediment was found to be the main factor controlling the organic matter accumulation in the sediments of the study region. The study revealed a strong correlation of organic carbon with silt and clay (Table 4.3) which could be attributed to the relatively high adsorptive capacity of fine particles for organic matter (Cotano and Villate, 2006; Ramaswamy et al., 2008). Fine grained fractions of the sediment (silt and clay) exhibited highly significant positive correlation with various biochemical constituents, which reflected the influence of granulometry on their distribution. The strong relationships of TOC (with CHO, PRT, LPD, tannin and lignin, chlorophyll a,b, c and phaeophytin), TN (with LPD and CHO) and total sulphur (with LPD and CHO and other variables) pointed towards the adsorption and diagenetic process which control the distribution of the biochemical components in the estuarine sedimentary environment. In the present study, chlorophyll pigments exhibited highly significant positive correlation with fine grained sediments (silt+clay) (Table 4.3) implied the effect of grain size on the pigment distribution in sediments (Colijn and Dijkema, 1981; Moreno and Niell, 2004). Strong correlations between biochemical constituents and chloropigments in the sediments (Table 4.3) indicated a major contribution of phytobenthic populations and their associated detritus to the bulk organic matter (Fabiano and Danovaro, 1994; Danovaro et al., 2000). The interrelationship among the biochemical components with each other pointed towards a common origin and the similar behaviour in the estuarine environment.

4.3.8 Principle component analysis

In aquatic environments, biogeochemical processes are responsible for the variation in sedimentary variables and therefore statistical tools such as

principal component analysis (PCA) can be utilised to examine the controlling factors. Significant biogeochemical processes that can operate on the organic matter in sedimentary environment include diagenesis, bioturbation, allochthonous and autochthonous additions and sorption/desorption. As shown in Figure 4.7, PCA revealed three factors that accounted for 69 % of the total variance. The first factor consisting of TOC, LPD, chlorophyll-a and total sulphur describe the productive nature of the estuarine system and the preservation of lipid compounds in the periodically fluctuating anoxic environment. Strong positive loadings for clay, silt, TOC, TN, TS, PRT, CHO, LPD, tannin and lignin, Eh and pH observed in factor 2, explained 23% of total variance, inferred sorption of organic matter on fine grained sediments. The relation of sedimentary parameters to the grain size gives indication of the sorption/desorption processes. Significant positive loadings (factor 3) for redox indicators like total sulphur, organic carbon and total nitrogen revealed that the major process that can operate in the system can be attributed to diagenesis (Joseph et al., 2008).

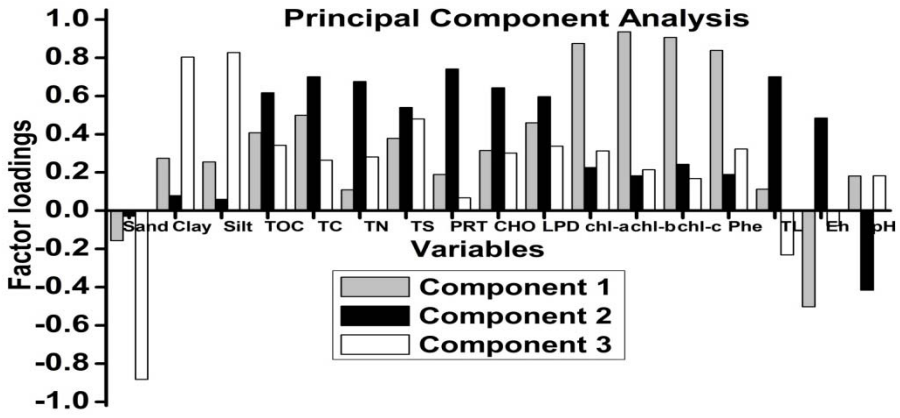

Figure 4.7 Principal component analysis representing loading pattern of different sedimentary parameters in the estuary

4.4 Conclusion

The higher lipid content and LPD/CHO ratio pointed towards the food quality that supports benthic fauna and accumulation of increased levels of lipid compounds in the sedimentary environment. Lower PRT/CHO ratio estimated in the sediments pointed towards a detrital heterotrophic environment and the addition of carbohydrates from terrigeneous input. The higher values of this ratio observed at various stations indicated freshly deposited OM in sediments. Biopolymeric carbon and the algal contribution to BPC provided significant information on the better understanding of the trophic status of the estuarine system.TOC/TS ratio inferred periodic anoxia and the estimated TOC/TN ratios implied the combined input of both terrestrial and autochthonous organic matter to sediments. TOC and TN concentrations strongly depend on the grain size of the sediments in the study region. Ratio of chlorophyll-a / (chlorophyll-a + phaeopigment) revealed rapid and recent deposition of phytoplankton detritus to sediments. The contribution of labile organic matter to total organic matter varied from 7.05 to 45.14 %. The predominance of carbohydrates over sedimentary protein indicates faster mineralisation of proteinaceous organic matter in sediments and the estuary behaves as a detrital trap for the accumulation of aged organic matter. The depleted $\delta^{13}C$ values in sediments indicated a combined input of autochthonous as well as terrestrial organic matter and this fact was confirmed by the higher concentration of tannin and lignin in sediments. The overall analysis revealed the fact that the combined input of organic matter from organic detritus generated by in situ primary production land runoff, industrial, agricultural and domestic sectors resulting in the accumulation of bulk quantities of organic matter in the estuarine sediments. Over all bulk parameters analysis pointed out the fact that molecular level indices have to be employed to achieve more specific information on origin and fate of organic matter in complex ecosystems like Cochin estuary.

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Chapter 5

SPATIO-TEMPORAL VARIABILITY OF FREE SUGAR DISTRIBUTION: IMPLICATIONS ON PRIMARY PRODUCTIVITY

Contents

5.1 Introduction

Free sugars are constituents of carbohydrate class of organic compounds occurring in water column as well as sedimentary phase, and represent an important fraction oforganic matter. These form a significant fraction of carbohydrates which are delivered to the estuaries via land run off (allochthonous) and in situ primary production (autochthonous). Monosaccharides serves as an important fraction of labile organic matter (Ittekkot and Arain, 1986) and their composition in sediments could reflect the biological origin of organic matter (Cowie and Hedges, 1984; Nierop et al., 2001; da Cunha et al., 2002).They may be originated directly via insitu production or through hydrolysis of structural and storage polysaccharides. In marine sediments, carbohydrates typically account for 3 to 10 % of TOC (Skoog and Benner, 1997; Bergamaschi et al., 1999; Burdige et al., 2000; Kerherve et al., 2002). According to Benner and Opsahl (2001) and Keil et al (1998)molecular composition and quantification of these groups of carbohydrates in estuarine sediments would provide insights into the biogeochemical processes operating in the sediments.

Eventhough free sugars constitute a significant fraction of total organic matter, no systematic study on the spatio-temporal distribution pattern has been reported in the sediments of Cochin estuary till now. Chlorophyll containing organisms are responsible for the photosynthetic production of organic matter and therefore, chlorophyll pigments in the water column can be used as a suitable indicator for the assessment of the productivity of estuarine systems and earlier reports of Moreno and Niell (2004) were available in this context. Therefore, in this chapter, an attempt was made to identify and quantify the free sugars to study their distribution and assess the implications on ecosystem productivity.

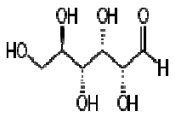
5.2 Results

Sediment samples were extracted with aqueous ethanol (70%, 2 h), centrifuged and deionised to remove cationic as well as anionic contaminants. The extract were dissolved in a known volume of distilled water, and analysed by HPLC method (detailed procedure for the extraction and analysis are furnished in Chapter 2).

HPLC technique revealed the presence of different types of free sugars in the sediment extracts of the study area. The chromatograms of the various selected samples are furnished in Appendix 2.1. A total of ten free sugars viz., ribose (Rib), xylose (Xyl), arabinose (Ara), fructose (Fru), mannose (Man), glucose (Glu), galactose (Gal), sucrose (Suc), maltose (Mal) and lactose (Lac) were estimated in the sediment extracts. These free sugars were categorised as aldohexoses (glucose, galactose, and mannose), aldopentoses (arabinose, xylose, and ribose), ketohexoses (fructose) and disaccharides (sucrose, maltose, lactose). The concentration and spatio-temporal variation of different classes of free sugars are illustrated below.

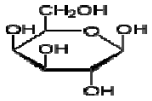
5.2.1 Aldohexoses

Glucose



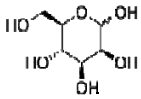
During the study period, glucose recorded its maximum concentration at station S5 (2.63±0.09 µg/g) in MON09. Meanwhile in both pre monsoons of the years as: PRM09 and PRM10, exhibited the highest concentration at stations S6 (1.29±0.02 µg/g) and S9 (0.07±0.45 µg/g) respectively. During both seasons, POM09 (1.86±0.06µg/g) and MON12 (0.77±0.03 µg/g), the highest concentration of glucose was recorded at the same station S4 (Table 5.1).

Galactose



Maximum concentration of galactose ($C_6H_{12}O_6$), was found at S4 (2.0±0.07 µg/g, PRM09) during the study period and also it was observed to be high during all seasons. Among the monsoon seasons, MON09 and MON12, galactose recorded the higher concentrations at S8 (1.33±0.08 µg/g) and S3 (0.43±0.01 µg/g) respectively. Furthermore, during POM09 (1.43±0.06 µg/g) and PRM10 (0.89±0.03 µg/g) the maximum content of galactose was also observed at station S4 (Table 5.1).

Mannose



In the sediments of Cochin estuary, maximum concentration for mannose was observed at S5 (10.92±0.15 µg/g, PRM10) during the study period (Table 5.1). Eventhough in the consecutive seasons, PRM09 and MON09, mannose was abundant at station S3 (0.52±0.02 µg/g) and S5 (8.57±0.13 µg/g) respectively. In POM09, it was higher at S6 (1.25±0.10 µg/g), while during MON12, highest content was recorded at S6 (0.13±0.01 µg/g).

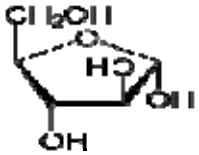
Table 5.1 Distribution of aldohexosesin surface sediments of CES

Free sugars (µg/g)	Stations	PRM09	MON09	POM09	PRM10	MON12
Glucose	S1	Nd	Nd	0.18±0.02	Nd	Nd
	S2	Nd	Nd	Nd	Nd	0.18±0.02
	S3	0.29±0.03	Nd	Nd	Nd	0.08±0.01
	S4	0.29±0.02	Nd	1.86±0.06	Nd	0.77±0.03
	S5	0.13±0.01	2.63±0.09	Nd	Nd	Nd
	S6	1.29±0.02	Nd	0.47±0.02	Nd	0.01±0.001
	S7	Nd	0.06±0.001	Nd	Nd	Nd
	S8	Nd	Nd	0.05±0.03	0.01±0.001	0.15±0.02
	S9	Nd	Nd	0.01±0.001	0.07±0.45	Nd
Galactose	S1	1.18±0.05	0.23±0.02	0.31±0.02	Nd	0.18±0.02
	S2	0.87±0.03	0.62±0.03	0.19±0.6	Nd	0.34±0.01
	S3	0.96±0.02	0.4±0.01	0.05±0.29	Nd	0.43±0.01
	S4	2.0±0.07	0.16±0.02	1.43±0.06	0.89±0.03	0.31±0.04
	S5	0.35±0.02	Nd	0.6±0.03	Nd	0.18±0.50
	S6	0.8±0.40	0.44±0.03	Nd	Nd	0.11±0.02
	S7	0.57±0.45	0.1±0.001	0.41±.04	0.39±0.05	0.12±0.03
	S8	1.6±0.05	1.33±0.08	Nd	0.39±0.04	0.23±0.02
	S9	0.15±0.01	0.1±0.01	0.07±0.25	0.1±0.001	0.08±0.002
Mannose	S1	Nd	0.01±0.001	0.24±0.02	Nd	0.02±0.001
	S2	Nd	0.45±0.04	0.22±0.01	0.3±0.02	Nd
	S3	0.52±0.02	0.01±0.001	Nd	0.69±0.04	0.02±0.001
	S4	Nd	0.04±0.007	Nd	Nd	0.04±0.02
	S5	Nd	8.57±0.13	0.13±0.02	10.92±0.15	0.03±0.01
	S6	Nd	Nd	1.25±0.10	Nd	0.13±0.01
	S7	0.22±0.03	Nd	Nd	0.47±0.03	Nd
	S8	Nd	0.47±0.02	0.4±0.40	0.1±0.001	Nd
	S9	0.01±0.001	0.01±0.001	Nd	0.09±0.01	0.03±0.01

Nd-not detected

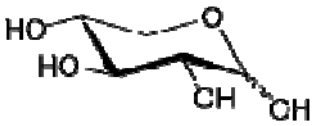
5.2.2 Aldopentoses

Arabinose



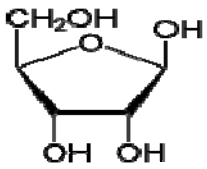
In the study period, arabinose exhibited its maximum abundance at S6 (6.88±0.34 µg/g) during PRM10 (Table 5.2) and exhibited lack of significant seasonal variation ($p>0.01$) as observed from ANOVA. Table 5.2, reveals both monsoon seasons (MON09 and MON12), highest abundance of arabinose was noticed at S5 (6.20±0.33 µg/g) and S8 (0.72±0.33 µg/g) respectively, while in PRM09 and POM09 seasons, the maximum content was recorded at S2 (3.75±0.29 µg/g) and S4 (4.92 ±0.35µg/g).

Xylose



The spatio-temporal variation of xylose is depicted in Table 5.2.Xylose, recorded its maximum concentration at S4 (4.75±0.23 µg/g; POM09), while in both monsoon seasons the maximum concentration of xylose was observed at S5 (3.74±0.33µg/g; MON09) and S2 (2.07±0.24 µg/g; MON12). It can be observed from Table 5.2, that during the pre monsoon seasons (PRM09 and PRM10), the maximum content was recorded at station S4 (2.15±0.24µg/g) and S2 (0.85±0.04µg/g) respectively.

Ribose



During the study period, maximum abundance of free sugar, ribose was found at S4 (19.84±0.35 µg/g, POM09). In both pre monsoon seasons, PRM09 and PRM10 highest concentration of ribose was recorded at stations S1 (4.22±0.32 µg/g) and S5 (16.04±0.45 µg/g) respectively. Further, among the

two monsoon seasons, MON09 and MON12, the higher abundance of ribose was observed at S5 (9.82±0.61 µg/g) and S2 (2.72±0.23 µg/g) respectively.

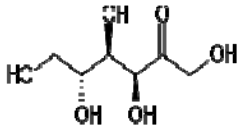
Table 5.2 Spatio-temporal variation of aldopentoses in surface sediments of CES

Free sugars (µg/g)	Stations	PRM09	MON09	POM09	PRM10	MON12
Arabinose	S1	Nd	0.6±0.02	Nd	3.23±0.13	0.46±0.08
	S2	3.75±0.29	1.24±0.22	0.51±0.11	Nd	Nd
	S3	1.21±0.12	1.23±0.16	Nd	1.19±0.22	0.45±0.09
	S4	Nd	0.4±0.04	4.92 ±0.35	Nd	0.44±0.05
	S5	0.72±0.02	6.20±0.33	0.54±0.01	Nd	Nd
	S6	2.54±0.13	Nd	0.81±0.12	6.88±0.34	Nd
	S7	1.53±0.14	Nd	Nd	0.88±0.14	0.46±0.08
	S8	1.44±0.23	2.02±0.22	Nd	1.08±0.08	0.72±0.33
	S9	Nd	0.28±0.32	Nd	0.21±0.08	Nd
Xylose	S1	1.58±0.12	0.21±0.24	Nd	Nd	0.2±0.01
	S2	Nd	0.36±0.04	Nd	0.85±0.04	2.07±0.24
	S3	0.98±0.01	Nd	0.12±0.001	0.49±0.17	1.45±0.08
	S4	2.15±0.24	0.14±0.19	4.75±0.23	Nd	1.23±0.12
	S5	0.64±0.01	3.74±0.33	Nd	Nd	0.23±0.23
	S6	Nd	Nd	0.66±0.12	Nd	0.11±0.12
	S7	0.69±0.04	0.1±0.001	Nd	Nd	0.21±0.09
	S8	0.4±0.02	Nd	0.3±0.001	0.35±0.07	Nd
	S9	0.17±0.03	0.14±0.01	0.18±0.12	Nd	Nd
Ribose	S1	4.22±0.32	Nd	Nd	Nd	0.38±0.03
	S2	Nd	0.74±0.017	1.1±0.13	Nd	2.72±0.23
	S3	0.87±0.04	Nd	0.26±0.11	Nd	1.67±0.14
	S4	Nd	0.28±0.16	19.84±0.35	Nd	1.34±0.12
	S5	0.75±0.05	9.82±0.61	0.73±0.04	16.04±0.45	0.74±0.13
	S6	Nd	Nd	1.54±0.03	Nd	Nd
	S7	2.9±0.06	Nd	8.49±0.12	Nd	0.43±0.22
	S8	1.02±0.13	Nd	Nd	0.73±0.08	0.44±0.21
	S9	0.37±0.12	0.19±0.11	Nd	0.16±0.02	0.5±0.26

Nd-not detected

5.2.3 Ketohexose

Fructose



The maximum concentration of fructose during the course of study was recorded at S4 (4.50±0.22 µg/g; POM09) (Table 5.3). During MON09, its presence was detected only at S8 (0.31±0.32 µg/g), while during MON12 maximum concentration of fructose was observed at S2 (1.01±0.23 µg/g). Comparing both the pre monsoon seasons (PRM09 and PRM10), its highest concentration was observed at stations S6 (0.48±0.21 µg/g) and S4 (0.49±0.34 µg/g) respectively.

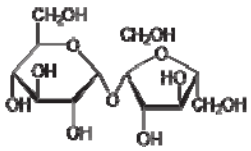
Table 5.3 Distribution of ketohexose in surface sediments of the study area

Free sugar (µg/g)	Stations	PRM09	MON09	POM09	PRM10	MON12
Fructose	S1	Nd	Nd	0.05±0.002	0.40±0.01	Nd
	S2	0.04±0.001	Nd	0.32±0.12	0.08±0.01	1.01±0.23
	S3	0.12±0.11	Nd	0.09±0.001	Nd	0.87±0.13
	S4	0.00	Nd	4.50±0.22	0.49±0.34	0.91±0.11
	S5	0.04±0.01	Nd	Nd	Nd	Nd
	S6	0.48±0.21	Nd	Nd	0.35±0.13	Nd
	S7	Nd	Nd	0.12±0.11	Nd	0.31±0.12
	S8	Nd	0.31±0.32	Nd	Nd	Nd
	S9	Nd	Nd	0.03±0.001	Nd	Nd

Nd-not detected

5.2.4 Disaccharides

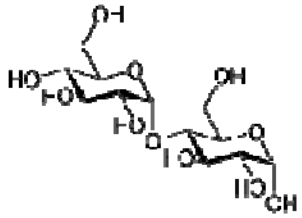
Sucrose



Sucrose, in surface sediments displayed its maximum abundance at S5 (24.81±0.45 µg/g, PRM10). Sediments at stations S1, S5, S6 and S9 recorded

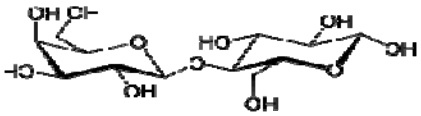
sucrose during all the seasons (Table 5.4). In both monsoon seasons (MON09 and MON12), the higher concentration of sucrose was observed at S5 (3.66±0.44 µg/g) and S4 (0.42±0.13 µg/g), while in PRM09 (1.34±0.23 µg/g) and POM09 (2.75±0.14µg/g) the peak concentration of sucrose was observed at S4 and S6 respectively (Table 5.4).

Maltose



Maximum concentration (Table 5.4) of maltose was recorded at S5 (3.34±0.34 µg/g; PRM10) during the study period. In PRM09 and POM09, highest content of this disaccharide was noticed at S6 (2.18±0.24 µg/g) and S7 (1.17±0.31 µg/g) respectively. Meanwhile, in MON09 and MON12, its highest concentration was observed at S5 (2.82±0.35 µg/g) and S8 (0.29±0.25 µg/g) respectively.

Lactose



During the study period, concentration maximum (1.47±0.13 µg/g, PRM10) of lactose was recorded at S6. In the case of monsoon seasons (MON09 and MON12), the enhanced contents of lactose was recorded at S6 and S3 respectively. While during PRM09 and POM09, the higher levels was recorded at S2 (1.3±0.22µg/g) and S7 (1.08±0.21µg/g) respectively (Table 5.4).

Table 5.4 Concentration of disaccharides in the surface sediments of CES

Carbohydrates (µg/g)	Stations	PRM09	MON09	POM09	PRM10	MON12
Sucrose	S1	1±0.02	0.19±0.01	0.55±0.01	0.29±0.04	0.11±0.001
	S2	0.6±0.04	0.31±0.02	0.15±0.03	0.27±0.05	Nd
	S3	0.4±0.01	0.31±0.019	0.04±0.009	0.52±0.06	Nd
	S4	1.34±0.23	Nd	0.33±0.01	2.25±0.02	0.42±0.13
	S5	0.4±0.01	3.66±0.44	0.64±0.04	24.81±0.45	0.11±0.001
	S6	0.9±0.03	0.41±0.03	2.75±0.14	4.49±0.05	0.1±0.002
	S7	Nd	0.09±0.03	0.27±0.07	0.14±0.09	0.32±0.004
	S8	0.2±0.01	0.48±0.04	Nd	Nd	0.19±0.008
	S9	0.1±0.001	0.07±0.001	0.04±0.001	0.06±0.001	0.05±0.001
Maltose	S1	1.3±0.03	0.24±0.01	0.17±0.01	0.56±0.01	0.21±0.003
	S2	0.9±0.01	0.47±0.02	0.16±0.001	0.32±0.02	Nd
	S3	0.5±0.02	0.87±0.03	0.06±0.003	1.53±0.08	0.13±0.002
	S4	1.8±0.01	0.16±0.001	0.82±0.01	0.33±0.19	0.22±0.001
	S5	0.4±0.009	2.82±0.35	0.66±0.02	3.34±0.34	0.23±0.004
	S6	2.18±0.24	0.79±0.02	0.73±0.03	2.18±0.06	0.15±0.001
	S7	0.7±0.01	0.13±0.05	1.17±0.31	0.12±0.001	0.22±0.002
	S8	0.4±0.02	1.04±0.07	0.2±0.004	0.61±0.01	0.29±0.25
	S9	0.2±0.01	0.12±0.01	0.04±0.003	0.1±0.001	0.1±0.001
Lactose	S1	1.1±0.01	0.12±0.04	0.15±0.001	0.23±0.002	Nd
	S2	1.3±0.22	0.23±0.001	0.08±0.001	Nd	Nd
	S3	Nd	0.21±0.01	0.05±0.004	0.38±0.004	0.33±0.12
	S4	0.8±0.01	0.07±0.001	0.49±0.2	0.44±0.001	0.21±0.001
	S5	0.2±0.001	Nd	0.82±0.07	1.23±0.02	0.11±0.008
	S6	1±0.01	0.29±0.22	0.35±0.03	1.47±0.13	0.06±0.007
	S7	0.7±0.01	0.06±0.001	1.08±0.21	0.09±0.001	Nd
	S8	0.2±0.001	0.25±0.01	0.13±0.01	0.22±0.005	0.13±0.005
	S9	Nd	0.05±	0.09±0.001	0.05±0.001	0.08±0.006

Nd-not detected

5.2.5 Chlorophyll pigments in water column

The spatio-temporal variation of chlorophyll pigments in the water samples collected from the various stations of the Cochin estuary is furnished in Table 5.5. Among the various seasons, during PRM09 chlorophyll-a (chl-a)

content ranged between 0.7±0.05 (S1) and 5.4±0.14 µg/l (S4). Meanwhile, in MON09 it ranged from 1.63±0.27 (S9) to 4.75±0.31 µg/l (S3). In POM09, the maximum content of chl-a, was observed at S7 (19.13±1.12µg/l) and minimum was recorded at S5 (3.11µg/l). However during PRM10, it fluctuated from 3.37±0.32 (S2) to 21.38±0.18 µg/l (S4). While during MON12, maximum concentration of chl-a was found at S7 (4.12±0.26µg/l) and minimum was observed at S1 (1.4±0.25µg/l). During the entire investigation period, chl-a exhibit highly significant spatio-temporal variability according to ANOVA.

Chlorophyll-b (chl-b) ranged from 0.72±0.15to 2.88±0.31 µg/l in PRM09 at S5 and S7 respectively, meanwhile during MON09 it varied from 0.79±0.11 (S1) to 16.23±1.24µg/l (S5). In the case of POM09, it ranged between 1.12±0.19(S8) and 4.78±0.21µg/l (S5), however, during PRM10, it displayed maximum at S4 (2.95µg/l) and minimum at S5 (0.81±0.19µg/l). During MON12, it varied from 0.69±0.15(S1) to 2.56±0.29µg/l (S7).

During the study period chlorophyll-c (chl-c) ranged from 1±0.18µg/l (S5) to 2.97±0.15 µg/l (S6) in PRM09, meanwhile during MON09 it ranged between 0.34±0.21 µg/l (S4) and 6.75±0.17µg/l (S2). However during POM09, it recorded minimum at S9 (0.24±0.15µg/l) and maximum at S4 (6.38±0.9µg/l), however, during PRM10, it displayed the maximum content at S4 (10.03±1.16µg/l) and minimum at S2 (0.56±0.09µg/l). During MON12, it varied from 0.68±0.03µg/l(S4) to 7.09±0.22µg/l (S2).

Phaeophytin (Phe) in the water samples, during PRM09 varied from 0.85±0.32 µg/l (S4) to 2.67±0.17 µg/l (S3). During MON09, it exhibited minimum value of 1.05±0.11 µg/l and a maximum of 7.66±0.27 µg/l at stations S2 and S9 respectively. Meanwhile, in POM09 it ranged between 0.86±0.37 µg/l (S3) and 3.2±0.11µg/l (S6) in the study area. However, during PRM10, it displayed minimum at station S1 (0.85±0.21 µg/l) and maximum at station S8 (11.29±1.92µg/l). While during MON12, minimum content of phaeophytin recorded as 0.94±0.32 µg/l (S4) and maximum as 3.98±0.31 µg/l (S1).

Table 5.5 The spatio-temporal distribution of chlorophyll pigments (μg/l) in water samples

Component	Stations	PRM09	MON09	POM09	PRM10	MON12
chl-a	S1	0.70±0.05	1.80±0.17	4.81±0.22	6.24±0.21	1.40±0.25
	S2	1.21±0.29	2.15±0.17	6.29±0.23	3.37±0.32	1.89±0.18
	S3	1.82±0.27	4.75±0.31	6.94±0.22	7.49±0.19	3.50±0.15
	S4	5.40±0.14	2.57±0.11	9.91±0.15	21.3±0.18	2.12±0.20
	S5	1.83±0.11	3.08±0.18	3.11±0.21	3.55±0.27	3.44±0.18
	S6	4.68±0.16	2.65±0.21	5.36±0.23	13.33±1.17	2.23±0.11
	S7	2.17±0.23	4.38±0.29	19.13±1.12	9.31±0.73	4.12±0.26
	S8	1.27±0.52	2.41±0. 16	5.24±0.43	4.75±0.14	2.60±0.15
	S9	1.81±0.22	1.63±0.27	4.36±0.24	4.12±0.18	1.45±0.17
chl-b	S1	1.27±0.22	0.79±0.11	3.64±0.16	1.48±0.32	0.69±0.15
	S2	0.93±0.18	1.03±0.41	2.35±0.21	1.55±0.33	0.98±0.20
	S3	1.47±0.43	1.00±0.21	3.86±0.49	1.41±0.22	1.20±0.72
	S4	2.12±0.19	2.50±0.26	2.40±0.17	2.95±0.61	2.00±0.33
	S5	0.72±0.15	16.23±1.24	4.78±0.21	0.81±0.19	0.71±0.11
	S6	1.18±0.21	2.32±0.14	3.09±0.29	1.21±0.27	1.30±0.22
	S7	2.88±0.31	2.36±0.29	1.40±0.18	1.56±0.38	2.56±0.29
	S8	1.21±0.65	1.49±0.34	1.12±0.19	1.53±0.22	1.18±0.38
	S9	2.00±0.43	1.83±0.28	1.74±0.71	0.96±0.27	1.89±0.22
chl-c	S1	1.13±0.18	1.28±0.12	5.56±0.27	2.49±0.44	1.62±0.32
	S2	1.06±0.31	6.75±0.17	3.51±0.15	0.56±0.09	7.09±0.22
	S3	1.91±0.34	0.44±0.07	5.23±0.18	3.69±0.26	0.78±0.18
	S4	1.61±0.22	0.34±0.21	6.38±0.9	10.03±1.16	0.68±0.03
	S5	1.00±0.18	0.45±0.28	5.66±0.15	1.31±0.26	0.79±0.22
	S6	2.97±0.15	4.25±0.27	0.32±0.11	4.89±0.27	4.59±0.73
	S7	2.21±0.17	0.88±0.11	3.36±0.43	3.13±0.21	1.22±0.20
	S8	1.56±0.29	0.57±0.19	0.49±0.11	1.74±0.28	0.91±0.25
	S9	2.34±0. 51	0.65±0.14	0.24±0.15	1.22±0.64	0.99±0.43
Phe	S1	1.30±0.35	4.03±0.42	2.88±0.27	0.85±0.21	3.98±0.31
	S2	2.22±0.19	1.05±0.11	1.81±0.15	2.67±0. 42	2.00±0.15
	S3	2.67±0.17	2.67±0.21	3.20±0.11	1.63±0.24	2.40±0.18
	S4	0.85±0.32	7.21±0.28	1.61±0.42	1.31±0.26	0.94±0.32
	S5	1.31±0.41	4.03±0.43	1.85±0.45	1.76±0.41	1.20±0.34
	S6	1.31±0.54	1.39±0.52	0.86±0.37	1.27±0.31	1.11±0.27
	S7	2.22±0.24	1.73±0.41	1.50±0.21	1.05±0.32	2.12±0.54
	S8	1.63±0.28	1.94±0.38	0.94±0.45	11.29±1.92	1.43±0.21
	S9	2.53±0.31	7.66±0.27	2.71±0.21	2.67±0.54	2.12±0.22

5.3 Discussion

5.3.1 Distribution of free sugars in sediments

In the present study, the seasonal distribution of the average free sugar content (Figure 5.1) in the sediments followed the trend:

PRM09: Arabinose > Ribose >Galactose> Maltose > Fructose > mannose > Xylose > Lactose > Sucrose > Glucose.

MON09: Arabinose > Ribose > mannose > Maltose > Sucrose > Xylose >Galactose> Glucose > Lactose > Fructose.

POM09: Ribose > Arabinose > Xylose > Fructose > Sucrose > Maltose > Lactose >Galactose> Glucose > mannose.

PRM10: Sucrose > Ribose > Arabinose > Mannose > Maltose > Lactose >Galactose> Xylose > Fructose > Glucose.

MON12: Ribose > Xylose > Fructose > Arabinose >Galactose> Maltose > Sucrose > Glucose > Lactose > Mannose.

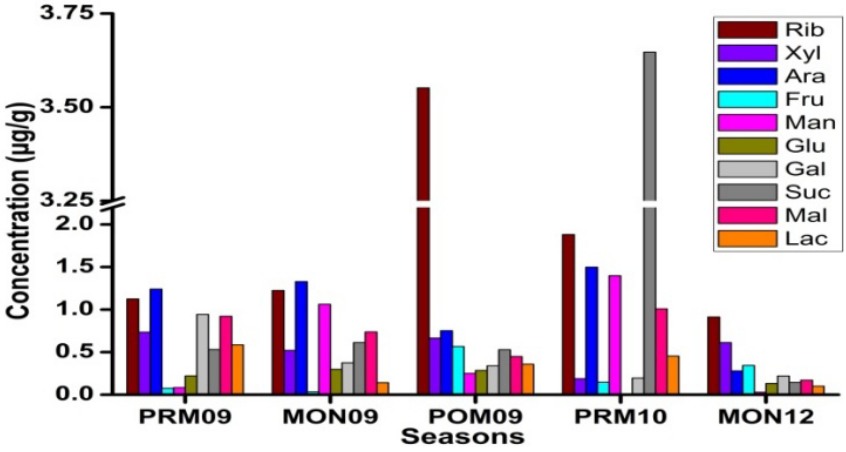


Figure 5.1 Average concentrations of free sugars occuring in the surface sediments of CES

Aldohexose: During the study period, abundance of glucose was observed at station S5 during MON09 (Figure 5.2). It has been established that the occurrence of glucose, the most important carbohydrate, has been recorded in both terrestrial and marine derived organic matter (Cowie and Hedges, 1984). The observations recorded in the present study implies both autochthonous as well as allochthonous sources contribute to the estuarine sedimentary organic matter wich correlates well with the above statement. Generally, the yield of carbohydrate from terrestrial plant tissue is higher than that of aquatic organisms (Cowie and Hedges,1984; Kogel-Knabner, 2002). The high productivity enables estuaries to act as a major source of these organic compounds to sediments. Neutral aldohexoses such as glucose are typical exudates of recent photosynthesis (Biersmith and Benner, 1998; Kirchman et al., 2001). Aquatic organisms and microbes contain significantly higher levels of these monosaccharides than terrestrial plants (Ogier et al., 2001). Most of the stations recorded depleted levels of glucose during all seasons on account of bacterial respiration process.In the course of sedimentogenesis, the reserve carbohydrate of marine organisms are more degradable compared to the structural carbohydrates (Kodina and Galimov, 1984), but they may be partially preserved in the sediments under the protection of a mineral matrix. The carbohydrates of the bottom sediments interact with each other and with the products of the destruction of other biopolymers to form geopolymers under natural conditions (Lazareva and Romankevich, 2012). Moreover the decrease in concentration of glucose was associated with increase in higher concentration of arabinose, xylose, mannose and galactose. These are combined with structural hetero-polysaccharides of microorganisms, including diatoms (Hecky et al., 1973; Haug and Myklestad, 1976), and terrestrial plants (Opsahl and Benner, 1999). Meanwhile, at S6 (PRM09), S5 (MON09) and S4

(POM09), a significantly higher concentration of glucose was observed (Figure 5.2). This might have been derived from the free sugars of higher plant tissues or else from the breakdown of polysaccharides of either algae or higher plants (Vallentyne and Bidwell, 1956); while not found any spatio-temporal variation ($p>0.05$). Glucose displayed a significant positive correlation with silt ($r=0.37$) and chlorophyll ($r=0.34$) indicating a close relationship of their formation by primary productivity as well as adsorption on fine grained sediments. Whereas highly significant positive correlation with other free sugars occurring in the sediment except sucrose, lactose and galactose indicates a common origin, behavioural resemblances and common pattern of distribution (Table 5.6).

The free sugar mannose exhibited higher abundance at S5 during both seasons of PRM10 (10.92±0.15 µg/g) and MON09 (8.57±0.13 µg/g), (Figure 5.2) and varied among the sampling sites ($p<0.01$). Angiosperm-derived hemicellulose is characterised by high concentration of xylose units, although this macromolecule derived from gymnosperm is relatively rich in mannose (Cowie and Hedges, 1984; Hedges, 1990). The mannose is a structural component of hetero polysaccharides in microorganisms, including diatoms (Hecky et al., 1973; Haug and Myklestad, 1976), and terrestrial plants (Opsahl and Benner, 1999). Mannose displayed significant positive correlation (Table 5.6) with ribose ($r=0.60$), glucose($r=0.40$), sucrose($r=0.83$), maltose($r=0.70$) and chl-a ($r=0.29$), indicating involvement of pigments in the enhancement of the productivity and subsequent formation of carbohydrates (Devassy and Goes, 1989).

During PRM09 galactose exhibited maximum concentration at station S4 (2.0±0.07 µg/g) and higher content were obtained at stations S1 and S8. Similar trend was also observed during MON09 (S8) and POM09 (S4); while

sediments collected from some stations, galactose was not detected (Figure 5.2). The galactose recorded highly significant ($p<<0.01$) seasonal and spatial variations. Pectin is found in non-woody plant materials (Sjostrom, 1981; Kogel-Knabner, 2002), which is a source of galactose; contributedto the increased content in the sediments. Another important source ofgalactose is the structural hetero polysaccharides of microorganisms, including diatoms (Hecky et al., 1973; Haug and Myklestad, 1976), and terrestrial plants (Opsahl and Benner, 1999; Stibal et al., 2010). In the present study galactose displayed significant positive correlation (Table 5.6) with silt ($r=0.32$), xylose ($r=0.35$), fructose ($r=0.31$) and lactose ($r=0.32$); describes the adsorption on fine grained sediments as well as similar behavioral resemblance with these sugars.

Among the aldohexoses(glucose, galactose and mannose), galactosefound to exhibit higher content in most of the stations. While in MON09, majority of stations recorded lower levels of aldohexoses, except S5 (where mannose and glucose was relatively higher). In the case of POM09, glucose (S4), galactose (S1, S3, S7 and S9) and mannose (S2, S6 and S8) were enriched at respective stations. During PRM10, most of the stations exhibited lower levels of aldohexoses, while at S5, the concentration of mannose was projected. From the Figure 5.2, it is clear that during MON12, among the aldohexoses, galactose was high in all station except S4, where glucose was the most abundant free sugar.

Table 5.6 Correlation matrix of free sugars with various sedimentary parameters in surface sediment of CES (n=45)

	Sand	Silt	Clay	TOC	PP	CHO	Rib	Xyl	Arb	Fru	Man	Glu	Suc	Malt	Lac	Gal	Chl-a	Chl-b	Chl-c	Phe	TS	TN	Salinity	TP
Sand	1																							
Silt	-0.96 (**)	1																						
Clay	-0.90 (**)	0.76 (**)	1																					
TOC	-0.43 (**)	0.38 (**)	0.44 (**)	1																				
PP	-0.02	0.01	0.05	0.33 (*)	1																			
CHO	-0.33 (*)	0.24	0.43 (**)	0.82 (**)	0.41 (**)	1																		
Rib	-0.22	0.34 (*)	-0.01	-0.01	-0.13	-0.15	1																	
Xyl	-0.30 (*)	0.42 (**)	0.04	-0.03	-0.23	-0.03	0.62 (**)	1																
Arb	-0.22	0.24	0.16	0.09	-0.07	0.02	0.32 (*)	0.40 (**)	1															
Fru	-0.18	0.31 (*)	-0.05	-0.01	-0.13	-0.06	0.62 (**)	0.67 (**)	0.37 (*)	1														
Man	-0.18	0.18	0.14	0.03	-0.143	-0.05	0.60 (**)	0.23	0.22	-0.08	1													
Glu	-0.28	0.37 (*)	0.07	0.05	-0.13	0.01	0.55 (**)	0.75 (**)	0.58 (**)	0.50 (**)	0.40 (**)	1												
Suc	-0.16	0.13	0.18	-0.01	-0.16	-0.13	0.55 (**)	-0.02	0.06	-0.05	0.83 (**)	0.05	1											
Malt	-0.36 (*)	0.37 (*)	0.30 (*)	0.04	-0.08	-0.10	0.50 (**)	0.25	0.51 (**)	0	0.70 (**)	0.43 (**)	0.67 (**)	1										
Lac	-0.20	0.19	0.19	-0.11	-0.07	-0.24	0.31 (*)	-0.01	0.36 (*)	0.06	0.18	0.01	0.43 (**)	0.67 (**)	1									
Gal	-0.29	0.32 (*)	0.19	0.10	-0.04	-0.01	0.14	0.35 (*)	0.11	0.31 (*)	-0.2	0.13	-0.12	0.16	0.32 (*)									
Chl-a	-0.58 (**)	0.57 (**)	0.51 (**)	0.62 (**)	0.02	0.37 (*)	0.28	0.23	0.32 (*)	0.22	0.29 (*)	0.34 (*)	0.23	0.37 (*)	0.06	0.11	1							
Chl-b	-0.62 (**)	0.62 (**)	0.53 (**)	0.59 (**)	-0.02	0.30 (*)	0.26	0.24	0.37 (*)	0.21	0.25	0.39 (**)	0.18	0.40 (**)	0.13	0.14	0.97 (**)	1						
Chl-c	-0.63 (**)	0.62 (**)	0.55 (**)	0.61 (**)	0.01	0.33 (*)	0.21	0.24	0.36 (*)	0.17	0.21	0.36 (*)	0.15	0.41 (**)	0.16	0.16	0.94 (**)	0.98 (**)	1					
Phe	-0.50 (**)	0.49 (**)	0.44 (**)	0.55 (**)	-0.01	0.29 (*)	0.25	0.21	0.28	0.20	0.27	0.33 (*)	0.21	0.33 (*)	0.03	0.05	0.94 (**)	0.93 (**)	0.90 (**)	1				
TS	-0.46 (**)	0.44 (**)	0.41 (**)	0.65 (**)	0.04	0.39 (**)	0.20	0.07	0.32 (*)	0.22	0.12	0.21	0.15	0.20	0.10	0.22	0.68 (**)	0.68 (**)	0.67 (**)	0.61 (**)				
TN	-0.13	0.08	0.20	0.36 (*)	0.14	0.32 (*)	-0.03	-0.11	0.14	0.05	-0.01	-0.01	-0.02	-0.08	-0.12	0.04	0.30 (*)	0.28	0.30 (*)	0.26	0.59 (**)	1		
Salinity	0.43 (**)	0.45 (**)	0.33 (*)	-0.06	-0.24	-0.33 (*)	0.32 (*)	0.16	0.24	0.07	0.39 (**)	0.30 (*)	0.45 (**)	0.57 (**)	0.41 (**)	0.11	0.47 (**)	0.55 (**)	0.55 (**)	0.48 (**)	0.24	-0.10	1	
TP	-0.33 (*)	0.30 (*)	0.34 (*)	0.48 (**)	0.42 (**)	0.35 (*)	-0.01	-0.03	0.12	-0.08	0.05	0.09	0.01	0.09	-0.01	0.145	0.31 (*)	0.33 (*)	0.32 (*)	0.24	0.39 (**)	0.26	0.07	1

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

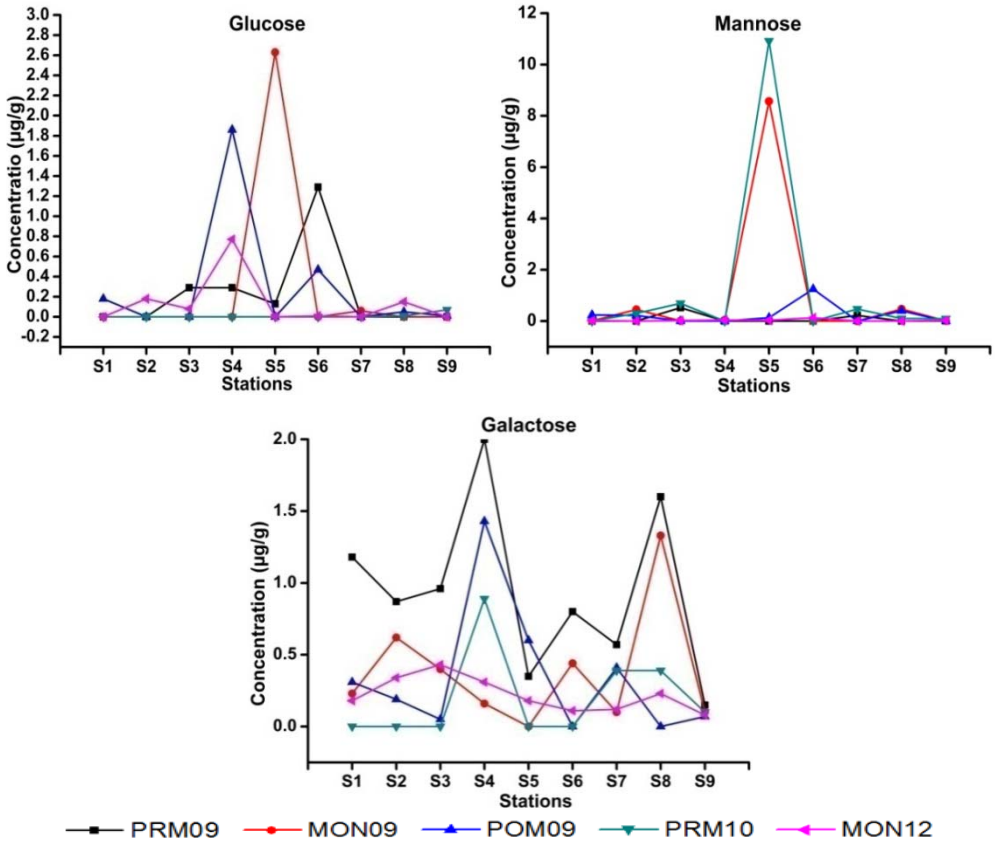


Figure 5.2 Variation of aldohexose (glucose, mannose and galactose) in the sediments

Aldopentoses: The observed free sugars such as arabinose exhibited its maximum concentration during PRM10 at S6 (6.88±0.34 µg/g), while a remarkable concentration was also observed during POM09 (S4; 4.92 ±0.35 µg/g) and MON09 (S5; 6.20±0.33 µg/g) (Figure 5.3). These stations were noted for domestic waste disposals and sewage outfall. These waste materials contain non-woody plant materials which is typically rich in pectin compared to woody plant materials. Pectinconstitute amajor portion ofarabinose composition (Sjostrom, 1981; Kogel-Knabner, 2002). No significant spatial or seasonal variation was observed during the investigation. Arabinose displayed strong positive correlation with ribose (r=0.32), fructose (r=0.37), lactose

(r=0.36), chl-a (r=0.32), chl-b (r=0.37), chl-c (r=0.36), xylose(r=0.40), glucose (r=0.58) and maltose (r=0.51) which implied the potential of photosynthetic activity (Table 5.6).

Xylose recorded its maximum value at S4 during POM09 (4.75±0.23 µg/g) and its spatio-temporal variation was insignificant ($p>0.05$). Enhanced level of xylose was observed during MON09 at station S5 and this indicated the presence of terrestrial organic matter derived from angiosperm plants (Guggenberger et al., 1994b; Biersmith and Benner, 1998; Khodse andBhosle, 2012). Terrestrial materials enriched with carbohydrates can be derived from angiosperm and gymnosperm plants. Xylose is more enriched in angiosperm tissues as compared to gymnosperm tissues (Cowie and Hedges, 1984). It is well known that in continental aquatic ecosystems, pentose is mainly derived from vascular plants (da Cunha et al., 2002). Sugars such as rhamnose, fucose, arabinose, xylose, mannose and galactose are associated with structural hetero polysaccharides of microorganisms, including diatoms (Hecky et al., 1973; Haug and Myklestad, 1976), and terrestrial plants (Opsahl and Benner, 1999). This site (S4) was found to be severely deteriorated with sewage out fall. A number of fish peeling and processing units are situated on the banks of the Cochin estuary delivering organic rich wastematerialsin to the water body (Vasudevan, 2000; Balasubramanian et al., 2012). Xylose exhibited significant negative correlation with sand, while displayed highly significant positive correlation with silt (r=0.42) and ribose (r=0.62), reflecting the influence of granulometry.

Higher concentration of ribose was observed at S4 (19.84±0.35µg/g) and S5 (16.04±0.45 µg/g) during POM09 and PRM10 respectively (Figure

5.3). These are abundant constituents of storage and structural polysaccharides in bacteria, fungi and diatoms (Percival, 1970; Cowie and Hedges, 1984; Moers et al., 1989). Bacteria, fungi and phytoplankton are enriched with rhamnose, fucose and ribose (Cowie and Hedges, 1984; Moers et al., 1989; Hicks et al., 1994; D'Souza, 2004). Aldose sugars such as arabinose, fucose, galactose, glucose, mannose, rhamnose, ribose and xylose are commonly found in dissolved and particulate organic matter and in terrestrial and marine organisms (Cowie and Hedges, 1984; Mopper et al., 1995; Borch and Kirchman, 1997; D' Souza and Bhosle, 2001), which results in the elevated levels. Ribose displayed significant positive correlation with silt and all other free sugars except lactose (Table 5.6).

Among the three aldopentoses, arabinose (S2, S3 and S8) and ribose (S1, S5, S7 and S9) were enriched at respective stations in PRM09, while at S4, xylose was enriched (Figure 5.3). Meanwhile, in MON09 and POM09, a noticeable concentration of three aldopentoses was found only at stations S5 and S4 respectively (Figure 5.3), among three aldopentoses, ribose was relatively enriched in both stations. During PRM10, ribose and arabinose was more abundant at respective stations S5 and S6. Ribose content was dominant at S2 to S5 during MON12, while arabinose was enriched at S1, S7 and S8.

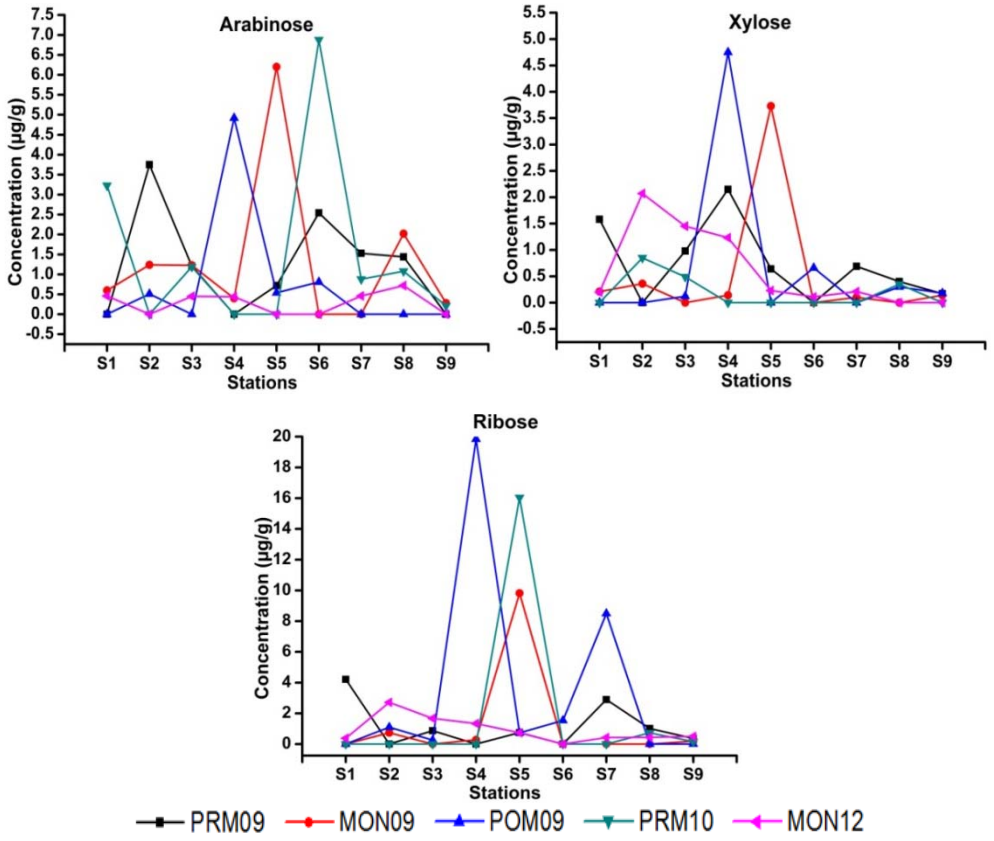


Figure 5.3 Concentration of arabinose, xylose and ribose in surface sediment of CES

Ketohexose: ANOVA revealed that the free sugar, fructose possess no spatial or seasonal variation and the maximum concentration was observed at station S4 during POM09. Figure 5.4, revealed that fructose exhibit non-detectable levels in many of the stations. On the basis of a sestonic origin, sedimentary sugars like glucose and fructose could originate directly from the free sugars of living plants or polysaccharide breakdown (Vallentyne and Bidwell, 1956). Fructose exhibited strong positive correlation with silt (r=0.31), arabinose (r=0.37), galactose (r =0.31), ribose (r=0.62), xylose (r=0.67) and glucose (r=0.50) implied its dependence on grain size of sediments and common behaviour similar to other carbohydrates.

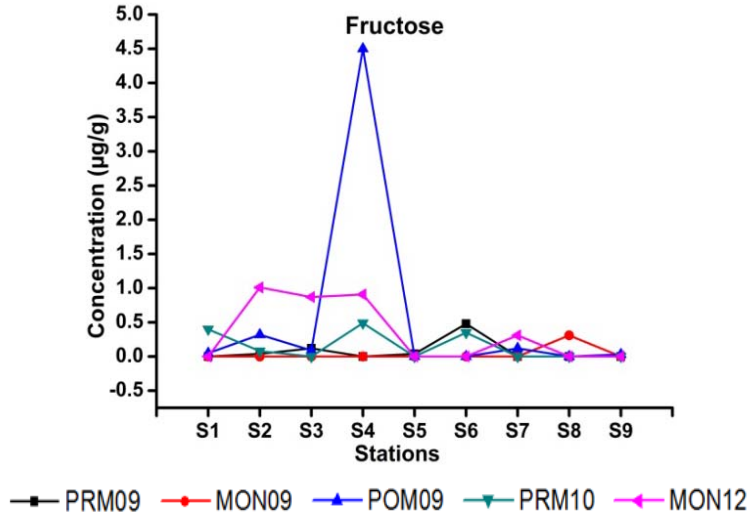


Figure 5.4 Distribution of ketohexose (fructose) in surface sediment of CES

Disaccharides: The highest concentration of maltose was observed at station S5 (3.34±0.34 µg/g) in PRM10. The Figure 5.5, suggested that maltose display a remarkable concentration at all stations compared to other disaccharides. Minor concentrations of maltose has been reported in *Haematococcus and Spirogyra*, and larger content in *Pontidis*(Norris et al., 1955). Granulometric dependence on the distribution and similar behaviour was detected by correlation; significant negative correlation with sand (r=-0.36), while positive correlation with silt (r=0.37), clay (r=0.30), ribose (r=0.50) and arabinose (r=0.51).

Among the free sugars, sucrose displayed its highest abundance at S5 (24.81±0.45µg/g) during PRM10. The peculiarity of this free sugar was that during all the seasons, majority of stations recorded surplus levels (Figure 5.5). Origin of sucrose in sediments is attributed mainly to the living plants (Oades, 1984; Guggenberger et al.,1994a), recorded strong positive correlation with ribose (r=0.55), mannose (r=0.83), maltose (r=0.67) and lactose (r=0.43), indicating the similarity in origin and behavior.

The concentration of lactose in the study period, was maximum (1.47±0.13µg/g) at S6 during PRM10 (Figure 5.5). From the Figure 5.5, it is clear that abundance of lactose was observed at the estuarine region (S3 to S7). The concentration of lactose displayed seasonal variations ($p<0.01$) but did not exhibit any variation among the sampling sites. Lactose, a hetero-disaccharide of glucose and galactose observed in sediment samples (Cowie and Hedges, 1984; Hamilton and Hedges, 1988) implies inputs from both microorganisms such as diatoms and terrestrial plants (Hecky et al., 1973; Haug and Myklestad, 1976; Opsahl and Benner, 1999). Lactose was found to exhibit positive relationships with ribose ($r=0.31$), arabinose ($r=0.36$), galactose ($r=0.32$), sucrose ($r=0.43$) and maltose ($r=0.67$), indicate behavior resemblances.

Among the three disaccharides, maltose was enriched in most of the stations during POM09, except stations S2 and S7. While during MON09, maltose was enriched in most of the stations (except S5). In POM09, high concentrations of sucrose (S1 and S6), maltose (S2, S3, S4, S7 and S8) and lactose (S5 and S9) were observed among the three disaccharides in the respective stations of the study area (Figure 5.5). Meanwhile, during PRM10, a remarkable concentration of these three disaccharides were observed only at S5 and S6, since sucrose was enriched in the sediments of those stations compared to other disaccharides. Among three disaccharides, maltose was more concentrated in majority of stations during MON12; while sucrose was enriched at S4 (Figure 5.5).

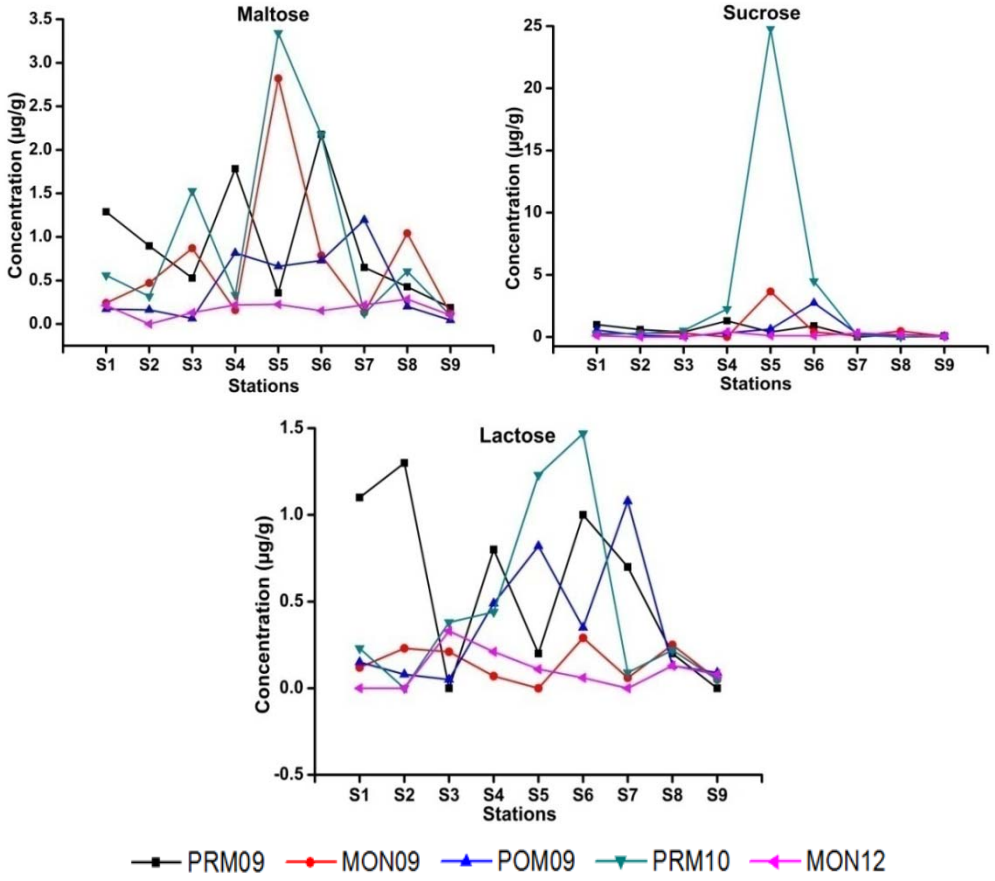


Figure 5.5 Concentration and variation of disaccharides in the sediments of CES

5.3.2 Free sugars in sediments and the primary productivity

Photosynthetic fixation of inorganic carbon and nutrients into plant biomass is the primary source of organic matter within estuaries. Carbohydrates in aquatic system (Kirchman et al., 2001) are associated with recent production by photosynthesis or chemosynthesis (Jansen et al., 1982; Biersmith and Benner, 1998).These class of organic compounds serve as an important energy source for heterotrophic organisms in the water column and sediments (Decho, 1990). Primary productivity is an important factor for controlling the distribution of organic molecules in sediments. Carbohydrates

synthesised by primary producers are classified as storage and structural polymers. Storage carbohydrates are labile and gets rapidly utilisedinsitu by heterotrophic organisms. The rate of photosynthetic activity depends on distribution of phytoplanktons which represent an important source of sedimentary organic matter. The dominant classes of phytoplankton in Cochin estuary includes: *Bacillariophyceae, Dinophyceae, Chlorophyceae, Cyanophyceae, Chrysophyceae, Dictyophyceae and Zygnematophycea*(Radhika, 2013; Dayala et al., 2014). Benthic macroalgae and microphytobenthos are also another important sources of primary production in estuaries (Bianchi, 1988; Pinckney and Zingmark, 1993; de Jonge and Colijn, 1994).

During the study period, gross primary productivity of the estuary found to vary from 0.02 to 0.63 $gC/m^3/day$, with significant seasonal and spatial variations ($p<<0.01$) (Figure 5.6). The comparison of the data on productivity recorded only slight deviations from previous studies (0.24-3.0 $gC/m^3/day$- Meera and Bijoy, 2010; 0.753 $gC/m^3/day$-Selvaraj et al., 2003). In general, monsoon recorded lower productivity due to decreased light penetration and greater turbidity in water column generated by land runoff. Enhanced turbidity in shallow regions from resuspension events can limit light penetration; thus, the most effective time for primary production occurs during daytime exposure periods (Guarini et al., 2000; 2002). Sudden changes in the hydrographic features and the mixing process caused by strong wave action during premonsoon, results in declining the primary productivity (Selvaraj et al., 2003). Seasonal changes in phytoplankton abundance and composition in estuaries are governed by changes in riverine inputs, nutrients, tidal variability, algal respiration, light availability, horizontal exchanges, and consumption by grazers (O'Donohue and Dennison, 1997; Thompson, 1998; Lucas and Cloern, 2002).Eventhough, surplus amount of nutrients is available via anthropogenic

input; the growth of phytoplanktons might have been declined due to the reduced light availability as a result of dredging (Cole and Cloern, 1987). The nutrient distribution in the sedimentary phase revealed sufficient levels of bioavailable forms to sustain an optimum rate of primary production in the estuary.

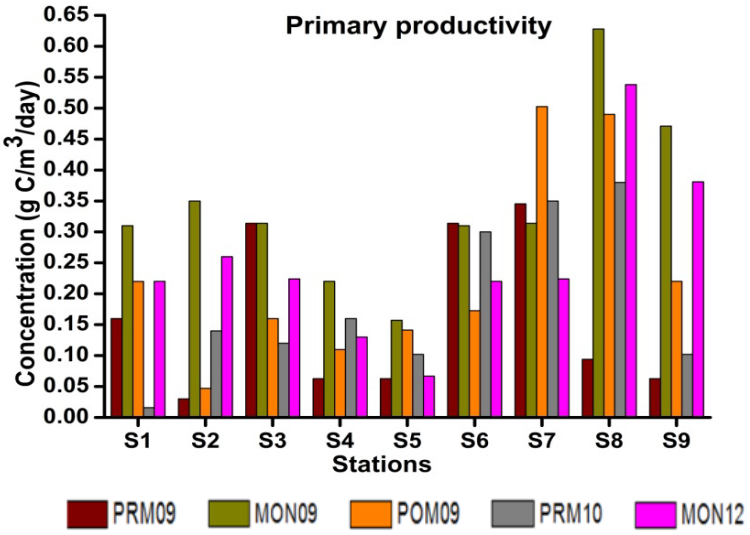


Figure 5.6 Spatio-temporal variation of gross primary productivity in CES

The increased total carbohydrate content and their strong correlation (r=0.41) with primary productivity (PP) is a direct indication of the enhanced productivity of this ecosystem. Strong positive correlation of PP with chlorophyll pigments (Table 5.6) also inferred the increased rate of photosynthesis taking place in the estuarine system. The unusual hike in the chlorophyll-a values observed during May-June, which was proportionately reflected in the primary productivity values. Photosynthesis by primary producers have generated carbohydrates consisting of monosaccharides/ structural polysaccharides. Each of the free sugars estimated in the sediments of the study area might be generated via autochthonous or allochthonous pathway. Allochthonous input of organic matter is evident from the characteristic TOC/TN ratios, and depleted $\delta^{13}C$ values

(described in chapter 4). It is well accepted that river derived nutrients are important in controlling phytoplankton abundance and composition;enhanced phytoplankton productivity arisedue to an import of coastal phytoplankton associated with high salinity (Hickey and Banas, 2003). Significant correlation of nutrients [TP with PP (r=0.42) and TOC (r=0.33)] indicated the involvement of nutrients in the formation of organic matter. The increase in anthropogenic loading of nutrients results in uncontrolled proliferation of primary producers as per previous observations (Rosenberg and Ramus, 1984; Duarte, 1995; Kamer et al., 2001).

5.3.3 Chlorophyll pigments as indicator of primary productivity

The lower values of primary productivity observed during MON12 at most of the stations (Figure 5.6) was due to freshwater discharges from the rivers, land runoff, leading to turbidity and less availability of light(Kawabata et al., 1993; Godhataraman, 2002; ThillaiRajasegar et al., 2005). Moreover, the wash out of phytoplankton by the monsoonal floods followed by reduction in salinity influence the phytoplankton population and lowers the PP during the monsoon season (MON12), similar phenomena was observed in previous investigations (Rajasegar et al., 2000, Gowda et al., 2001; ThillaiRajasekar et al., 2005). ANOVA revealed significant spatial and seasonal variation ($p<0.01$) in surface waters. The higher primary productivity at S7, S8 and S9 during the study period (Figure 5.6) could be attributed to high light intensity, clarity of water column and nutrient availability as reported in the previous investigations (Gopinathan et al., 1994; ThillaiRajasegar et al., 2005).

Chlorophyll pigments have been recognised as a marker of organic carbon (Bianchi et al., 1995) and could be used as a measure of changes in their biomass. Phytopigments such as chl-a, chl-b, chl-c and phaeophytin are useful indicators of different processes taking place in the water column (Welschmeyer and Lorenzen, 1985). Phytoplankton biomass contains chl-a,

chl-b, chl-c and its degradation products, phaeophytin. The depleted levels of pigments observed during monsoon seasons (MON09 and MON12, Figure 5.7) at most of the stations might also be due to high flushing which results in the faster removal of phytoplankton to the coastal regions (Jyothibabu et al., 2006). Enriched levels of nutrients in water columnfavoursthe growth of large phytoplankton, while the production of small phytoplankton is mainly controlled by grazing of microzooplankton (Jyothibabu et al., 2006).

Chlorophyll-a is a ubiquitous pigment and can be used as a global algal biomass indicator. It occurs in all groups of photosynthetic organisms except some bacteria (Moss, 1968). Content of chlorophyll-awas maximum during PRM10 at S4, which is a thickly populated semi urban area influenced by sewage disposal and noted lowest value at S1 (riverine area) during PRM09 (Figure 5.7). The variation in concentration of this pigment in the present study was generally associated with environmental factors, especially inflow of freshwater during rainy season, light and nutrient availability. Earlier studies revealed that mean chl-a concentration in the estuarine and coastal waters vary with respect to other aspects (Selveraj et al., 2003; Renjith, 2006). Chlorophyll-a content recorded in the Cochin estuary include: 2 to 21µg/l (Nair et al., 1975), 4.93 to 8.93 µg/l (Selveraj et al., 2003) and 1 to 34.61 µg/l (Renjith, 2006), 2.75 to 17.97 µg/l (Aneeshkumar, 2009). The estimated chl-a value recorded in the present study revealed comparatively lower values with the earlier reports (Figure 5.7). The higher chlorophyll content during POM09 and PRM10 seasons (Figure 5.7) was comparable with earlier observations recorded in Cochin estuary (Gopinathan, 1972; Devessy and Bhattathiri,1974; Martin et al., 2012). In addition to these reports Nair et al (1975) have estimated an overall range of 1.5-18 mg/m^3 for chl-a. Maximum content for chl-a in pelagic flora occurs during monsoon (Joseph and Pillai,1975); whereas for benthic microflora the maximum were reported during

premonsoon and monsoon (Sivadasan and Joseph, 1995). The higher chl-a in the study area (S3 to S7; Figure 5.7) can be attributed to the enrichment of nutrients contributed by industrial and domestic activities (Jyothibabu et al., 2006; Madhu et al., 2007). Moreover, the higher organic productions are not transferred to the higher trophic level due to the lack of effective grazers, which leads to settling of the excess chlorophyll to the sediments (Jyothibabu et al., 2006).

Chlorophyll-b, also exhibited comparatively similar pattern of distribution, in the entire sampling sites with slight variations. Moderately higher concentration was observed at all stations during all seasons under investigation. The environmental factors that strongly influence pigment composition of phytoplankton are irradiance, spectral distribution of light, day length, diurnal cycle, nutrient status (Partensky et al., 1993; Schluter et al., 2000; Henriksen et al, 2002; Tukaj et al., 2003), Fe content (Van Leeuwe et al., 1998) and growth phase (Schluter et al., 2000). The concentration of pigments and nutrients in the water column are the key factors for determining the biological productivity and potential resources. The transparency and the nutrient level indicated the fertility of the water body for the enhancement of primary productivity and the availability of the photosynthetic pigments and phytoplankton.Correlation between nutrients and primary production was insignificant suggesting that instantaneous concentration of nutrients has lesser influence (Varshneyet al., 1982). Insignificant correlation between nitrogen and primary production pointed out the fact that in spite of the higher concentration of available nutrients in water column; the productivity was declined by factors like transparency and other meteorological factors.

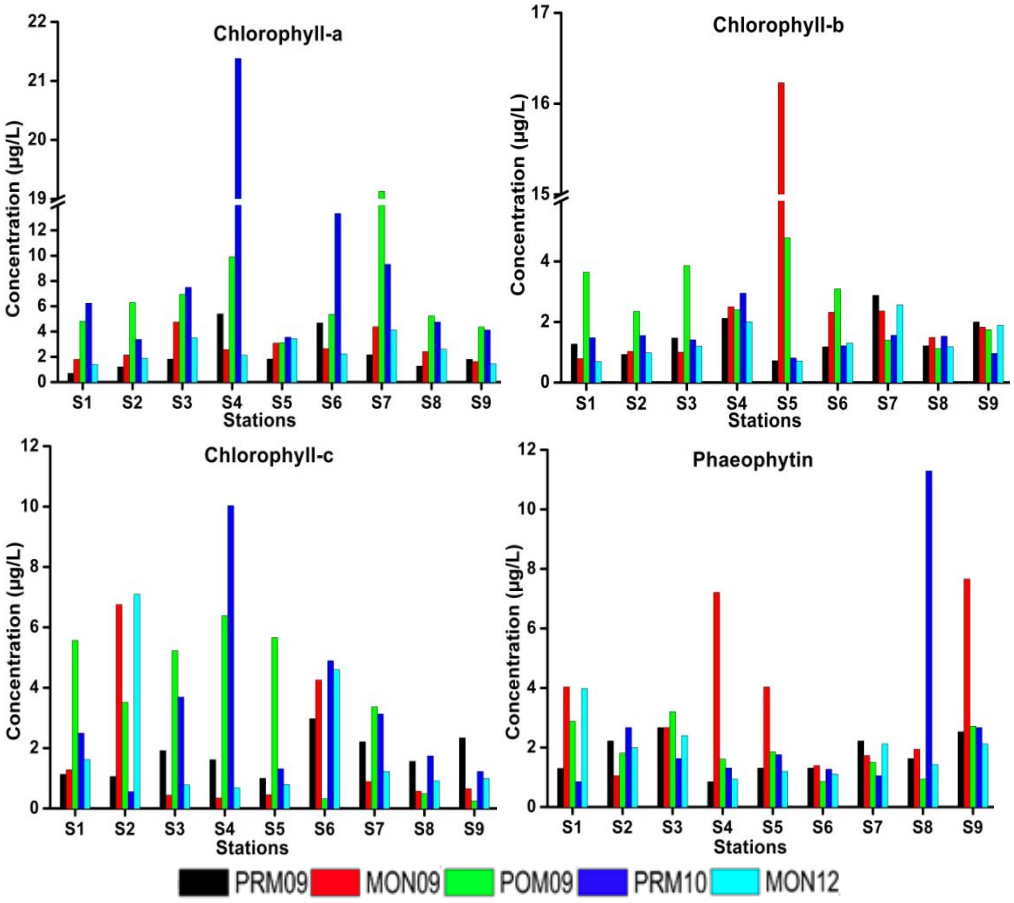


Figure 5.7 Distribution of chlorophyll-a,b,c and phaeophytin in water samples of the study area

Principal Component Analysis: PCA of the estimated variables (Table 5.7) established four components explaining 75.17% of total variance. First component account for 42.48% of total variance and exhibited positive loadings on TOC, chlorophyll pigments, TS and TN. It denoted autochthonous/allochthonous addition of photosynthetic pigments to the sedimentary phase. Since the factor contains TOC, TS and TN, the major process that can operate in the system is the diagenesis (Joseph et al., 2008). Variables included in component 2 exhibited strong positive loading on silt, clay, salinity, chlorophyll-a, b, c and accounted for 15.92% of total variance. It

also indicated adsorption of chlorophyll pigments on fine grained sediments which was assisted by the periodically varying salinity gradient. Component 3 explained 9.1% of total variance and consisted of PP, CHO, TS, TN and TP suggested enhanced primary production occurring in the estuary promoted by the surplus nutrients input through the river run-off. Meanwhile, component 4 explained 7.67% of total variance and consisted of Eh, pH, salinity and total free sugars. The concentration and spatial-temporal distribution of free sugars in the study area are mainly controlled by granulometry of sediments and salinity of the water column.

Table 5.7 Results of principal component analysis

Parameters	Components			
	1	2	3	4
pH	0.047	0.009	0.055	0.743
Eh	0.017	-0.593	0.155	-0.476
Sand	-0.361	-0.885	-0.165	-0.022
Silt	0.355	0.871	0.092	0.051
Clay	0.317	0.778	0.265	-0.030
Salinity	0.348	0.484	-0.456	0.529
TOC	0.600	0.174	0.643	-0.138
PP	-0.134	-0.015	0.776	0.152
CHO	0.324	0.131	0.753	-0.321
Chl-a	0.911	0.310	0.069	0.053
Chl-b	0.889	0.384	0.019	0.141
Chl-c	0.870	0.395	0.048	0.149
Phe	0.898	0.257	-0.021	0.046
TS	0.764	0.131	0.306	0.104
TN	0.490	-0.211	0.405	-0.011
TP	0.173	0.284	0.627	0.162
Sum of free sugars	0.099	0.049	0.056	0.709
% of variance	42.48	15.92	9.10	7.67

5.4 Conclusion

The seasonal average concentration of free sugars in the sediments was as follows: arabinose (PRM09 and MON09), ribose (POM09 and MON12) and sucrose (PRM10). During the study period, most of the stations recorded depleted levels of glucose during all seasons, while at S5 maximum content was observed during MON09. In the case of mannose, its higher abundance was observed at S5 (both PRM10 and MON09). Among three aldohexoses (glucose, galactose and mannose), galactose was found to be higher in most of the stations. Among the three aldopentoses (ribose, xylose and arabinose), arabinose (S2, S3 and S8) and ribose (S1, S5, S7 and S9) were enriched at respective stations in PRM09, while at station S4, xylose was enriched. It is clear that fructose was almost absent in many of the stations. Among the three disaccharides-sucrose, maltose and lactose, maltose was enriched in most of the stations during POM09. The significant positive correlation of glucose with silt and chlorophyll pigments, suggests a strong relation to their formation by primary productivity as well as adsorption on fine grained sediments. Meanwhile, a highly significant positive correlation with other free sugars present in the sediment except sucrose, lactose and galactose which reveals a common origin and similarity in behaviour and distribution pattern. The general positive relationship between phosphate and chlorophyll pigments confirmed the fact that nutrient availability of the water column governs the instantaneous rates of chlorophyll and carbon production. The surplus levels of chlorophyll pigments in water column have imparted high rate of productivity of the estuarine system. The estimated contents of free sugar in sediments of the study area has been attributed to both autochthonous as well as allochthonous input as evident from stable carbon isotope ratio and TOC/TN ratio.The overall examination implied that the biogeochemistry of free sugars

and the productivity of Cochin estuary were influenced by the interactions between nutrient, chlorophyll, TOC and other physicochemical variables. The concentration and spatio-temporal distribution of free sugars in the study area are mainly controlled by granulometry of sediments, salinity of the water column, in situ production as well as terrestrial allochthonous inputs.

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Chapter 6

DISTRIBUTION AND DEGRADATION STATUS OF AMINO ACIDS IN ESTUARINE SEDIMENTS

Contents

6.1 Introduction

Amino acids (AAs) are the structural components of proteins which have been recognised as the largest reservoir of organic nitrogen in marine organisms (Kaiser and Benner, 2008). This particular class of organic compounds are important constituents of living and dead organic matter (Cowie and Hedges, 1992), and represent a major fraction of bioactive OM preserved in the marine sediments (Keil et al., 2000; Vandewiele et al., 2009). Moreover amino acids play an important role as an intermediate in the marine nitrogen cycle (Capone et al., 2008) and act as one of the most labile fraction of OM in marine sediments (Ittekkot and Zhang, 1989; Spitzy and Ittekkot, 1991; Duan and Bianchi, 2007).

A better understanding ofabundance of amino acids and their composition will provide an insight into the sources and biogeochemical cycling of organic matter (Ittekkot and Arian, 1986; Hedges et al., 1994; Aufdenkampeet al., 2001; Jianfang Chen et al. 2004). The degradation state of the estuarine sedimentary environment can be evaluated using the contribution of amino acid carbon to total organic carbon (THAA-C%) and amino acid

nitrogen to total nitrogen (THAA-N%) (Cowieand Hedges, 1994; Dauwe et al., 1999; Davis et al., 2009). The content and compositions of total hydrolysable amino acids (THAA) have been utilised as diageneticindicators on account of their selective decomposition or preferential preservation during diagenesis (Keil et al., 2000; Amon et al., 2001; Vandewiele et al., 2009). Amino acid-based degradation index (DI) has been successfully employed to characterise the diagenetic status of OM in estuarine sediments (Dauwe and Middelburg, 1998; Lomstein et al., 2006).

A mixture of nitrogen compounds like proteins and amino acid degradation products occur in the estuarine sediments (Nunn and Keil, 2004).Autochthonous as well as allochthonous processes can contribute nitrogen in the form of amino acids, and hence sediments can act as a large reservoir of amino acids.Previous studies in Cochin estuary focussed mainly on the distribution and source characterisation of organic matter (Balachandran et al., 2005; Joseph et al., 2008; Martin et al., 2010; Gireeshkumaret al., 2012; Renjith et al., 2012; Akhilet al., 2013); but evaluation of the degradation state of organic matter using amino acids is not attempted yet. Therefore, the present study investigates the spatio-temporal distribution pattern and degradation status of amino acids to evaluate the quality of organic matter and diagenetic process in sediments sampled from Cochin estuarine system.

6.2 Results

Total hydrolysable amino acid (THAA) were extracted by adding HCl (6M) to freeze dried homogenised sediment in pre-cleaned and muffled glass vials, and purging the headspace with N_2. The individual amino acids were quantified according by HPLC method (detailed procedure for the extraction and analysis are furnished in Chapter 2).

A total of seventeen amino acids were identified from the surface sediment extracts of the study area. The chromatograms of the various selected samples in each season are presented in Appendix 3.1. The detected AAs included aspartic

acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine(Val), methionine (Met), cysteine (Cys), isoleucine (Ile), leucine (Leu), phenylalanine (Phen) and lysine (Lys). The sum of the concentrations of all the identified amino acids expressed as total hydrolysable amino acid (THAA), and the relative abundance of individual amino acids is expressed as mole% of THAA [percentage of (concentration of individual amino acid/ sum of total amino acids in sediment extract)] is presented in Table 6.1.

Table 6.1 Distribution of THAA, THAA-N and THAC-C in surface sediments of CES

Parameter	Stations	PRM09	MON09	POM09	PRM10	MON12
THAA-N%	S1	43.71	47.21	10.58	15.36	39.08
	S2	50.12	74.43	8.69	42.93	73.21
	S3	28.69	86.72	34.74	38.78	26.07
	S4	64.57	87.71	20.67	25.39	27.81
	S5	8.40	39.87	30.62	70.45	24.39
	S6	72.22	75.72	25.91	30.66	15.91
	S7	34.52	18.95	42.49	6.03	5.09
	S8	62.81	30.06	22.53	16.23	16.76
	S9	62.54	41.49	15.50	14.43	16.24
THAA-C%	S1	16.23	7.25	5.05	19.99	4.38
	S2	20.60	23.61	9.99	33.52	24.48
	S3	7.42	24.33	37.51	7.74	7.62
	S4	12.31	37.00	9.08	29.24	18.19
	S5	2.75	10.38	12.24	23.59	10.59
	S6	19.35	20.73	6.66	7.96	5.55
	S7	18.14	4.73	6.55	33.89	2.99
	S8	14.47	7.44	5.68	3.69	5.52
	S9	27.02	29.94	6.82	8.63	4.44
THAA (µmol/g)	S1	36.90	54.79	40.78	57.65	33.06
	S2	22.55	51.64	14.08	69.36	69.10
	S3	32.18	38.87	41.50	39.42	18.21
	S4	17.88	41.32	38.56	62.20	32.90
	S5	11.77	45.99	41.50	92.20	57.70
	S6	66.41	87.32	26.74	43.30	32.24
	S7	12.03	10.04	16.80	32.45	9.56
	S8	124.54	65.02	48.70	29.90	58.70
	S9	18.27	10.00	3.72	8.84	4.14

The maximum concentration of THAA was recorded at S8 during PRM09 (124.54 µmol/g) while the minimum content was observed at S9 during

POM09 (3.72µmol/g). THAA concentration exhibited highly significant spatial ($p<0.01$) differences with lack of seasonal variation. THAA-C% and THAA-N% (Table 6.1) varied from 2.75 % (S5; PRM09) to 37.51% (S3; POM09) and 5.09% (S7; MON12) to 87.71% (S4; MON09) respectively. Meanwhile, THAA-N% recorded a significant seasonal ($p<0.01$) variation.

The relative abundance of various individual amino acids in the sediment extracts of the study area are summarised here.

6.2.1 Aliphatic (glycine, alanine, proline, valine, isoleucine, leucine) and aromatic (tyrosine, phenylalanine) neutral amino acids

Distribution of various aliphatic and aromatic neutral amino acids in surface sediments of Cochin estuary is depicted in Figure 6.1

Glycine: Maximum relative abundance of glycine was noted at S8 (19.10±0.07 mole%; PRM09). During PRM10, higher abundance was recorded at station S1 (13.94±0.52 mole%). In MON09 and MON12, higher abundance of glycine was observed at stations S2 (14.05±0.32 mole%) and S6 (16.47±0.33 mole%); while during POM09, relative abundance of glycine was observed at station S3 (3.13±0.24 mole%).

Alanine: It recorded maximum relative abundance at station S8 (9.36±0.47 mole%) during PRM10. During MON09 and MON12,the higher abundance of alanine was recorded at stations S5 (4.13±0.09 mole%) and S7 (7.96±0.54 mole%) respectively. Meanwhile during PRM09 and POM09, station S4 recorded its higher abundance.

Proline: Maximum relative abundance of proline was observed at station S3 (24.10±0.49 mole%) during POM09. Station S2 exhibited maximum concentration of proline (23.07±0.47 mole%) during PRM10; while, during PRM09, MON09 and MON12 relatively high abundance of proline was recorded at stations S9 (5.70±0.49 mole%), S2 (7.86±0.26 mole%) and S8 (12.44±0.31 mole%) respectively.

Valine: Maximum mole% of valine was noted at station S6 (46.19±0.35 mole%) during PRM10. Higher abundance of valine was recorded at station S6 (22.59±0.42 mole%) and S3 (9.88±0.62 mole%) during PRM09 and POM09 respectively. Meanwhile during MON09 and MON12, stations S5 (0.83±0.23 mole%) and S2 (36.18 ±0.55 mole%) recorded its higher abundance.

Isoleucine: Maximum relative abundance of isoleucine was recorded at station S4 during MON09 with a strong spatial variation ($p<0.01$). At stations S5 (3.32±0.22 mole%) and S4 (4.50±0.30 mole%),the maximum relative abundance was recorded during PRM09 and PRM10. Meanwhile, in the case of POM09 and MON12, stations S6 (1.05±0.17mole%) and S4 (3.71±0.50 mole%) recorded higher relative abundance of isoleucine respectively.

Leucine: In sediments collected fromstations S1 and S9, leucine was identified in all seasons and maximum abundance was found at station S3 (63.43±0.31mole%: PRM09). In MON09 and MON12, leucine recorded high relative abundance at station S5 (13.23±0.16 mole%) and S2 (7.96±0.54 mole%) respectively. Meanwhile, comparing POM09 and PRM10, the highest abundance of leucine was recorded as 27.88±0.62 mole% (S2) and 16.10±0.64 mole% (S3) respectively.

Tyrosine: During the present investigation, stations S5, S7 and S9 recorded tyrosine at all seasons. Maximum abundance of tyrosine was observed at station S4 (MON09). In PRM09 (6.41±0.29 mole%) and PRM10 (10.70±0.50 mole%), highest abundance was observed at stations S9 and S8 respectively. Meanwhile, maximum abundance of tyrosine was observed at station S7 during two seasons viz.,POM09 (12.50±0.57mole%)and MON12 (25.13±0.45 mole%).

Phenylalanine: The maximum abundance of phenylalanine was observed at station S1 (51.44±0.31 mole%) during MON12 and exhibited seasonal variations ($p<0.01$). In the case of PRM09 and PRM10, the higher abundance of phenylalanine was recorded at stations S7 and S9 respectively. During MON09 and POM09, highest abundance of phenylalanine was recorded at stations S7 (35.89±0.63 mole%) and S5 (34.22±0.51 mole%) respectively.

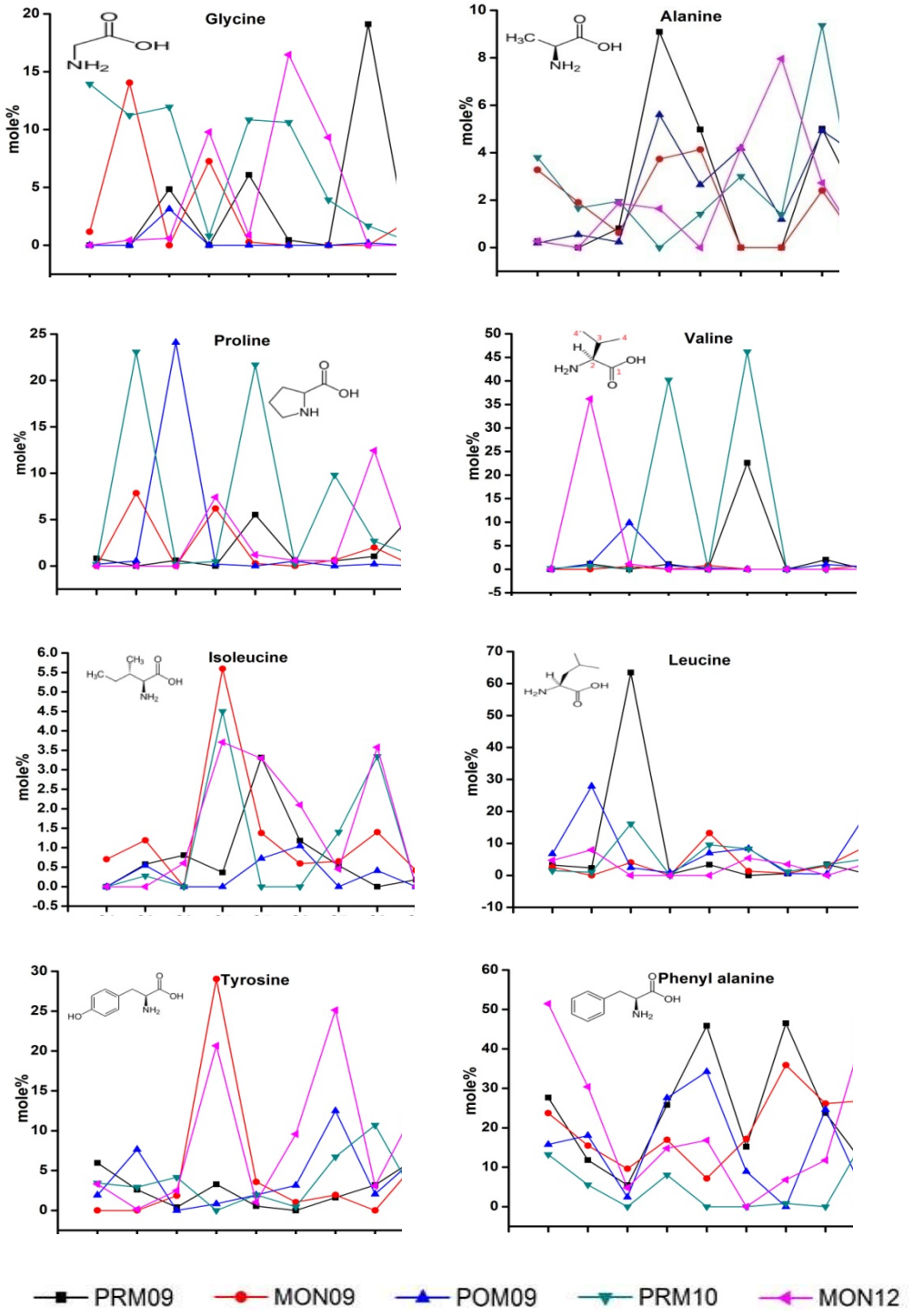

Figure 6.1 Spatial and seasonal variations of aliphatic and aromatic neutral amino acids in the sediments of CES

6.2.2 Acidic amino acids (aspartic acid and glutamic acid)

Aspartic acid: The spatio-temporal variation of aspartic acid is depicted in Figure 6.2, and the maximum concentration was noticed at station S5 (47.49±0.34 mole%) during MON12. The seasons PRM09 and PRM10 recorded higher abundance of aspartic acid at stations S6 (21.85±0.60 mole%) and S9 (44.20±0.50 mole%) respectively. MeanwhileduringMON09 and MON12,the higher abundance of this amino acid was observed at stations S6 (38.94±0.67 mole%) and S5 (47.49±0.34 mole%) respectively. Moreover, comparing the abundance during the entire period, an increased level of aspartic acid was observed during monsoon seasons. Figure 6.2, revealed that during POM09 higher abundance was observed at station S5 (29.16±0.46 mole%).

Glutamic acid: Figure 6.2 illustrates the variations of glutamic acid at different seasons. Higher relative abundance of glutamic acid was noticed at stations S8 (4.80±0.28 mole%) and S2 (3.47±0.33 mole%) during MON09 and MON12 respectively. During PRM09, maximum abundance was noticed at station S5 and exhibited significant seasonal variation ($p<0.01$). Besides, the seasons POM09 (7.14±0.24 mole%) and PRM10 (16.25±0.18 mole%), exhibited remarkably higher abundance of glutamic acid at station S7.

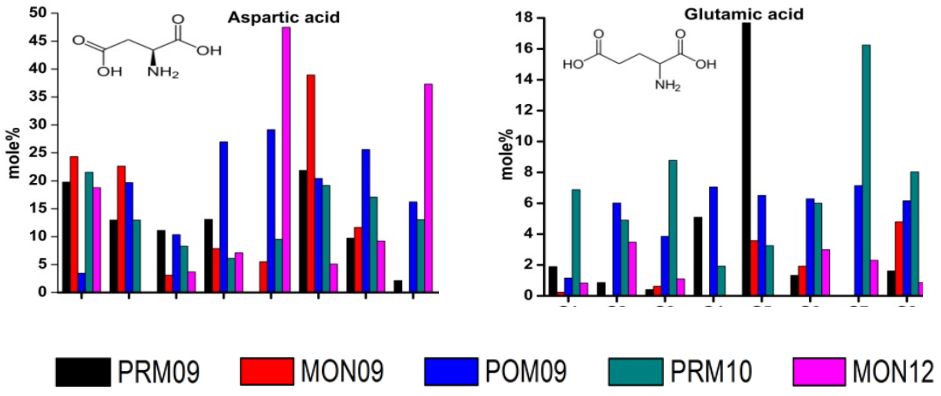

Figure 6.2 Distribution of aspartic acid and glutamic acid in surface sediments of Cochin estuary

6.2.3 Basic amino acids (histidine, lysine and arginine)

Histidine: The spatio-temporal variation ofhistidine during the study period is depicted in Figure 6.3. The maximum abundance was recorded at station S7 during POM09 (48.21±14.65 mole%). MON09 and MON12 reveled higher abundance of histidine at stations S9 (32.41±0.29 mole%) and S6 (43.73± 0.51 mole %) respectively. While in PRM09 and PRM10 higher mole% abundance was recorded at stations S8 (7.93 mole%) and S3 (36.34±0.81 mole%) respectively.

Lysine: Lysine recorded its maximum abundance at station S1 (64.62±0.44 mole%) during POM09. Both MON09 and MON12 recordedhigher abundance of lysine at stations S5 (41.32±0.23 mole%) and S3 (54.91±0.65 mole%) respectively. Meanwhile in both pre monsoon seasons (PRM09 and PRM10), it recorded higher abundance (Figure 6.3) at stations S9 (33.46±0.33 mole%) and S2 (17.30±0.57 mole%) respectively.

Arginine: The spatio-temporal variationsof arginine is depicted in Figure 6.3. During both monsoon seasons, highest abundance of arginine was observed at stations S8 (24.79±0.56 mole%; MON09) and S7 (4.50±0.36 mole%; MON12) while, recorded its maximum abundance at station S9 (41.65±0.46 mole%) during PRM09 (Figure 6.3). However POM09 (8.01±0.29 mole%) and PRM10 (14.38±0.48 mole%), recorded its higher abundance at same station S8.

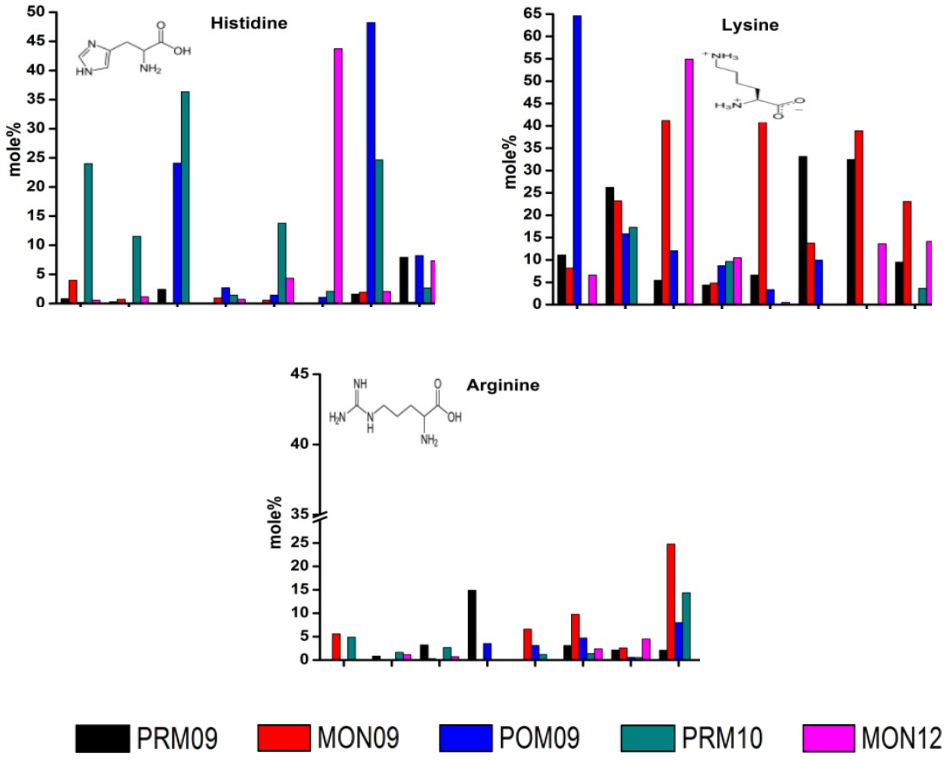

Figure 6.3 Spatial and seasonal variations of histidine, lysine and arginine in the sediments of the study area

6.2.4 Hydroxy amino acids (serine and threonine)

Serine: The maximum abundance of serine during the study period was observed at station S8 (27.09±0.70 mole%; PRM10). It was observed that during consecutive seasons PRM09 and MON09, serine was abundant at stations S4 (20.37±0.26 mole%) and S1 (11.47±0.33 mole%) respectively. Meanwhile, during MON12, serine recorded its highest abundance (16.29±0.56mole%**)** at station S5 (Figure 6.4).

Threonine: Threonine displayed seasonal variation ($p<0.05$), with its maximum abundance (3.17±0.12 mole%; PRM09)noticed at station S2 during the course of study (Figure 6.4). Meanwhile, during POM09, it was not detected in sediments of the study area. Comparing the both monsoon seasons, the presence of threonine was noticed (2.00±0.21 mole%) only at station S8

during MON09; whileamaximum abundance (2.70±0.49 mole%) was recorded at station S6 for MON12.

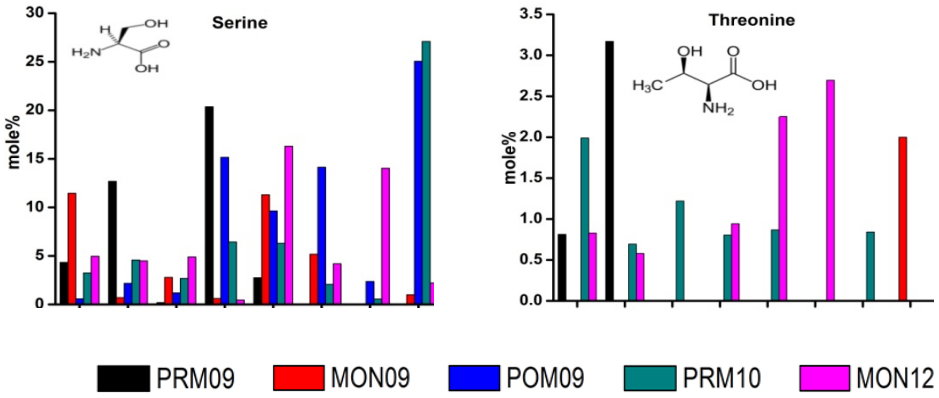

Figure 6.4 Distributions of serine and threonine in sediments of Cochin estuary

6.2.5 Sulphur containing amino acids (methionine and cysteine)

Methionine: Figure 6.5, clearly illustrates the variations of methionine during the study period. Maximum abundance of this amino acid was found in station S3 (35.30±0.50 mole%; MON09). During all seasons, the occurrence of methionine was observed at station S1. Among the premonsoon seasons, PRM09 and PRM10, methionine recorded its highest abundance at stations S1 (23.04±0.17 mole%) and S5 (19.52±0.37 mole%) respectively. Meanwhile, during POM09 and MON12 the higher abundance was found at stations S9 (26.88±0.62 mole%) and S3 (23.23±0.59 mole%) respectively.

Cysteine: Maximum relative abundance of cysteine, during the investigation was observed at station S7 (14.85±0.60 mole%; PRM10). In the case of monsoon seasons, cysteine recorded its higher relative abundance at stations S1 (0.70±0.14 mole%; MON09) and S8 (1.53±0.38 mole%; MON12).Besides PRM09 and POM09recorded its maximum abundance (Figure 6.5) at stations S2 (2.31±0.22mole%) and S3 (6.27±0.19mole%) respectively.

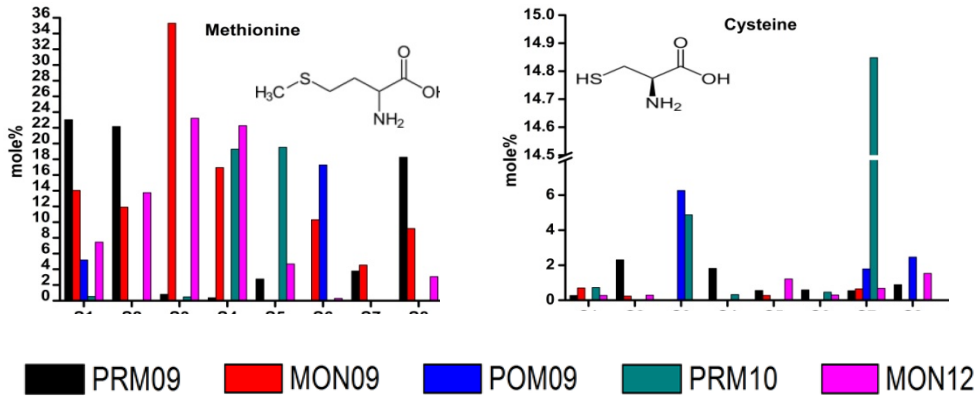

Figure 6.5 Spatial and seasonal variations of methionine and cysteine in surface sediments of study area

6.3 Discussion

6.3.1 Seasonal variability and biogeochemical implications of amino acids in sediments

Seventeen amino acids (AAs) were identified in the sediment extracts of the study area, did not differ much in their composition, but differed in their relative abundance. The observed higher abundance of these AAs and low mole% of glycine during the study period may be due to the presence of terrestrial OM in sediments (Fernandes, 2011). These amino acids are found to be more abundant in vascular plant tissues as compared to phytoplankton (Cowie and Hedges, 1992; Wu et al., 2007). Moreover amino acids such as glutamic acid, aspartic acid, isoleucine, valine, tyrosine, and phenylalanine are usually enriched in the cell plasma of diatoms (Hecky et al., 1973; Dauwe and Middelburg, 1998). These AAs are abundant in freshly derived marine OM and are found to be easily susceptible to degradation. These amino acids exhibited strong depletion with increasing state of decomposition. Most of the AAs are enriched in the cell wall protein of the diatoms and hence considered to be selectively preserved by the protein-silica complex of diatom cell walls.

In the overall distribution, the higher abundance of AAs in sediments of CES (Table 6.2) followed the trend:

PRM09-Leucine > Phenylalanine > Arginine > Lysine

MON09-Lysine > Aspartic acid >Histidine> Tyrosine > Phenylalanine

POM09-Lysine >Histidine> Phenylalanine >Leucine> Methionine > Serine >Proline> Aspartic acid

PRM10-Valine > Aspartic acid >Histidine> Phenylalanine > Serine >Proline

MON12-Lysine > Phenylalanine > Aspartic acid >Histidine>Valine> Tyrosine > Methionine

A comparison of these results were made with otherimportant worldwide studies and are presented in Table 6.3.

Amino acid such as glycine, serine and threonine are known to be enriched in cell walls of diatoms (Hecky et al., 1973). The association of these amino acids with the cell wall protects them from degradation, resulting in their accumulation in the degraded organic matter (Dauwe and Middelburg, 1998). The photorespiration also causes the release of glycine and serine by growing algal cells (Ogren and Chollet, 1982). Generally higher content of glycine in sediments could be due to the fact that it is a short chain amino acid, having minor food value and synthesis from other AAs during heterotrophic metabolism (Fernandes et al., 2014). According to Dauwe et al (1999), the observed elevated levels of glycine and alanine in the sediments implied degraded state of OM. Hence, the presence of glycine along with alanine in POM09 (stations S5 and S8), MON09 (stations S2 and S4), POM09 (S3), PRM10 (majority of the stations except S4 and S9) and MON12 (stations S4, S6 and S7) indicates the presence of partially degraded OM in the estuary (Table 6.2).

Table 6.2 Spatio-temporal variations of sedimentary amino acids (mole%) in the study area

Seasons	Stations	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Cys	Ile	Leu	Phen	Lys	Maximum
PRM09	S1	19.78± 0.46	1.90± 0.35	4.34± 0.24	Nd	0.81± 0.19	Nd	0.81± 0.15	0.27± 0.03	0.81± 0.12	5.96± 0.19	Nd	23.04± 0.17	0.27± 0.01	Nd	3.25± 0.04	27.64± 0.17	11.11± 0.08	27.64± 0.17
	S2	12.97± 0.05	0.86± 0.05	12.68± 0.26	Nd	0.29± 0.04	0.86± 0.02	3.17± 0.12	Nd	Nd	2.59± 0.03	1.15± 0.11	22.17± 0.12	2.31± 0.22	0.58± 0.05	2.31± 0.22	11.82± 0.58	26.23± 0.09	26.23± 0.09
	S3	11.11± 0.08	0.40± 0.05	0.20± 0.04	4.85± 0.13	2.42± 0.16	3.23± 0.16	Nd	0.81± 0.11	0.61± 0.11	0.40± 0.07	Nd	0.81± 0.07	Nd	0.81± 0.06	63.43± 0.31	5.45± 0.17	5.45± 0.15	63.43± 0.31
	S4	13.09± 0.22	5.09± 0.16	20.37± 0.26	Nd	Nd	14.91± 0.15	Nd	9.09± 0.28	Nd	3.27± 0.19	1.09± 0.14	0.36± 0.05	1.82± 0.26	0.36± 0.05	0.36± 0.05	25.83± 0.58	4.36± 0.19	25.83± 0.58
	S5	Nd	17.69± 0.34	2.76± 0.30	6.08± 0.29	Nd	Nd	Nd	4.97± 0.37	5.53± 0.21	0.55± 0.04	Nd	2.76± 0.26	0.55± 0.04	3.32± 0.22	3.32± 0.22	45.88± 0.62	6.63± 0.45	45.88± 0.62
	S6	21.85± 0.60	1.33± 0.17	Nd	0.44± 0.03	Nd	3.10± 0.16	Nd	Nd	0.59± 0.06	Nd	22.59± 0.42	Nd	0.59± 0.06	1.18± 0.13	Nd	15.21± 0.36	33.13± 0.09	33.13± 0.09
	S7	9.73± 0.52	Nd	Nd	Nd	1.62± 0.16	2.16± 0.12	Nd	Nd	0.54± 0.03	1.62± 0.23	Nd	3.79± 0.20	0.54± 0.03	0.54± 0.03	0.54± 0.03	46.51± 0.36	32.45± 0.32	46.51± 0.36
	S8	2.14± 0.10	1.62± 0.22	Nd	19.10± 0.07	7.93± 0.52	2.09± 0.14	Nd	5.01± 0.24	1.04± 0.05	3.18± 0.13	2.04± 0.10	18.27± 0.19	0.89± 0.06	Nd	3.44± 0.31	23.75± 0.53	9.50± 0.35	23.75± 0.53
	S9	Nd	Nd	Nd	0.18± 0.03	Nd	41.65± 0.46	Nd	1.60± 0.11	5.70± 0.49	6.41± 0.29	Nd	Nd	Nd	0.18± 0.02	0.53± 0.02	10.32± 0.23	33.46± 0.33	41.65± 0.46
MON09	S1	24.34± 0.24	0.23± 0.04	11.47± 0.33	1.17± 0.12	3.98± 0.34	5.62± 0.15	Nd	3.28± 0.20	Nd	Nd	Nd	14.04± 0.38	0.70± 0.14	0.70± 0.14	2.57± 0.19	23.73± 0.51	8.19± 0.13	24.34± 0.24
	S2	22.63± 0.45	Nd	0.71± 0.05	14.05± 0.32	0.71± 0.01	Nd	Nd	1.91± 0.22	7.86± 0.26	Nd	Nd	11.91± 0.36	0.24± 0.03	1.19± 0.14	Nd	15.48± 0.34	23.22± 0.30	23.22± 0.30
	S3	3.10± 0.16	0.62± 0.12	2.79± 0.20	Nd	Nd	0.31± 0.04	Nd	0.62± 0.08	Nd	1.86± 0.18	0.62± 0.12	35.30± 0.50	Nd	Nd	4.03± 0.37	9.60± 0.28	41.16± 0.12	41.16± 0.12
	S4	7.89± 0.35	Nd	0.62± 0.06	7.26± 0.19	0.93± 0.07	Nd	Nd	3.73± 0.31	6.20± 0.35	29.05± 0.67	Nd	16.95± 0.67	Nd	5.60± 0.28	Nd	16.95± 0.46	4.84± 0.45	29.05± 0.67

POM09	S5	5.51± 0.29	3.58± 0.27	11.30± 0.21	0.28± 0.04	0.55± 0.04	6.61± 0.43	Nd	4.13± 0.09	0.28± 0.05	3.58± 0.27	0.83± 0.23	Nd	0.28± 0.07	1.38± 0.27	13.23± 0.16	7.16±	41.32± 0.23	41.32± 0.23
	S6	38.94± 0.67	1.92± 0.30	5.18± 0.13	Nd	Nd	9.77± 0.26	Nd	Nd	Nd	1.04± 0.17	Nd	10.31± 0.22	Nd	0.59± 0.07	1.33± 0.23	17.18± 0.34	13.74± 0.31	38.94± 0.67
	S7	11.67± 0.47	Nd	Nd	Nd	1.94± 0.17	2.59± 0.35	Nd	Nd	0.65± 0.03	1.94± 0.17	Nd	4.54± 0.38	0.65± 0.18	0.65± 0.18	0.65± 0.18	35.89± 0.63	38.88± 0.62	38.88± 0.62
	S8	Nd	4.80± 0.28	1.00± 0.10	Nd	0.20± 0.04	24.79± 0.56	2.00± 0.21	2.40± 0.14	2.00± 0.21	Nd	Nd	9.20± 0.14	Nd	1.40± 0.28	3.00± 0.42	26.15± 0.10	23.07± 0.26	26.15± 0.10
	S9	16.96± 0.68	2.07± 0.26	1.65± 0.18	2.07± 0.26	32.41± 0.29	Nd	Nd	Nd	Nd	5.79± 0.21	0.83± 0.16	2.07± 0.19	Nd	0.41± 0.08	9.10± 0.28	26.84± 0.59	Nd	32.41± 0.29
	S1	3.46± 0.24	1.15± 0.11	0.58± 0.05	0.16± 0.04	0.19± 0.01	Nd	Nd	0.19± 0.04	0.19± 0.04	1.92± 1.26	Nd	5.19± 0.14	Nd	Nd	6.73± 0.52	15.77± 0.55	64.62± 0.44	64.62± 0.44
	S2	19.68± 0.48	6.01± 0.29	2.19±	Nd	Nd	0.05± 0.32	Nd	0.55± 0.03	0.55± 3.15	7.65± 0.46	1.09± 0.14	Nd	Nd	0.55± 0.03	27.88± 0.62	18.04± 0.24	15.85± 0.60	27.88± 0.62
	S3	10.36± 0.26	3.86± 0.39	1.20± 0.22	3.13± 0.24	24.10± 0.56	Nd	Nd	0.24± 0.04	24.10± 0.49	Nd	9.88± 0.62	Nd	6.27± 0.19	Nd	2.41± 0.29	2.41± 0.43	12.05± 0.39	24.10± 0.49
	S4	26.97± 0.69	7.05± 0.25	15.15± 0.24	Nd	2.70± 0.49	3.53± 0.37	Nd	5.60± 0.43	0.21± 0.04	0.83± 0.09	0.83± 0.09	Nd	Nd	Nd	0.83± 0.04	27.59± 0.42	8.71± 0.50	27.59± 0.42
	S5	29.16± 0.46	6.51± 0.36	9.64± 0.45	Nd	1.45± 0.32	3.13± 0.24	Nd	2.65± 0.46	Nd	1.93± 0.30	0.24± 0.06	Nd	Nd	0.72± 0.05	6.99± 0.70	34.22± 0.51	3.37± 0.26	34.22± 0.51
	S6	20.42± 0.30	6.28± 0.20	14.14± 0.38	Nd	1.05± 0.17	4.71± 0.50	Nd	4.19± 0.27	0.52± 0.09	3.14± 0.10	Nd	17.28± 0.20	Nd	1.05± 0.17	8.38± 0.27	8.90± 0.64	9.95± 0.32	20.42± 0.30
	S7	25.60± 0.42	7.14± 0.24	2.38± 0.27	Nd	48.21± 14.65	0.60± 0.10	Nd	1.19± 0.23	Nd	12.50± 0.57	Nd	Nd	1.79± 0.27	Nd	0.60± 0.14	Nd	Nd	48.21± 14.65
	S8	16.22± 0.30	6.16± 0.40	25.05± 0.32	0.21± 0.03	8.21± 0.36	8.01± 0.29	Nd	4.93± 0.12	0.21± 0.05	2.05± 0.18	1.03± 0.23	Nd	2.46± 0.33	0.41± 0.07	0.41± 0.05	24.64± 0.45	Nd	25.05± 0.32
	S9	2.69± 0.27	2.15± 0.11	11.83± 0.59	Nd	17.74± 0.52	3.76± 0.47	Nd	3.76± 0.40	Nd	6.45± 0.32	0.54± 0.14	26.88± 0.62	Nd	Nd	19.35± 0.25	2.69± 0.42	2.15± 0.25	26.88± 0.62

PRM10	S1	21.54± 0.38	6.88± 0.62	3.26± 0.18	13.94± 0.52	24.00± 0.56	4.89± 0.63	1.99± 0.56	3.80± 0.50	0.18± 0.04	3.44± 0.31	0.18± 0.01	0.54± 0.07	0.72± 0.05	Nd	1.45± 0.25	13.21± 0.15	Nd	24.00± 0.56
	S2	12.98± 0.48	4.90± 0.50	4.57± 0.41	11.23± 0.44	11.53± 0.38	1.66± 0.33	0.69± 0.11	1.66± 0.33	23.07± 0.47	2.91 ± 0.43	0.69± 0.11	Nd	Nd	0.28± 0.05	0.97± 0.12	5.55± 0.39	17.30± 0.57	23.07± 0.47
	S3	8.29± 0.21	8.78± 0.55	2.68± 0.41	11.95± 0.67	36.34± 0.81	2.68± 0.41	1.22± 0.16	1.95± 0.53	0.24± 0.08	4.15± 0.32	Nd	0.49± 0.10	4.88± 0.62	Nd	16.10± 0.64	Nd	0.24± 0.03	36.34± 0.81
	S4	6.11± 0.36	1.93± 0.59	6.43± 0.30	0.80± 0.21	1.45± 0.32	Nd	0.80± 0.14	Nd	0.48± 0.17	Nd	40.19± 0.84	19.29± 0.21	0.32± 0.04	4.50± 0.35	Nd	8.04± 0.31	9.65± 0.46	40.19± 0.84
	S5	9.54± 0.38	3.25± 0.18	6.29± 0.42	10.85± 0.60	13.77± 0.55	1.19± 0.14	0.87± 0.19	1.41± 0.29	21.69± 0.49	1.95± 0.18	0.11± 0.02	19.52± 0.37	Nd	Nd	9.54± 0.38	Nd	Nd	21.69± 0.49
	S6	19.17± 0.33	6.00± 0.36	2.08± 0.34	10.62± 0.44	2.08± 0.27	1.39± 0.27	Nd	3.00± 0.21	0.23± 0.04	0.46± 0.06	46.19± 0.35	Nd	0.46± 0.04	Nd	8.31± 0.22	Nd	Nd	46.19± 0.35
	S7	17.09± 0.20	16.25± 0.18	0.56± 0.08	3.92± 0.51	24.65± 0.46	0.56± 0.06	0.84± 0.06	1.40± 0.28	9.81± 0.57	6.72± 0.51	Nd	Nd	14.85± 0.60	1.40± 0.28	1.12± 0.16	0.84± 0.17	Nd	24.65± 0.46
	S8	13.04± 0.38	8.03± 0.51	27.09± 0.70	1.67± 0.33	2.68± 0.48	14.38± 0.48	Nd	9.36± 0.47	2.68± 0.48	10.70± 0.50	Nd	Nd	Nd	3.34± 0.24	3.34± 0.24	Nd	3.68± 0.48	27.09
	S9	44.20± 0.50	Nd	8.04± 0.66	0.37± 0.05	6.94± 0.67	Nd	2.19± 0.28	Nd	1.10± 0.21	1.83± 0.37	Nd	4.38± 0.27	0.73± 0.09	Nd	5.11± 0.51	17.54± 0.38	7.67± 0.48	44.20
MON12	S1	18.77± 0.55	0.83± 0.23	4.97± 0.54	Nd	0.55± 0.11	Nd	0.83± 0.13	0.28± 0.05	Nd	3.31 ± 0.22	Nd	7.45± 0.32	0.28± 0.05	Nd	4.69± 0.49	51.44± 0.31	6.63± 0.44	51.44± 0.31
	S2	Nd	3.47± 0.33	4.49± 0.34	0.43± 0.08	1.16± 0.11	1.16± 0.11	0.58± 0.06	Nd	Nd	0.14± 0.04	36.18± 0.55	13.75± 0.53	0.29± 0.06	Nd	7.96± 0.54	30.39± 0.28	Nd	36.18± 0.55
	S3	3.68± 0.48	1.10± 0.14	4.89± 0.63	0.60± 0.11	Nd	0.71± 0.15	Nd	1.87± 0.33	Nd	2.47± 0.33	1.15± 0.11	23.23± 0.59	Nd	0.60± 0.14	Nd	4.78± 0.55	54.91± 0.65	54.91± 0.65
	S4	7.11± 0.08	Nd	0.46± 0.07	9.79±	0.70± 0.07	Nd	0.94± 0.10	1.64± 0.38	7.42± 0.29	20.67± 0.47	Nd	22.28± 0.76	Nd	3.71 ± 0.50	Nd	14.80± 0.57	10.49± 0.34	22.28
	S5	47.49± 0.34	Nd	16.29± 0.56	0. 87± 0.05	4.33± 0.24	Nd	2.25± 0.18	Nd	1.21± 0.15	1.04± 0.17	Nd	4.68± 0.48	1.21± 0.15	3.29± 0.21	Nd	16.81 ± 0.57	0.52± 0.05	47.49± 0.34
	S6	5.09± 0.21	2.99± 0.70	4.19± 0.14	16.47± 0.33	43.73± 0.51	2.40± 0.28	2.70± 0.49	4.19± 0.28	0.60± 0.09	9.58± 0.41	Nd	0. 30± 0.07	0.30± 0.07	2.10± 0.35	5.39± 0.28	Nd	Nd	43.73± 0.51
	S7	9.21± 0.36	2.30± 0.21	14.03± 0.38	9.32± 0.23	2.04± 0.17	4.50± 0.36	Nd	7.96± 0.54	0.57± 0.08	25.13± 0.45	Nd	Nd	0.68± 0.13	0.45± 0.04	3.46± 0.32	6.81 ± 0.57	13.61 ±	25.13± 0.45
	S8	37.31± 0.57	0.85± 0.06	2.21 ± 0.29	Nd	7.33± 0.23	Nd	Nd	2.73± 0.51	12.44± 0.31	3.07± 0.19	Nd	3.07± 0.26	1.53± 0.38	3.58± 0.41	Nd	11.75± 0.53	14.14± 0.45	37.31± 0.57
	S9	20.97± 0.69	1.57± 0.41	9.96± 0.61	Nd	2.10± 12.16	Nd	1.57± 0.41	Nd	Nd	12.58± 0.41	Nd	Nd	0.52± 0.13	Nd	3.67± 0.47	45.62± 0.44	1.57± 0.33	45.62± 0.44

Nd – Not detected

The presence of phenylalanine during PRM09 (at all stations), MON09 (at all stations), POM09 (except S7),PRM10 (except S3, S5, S6 and S8) and MON12 (except S6) reflects inputs from cell plasma (Haugen and Lichtentaler, 1991; Cowie and Hedges, 1992). Meanwhile, higher mole% of lysine observed at stations S2, S6, S7 and S9during PRM09 might be due to the presence of phytoplankton, zooplankton and bacteria (Larsen et al., 2015). The higher abundance of lysine in sediment was observed at stations (S2, S3, S5 and S7; MON09), (S1; POM09) and (S3; MON12)**,** pointed out the influence of marine organisms in the surface sediments and similarobservations has already been quoted (Flynn, 1990; Cowie and Hedges, 1992).

In the present study, aspartic acid dominated at stations S1 and S6 during MON09. The high relative abundance of aspartic acid (S6) and serine (S8) recorded in POM09 may be due to the input from vascular plant tissues into the sediments (Cowie and Hedges, 1992). Meanwhile serine and aspartic acid dominated at stations S8 and S9 respectively during PRM10, however aspartic acid was enriched at stations S5, S8 and S9 during MON12. This might be attributed either to phytoplankton contribution or high plant input, because amino acids like aspartic acid, serine, arginine, alanine and leucine occur in plants as well as phytoplankton according to earlier research reports (Cowie and Hedges, 1992; Dauwe and Middelburg, 1998).

The variation of individual amino acid abundance depends on their association with cell wall and cytoplasm (Cowie and Hedges, 1996). Previous investigations suggest that AAs such as glutamic acid, glycine, alanine, leucine, arginine and lysine are abundant in marine phytoplankton, zooplankton and bacteria (Flynn, 1990; Cowie and Hedges, 1992; Unger et al., 2005). The high relative abundance of leucine at stations S3 (PRM09) and S2 (POM09) and

arginine at station S9 during PRM09 which confirms the input of these AAs from marine phytoplankton, zooplankton and bacteria.

The higher relative abundance of tyrosine, histidine and phenylalanine was noticed during MON09 at stations S4, S9 and S8 respectively. During POM09,the enrichment of AAs such as lysine, leucine and prolinewere recorded at stations S1, S2 and S3 respectively. Meanwhilethe dominance of histidine at station S7 (POM09) and at stations S1, S3 and S7 (PRM10) can be attributed to the input of microorganisms (Dauwe and Middelburg, 1998). From the Table 6.2, it is clear that aspartic acid, glutamic acid, serine, alanine and leucineoccured at all stations during POM09. The increase in the mole % of proline at stations S2 and S5 during PRM10 might be due to the presence of marine bacteria **(**Brown and Stanley, 1972; Stanley and Brown, 1976;Henrich et al., 1984). Moreover the presence of AAs like aspartic acid, serine, glycine, histidine and proline in the study area (Table 6.2), might be due to the combined input of heterotrophic organisms as well as microorganisms (Dauwe andMiddelburg, 1998). Previous investigations suggests that significant quantities of AAs like aspartic acid, lysine, glutamic acid, isoleucine, valine, tyrosine occurs in the cells of aquatic organisms (Dauwe and Middelburg, 1998). This might be the reason for elevated abundance of valine (S2) and lysine (S3) during MON12. The similar implications isalso applicable to the higher abundance of valine at stations S4 and S6 during PRM10.

Table 6.3 Sedimentaryamino acids reported in various investigations

Sl.No.	Study site	Amino acid abundance	THAA	THAA-C%	THAA-N%	DI	Reference
1	Surface sediments of an intertidal mud flat, Lowescove, central coastal Maine	Glu > Ala> Asp> Gly					Mayer et al., 1995
2	Sediment cores (15cm depth) from Itacuruca mangrove forests, south eastern Brazil.	Asp> Glu > Gly> Val > Thr (in avicennia soils)					Lacerda et al., 1995
3	The sediments from the N.W European Continental Margin (47-50^{0}N)	Asp > Gly > Glu > Ser > Ala					Boski et al 1998
4	Sediment, Equatorial	Asp> Gly > Ala> Glu					Lee et al., 2000
5	Vembanad Lake, west coast of India	Gly > Asp > Ala > Glu > Leu > Ser > Val > Thr > Arg > Ilu > Phen > Lys > His > Tyr > Met	157.5-9847.3 µg/g	2.1-20.8	22.4-85.5		Verma and Subramanian, 2002
6	Pearl River Estuary	Gly > Asp ~Ala > Glu > Thr~Pro > Val~ Ser > Leu > Lys > Ileu > Arg > Phen >His > Tyr > Met > Cys		11.7-16.2			Jianfang Chen et al., 2004
7	Lake Zug, Switzerland	Gly > Ala > Asp > Thr > Glu > Lys > Val > Leu > Ser > Ilu > Arg > Phen > Tyr > Met > His	18.7-21.5 wt %	1.7-3.1		0.13 to 0.24	Meckler et al., 2004
8	Ob andYenisei rivers and Kara Sea	Gly > Asp > Glu > Ala > Ser >Thr > Val > Leu > Lys > Ilu > Arg > Phen > His > β-Ala> ϒ-Ala > Tyr > Ornithine > Met (surface sediment) Gly > Asp > Glu~Ala > Ser > Thr > Val > Leu > Lys > Arg~Ilu > Phen > β-Ala > His > ϒ-Ala > Ornithine > Tyr > Met (Sediment, North of 74.5 ^{0}N), Gly > Asp > Ala > Glu > Ser > Thr > Val > Leu > Lys > Ilu > Arg > Phen > His > β-Ala> ϒ-Ala > Tyr > Ornithine > Met (Sediment, South of 74.5 ^{0}N)					Unger et al., 2005

9	Mangrove sediments of Kochi, Kerala, India	Ser > Thr > Asp > Glu					Zeena , 2005
10	Pearl River Estuary , Coarse particulate organic matter (CPOM) in the sediment	Lys > Arg > Phen > Ornithine > ϒ-amino butyric acid > Tyr > Leu > Val >Asp > Cys > Met > His (station-S2)		8.18	50.46		Zhang et al., 2012
		Gly > Ala > Leu > Val > Glu > Ser > Thr > Lys > Phen > Ile > Arg > Tyr > β-Ala > Ornithine > ϒ-amino butyric acid > Asp > His > Met (station-S5)		17.14	46.08		
		Gly > Ala > Pro > Ser > Thr > Val > Leu > Lys > Ile > Phen > Arg > Glu > His > Asp > Met > Tyr (station-S9)		2.26	20.30		
		Arg > Lys > Phen > Ornithine > Tyr >His > Leu > β-Ala > Ile> ϒ-amino butyric acid > Val > Glu > Met > Gly > Asp (station-S12)		9.18	28.51		
11	St. Lawrence Estuary	Gly > Asp > Glu > Ala > Thr > Val > Lys > Leu > Arg > Ilu > Phen > His > Tyr > Met,	41-58 µmol/g	11-19	42-69	-1.02 to 0.18	Alkhatib et al., 2012a
12	Core sediment from Bay of Bangal	Gly > Ala >Val > Leu > Thr > Glu > Asp > Ser~Ile > Phen > Arg > Lys > ϒ-amino butyric acid > β-Ala > Ornithine > Tyr (Bay of Bangal core-1, BOB-1 (surface sediment 2 cm)	1.30 mg/g	5.6	14.6	1.4	Fernandes et al., 2014
		Gly > Glu >Ala~Asp > Lys > Thr > Leu> Ser > Ile > Arg > Phen > β-Ala~ϒ-amino butyric acid > Ornithine> Tyr (Bay of Bangal core-2, BOB-2 (surface sediment 2 cm)	1.33 mg/g	3.0	9.6	0.9	
13	Cochin estuary	PRM09-Leu > Phen > Arg > Lys MON09-Lys > Aspa > His > Tyr > Phen POM09-Lys > His > Phen > Leu > Met > Ser > Pro > Asp PRM10-Val > Asp > His > Phen > Ser > Pro MON12-Lys > Phen > Asp > His >Val >Tyr > Met	3.72-124.54 µmol/g	2.75 - 37.51	5.09-87.71	-1.81 to 2.35	Present study

The sum of basic AAs (Arg+His+Lys) were found to be more abundant than the acidic amino acids (Asp+Glu) in PRM09 at all stations except S1, S3 and S5, while acidic AAs were high during MON09 at S1, S4 and S6. During POM09, the content of acidic amino acids was found to be higher than the basic AAs except at S1, S3, S7 and S9. From S1 to S5, it can be observed that, the basic AAs were found to be enriched compared to the acidic amino acids during PRM10. Meanwhile, during MON12, the concentration of basic AAs were found to be more enhanced than the acidic amino acids (Figure 6.6) at all stations except S1, S2, S5, S8 and S9.

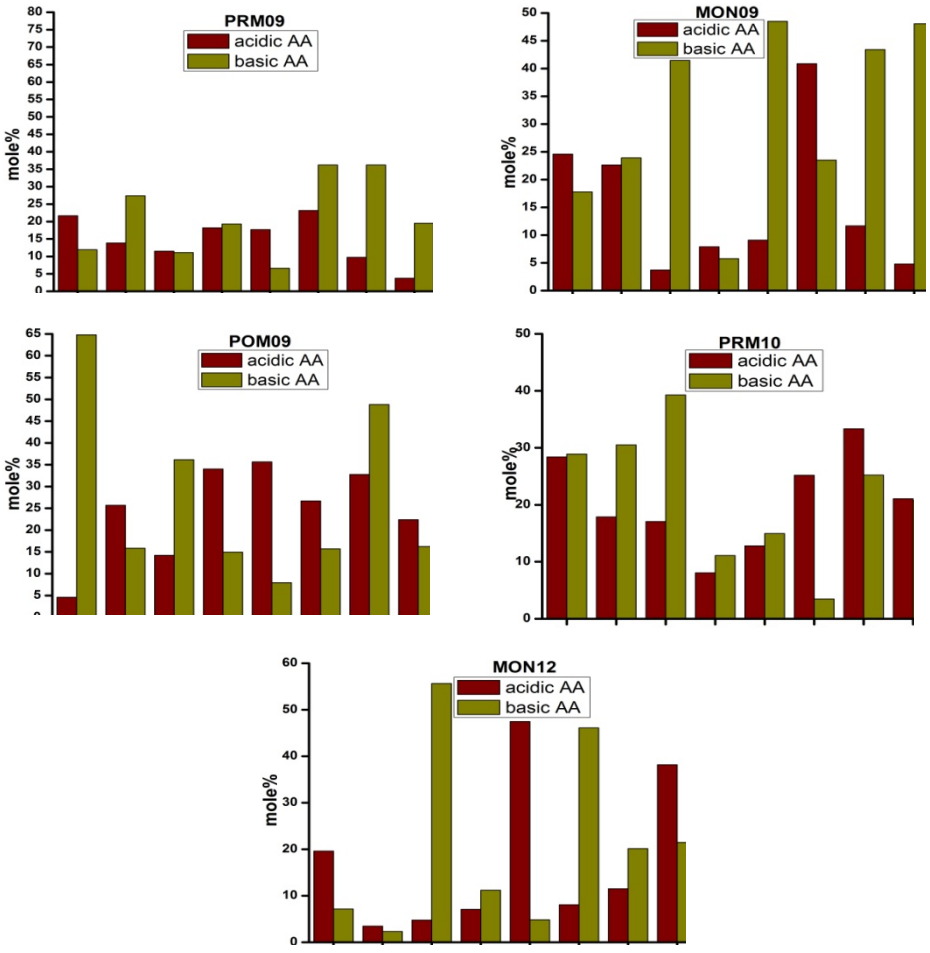

Figure 6.6 Sum of acidic (Asp+Glu) and basic (Arg+His+Lys) amino acids in sediments of the study area

Among the acidic amino acids, aspartic acid was relatively more enriched in sediments of all the stations during PRM09, MON09, PRM09, PRM10 (except S3) and MON12 (Table 6.2). Of the three basic amino acids (Arg, His, Lys), lysine was found to be relatively abundant except at stations S4 and S9 during PRM09. MON09 was characterised by the enriched levels of lysine content (except at stations S8 and S9) in the sediment extracts. Amongthe basic AAs, during POM09, lysine was found to be highly abundant (except at S3, S7, S8 and S9). Meanwhile during PRM10, among these basic AAs in the sediments, histidine was found to be more enriched (except at S2, S4, S8 and S9). MON12 was characterised by remarkable occurrence of all basic amino acids at station S7; among these lysine was found to be more dominating (Table 6.2).

The average contribution of the neutral aliphatic AAs (valine, proline, leucine, isoleucine, glycine and alanine) to the estuarine sediments were: 19.81 mole% (PRM09), 17.77 mole% (MON09), 15.81 mole% (POM09), 32.25 mole% (PRM10) and 17.18 mole% (MON12). While the average contribution of neutral aromatic AAs (tyrosine and phenylalanine) were: (PRM09-26.27 mole %);(MON09-24.69mole%);(POM09-18.97 mole%); (PRM10-8.59 mole%) and (MON12-28.93 mole%). However, the average contribution of other AAs (serine, threonine, cysteine and methionine) accounted in each seasons were: 13.61 mole % (PRM09), 15.87 mole % (MON09), 15.78 mole % (POM09), 15.10 mole % (PRM10) and 16.65 mole % (MON12).

In order to simplify and create a generalized outline of the distribution pattern of AAs, the entire study area was categorised into three zones based on salinity. They are Fresh water zone (S1, S2), Estuarine zone (S3, S4, S5, S6) and Riverine /Industrial zone (S7, S8, S9). The average relative abundance of AAs in each zones of CES, during the study period followed the trend:

Fresh water zone:-Phenylalanine > Lysine > Aspartic acid > Methionine >Valine ˜ Leucine>Proline>Histidine> Glycine > Serine > Glutamic acid > Tyrosine > Arginine > Alanine > Threonine > Cysteine > Isoleucine.

Estuarine zone:-Lysine > Aspartic acid >Phenylalanine >Leucine>Valine>Histidine> Methionine >Tyrosine > Serine > Glutamic acid >Proline>Glycine > Arginine > Alanine > Isoleucine > Cysteine > Threonine.

Riverine /Industrial zone:-Phenylalanine > Lysine > Aspartic acid >Histidine> Serine > Arginine > Tyrosine >Leucine> Methionine > Glutamic acid > Alanine > Glycine > Cysteine >Proline> Isoleucine > Threonine >Valine.

6.3.2 Degradation state of estuarine sedimentary organic matter

The organic carbon in marine sediments has a profound influence on biogeochemical cycles, and acts as a sink of greenhouse gases such as CO_2 and CH_4 (Larsen et al., 2015). The formation and degradation of OM in sediments are an integral process of estuarine ecosystem dynamics, preferably at the intermediate stage of degradation (Cowie and Hedges, 1994).A major portion of the sedimentary organic matter derived from primary production is ultimately oxidised to CO_2 and a significant fraction gets preserved (Cowie and Hedges, 1994). Moreover compared to bulk sedimentary organic matter, amino acids undergo degradation at a faster rate (Cowie and Hedges, 1992; 1994; Wakeham and Lee, 1993). Preferential consumption of amino acids results in the contribution of amino acid nitrogen to total nitrogen in the sedimentary OM (Lee, 1988). The organic C and N supplied to the benthic boundary layers are associated in a complex mixture of living and dead organic material that undergoes continuous alteration and degradation (Boski et al., 1998). These degradations support both microbial production and

regeneration of nitrogenous nutrient fractions such as NO_3^-, NO_2^- and NH_4^+ in the estuarine sediments (Middelboe et al., 1995; Burdige and Zheng, 1998; Stepanauskas and Leonardson, 1999). The labile amino acids undergo decomposition easily, whereas refractory ones will remain in the system. These refractory AAs are assumed to be either nonreactive or degraded at slower rates than labile amino acids. Selective microbial utilisation of individual amino acids will also affect the concentration of AAs in the sediments (Burdige and Martens, 1988). The overall analysis revealed the fact that a major fraction of the organic matter in the sediments of the study region is at intermediate stage of degradation (Zhang et al., 2012).

Information on the degradation state of sedimentary organic matter is provided by the relationship between degradation index (DI) with total hydrolysable AAs. The contribution of amino acid carbon to total organic carbon (THAA-C%) and amino acid nitrogen to total nitrogen (THAA-N%) and relative proportion of individual amino acids are useful diagenetic indicators (Cowie and Hedges, 1994; Dauwe et al., 1999; Davis et al., 2009). By applying THAA-C%, THAA-N%, and DI and its relation with THAA,a better knowledge on the relative diagenetic stage and reaction potential of sedimentary organic matter can be achieved (Cowie and Hedges, 1994; Keil et al., 2000;Pantoja and Lee, 2003; Lomstein et al., 2006; Fernandes et al., 2014). In this investigation, THAA-C% yield in surface sediments of CES, recorded a fluctuating trend (Figure 6.7). The percentage contributions of AAs carbon (THAA-C) to TOC in surface sediments of the study area varied significantly from 2.75 % (S5; PRM09) to 37.51 % (S3; POM09). During PRM09, most of the stations recorded high yield of THAA-C%. While, during MON09, low yield ofTHAA-C% was noticed at stations S1, S5, S7 and S8 and remaining stations recorded higher yield. During POM09, high yield of THAA-C% was observed at S3. Meanwhile in PRM10 higher yield of THAA-

C% was pronounced at stations S1, S2, S4, S5 and S7, but the other stations exhibited lower yield. MON12, recorded higher yield of THAA-C% atstations S2 and S4 with a decreasing trend towards the northern arm of the estuary.Low THAA-C% levels are indicative of terrigenous OM, which consist of high lignin or carbohydrate content than marine OM (Rashid, 1985; Verma and Subramanian, 2002). According to Cowie and Hedges (1992), the fluctuated yield in THAA-C% during the course of study might be due to the selective removal of amino acids relative to TOC. This result indicated that amino acids comprises of the labile fraction present in OM in sediment; whereas THAA-C% are generally indicative of the proportion of OM that has undergone biological degradation at certain stations (Figure 6.7) of CES (Yamashita and Tanoue, 2003b).

It has been established that percentages of total N as THAA are diagenetically sensitive and have generally been observed to be declined with progressive degradation (Henrichs et al., 1987; Keil et al., 2000; Pedersen et al., 2001; Lomstein et al., 2006). During the study period, percentage of AAs nitrogen to TN (THAA-N%) was found to vary from 5.09 % (S7, MON12) to 87.71% (S4, MON09) with significant seasonal variations (Figure 6.7). In the surface sediments collected from CES, THAA-N % was in the range: 8.40 to 72.22 % (PRM09), 18.94 to 80.70 % (MON09), 8.68 to 42.49 % (POM09), 6.03 to 70.45 % (PRM10) and 5.08 to 73.21 % (MON12) in the respective seasons (Figure 6.7). From the Figure 6.7, it is clear that during MON09, atstations S3 and S4, THAA-N% increased drastically. This may be due to nitrogen fixation in the sediments by adsorption/absorption into the brackish and marine regions of the estuary (Verma and Subramanian, 2002).The Figure 6.7, revealed that decreasing trend as well as higher yield for THAA-N% was observed during the study period. This fluctuated yield of THAA-N% indicated selective removal of amino acids relative

to the other organic nitrogen compounds. Meanwhile, fluctuating trendsof THAA-N% and THAA-C% occurs as a result of slight degradation of OM in sediments (Alkhatib et al., 2012a). THAA-C% and THAA-N% (Figure 6.7) recorded higher ranges compared to the similar investigations in sediments of Taihu River (China) and reported values of 18.04-33.05% for THAA-N% and 4.6-12.6% for THAA-C% (Yao et al., 2012). According to Suthhofet al (2000) the observed range of THAA-N% was 23- 38%; meanwhile THAA-C% recorded a range of 10-18% in the surface sediments of continental margin of Pakistan. Similarly, THAA-C/TOC reported from Southern Ocean varied from 6 to 23%. Moreover, values of 20-70% of THAA-N% in sediments wasreported from Peruvian upwelling region (Henrichs et al., 1984). In view of the overall observations, the percentage of AAs-N to TN and AAs-C to TOC in most of the stations in the present study were higher, indicating the fact that OM in sediments of CES was not significantly degraded.

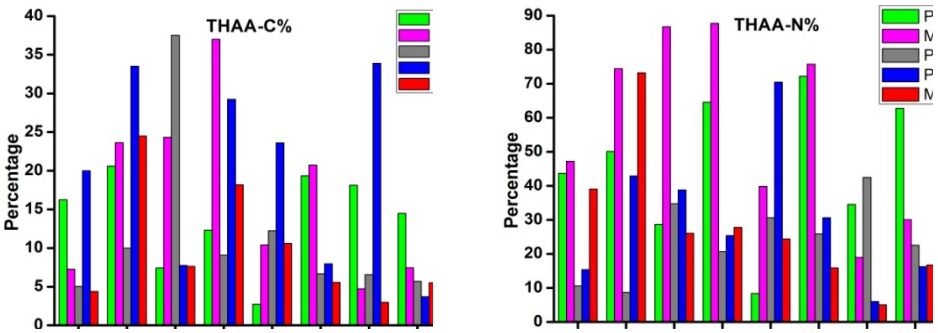

Figure 6.7 Spatial and seasonal variations of THAA-C% and THAA-N% in sediment extracts of the study area

Degradation index (DI): The molar percentage of the seventeen AAs is used to calculate the degradation index (Dauwe and Middelburg, 1998; Dauwe et al., 1999) to assess the diagenetic alteration of a sample by comparing it to a set of 45 samples of different degradation states and environments. Molar percentages of individual AAs are standardised by the mean and standard

deviations of the 45-sample data set. The DI then integrates the AAs weighed by the factor coefficients for the first axis of the principal component analysis (PCA) of Dauwe et al (1999) according to the formula:

$$DI = \sum_i \frac{var_i - AVG\ var_i}{STD\ var_i} \times fac.coef._i$$

Where var_i = mole% of amino acid; $AVGvar_i$=mean amino acid mole%; $STDvar_i$= standard deviation of amino acid mole%; and $fac.coef._i$ = PCA derived loading of amino acids (factor coefficient of the first axis). The more negative the DI value, the more degraded the sample, while positive DI values are indicative of fresh materials (Dauwe et al., 1999; Duan and Bianchi, 2007). The amino acid based degradation index indicated that OM of the surface sediments of shallow stations was relatively fresher than that of deeper stations (Fernandes et al., 2014).

A number of studies have proved that, DI is a reliable and robust index to unravel the extent of diagenetic alteration in sediments (Dauwe and Middelburg, 1998; Keil et al., 2000; Lomstein et al., 2006; Vandewiele et al., 2009). The consistent trend of DI and TOC/TN ratio in the OM revealed that the bulk elemental parameter could also be usedto indicate the diagenetic history of OM. Therefore, the study employed amino acid based DI to assess the quality of the sedimentary OM in CES**.** In contrast, other amino acids such as phenylalanine, glutamic acid, tyrosine, leucine, and isoleucine become depleted with increasing degradation state. The DI values reported by Dauwe and Middelburg (1998) and Lomstein et al (2006), ranged from -2.2 in strongly degraded sediment to 1.5 for fresh phytoplankton. In the present investigation, most of the stations were categorised under this range (Figure 6.8) and varied from -1.81 (MON12, S5) to 2.35 (PRM10, S8). The positive DI indicated more fresh organic matter in the estuarine sediments (Dauwe et al., 1999; Duan and Bianchi, 2007). In CES, a distinct seasonal difference ($p<0.01$) for DI was observed during the study period (Figure 6.8).

The yields and compositions of total hydrolysable amino acid (THAA) have been widely utilised as diagenetic indicators, because they are selectively decomposed or preferentially preserved during diagenesis (Keil et al., 2000; Amon et al., 2001; Vandewiele et al., 2009). The changes in the amino acid composition during OM decomposition depend primarily on the amino acids and their association with cell wall, cell membrane or cell plasma, and/or sorption onto mineral surfaces (Aufdenkampe et al., 2001). Most of the organic matter in the sediments of the study area is at intermediate stage of degradation (Cowie and Hedges, 1994). In addition, several studies of amino acids in sediments (Henrichs et al., 1984; Cowie and Hedges, 1992; Sugai and Henrichs, 1992; Boski et al., 1998; Dauwe and Middelburg, 1998) have indicated that the relative contribution of individual amino acids to total hydrolysable amino acids (THAA) changes during OM mineralisation.

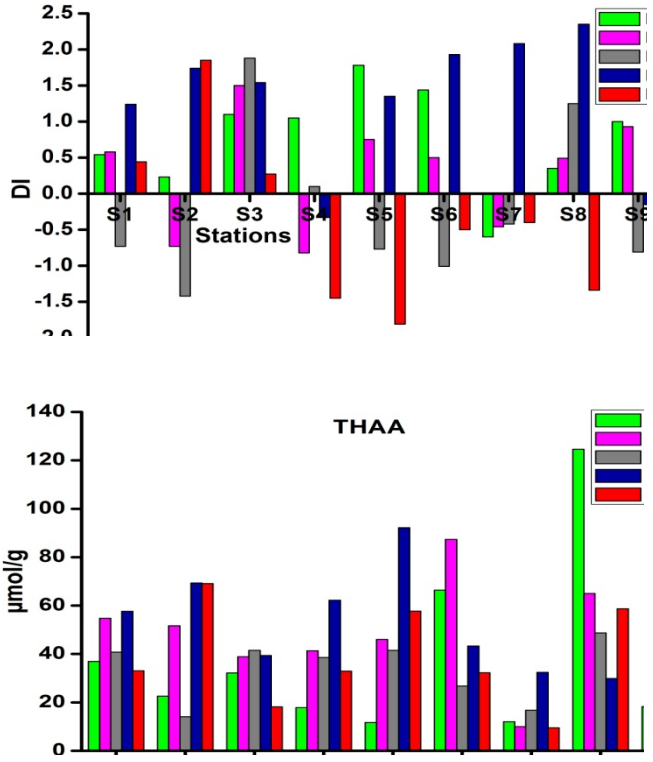

Figure 6.8 Degradation index and Total hydrolysable amino acid in the sediments ofthestudy area

Positive DI and high THAA, suggested the presence of fresh OM in surface sediments of the estuarine system while, negative DI and low THAA yields,indicated the presence of degraded OM in surface sediments of estuarine system (Pantoja and Lee, 2003; Lomstein et al., 2006; Fernandes et al., 2014). In this study, during PRM09, the stations S1, S3, S6 and S8 exhibited positive DI (ranged from -0.60 to 1.78) and high THAA yield, which reflects the presence of fresh OM deposition at these stations during the study period. Minimum value of THAA was observed at station S5 (11.77 μmol/g) and maximum was reported at station S8 (124.54μmol/g) (Figure 6.8) during PRM09. The positive DI with higher THAA yield was found at stations S1, S3, S5, S6 and S8 during MON09, indicated freshness of OM. Meanwhile, negative DI with low THAA observed atstations S2, S4 and S7 during the same period indicated those stations of the CES settled with aged OM. The observed decrease in concentration and yield of THAA in certain stations of the CES suggested their utilisation by heterotrophic microorganisms (Fernandes et al., 2014). During POM09 stations S2, and S6 provide low THAA with negative DI, which might be due to decomposition by benthic macro and microorganisms (Lomstein et al., 2006), while, stations S3, S4 and S8 recorded positive DI with high THAA (Figure 6.8). During PRM10, most of the stations except S4, S8 and S9, displayed positive DI with high THAA. Stations S1and S2exhibited positive DI with higher THAA yield during MON12, suggested newly deposited OM at these stations (Bourgoin and Tremblay, 2010).The sediments with low or negative DI values indicated more refractory organic matter dominated in certain stations (Figure 6.8) of the study area (Chen et al., 2004), and DI generally decreased with decreasing sediment grain sizes (Dauwe et al., 1999).

The negative DI with lower THAA value at various stations (Figure 6.8) revealed the presence of degraded OM in surface sediments of CES due to the continued preferential loss of planktonic material (Cowie and Hedges, 1992). In general, DI as a function of THAA concentration viewed a positive relationship; ie., for low values of DI, concentrations of THAA was lower. However as an exception, high THAA concentration with low DI values were also observed in the study area (Figure 6.8) and this could be explained by two possible causes. One is the input of the larger amount of allochthonous THAA derived from terrestrial organic matter or diffusion from sediment pore waters (Bauer and Druffel, 1998; Raymond and Bauer, 2001; Yamashita and Tanoue, 2003a).

Lower THAA yields at stations S7 and S9 (Figure 6.8) indicated that the OM was substantially degraded. The observed lower THAA yield during the study period was attributed to the presence of degraded terrestrial OM or retarded growth of phytoplankton (Cowie and Hedges, 1992; Hedges et al., 1997). Another reason for the lower THAA yield was the presence of terrestrial OM, which contains less amino acid compared to phytoplankton. In contrast, the improved light conditions, that favoured the growth of in situphytoplankton, resulted in the higher THAA concentrations of the study area. This reflects that OM was relatively fresh and of phytoplankton origin, during pre-monsoon season. This was also supported by a positive relationship (Table 6.4) between THAA and chl-a($r = 0.54$). Abundance of amino acid in the sediments of CES was significantly affected by the variations in the stations ($p < 0.01$). THAA account for a major fraction of the freshly produced OM, and are relatively labile compared to bulk OM (Ittekkot and Arain, 1986; Cowie and Hedges, 1994). During OM degradation THAA yields decreases and therefore highly

suitable to assess the degradation status of OM in the study area (Cowie and Hedges, 1992; Davis et al., 2009).

From the Figure 6.8, it is clear that, in most of the seasons of study site (S1-PRM09, MON09, PRM10 and MON12; S2-PRM10 and MON12; S3-PRM09, MON09, POM09 and PRM1O; S4-POM09; S5 -MON09 and PRM10; S6 -PRM09, MON09 and PRM10; S7-PRM10; S8-PRM09, MON09 and POM09) recorded positive DI and higher THAA concentration with significant spatial variation of THAA ($p<0.01$) and seasonal variation of DI ($p<0.01$). The presence of OM in surface sediments was relatively fresh in most of the seasons, which points towards bacterial influence in the stations (Jorgensen et al., 1990; Fernandes, 2011). From the support of above observations, it can be concluded that estuarine zone recorded fresh OM than fresh water zone and riverine/industrial zone. This fact was further supported by TOC/TN ratio, where higher TOC/TN values (evidence that degraded OM and low TOC/TN ratio), in turn supports the relatively fresh OM **(**Goni and Hedges, 1995; Meyers, 1997; Zimmerman and Canuel, 2001; Bashkin, 2002; Gordon and Goni, 2003**).** The high TOC/TN ratio (> 20) implies the terrestrial derived OM while, low TOC/TN ratio (<13) recorded marine OM (Meyers, 1997). In view of this, the low TOC/TN values strongly suggested the enrichment of bacterial-N in the sedimentary OM. The biodegradation of terrestrial OM results in bacterial enrichment (Tremblay and Benner, 2006**)** andmay reduce thedifferences in bacterial-N contribution during the study period.As discussed in Chapter 4, most of the stations recorded low TOC/TN ratios (<13) (Figure 4.6), supporting the freshness of OM in the sedimentary system. Apart from this, intermediate values of TOC/TN ratios signalled a combined input of both autochthonous and terrestrial OM to the estuarine sediments under investigation (Muriet al., 2004).

Table6.4 Correlations of amino acids with general sedimentary parameters (n=45)

	Sand	Silt	Clay	TS	TN	chl-a	TOC	THAA	THAA-N%	THAA-C%	AlinuAAs	AronuAAs	AcidAAs	BasAAs	HydAAs	SulfAAs
Sand	1															
Silt	-0.96 (**)	1														
Clay	-0.90 (**)	0.76 (**)	1													
TS	-0.46 (**)	0.44 (**)	0.41 (**)	1												
TN	-0.28	0.22	0.35 (*)	0.58 (**)	1											
chl-a	-0.58 (**)	0.57 (**)	0.51 (**)	0.68 (**)	0.35 (*)	1										
TOC	-0.43 (**)	0.38 (**)	0.44 (**)	0.64 (**)	0.46 (**)	0.63 (**)	1									
THAA	-0.44 (**)	0.38 (**)	0.47 (**)	0.48 (**)	0.33 (*)	0.54 (**)	0.45 (**)	1								
THAAN%	-0.10	0.10	0.09	-0.23	-0.46 (**)	-0.04	-0.16	0.45 (**)	1							
THAAC%	0.14	-0.17	-0.05	-0.25	-0.01	-0.22	0.53 (**)	0.28	0.52 (**)	1						
AlinuAAs	-0.04	-0.01	0.35 (*)	0.14	0.09	0.31 (*)	0.34 (*)	0.29 (*)	0.03	0.20	1					
AronuAAs	0.02	-0.01	0.32 (*)	-0.16	-0.24	-0.29 (*)	-0.01	-0.26	-0.02	-0.16	-0.44 (**)	1				
AcidAAs	-0.06	0.07	0.02	0.18	0.21	0.18	0.39 (*)	0.01	-0.22	-0.15	-0.22	-0.01	1			
BasAAs	0.16	0.41 (*)	-0.16	-0.09	0.03	-0.10	0.01	-0.12	0.04	0.04	-0.29 (*)	-0.33 (*)	-0.39 (**)	1		
HydAAs	-0.11	0.20	-0.05	0.17	0.01	0.09	0.37 (*)	-0.12	-0.21	-0.35 (*)	-0.24	-0.01	0.27	-0.29 (*)	1	
SulfAAs	-0.08	0.31 (*)	0.12	-0.21	-0.13	-0.21	0.29 (*)	0.17	0.35 (*)	0.28	-0.08	-0.12	-0.33 (*)	-0.07	-0.09	1

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

Note: Alinu AAs: aliphatic neutral amino acids, Aronu AAs: aromatic neutral amino acids, Acid AAs: acidic amino acids,Bas AAs: basic amino acids, Hyd AAs: hydroxy amino acids, Sulf AAs: sulphur containing amino acids.

The correlation analysis of various amino acids are given in (Table 6.4). TOC exhibited strong positive correlation with various classes of amino acids such as aliphatic neutral AAs (r = 0.34), acidic AAs (r = 0.39), hydroxy AAs (r = 0.38) and sulphur containing AAs(r = 0.29). The influence of granulometry on the distribution of AAs was evident from correlations of aliphatic neutral AAs and aromatic neutral AAs with clay as well as correlations of basicAAs and sulphur containing AAs with silt. The transport of lithogenic material brought about by five rivers flowing in to the estuary enhances sedimentation process. Induced primary production together with terrestrial material usually results in the increasedremoval of freshly generated organic matter from water column to the sediments (Jacob et al., 2008). Furthermore, factors like higher sinking speeds and clay minerals can also reduce the time for degradation in the water column (Sirocko and Ittekkot, 1992). The combined effects of sediment grain size, minerals, increased levels of TOC and periodically varying anoxic conditions accelerates the preservation of amino acids in the sediments of study area. It has already been established that OM complex with minerals is less degradable (Fernandes et al., 2014). Pearson correlation revealed strong positive correlation of aliphatic neutral AAs with THAA (Table 6.4). Significant negative correlation between aliphatic neutral AAs and aromatic neutral AAs (r = -0.44), aromatic neutral AAs and basic AAs (r = -0.33), basic AAs and hydroxy AAs (r = -0.29), sulphur containing AAs and acidic AAs (r = -0.33) indicated diverse origin and dissimilarity in their behaviour.

Principal component analysis (PCA)

PCA, which is widely used to evaluate the natural and anthropogenic processes, was applied to explore the origin and geochemical factors influencing AAs distribution in sediments. The Varimax rotation provided a

clear description of the overall behaviour of various parameters and the factors are given in Table 6.5. Component 1 explained 37.371 % of total variance which includes silt clay, total sulphur, acidic amino acids, basic and hydroxy amino acid. This indicated the role of granulometry (adsorption process) as the controlling factor for the distribution of variables. Meanwhile component 2, described 21.172 % of the total variance and composed of TS, TN, TOC reflected the origin of the AAs (via autochthonous or allochthonous) and form the part of organic matter and ultimately settled in sediments. This component also infers thediagenesis (since it contains redox indicators like TS and TN) involving microbial activity which ultimately controls the distributional characteristics of amino acids in sediments (Yamashita and Tanoue, 2003a; Zeena, 2005).

Table 6.5 Results of principal component analysis

Parameters	Components	
	1	2
Sand	-0.965	-0.129
Silt	0.917	0.098
Clay	0.902	0.163
TS	0.430	0.698
TN	0.246	0.681
TOC	0.452	0.643
Alinu AAs	0.018	0.887
AronuAAs	0.033	0.521
Acid AAs	0.709	0.335
Basic AAs	0.668	0.141
Hyd AAs	0.831	-0.019
Sulf AAs	0.298	-0.697
% of Variance	37.371	21.172

6.4 Conclusion

Season wise relative abundance of AAs in the estuarine sediments followed the trend:

PRM09- Leucine> Phenylalanine > Arginine > Lysine

MON09- Lysine > Aspartic acid >Histidine> Tyrosine > Phenylalanine

POM09- Lysine >Histidine> Phenylalanine >Leucine> Methionine > Serine >Proline> Aspartic acid

PRM10- Valine> Aspartic acid >Histidine> Phenylalanine > Serine >Proline

MON12- Lysine > Phenylalanine > Aspartic acid >Histidine>Valine> Tyrosine > Methionine.

Based on zones of the study area, relative abundance of AAs in the sediments were in the order:

Fresh water zone:- Phenylalanine > Lysine > Aspartic acid > Methionine >Valine ˜ Leucine>Proline>Histidine> Glycine > Serine > Glutamic acid > Tyrosine > Arginine > Alanine > Threonine > Cysteine > Isoleucine.

Estuarine zone:-Lysine > Aspartic acid > Phenylalanine >Leucine>Valine>Histidine> Methionine > Tyrosine > Serine > Glutamic acid >Proline> Glycine > Arginine > Alanine > Isoleucine > Cysteine > Threonine.

Riverine /Industrial zone:- Phenylalanine > Lysine > Aspartic acid >Histidine> Serine > Arginine > Tyrosine >Leucine> Methionine > Glutamic acid > Alanine > Glycine > Cysteine >Proline> Isoleucine > Threonine >Valine.

Glutamic acid, aspartic acid, isoleucine, valine, tyrosine, and phenylalanine are enriched in diatom cell plasma and are found to be easily susceptible to degradation and their abundance in sediments indicated freshly

derived organic matter. Vascular plant input to sedimentary OM was evident from the relative abundance of aspartic acid. Contribution of microorganisms was inferred from the dominance of histidine at S1 and S7. The sum of basic AAs (Arg+His+Lys) were found to be more abundant than the acidic amino acids (Asp+Glu) during PRM09, MON09 and PRM10, meanwhile during POM09 and MON12 acidic amino acids dominated in surface sediments. Among the two acidic amino acids, aspartic acid was found to be relatively more enriched than glutamic acid in all stations during PRM09, MON09, PRM09, PRM10 and MON12 seasons; while basic amino acid lysine was found to be relatively abundant during PRM09, MON09, POM09 and MON12. POM09 was characterised by higher abundance of arginine,while during PRM10 histidine was relatively enriched. The percentage contributions of THAA-C% and THAA-N% exhibited fluctuating trends as a result of slight degradation. The observed THAA-C% and THAA-N% in sediments were at higher ranges compared to other estuaries, indicating that OM in sediments of CES was not highly degraded. Stations of the study area recorded positive DI values and higher THAA concentration with remarkable spatial and seasonal variations during most of the seasons, indicating fresh input of OM with bacterial signals. The estuarine zone of the study area recorded fresh OM than fresh water and riverine/industrial zones.Thelower or negative DI values implied accumulation of refractory material in the estuarine sedimentary organic matter. DI generally decreased with decreasing sediment grain sizes suggesting the role of texture in the distribution and preservation of amino acids in sediments. Multivariate statistical analysis pointed out the involvement of sediment texture, TOC, redox state and microbial processes on the dispersal pattern of amino acids in the study area.

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SUMMARY

Cochin estuarine system is among the most productive aquatic environment along the Southwest coast of India, exhibits unique ecological features and possess greater socioeconomic relevance. Serious investigations carried out during the past decades on the hydro biogeochemical variables pointed out variations in the health and ecological functioning of this ecosystem. Characterisation of organic matter in the estuary has been attempted in many investigations. But detailed studies covering the degradation state of organic matter using molecular level approach is not attempted. The thesis entitled Provenance, Isolation and Characterisation of Organic Matter in the Cochin Estuarine Sediment-" a Diagenetic Amino Acid Marker Scenario" is an integrated approach to evaluate the source, quantity, quality, and degradation state of the organic matter in the surface sediments of Cochin estuarine system with the combined application of bulk and molecular level tools.

Sediment and water samples from nine stations situated at Cochin estuary were collected in five seasonal sampling campaigns, for the biogeochemical assessment and their distribution pattern of sedimentary organic matter. The sampling seasons were described and abbreviated as follows: April-2009 (pre monsoon: PRM09), August-2009 (monsoon: MON09), January-2010 (post monsoon: POM09), April-2010 (pre monsoon: PRM10) and September-2012 (monsoon: MON12). The objectives of the present study were: i) To find the nutrient enrichment in the estuarine sediments using phosphorous and nitrogen fractionation. ii) To assess the spatio-temporal variation, nature and quality of bulk sedimentary organic matter as well as the benthic trophic status of the estuary. iii) Extraction, quantification and distribution of free sugars in sedimentary organic matter and its implications on productivity. iv) Distribution

pattern and diagenetic process of amino acids in order to unravel the quality of estuarine sedimentary organic matter were also encountered.

In order to evaluate the general environmental conditions of the estuary, water samples were analysed for water quality parameters, chlorophyll pigments and nutrients by standard methods. Investigations suggested the fact that hydrographical variables and nutrients in Cochin estuary supports diverse species of flora and fauna. Moreover the sedimentary variables such as pH, Eh, texture, TOC, fractions of nitrogen and phosphorous were determined to assess the general geochemical setting as well as redox status. The periodically fluctuating oxic/ anoxic conditions and texture serve as the most significant variables controlling other variables of the aquatic environment. The organic matter in estuary comprise of a complex mixture of autochthonous as well as allochthonous materials. Autochthonous input is limited or enhanced by the nutrient elements like N and P (in their various fractions), used as a tool to evaluate their bioavailability. Bulk parameter approach like biochemical composition, stoichiometric elemental ratios and stable carbon isotope ratio was also employed to assess the quality and quantity of sedimentary organic matter in the study area. Molecular level charactersation of free sugars and amino acids were carried out by liquid chromatographic techniques. Carbohydrates are the products of primary production and their occurrence in sediments as free sugars can provide information on the estuarine productivity. Amino acid biogeochemistry provided implications on the system productivity, nature of organic matter as well as degradation status of the sedimentary organic matter in the study area.

pH of the sediments was slightly alkaline during the investigation and its maximum was observed at S8. Values of Eh in sediments remain oxic during the monsoon and exhibited reducing condition during the post monsoon and pre-monsoon. Analysis of sediment texture revealed the dominance of sand at the confluence of the riverine portion of the study area. In the case of silt and clay fractions enhanced levels towards the estuarine

stations were also observed. Total organic carbon content in the sediments of the study region was controlled mainly by the rate of supply of terrestrial materials, rate of deposition of organic to inorganic constituents, primary productivity, redox conditions as well as texture of sediments.

Sequential chemical extraction of P and N provided a better understanding of the nutrient enrichment in the estuary. An abrupt increase in the concentration of total phosphorous with increase in salinity was observed in the study region. The processes of reductive dissolution of iron hydroxides and formation of calcium carbonate minerals were the major factors governing the distribution of both Fe bound and Ca bound P in the estuary. Concentration of Ca bound P was more pronounced at the regions with higher salinity of the study area since the formation of $CaCO_3$ is favoured by the more alkaline pH. During the study period, nitrogen compounds followed the trend: residual-N> nitrate-N> nitrite-N> urea-N> ammonia-N. Among the P fractions, Fe bound P exhibited a distinct seasonal distribution pattern with maximum content displayed during the monsoon. Results of multivariate statistical analysis indicated that P fractions and N fractions supported the periodic interchange of oxic /anoxic character of the surface sediments. Intense land use change, unscientific agriculture practices and population growth have significantly altered river fluxes of nutrients. TOC/TS ratio inferred periodic anoxia, while TN/TP and TOC/TP ratios revealed enrichment of P in the Cochin estuarine system.

The predominance of carbohydrates over protein indicated faster mineralisation of proteinaceous organic matter in sediments and the estuary behaves as a detrital trap for the accumulation of aged organic matter. The higher lipid content and LPD/CHO ratio pointed towards the better food quality that supports benthic fauna and better accumulation of lipid compounds in the sedimentary environment. Allochthonous addition of carbohydrates via terrestrial run off was responsible for the lower PRT/CHO ratio estimated in the

sediments and the lower ratios also denoted a detrital heterotrophic environment. Biopolymeric carbon and the algal contribution to BPC provided important information on the better understanding the trophic state of the estuarine system and the higher values of chlorophyll-a to phaeophytin ratio indicated deposition of phytoplankton to sediment at a rapid rate.

The estimated TOC/TN ratios implied the combined input of both terrestrial and autochthonous organic matter to sediments. TOC and TN concentrations strongly depend on the grain size of sediments in the study region. The more depleted $\delta^{13}C$ value (-32.34 to -25.07 ‰) in the sediments indicated terrestrial input consisting of vascular plant debris. Terrestrial input was also testified by higher concentration of tannin and lignin in the sediments of the estuary. Rapid and recent deposition of phytoplankton detritus to sediments was inferred from chlorophyll-a / (chlorophyll-a + phaeopigment) ratios. Bulk parameter approach revealed a combined input of organic matter from in situ primary production, land runoff, industrial, agricultural and domestic sewage in the estuarine sediments.

Among the free sugars, depleted levels of glucose in sediments in most of the stations and abundance of mannose at station S5 was observed during the present investigation. Among aldohexoses, concentration of galactose was found to be higher in most of the stations. PRM09 was characterised by the abundance of aldopentose- arabinose (S2, S3 and S8) and ribose (S1, S5, S7 and S9) at respective stations, while enrichment of xylose was noted at S4. Enrichment of the disaccharide- maltose was noticed in most of the stations during POM09. Correlation analysis implied the role of primary productivity on organic matter production, similarity in behaviour and distribution pattern of free sugars. The strong relationship between phosphate and chlorophyll pigments confirmed the fact that nutrient availability in the water column governs the instantaneous rates of chlorophyll and organic matter production. The enhanced level of chlorophyll pigments in water column have imparted

higher rate of productivity of the estuarine system. The free sugar content has been attributed to both autochthonous as well as allochthonous input as evident from stable carbon isotope ratio and TOC/TN ratio. The overall examination revealed that the biogeochemistry of free sugars and the productivity of Cochin estuary were influenced by the interactions between nutrient content, chlorophyll, TOC and other physicochemical variables. Multivariate statistical analysis indicated that concentration and spatio-temporal distribution of frees sugars in the study area are regulated by grain size of sediments, salinity of the water column, in situ primary production, allochthonous input, nutrient levels and redox status.

Relative abundance of AAs in the estuarine sediments based on seasons followed the trend: PRM09-Leucine > Phenylalanine > Argine > Lysine, MON09-Lysine > Aspartic acid > Histidine > Tyrosine > Phenylalanine, POM09-Lysine > Histadine > Phenyalanine > Leucine > Methionine > Serine > Proline > Aspartic acid, PRM10-Valine > Aspartic acid > Histidine > Phenylalanine > Serine > Proline, MON12-Lysine > Phenylalanine > Aspartic acid > Histidine > Valine > Tyrsine > Methionine.

The classification of study area into three zones based on salinity was employed in the present study for the sake of simplicity and generalized interpretations. The distribution of AAs in the three zones followed the trend:

Fresh water zone (S1, S2):- Phenylalanine > Lysine > Aspartic acid > Methionine > Valine ˜ Leucine > Proline > Histidine > Glycine > Serine > Glutamic acid > Tyrosine > Arginine > Alanine > Threonine > Cysteine > Isoleucine.

Estuarine zone (S3, S4, S5, S6):- Lysine > Aspartic acid > Phenylalanine > Leucine > Valine > Histidine > Methionine > Tyrosine > Serine > Glutamic acid > Proline > Glycine > Arginine > Alanine > Isoleucine > Cysteine > Threonine.

Riverine /Industrial zone (S7, S8, S9):- Phenylalanine > Lysine > Aspartic acid > Histidine > Serine > Arginine > Tyrosine > Leucine > Methionine > Glutamic acid > Alanine > Glycine > Cysteine > Proline > Isoleucine > Threonine > Valine.

The abundance of AAs like glutamic acid, aspartic acid, isoleucine, valine, tyrosine, and phenylalanine in sediments of the study area indicated freshly derived organic matter. Vascular plant input to sedimentary OM was evident from the relative abundance of aspartic acid. The input of AAs from microorganisms was testified by the dominance of histidine at S1 and S7. The sum of basic AAs (Arg+His+Lys) were found to be more abundant than the acidic amino acids (Asp+Glu) during PRM09, MON09 and PRM10, besides, in POM09 and MON12 acidic amino acids dominated in surface sediments. Abundance of aspartic acid than glutamic acid was formed in all stations during PRM09, MON09, PRM09, PRM10 and MON12 seasons. Among the three basic amino acids, lysine was found to be relatively abundant during PRM09, MON09, POM10 and MON12. POM10 was characterised by higher mole% of arginine, while during PRM10 histidine was relatively abundant. The percentage contributions of THAA-C% and THAA-N% exhibited fluctuating trends as a result of slight degradation. Observed THAA-C% and THAA-N% in sediments were found at higher ranges as compared to other estuaries, indicating that OM in sediments of CES was not highly degraded. The positive degradation index and higher concentration of THAA in sediments implied the fact that OM was relatively fresh and had bacterial contributions at the studied area. According to the zone wise categorization, the estuarine zone of the study area recorded more recent OM than fresh water and riverine/industrial zones.

Within the time constraints, the investigation was effective in unraveling the nature, source, degradation state and various interrelated biogeochemical processes involved in the organic matter dynamics of the estuary. Source specific indices like molecular biomarkers, compound specific isotope analysis are recommended to achieve more information on origin and fate of organic matter in complex ecosystems like Cochin estuary.

PUBLICATIONS

Salas P M, Sujatha C H and Ratheesh Kumar C S (2015) Fate and source distribution of organic constituents in a river dominated tropical estuary; Journal of Earth System Science. (accepted and in press, D-14-00343R4).

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APPENDIX-1

Appendix 1.1: Spatio-temporal variation of biochemical components present in the study area

Parameters	Stations	PRM09	MON09	POM09	PRM10	MON12
TOC (%)	S1	1.57±0.21	4.78±0.23	5.77±0.44	1.68±0.13	5.28±0.24
	S2	0.72±0.04	1.31±0.17	1.02±0.16	1.21±0.16	1.81±0.22
	S3	2.74±0.18	1.12±0.12	0.74±0.13	3.02±0.11	1.62±0.18
	S4	0.96±0.03	0.75±0.06	2.87±0.12	1.26±0.12	1.25±0.14
	S5	2.95±0.19	2.93±0.16	2.38±0.21	2.24±0.21	3.43±0.25
	S6	2.21±0.29	2.96±0.22	2.67±0.32	2.88±0.25	3.46±0.22
	S7	0.50±0.06	1.58±0.11	1.68±0.12	0.61±0.04	2.08±0.24
	S8	5.44±0.27	6.39±0.14	5.56±0.10	4.98±0.22	6.86±0.21
	S9	0.56±0.04	0.16±0.02	0.36±0.05	0.72±0.05	0.66±0.04
LPD (mg/kg)	S1	1494.39±11.21	1313.27±18	1945.25±7.38	5018.69±7.39	717.41±5.58
	S2	357.91±9.34	756.84±7.92	764.26±9.76	3268.37±5.87	204.71±4.12
	S3	1475.27±7.48	594.32±5.95	1477.75±8.72	3454.82±7.63	335.67±2.91
	S4	1498.09±5.87	475.95±2.96	3169.81±6.94	2533.33±7.85	412.56±3.59
	S5	2591.79±8.94	1948.49±21	2964.81±7.38	2500.00±6.88	539.63±4.56
	S6	796.99±9.92	1754.92±12	1912.80±18.49	3371.95±7.38	440.48±5.18
	S7	239.66±5.72	2233.23±7.41	491.64±7.35	1143.10±6.98	564.23±4.17
	S8	3055.39±11	5795.62±10.58	1376.59±14.18	3981.37±11.12	757.02±3.06
	S9	755.95±6.19	461.02±9.92	115.29±4.25	688.72±8.93	134.56±4.55
CHO (mg/kg)	S1	2611.36±6.98	6185.29±7.59	9491.98±7.39	3310.27±5.94	4379.53±3.94
	S2	1002.45±9.93	1977.39±11.53	1487.40±6.92	3885.56±11	5384.11±9.37
	S3	2005.65±11.22	2159.61±6.99	500.00±9.89	1440.08±7.84	3425.43±8.09
	S4	1151.26±9.27	1653.79±7.72	1263.08±5.99	955.07±5.67	3988.78±7.32
	S5	1946.06±4.38	4550.48±7.92	887.14±6.93	891.88±6.69	5075.00±4.82
	S6	2451.43±5.44	3568.73±6.96	1327.83±9.82	3382.70±5.49	4626.42±6.84
	S7	2073.74±7.93	1925±7.17	1309.20±8.95	1523.21±9.87	4564.23±7.93
	S8	4700±8.32	8887.16±8.62	6130.00±10.11	6680±8.49	13285±10.33
	S9	1033.94±5.45	618.61±7.83	434.19±8.74	657.26±4.93	658.50±2.84

PRT (mg/kg)	S1	233.41±15.11	2355.41±98.12	15250.00±35.32	1280.48±56.11	6912.19±36.23
	S2	558.78±44.12	156.51±4.23	788.84±23.12	1221.43±34.12	6617.83±33.13
	S3	4628.74±19.12	442.78±1.12	850.00±11.32	765.87±3.34	5897.33±21.45
	S4	110.67±7.77	248.93±12.34	2953.43±5.65	329.83±2.85	4978.23±5.65
	S5	271.87±4.24	480.04±3.53	357.07±4.94	716.51±4.24	4887.83±15.23
	S6	490.86±4.12	775.16±20.12	2015.48±2.82	814.49±9.89	5438.83±5.65
	S7	1370.00±9.89	600.70±7.77	234.10±4.97	470.47±9.19	4544.43±7.07
	S8	4305.56±5.67	2642.71±10.78	1396.52±8.98	14000.00±16.34	11157.00±8.48
	S9	366.78±7.77	189.22±6.36	196.99±4.24	447.01±4.94	1163.46±6.36
BPC (%)	S1	0.16±0.03	0.25±0.03	0.94±0.04	0.57±0.04	0.57±0.03
	S2	0.06±0.01	0.08±0.01	0.12±0.02	0.39±0.07	0.55±0.04
	S3	0.37±0.03	0.08±0.03	0.19±0.03	0.38±0.12	0.45±0.05
	S4	0.16±0.02	0.06±0.04	0.46±0.04	0.27±0.11	0.43±0.12
	S5	0.27±0.05	0.22±0.10	0.31±0.06	0.29±0.07	0.48±0.17
	S6	0.10±0.02	0.21±0.05	0.29±0.10	0.38±0.03	0.48±0.20
	S7	0.09±0.03	0.25±0.04	0.06±0.02	0.14±0.04	0.40±0.15
	S8	0.52±0.02	0.71±0.11	0.21±0.02	1.08±0.21	1.13±0.03
	S9	0.09±0.03	0.06±0.01	0.02±0.01	0.09±0.02	0.09±0.01
TL (mg/kg)	S1	2025.74±9.54	3957.45±12.31	3326.19±10.32	4207.59±5.48	3859.45±10.54
	S2	644.63±6.75	1015.05±15.58	792.57±6.47	1666.87±8.73	917.05±9.93
	S3	495.26±9.92	572.56±6.98	341.58±4.81	1140.99±7.45	474.56±8.48
	S4	126.26±8.83	113.54±7.96	244.81±9.48	635.67±5.63	145.43±8.94
	S5	236.00±7.89	772.50±8.93	504.79±10.51	660.93±6.19	674.50±7.91
	S6	199.37±8.94	938.31±6.83	802.65±9.71	1244.62±5.92	840.31±9.65
	S7	254.51±7.48	1294.99±7.54	804.26±8.94	844.90±5.39	1196.99±9.47
	S8	2648.60±8.95	3668.89±6.73	2175.57±6.59	3126.35±6.97	3570.89±8.49
	S9	547.56±7.93	195.42±8.87	496.83±5.63	839.19±7.39	97.42±1.25

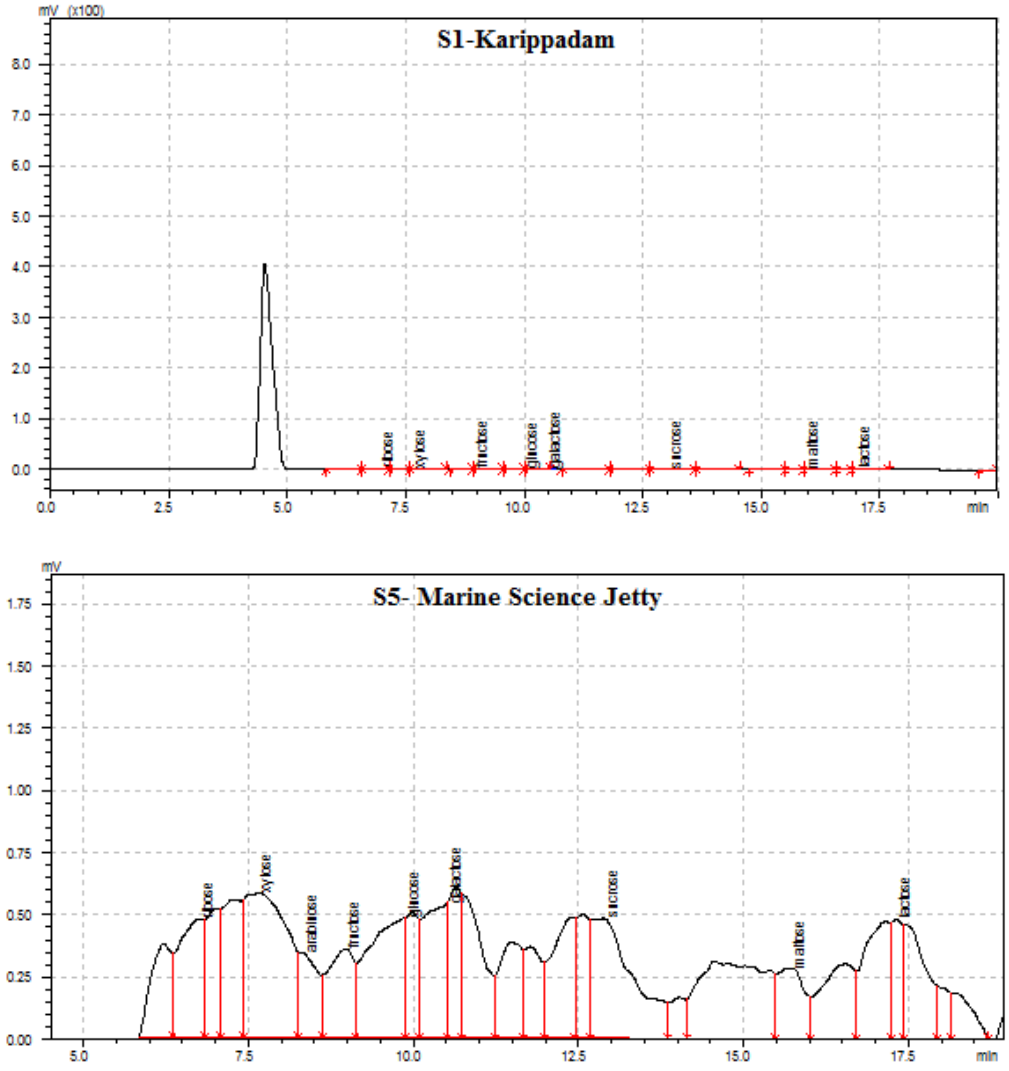
Appendix 1.2: Seasonal and spatial distribution of Chlorophyll pigments (µg/kg) present in the study area

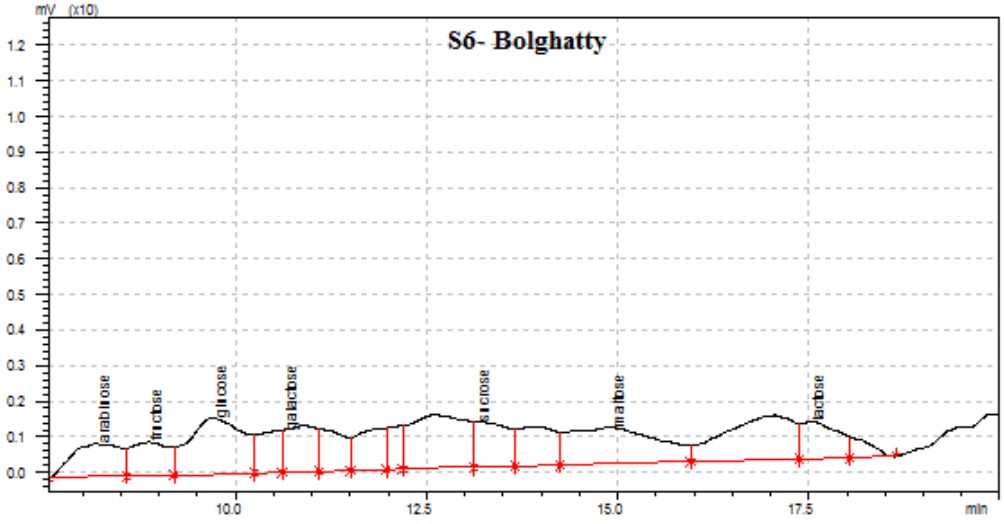
Parameters	Stations	PRM09	MON09	POM09	PRM10	MON12
Chl-a	S1	4.11±0.23	8.59±0.32	8.85±0.65	7.17±0.42	7.81±0.93
	S2	2.18±0.32	5.80±0.42	4.74±0.52	12.98±0.44	5.02±0.25
	S3	15.49±0.58	5.87±0.35	5.89±0.43	16.54±0.53	5.09±0.17
	S4	3.35±0.12	3.77±0.31	16.89±1.34	11.97±0.58	2.99±0.13
	S5	12.17±0.22	15.14±1.02	13.88±0.17	13.21±0.63	14.36±0.47
	S6	10.86±0.27	9.04±0.33	11.96±0.33	10.82±1.02	8.26±0.53
	S7	2.08±0.29	3.01±0.12	3.54±0.11	2.68±0.78	2.23±0.17
	S8	9.63±0.31	16.44±1.11	12.76±0.27	6.75±0.32	15.66±1.13
	S9	1.42±0.41	0.37±0.03	0.59±0.36	0.74±0.11	0.41±0.04
Chl-b	S1	2.14±0.21	3.49±0.32	3.29±0.22	2.99±0.28	3.04±0.72
	S2	1.20±0.11	2.16±0.15	2.40±0.18	5.47±0.17	1.71±0.25
	S3	5.77±0.17	2.39±0.21	3.30±0.31	6.72±0.31	1.94±0.16
	S4	2.26±0.25	2.00±0.16	7.34±0.43	4.81±0.33	1.55±0.11
	S5	5.73±0.15	7.01±0.24	6.60±0.23	4.83±0.21	6.56±0.31
	S6	6.02±0.22	4.28±0.31	6.55±0.17	5.59±0.43	3.83±0.18
	S7	1.23±0.31	1.58±0.17	2.32±0.14	1.89±0.15	1.13±0.08
	S8	5.49±0.56	6.60±0.24	5.83±0.22	3.02±0.31	6.15±0.43
	S9	0.87±0.18	0.24±0.06	0.75±0.04	0.54±0.22	0.23±0.01
Chl-c	S1	2.70±0.13	4.45±0.43	3.92±0.37	2.91±0.21	3.96±0.22
	S2	1.30±0.07	2.66±0.21	2.46±0.18	6.23±0.32	2.17±0.18
	S3	5.81±0.55	2.36±0.32	3.50±0.26	7.04±0.37	1.87±0.24
	S4	3.66±0.16	2.74±0.27	7.10±0.61	4.74±0.33	2.25±0.43
	S5	5.86±0.34	6.92±0.18	6.73±0.33	4.57±0.35	6.43±0.41
	S6	6.49±0.26	4.21±0.13	6.87±0.38	6.68±0.22	3.72±0.14
	S7	1.64±0.14	1.85±0.21	2.99±0.39	2.70±0.06	1.36±.12
	S8	5.77±0.28	6.58±0.24	6.42±0.17	4.05±0.26	6.09±0.51
	S9	1.03±0.22	0.23±0.07	0.89±0.03	0.51±0.31	0.22±0.05
Phe	S1	7.05±0.23	14.39±1.33	14.49±0.27	12.86±1.18	13.61±1.22
	S2	3.35±0.15	9.83±0.55	9.72±0.21	21.03±1.55	9.05±0.63
	S3	28.18±0.27	9.51±0.41	28.98±2.18	28.28±2.33	8.73±0.48
	S4	6.29±0.33	5.38±0.36	28.28±0.31	18.75±2.78	4.60±0.36
	S5	21.22±1.27	25.38±0.37	21.47±0.34	22.34±2.18	24.60±0.18
	S6	21.59±2.17	15.21±0.44	22.71±0.32	18.13±2.64	14.43±1.11
	S7	3.82±0.14	5.38±0.35	6.95±0.16	4.68±2.35	4.60±0.18
	S8	15.13±0.22	26.18±0.27	22.02±0.18	13.30±3.18	25.40±0.33
	S9	1.75±0.34	1.33±0.31	1.75±0.23	2.21±0.33	0.55±0.02

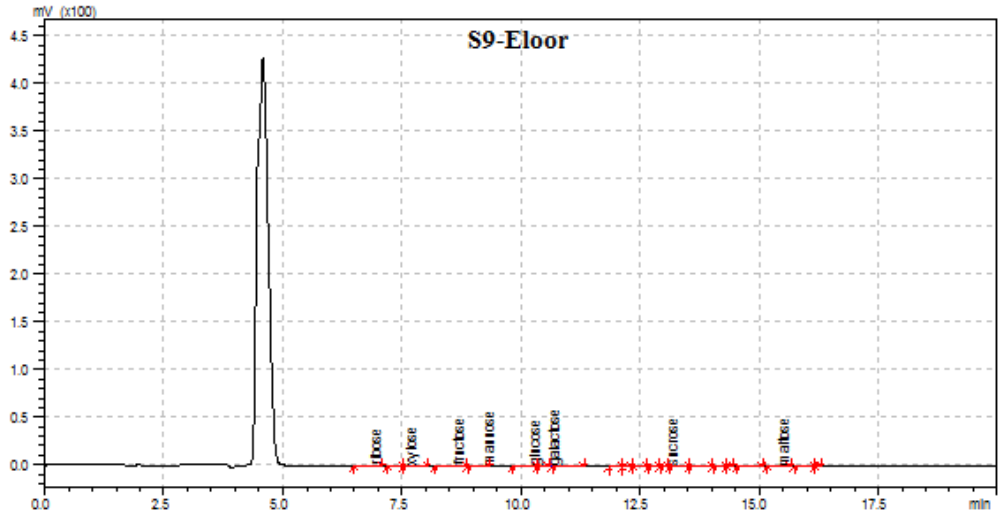
APPENDIX-2

Appendix 2.1 Chromatogram of free sugars in selected stations of Cochin estuary (season wise)

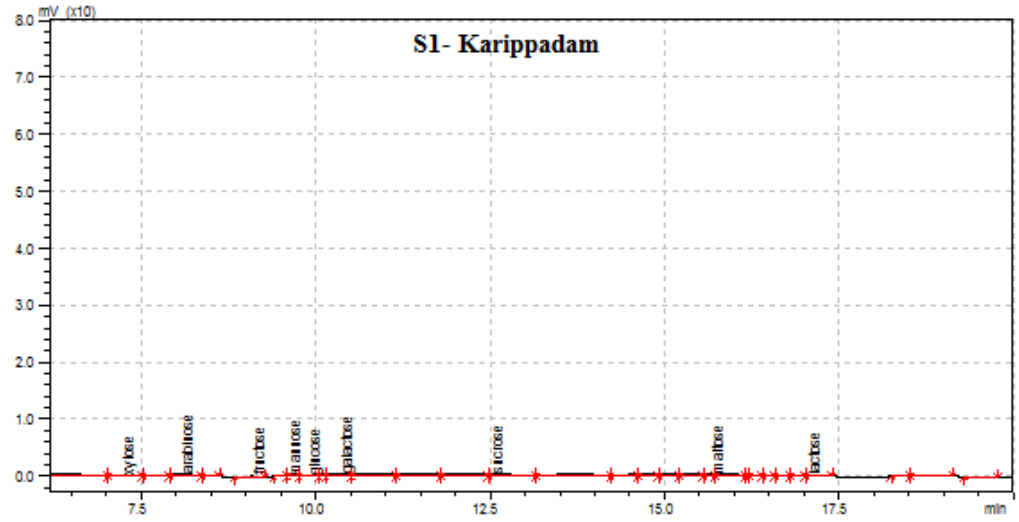
CHROMATOGRAM DURING PRM09

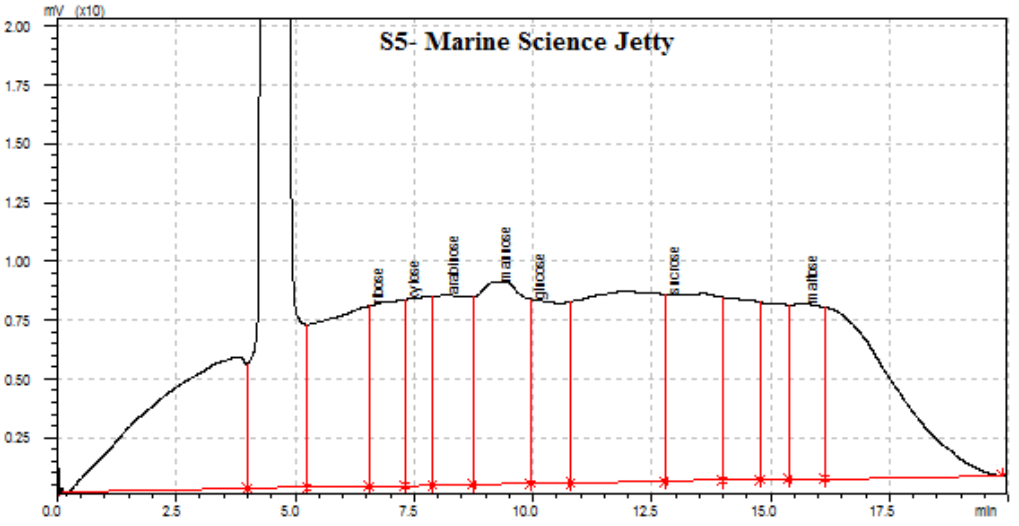


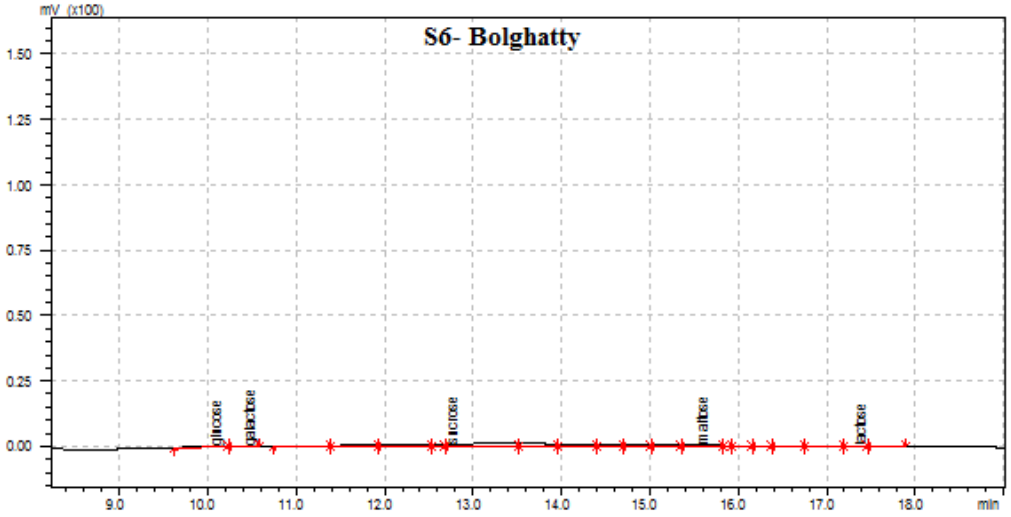

mV (x10)
S6- Bolghatty
arabinose
fructose
glucose
galactose
sucrose
maltose
lactose
min

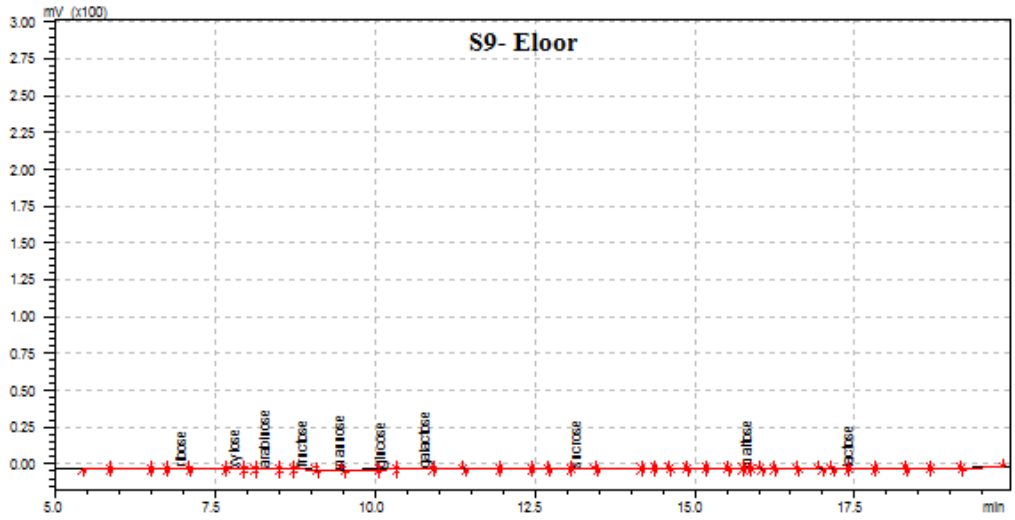

mV (x100)
S9-Eloor
xylose
fructose
mannose
glucose
galactose
sucrose
maltose
min

CHROMATOGRAM DURING MON09

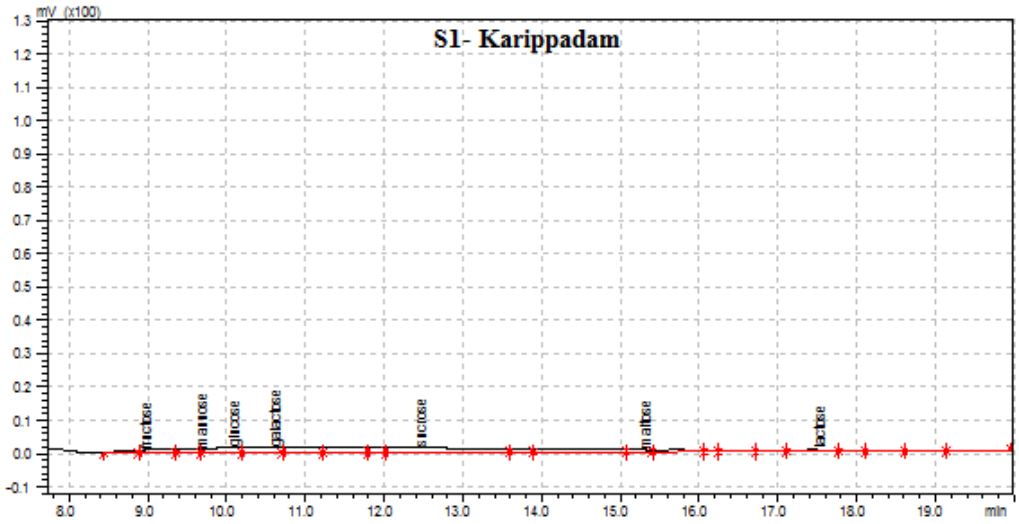


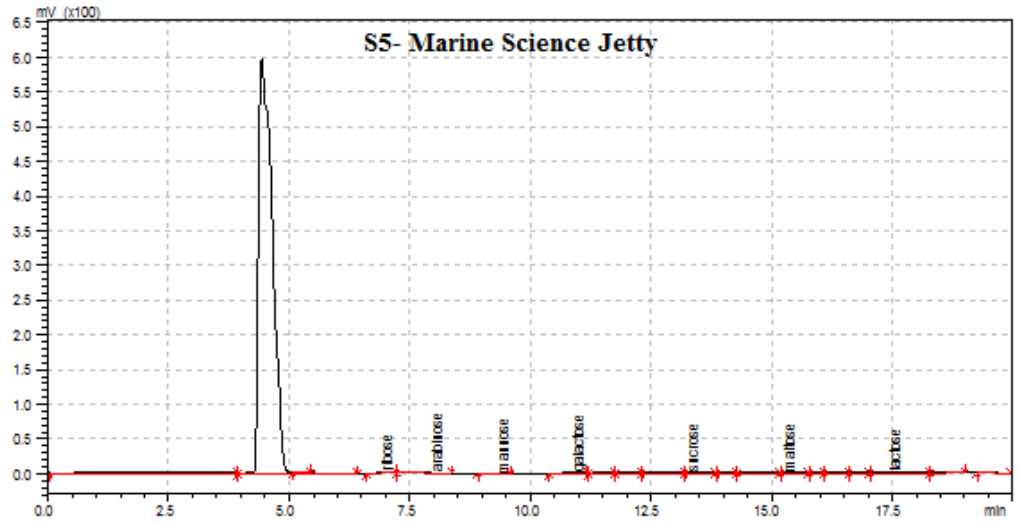


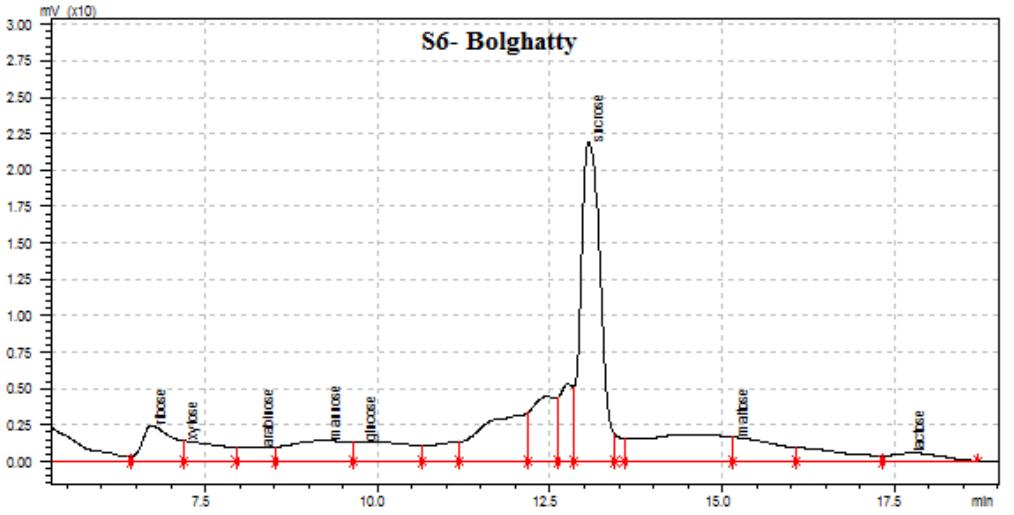

S6- Bolghatty
mV (x100)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
glucose
galactose
sucrose
maltose
lactose
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
min

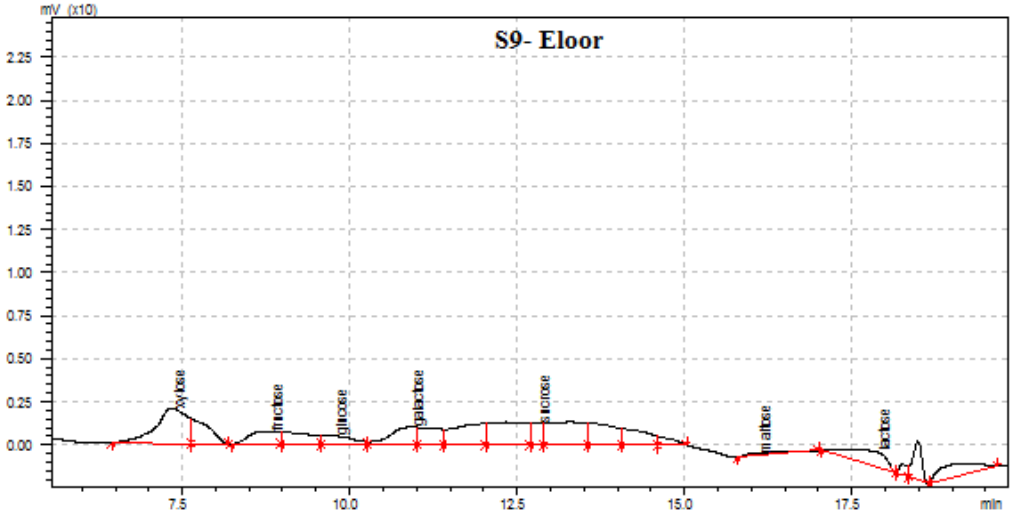

S9- Eloor
mV (x100)
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
ribose
xylose
arabinose
fructose
mannose
glucose
galactose
sucrose
maltose
lactose
5.0
7.5
10.0
12.5
15.0
17.5
min

CHROMATOGRAM DURING POM09

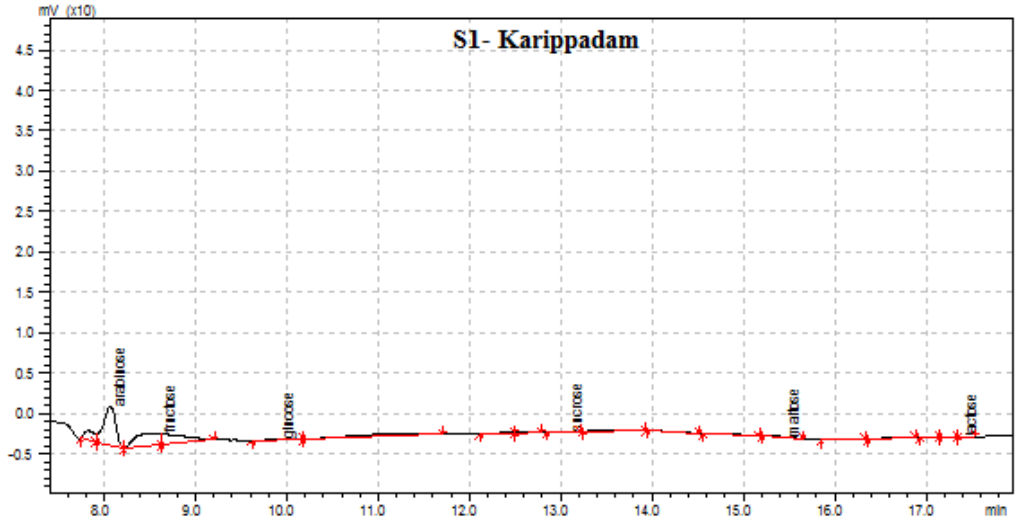


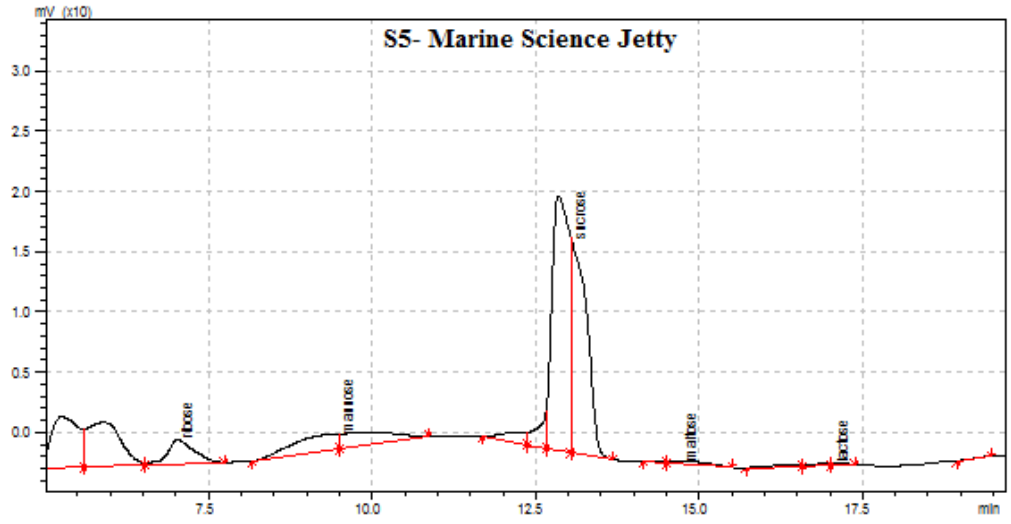


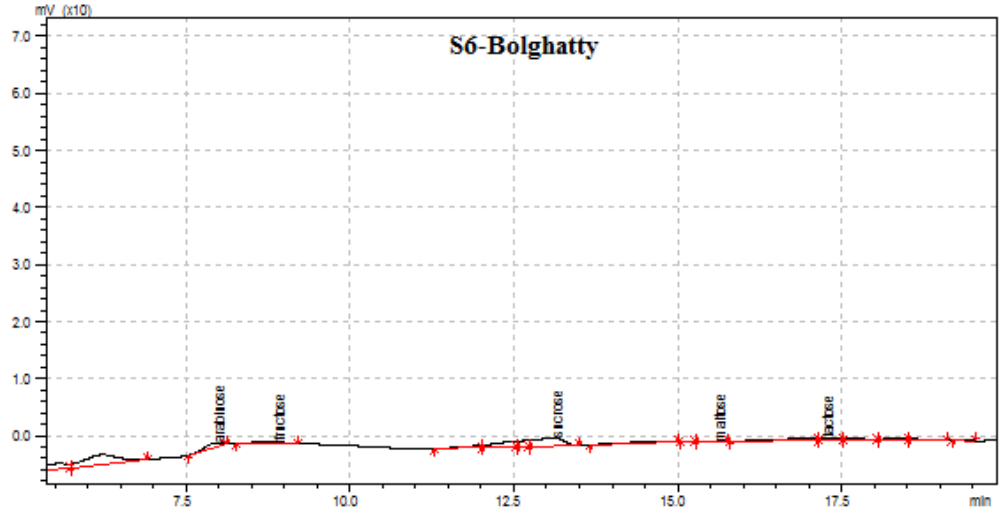

S6- Bolghatty
mV (x10)
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
ribose
xylose
arabinose
mannose
glucose
sucrose
maltose
lactose
7.5
10.0
12.5
15.0
17.5
min

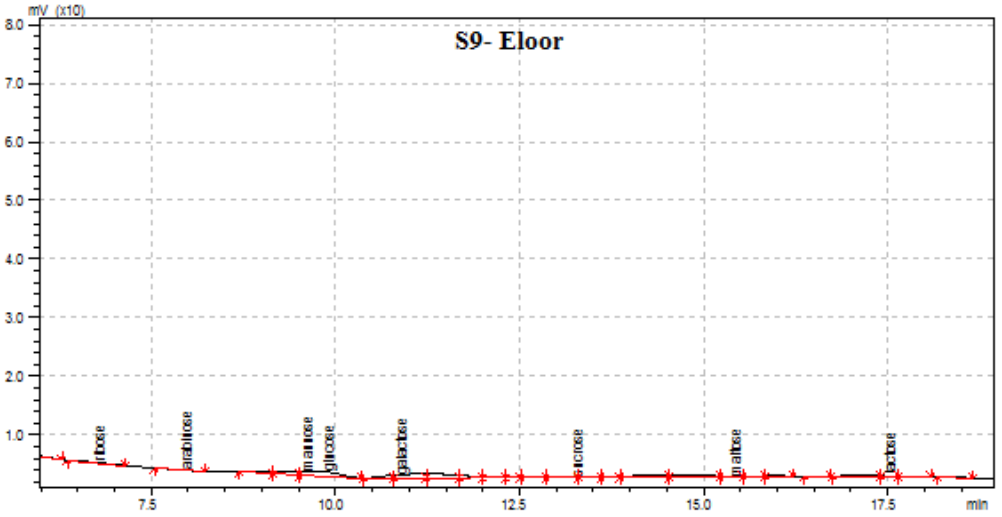

S9- Eloor
mV (x10)
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
xylose
fructose
glucose
galactose
sucrose
maltose
lactose
7.5
10.0
12.5
15.0
17.5
min

CHROMATOGRAM DURING PRM10

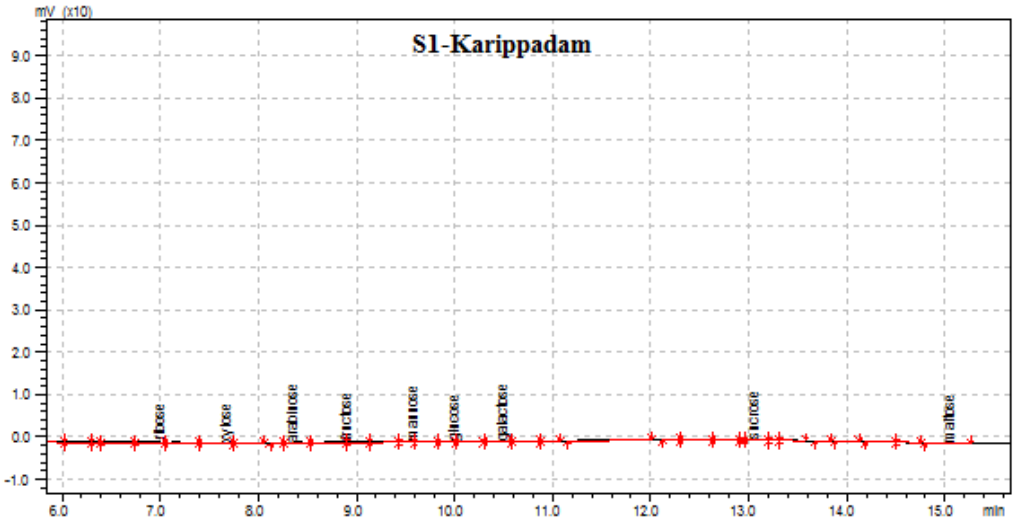


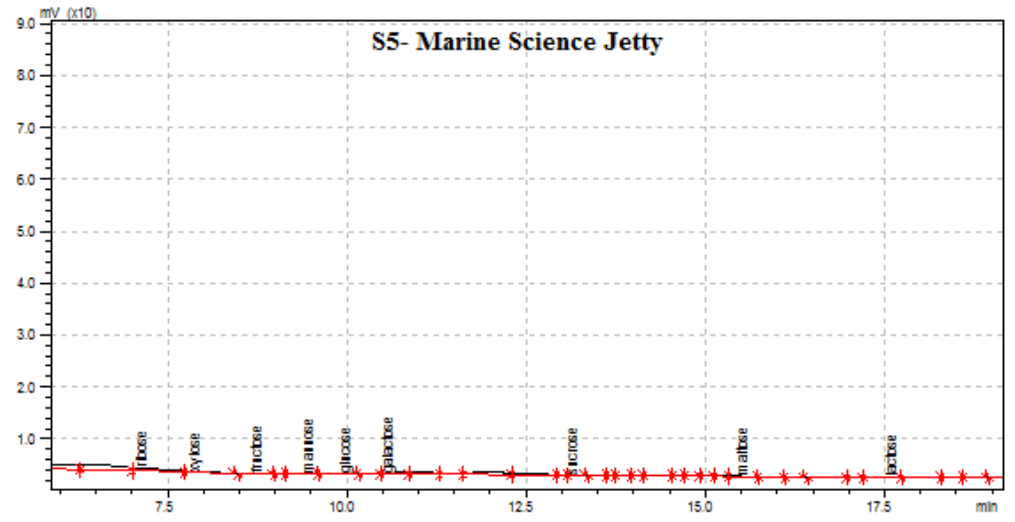


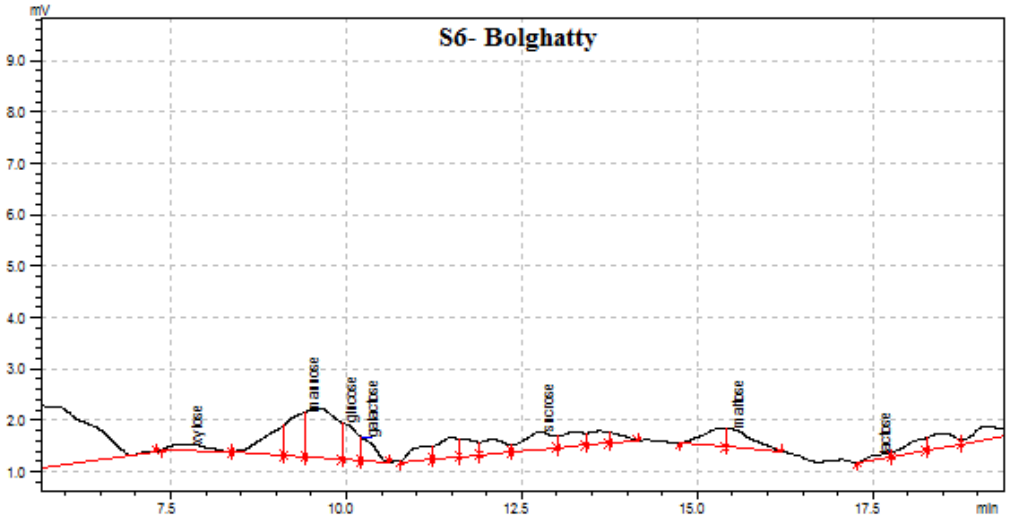

mV (x10)
S6-Bolghatty
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
arabinose
fructose
sucrose
maltose
lactose
7.5
10.0
12.5
15.0
17.5
min

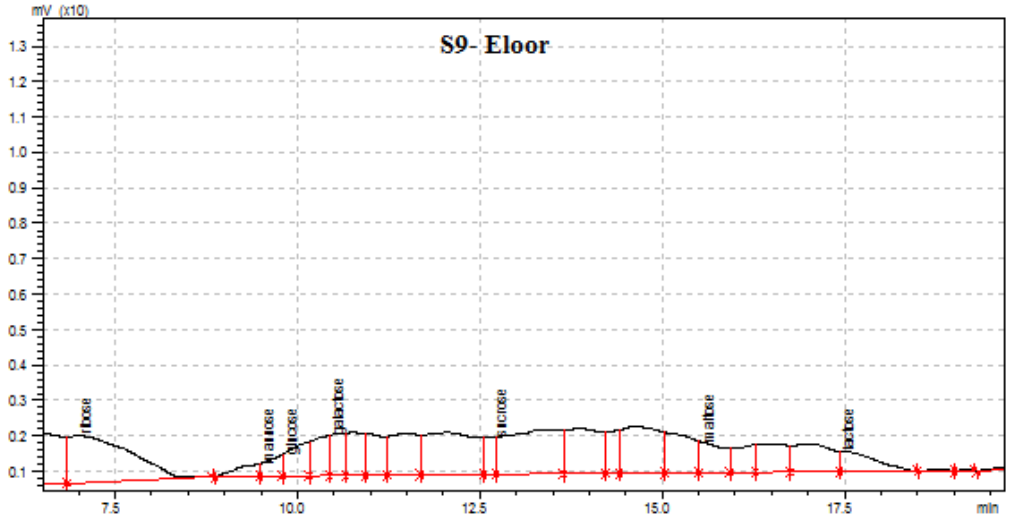

mV (x10)
S9- Eloor
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ribose
arabinose
mannose
glucose
galactose
sucrose
maltose
lactose
7.5
10.0
12.5
15.0
17.5
min

CHROMATOGRAM DURING MON12





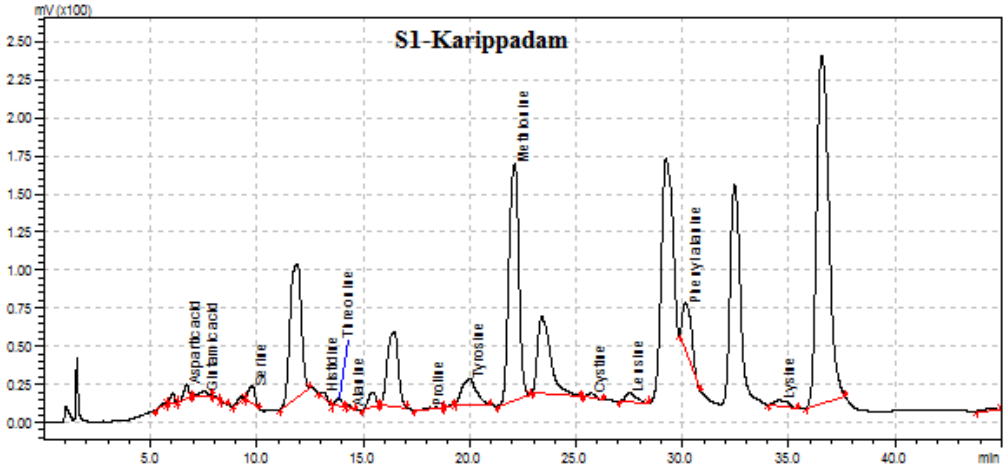

mV
S6- Bolghatty
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
xylose
mannose
glucose
galactose
sucrose
maltose
lactose
7.5
10.0
12.5
15.0
17.5
min

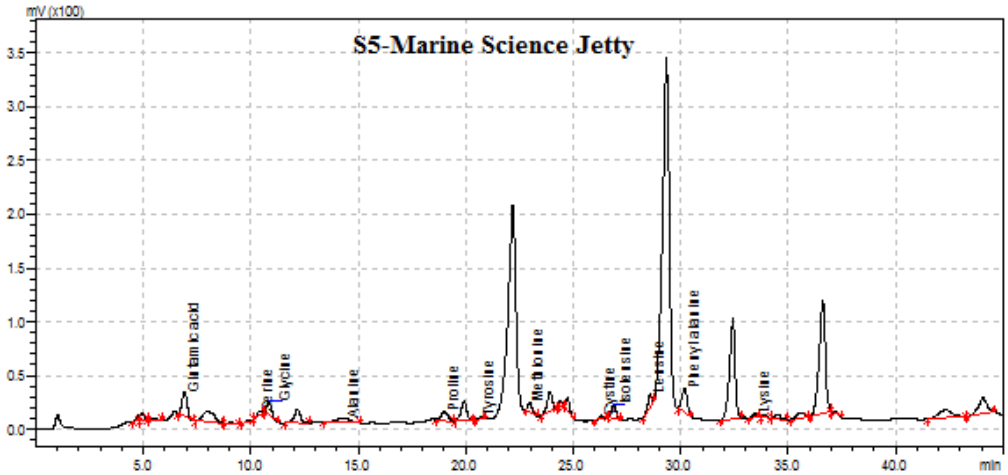

mV (x10)
S9- Eloor
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
xylose
mannose
glucose
galactose
sucrose
maltose
lactose
7.5
10.0
12.5
15.0
17.5
min

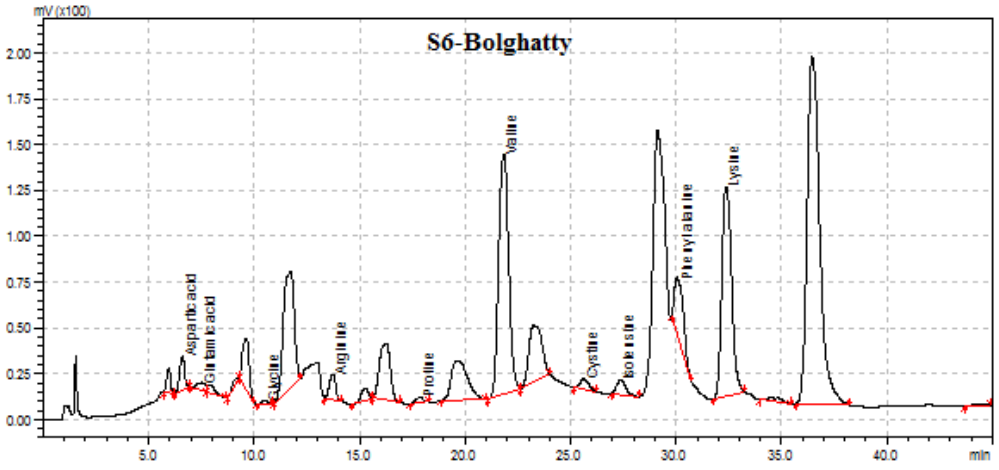
APPENDIX-3

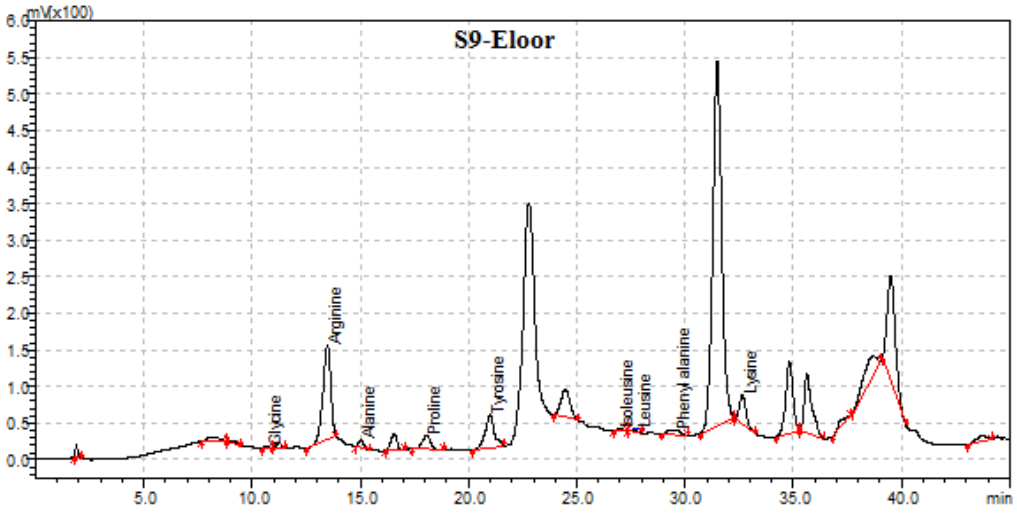
Appendix 3.1 Chromatogram of amino acids in selected stations of Cochin estuary (season wise)

CHROMATOGRAM DURING PRM09

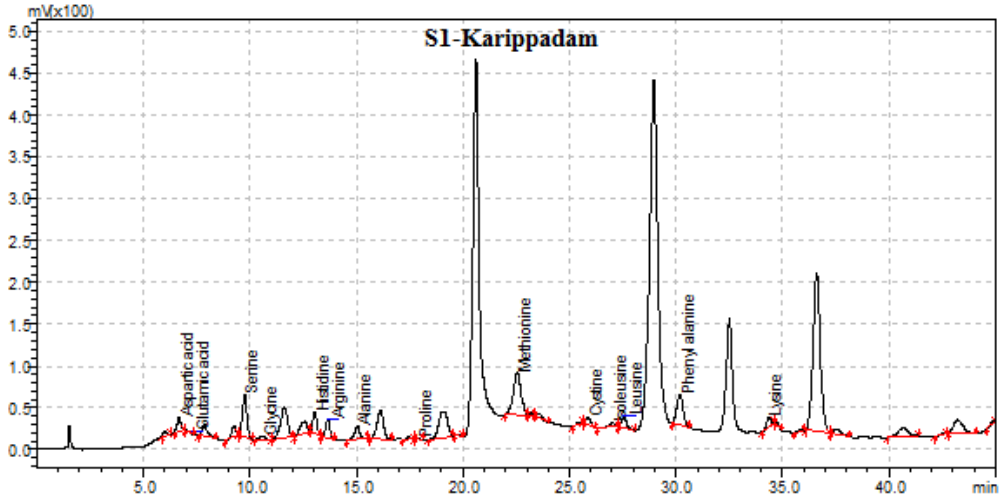


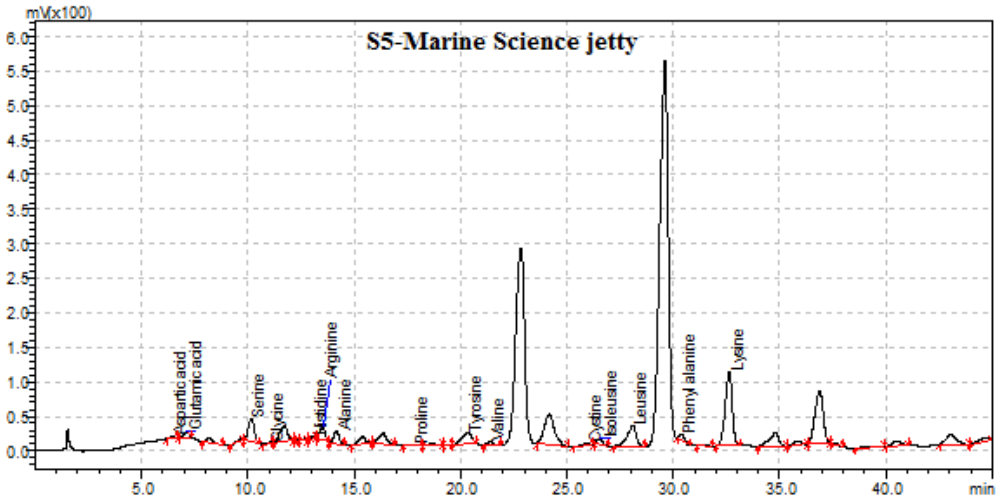


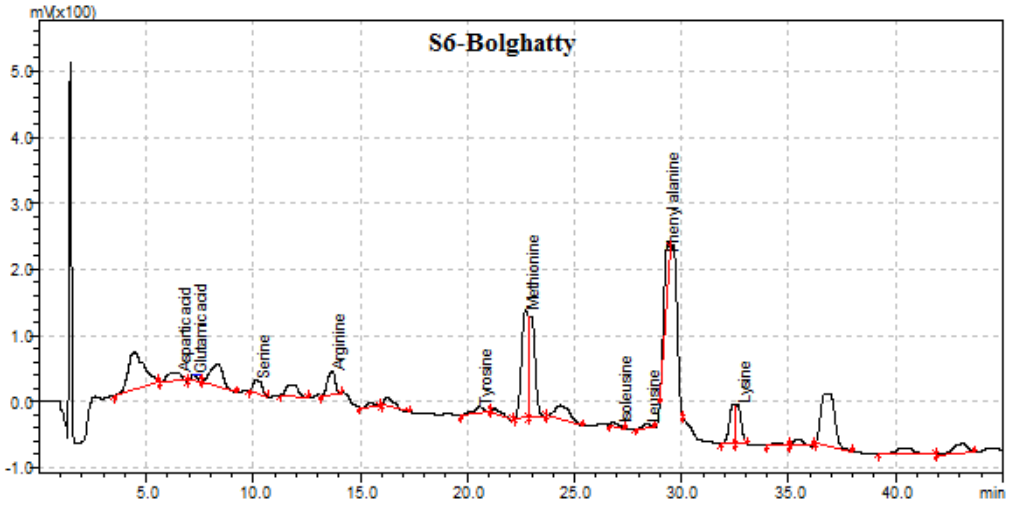

mV (x100)
S6-Bolghatty
Aspartic acid
Glutamic acid
Glycine
Arginine
Proline
Valine
Cystine
Isoleucine
Phenylalanine
Lysine
min

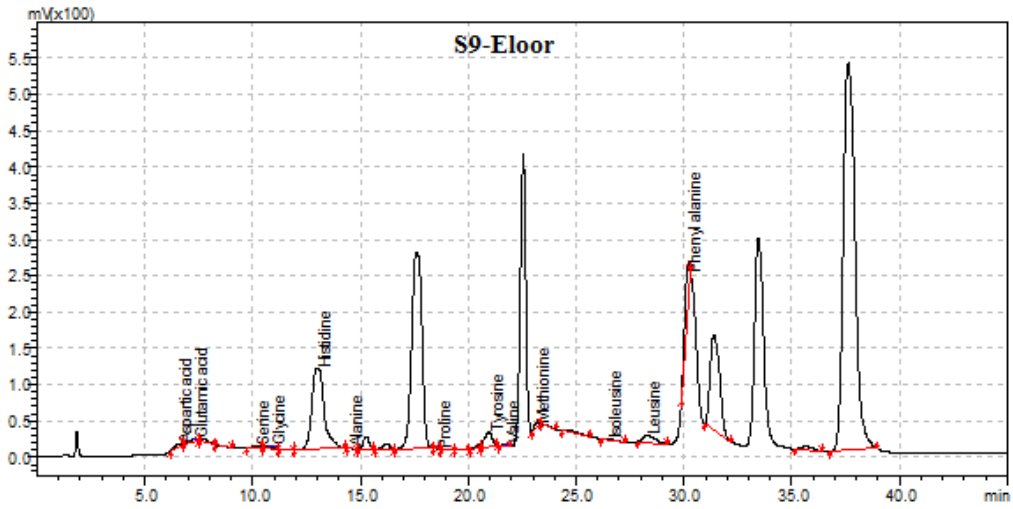

mV(x100)
S9-Eloor
Glycine
Arginine
Alanine
Proline
Tyrosine
Isoleucine
Leucine
Phenyl alanine
Lysine
min

CHROMATOGRAM DURING MON09

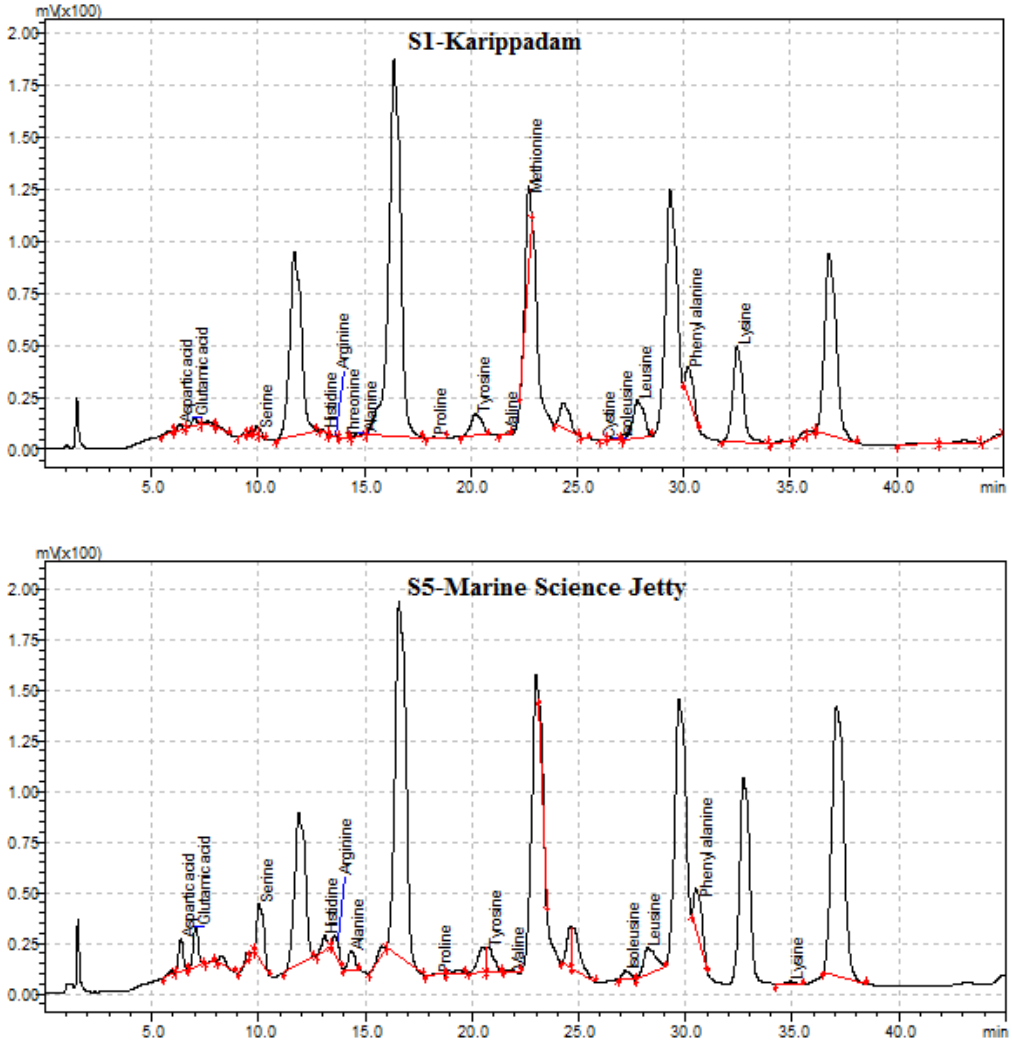


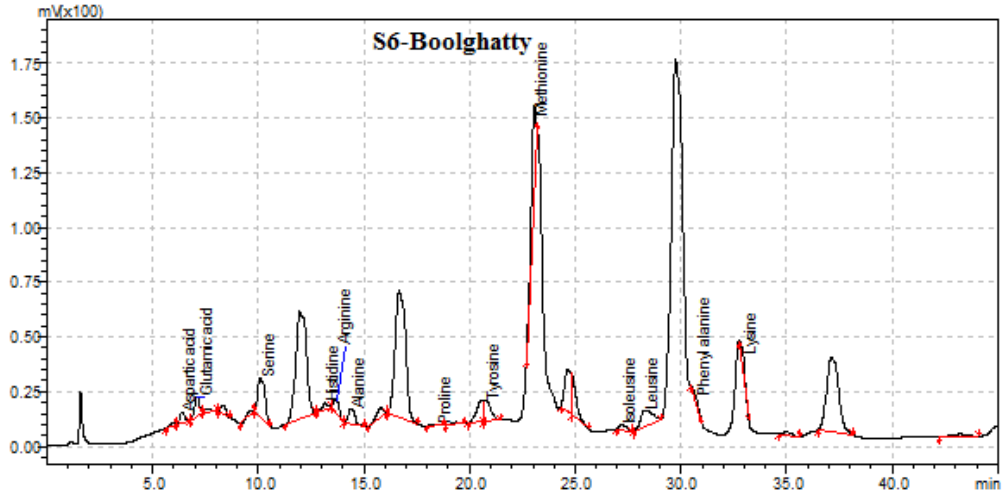


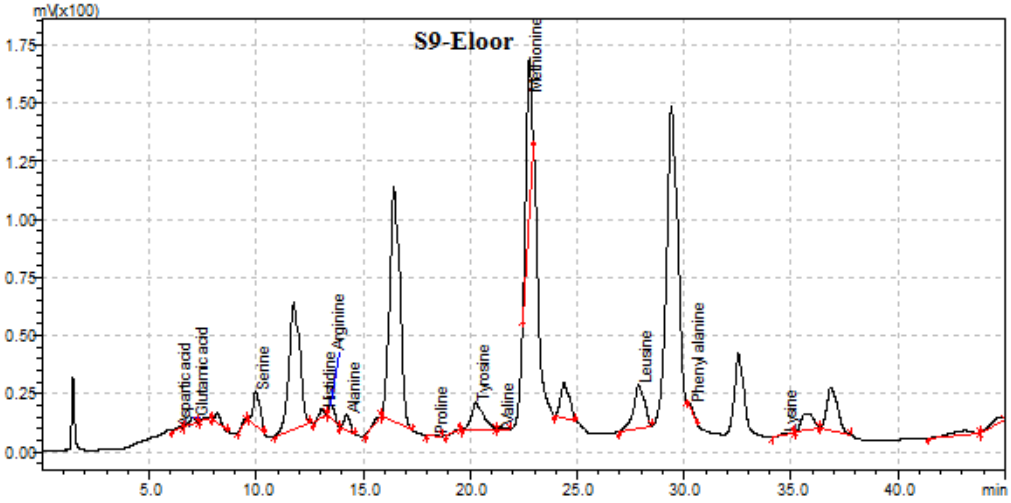

S6-Bolghatty
mV(x100)
Aspartic acid
Glutamic acid
Serine
Arginine
Tyrosine
Methionine
Isoleusine
Leusine
Phenyl alanine
Lysine
min


S9-Eloor
mV(x100)
Aspartic acid
Glutamic acid
Serine
Glycine
Histidine
Alanine
Proline
Tyrosine
Valine
Methionine
Isoleusine
Leusine
Phenyl alanine
min

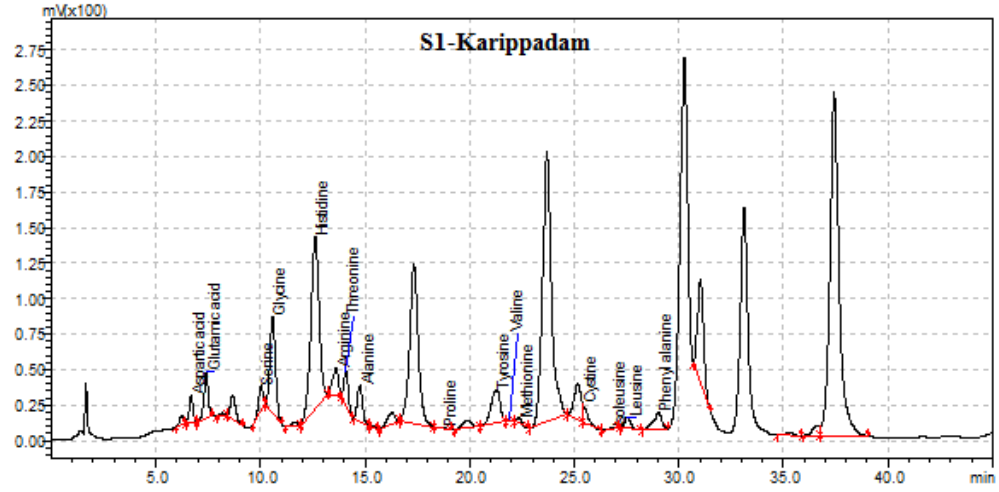
CHROMATOGRAM DURING POM09

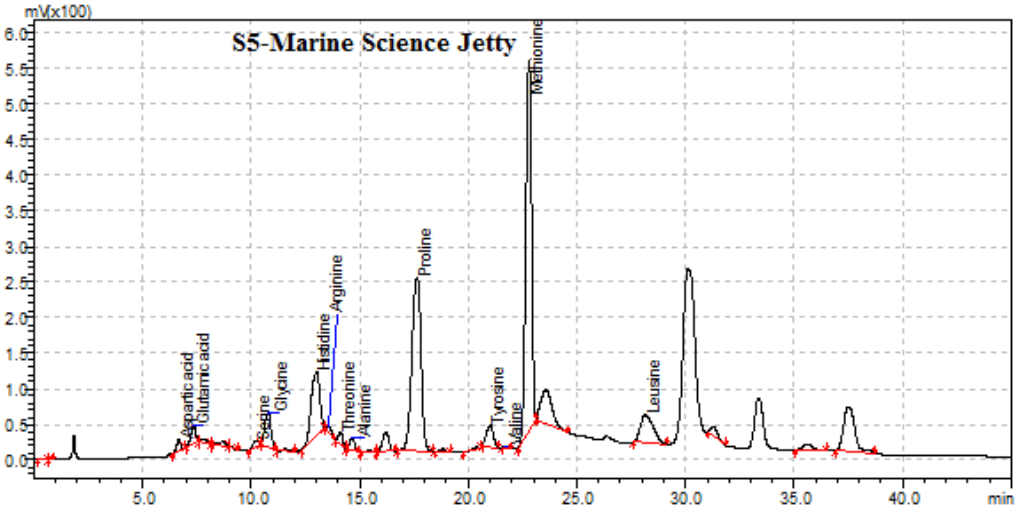


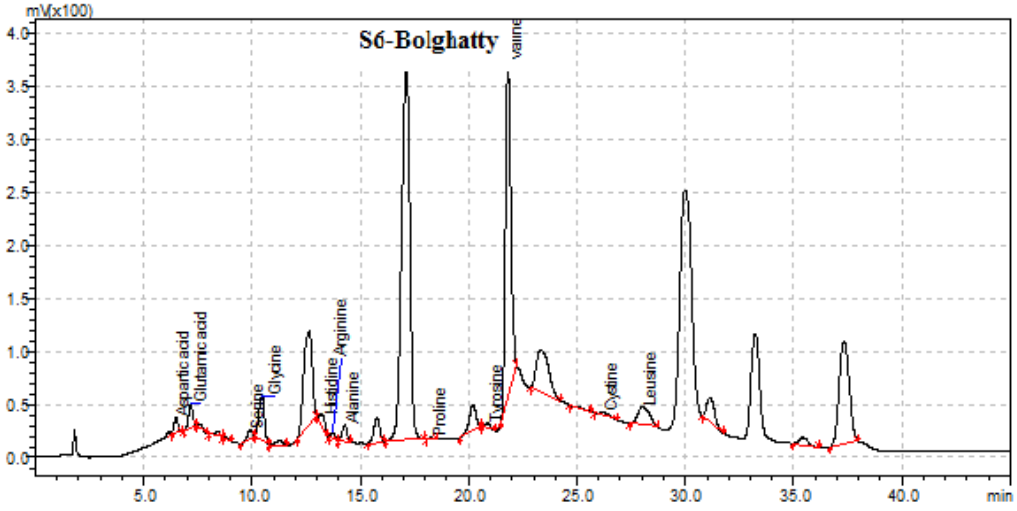

mV(x100)
S6-Boolghatty
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Aspartic acid
Glutamic acid
Serine
Histidine
Arginine
Alanine
Proline
Tyrosine
Methionine
Isoleusine
Leusine
Phenyl alanine
Lysine
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

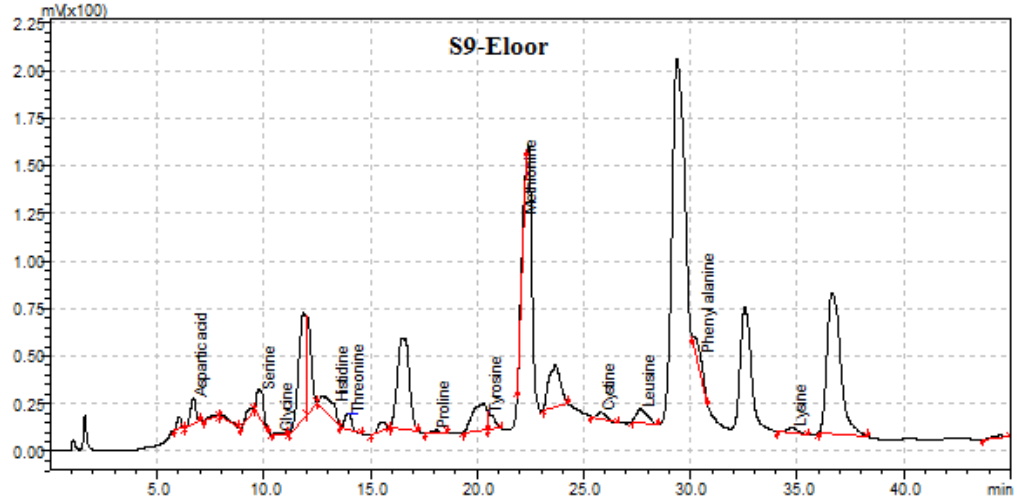

mV(x100)
S9-Eloor
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Aspartic acid
Glutamic acid
Serine
Histidine
Arginine
Alanine
Proline
Tyrosine
Valine
Methionine
Leusine
Phenyl alanine
Lysine
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

CHROMATOGRAM DURING PRM10

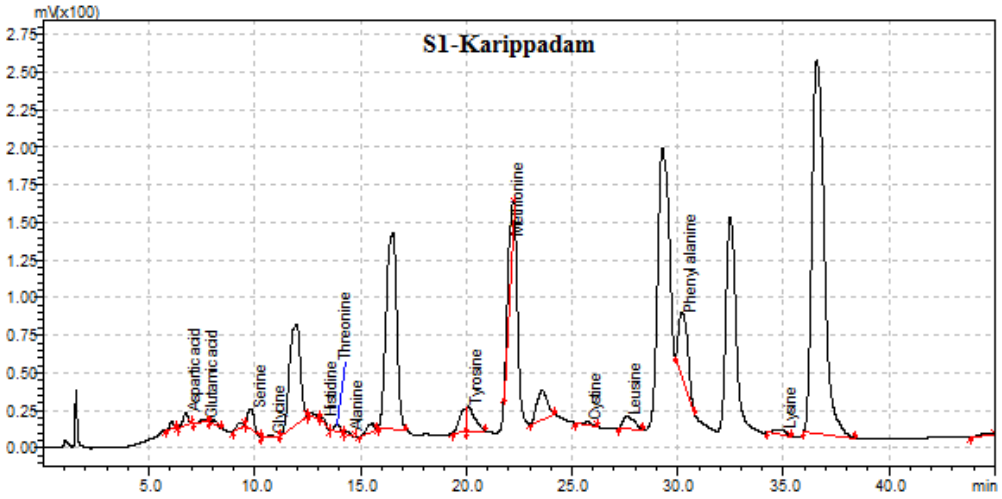


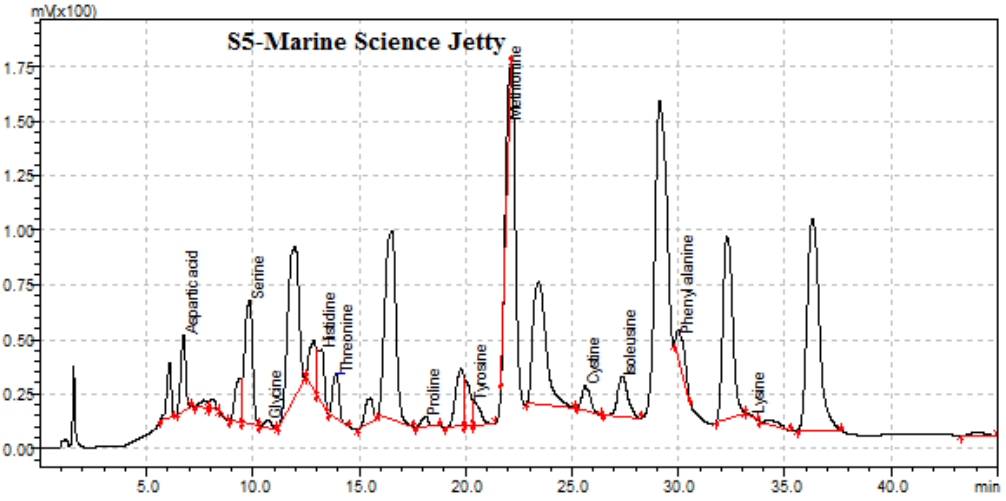


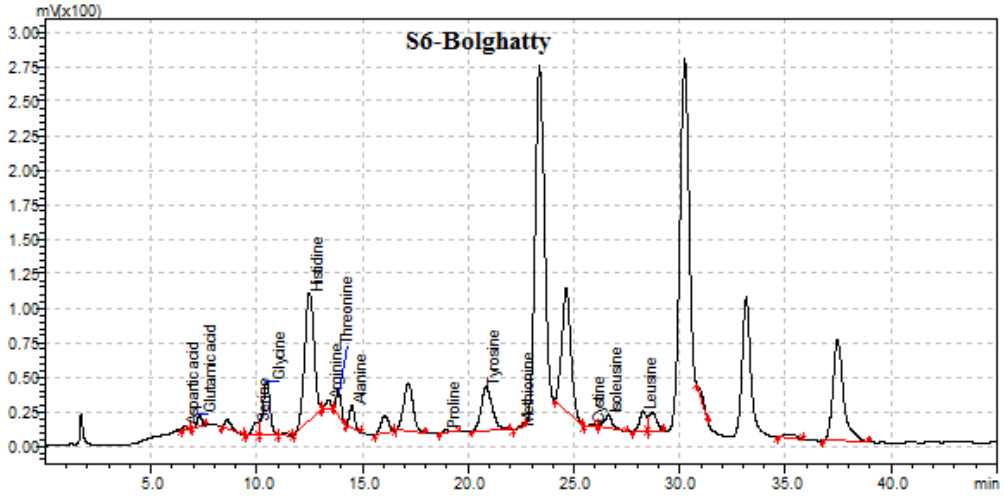

mV(x100)
S6-Bolghatty
Aspartic acid
Glutamic acid
Serine
Glycine
Histidine
Arginine
Alanine
Proline
Tyrosine
valine
Cystine
Leusine
min

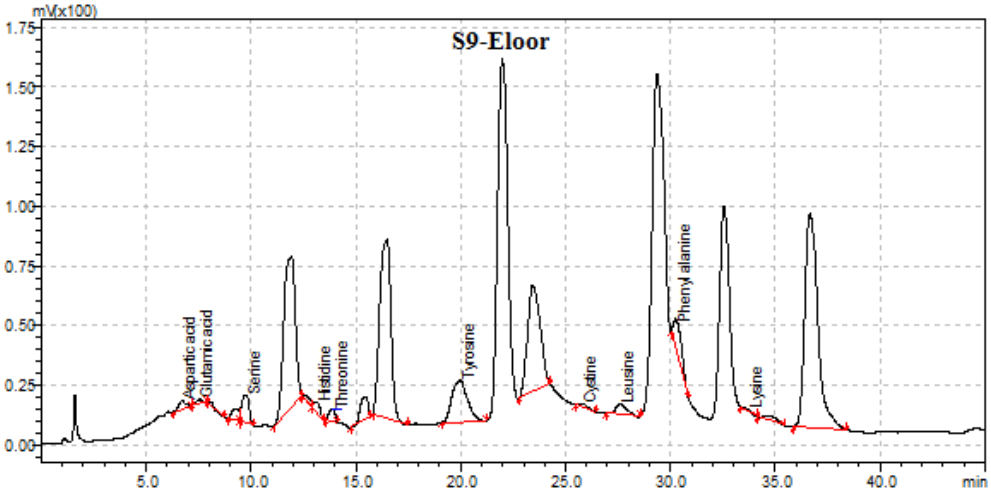

mV(x100)
S9-Eloor
Aspartic acid
Serine
Glycine
Histidine
Threonine
Proline
Tyrosine
Cystine
Leusine
Phenylalanine
Lysine
min

CHROMATOGRAM DURING MON12






mV(x100)
S6-Bolghatty
Aspartic acid
Glutamic acid
Serine
Glycine
Histidine
Arginine
Threonine
Alanine
Proline
Tyrosine
Methionine
Cystine
Isoleusine
Leusine
min


mV(x100)
S9-Eloor
Aspartic acid
Glutamic acid
Serine
Histidine
Threonine
Tyrosine
Cystine
Leusine
Phenyl alanine
Lysine
min