Characterization, Sources and Potential Risk Assessment of Aliphatic Hydrocarbons and Polycyclic Aromatic Hydrocarbons in Surface Sediments of Chitrapuzha River on the South West Coast of India

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Ph.D. Thesis under the Faculty of Marine Sciences

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Certificate

This is to certify that the thesis entitled "**Characterization, Sources and Potential Risk Assessment of Aliphatic Hydrocarbons and Polycyclic Aromatic Hydrocarbons in Surface Sediments of Chitrapuzha River on the South West Coast of India**" is an authentic record of the research work carried out by Mr. Sanil Kumar K. S under my supervision and guidance at the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Kochi-16, in partial fulfilment of the requirements for PhD degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any university. I further certify that all the relevant corrections and modifications suggested by the audience during the pre-synopsis seminar and recommended by the Doctoral Committee of the candidate have been incorporated in the thesis.

Kochi-682016
December, 2015

Dr. S.Muraleedharan Nair
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Declaration

I hereby declare that the thesis entitled "**Characterization, Sources and Potential Risk Assessment of Aliphatic Hydrocarbons and Polycyclic Aromatic Hydrocarbons in Surface Sediments of Chitrapuzha River on the South West Coast of India**" is an authentic record of the research work carried out by me under the guidance and supervision of Dr. S.Muraleedharan Nair, 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 an degree, diploma, associateship, fellowship or any other similar title or recognition.

Kochi -16 **Sanil Kumar K. S**

December, 2015

Dedicated to

My beloved mother……,

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Preface

Rivers are important vectors for the delivery of both organic and inorganic carbon to the coastal zone, thereby connecting the terrestrial carbon cycle to the marine carbon cycle. Terrestrial organic carbon, whose input to coastal margin sediments is mainly controlled by riverine sources, has been shown to represent a significant fraction of total organic carbon in coastal and estuarine sediments. During transportation, water and sediment undergo considerable changes in their physico-chemical properties depending on terrain characteristics and climate of the region through which the river flows. It is now well understood that river transport of particulates, nutrients and minerals plays a major role in maintaining the productivity of the coastal and the near shore environments. Thus, rivers and their estuaries provide connectivity between terrestrial and marine environments and also act as corridors for free movement of aquatic organisms among various sub-environments.

Unfortunately, extensive urbanization and industrialization consequent to the economic development in recent years have caused a worldwide increase in river input of geochemical constituents, especially nutrient elements to the coastal ecosystem leading to 'imposed eutrophication' incidences in many parts of the coastal areas. The situation is being complicated further by the massive discharge of toxic contaminants from point and non-point sources. Discharge of pollutants from urban, agricultural and industrial sources, indiscriminate mining, damming of rivers, inter-basin transfer of water, etc. have adversely affected the natural processes of these river systems. Moreover, these impacts of demographic changes clearly had detrimental effects on the overall biogeochemical cycling in estuarine sediments. Due to the aforementioned physical mixing and biological processes, the distribution and pathway of sedimentary organic matter in aquatic systems are rather complex.

A significant portion of organic matter sinks through the water column and subsequently deposited in sediments by the interaction of a series of physical, chemical and biological processes. The terrestrial organic matter represents a substantial fraction of organic matter in aquatic sediments and varies with production patterns, transport pathway and environmental conditions. The sediments are dominant sites for organic matter breakdown and nutrient regeneration in aquatic systems in which the sediment oxygen demand for organic matter degradation can induce hypoxic and anoxic conditions in bottom waters. The sediment oxygen demand and the quantities of nutrients regenerated is not only a simple function of the total quantity of organic matter, but also its quality. Hence, the study of spatial variability of sedimentary organic matter and its provenance, transport and degradation is important for understanding the contemporary global carbon cycle.

Basically, two general approaches are employed for studying the nature and sources of sedimentary organic matter in complex aquatic sediments. Organic geochemical studies employ a combination of bulk parameters and molecular biomarker techniques can effectively be utilised to assess the source, fate and seasonal variations of sedimentary organic matter. Like many rivers in the world, Chitrapuzha River is also under the severe threat of contaminants (both organic and inorganic compounds) of anthropogenic origin. The large and medium scale industries situated on the banks of the river have caused wide fluctuations in its biogeochemical characteristics and declined biodiversity of the aquatic environment. Therefore, a detailed investigation on the nature, concentration, distribution, spatio-temporal variations, as well as the contamination status of the sedimentary organic matter in Chitrapuzha River is highly relevant in the present scenario.

The present work is divided into 6 chapters:

Chapter **1- Introduction** provides basic information on the basic biogeochemical aspects of the riverine environment, methods of organic matter quality assessment and source characterization. The main objectives and scope of the study also furnished in this chapter.

The description of the study area and general methods adopted for the study are presented in Chapter **2 - Materials and methods**. It also illustrates the general hydrographical features of the study area.

The general sediment characteristics of the study area are described in Chapter **3 - General geochemical characteristics**. It also discusses the quality of organic matter using biochemical descriptors, and the preliminary source characterizations were carried out with the help of bulk indices (TOC/TN, $\delta^{13}C$ and $\delta^{15}N$). The relative fraction of terrestrial derived organic matter was calculated using two end member mixing.

Chapter **4 - Fatty acid composition as biomarkers** provides the spatial and seasonal distributions of the various straight chain, branched and unsaturated fatty acids, and the biomarkers data is applied to assess the natural sources of sedimentary organic matter.

Spatial and seasonal distributions of aliphatic hydrocarbons are explained in Chapter **5 - Aliphatic hydrocarbons**. Here an attempt is made to assess the biogenic and petrogenic sources of sedimentary organic matter.

Chapter **6 - Polycyclic aromatic hydrocarbons** characterize the organic matter in sediments in terms of PAHs and also describes the contamination status of the riverine environment. Sediment quality guidelines were used to make an initial assessment of toxicological significance of PAHs in surface sediments.

Finally, **Summary** of the results and the salient findings are presented with comprehensive explanations.

Appendix, and List of Publications are included at the end of the thesis.

Contents

List of Abbreviations

ACL	-	Average Chain Length
AHCs	-	Aiphatic Hydrocarbons
ANOVA	-	Analysis Of Variance
APHA	-	American Public Health Association
ATSDR	-	Agency for Toxic Substances and Disease Registry
BAFAs	-	Bacterial Fatty Acids
BDPP	-	Brahmapuram Diesel Power Plant
BFAs	-	Branched Fatty Acids
BPC	-	Bio Polymeric Carbon
BPCL	-	Bharat Petroleum Corporation Limited
CA	-	Cluster Analysis
Cchl-a	-	Algal Contribution to BPC
Chl-a	-	Chlorophyll-a
Chl-b	-	Chlorophyll-b
Chl-c	-	Chlorophyll-c
CHO	-	Total Carbohydrates
CPAH	-	Carcinogenic Polycyclic Aromatic Hydrocarbons
CPI	-	Carbon Preference Index
DO	-	Dissolved Oxygen
EPA	-	Environmental Protection Agency
ERL	-	Effects Range Low
ERM	-	Effects Range Median
EU	-	European Union
FACT	-	Fertilizers And Chemicals Travancore Limited
FAMEs	-	Fatty Acid Methyl Esters
FAs	-	Fatty Acids

GC-MS - Gas Chromatography-Mass Spectrometry

HCA - Hierarchical Cluster Analysis

HMW - High Molecular Weight

HOCL - Hindustan Organic Chemicals Limited

IARC - International Agency for Research on Cancer

ISQGs - Interim Sediment Quality Guidelines

IUPAC - International Union of Pure and Applied Chemistry

LCFAs - Long Chain Fatty Acids

LMW - Low Molecular Weight

LOM - Labile Organic Matter

LPD - Total Lipids

mg C/g - Milli gram Carbon per gram

mgg^{-1} - Milli gram per gram

MN - Monsoon

MUFAs - Monounsaturated Fatty Acids

ND - Not Detectable

ng/g - Nanogram per gram

NOAA - National Oceanic and Atmospheric Administration

OM - Organic Matter

PAHs - Polycyclic Aromatic Hydrocarbons

PCA - Principal Component Analysis

PCs - Principal Components

PEL - Probable Effect Level

Ph - Phytane

POM - Post-monsoon

PRM - Pre-monsoon

Pr - Pristane

PRT - Total Protein

PUFAs - Polyunsaturated Fatty Acids

SAFAs - Saturated Fatty Acids

SCFAs - Short Chain Fatty Acids

SPSS - Statistical Package for Social Sciences

SQGs - Sediment Quality Guidelines

TAR - Terrigenous/Aquatic Ratio

TC - Total Carbon

TEFi - Toxicity Equivalency Factor

TEL - Threshold Effect Levels

TFAs - Total Fatty Acids

TN - Total Nitrogen

TOC - Total Organic Carbon

TP - Total Phosphorus

T_rOM - Terrestrial Organic Matter

TS - Total Sulphur

UCM - Unresolved Complex Mixture

USEPA - U.S. Environmental Protection Agency

μgg^{-1} - Microgram per gram

Chapter 1

INTRODUCTION

Contents

1.1 General introduction

Estuaries are semi-enclosed coastal bodies of water, located at the interface between land and ocean, where seawater is measurably diluted by the inflow of fresh water (Pritchard 1967; Attrill and Rundle 2002; Wolanski 2007). The mixing of fresh water and sea water in the estuarine systems creates strong gradients of salinity, turbidity and concentration of bioactive elements, while, at the same time, a number of biological processes also acts at different time scales. Coastal waters, including estuaries and near shore oceanic environments, are among the most highly productive areas in the world. However, several man made changes have occurred in this sensitive ecological system in recent years, which affect the general hydrographic parameters of the estuary. Over the past three decades, there has been a rapid increase in human civilization along the coastal regions, because of the favourable features such as profuse vegetation, productive soil, access to navigational amenities, etc. Today, 61% of the world population is residing along the coastal outskirts (Alongi 1998) and it is estimated to be 75% by the end of 2025 (Hinrichsen 1998). Exploitation of marine environment for food, minerals, chemicals, recreational activities and waste disposal led to constant pollution of the aquatic environment by anthropogenic inputs. The word pollution generally refers to virtually any substance or energy released into the environment by human activities, which are detrimental to mankind or ecosystem. Human activities to improve the standard of living, have led to the introduction of many hazardous, non degradable chemicals into the aquatic ecosystem, which have attracted serious concern of the environmentalists. Similarly, deposition of harmful industrial by-products and contaminants into the surface waters poses serious problems to the environment. Organic effluents such as domestic sewages are a serious problem and the discharge of small quantities of sewages into the aquatic systems can actually increase the productivity of ecosystems, however, excessive quantities will deplete oxygen content, causing severe threat to aquatic life. As a result, the presence of organic and inorganic pollutants in aquatic system continues to be one of the most important environmental issues of the world today.

Rivers are important vectors for the delivery of both organic and inorganic carbon to the coastal zone, thereby connecting the terrestrial carbon cycle to the marine carbon cycle. Rivers carry materials derived from its catchments to estuaries. Terrestrial organic carbon, whose input to coastal margin sediments is mainly controlled by riverine sources (Hedges et al. 1997; Cole et al. 2006), has been shown to represent a significant fraction of total organic carbon (TOC) in coastal and estuarine sediments (Hedges and Parker 1976; Hedges et al. 1988; Prahl et al. 1994; Raymond and Bauer 2001). During transportation, water and sediments undergo considerable changes in their physico-chemical properties depending on terrain characteristics and climate of the region through which the river flows (Gibbs 1977; Lal 1977; Subramanian 1979; Walling 1999; Somayajulu et al. 2002; Ankers et al. 2003; Turner and

Rabalais 2004). River transport of particulates, nutrients and minerals plays a major role in maintaining the productivity of the coastal and the nearshore environments. Thus, rivers and their estuaries provide connectivity between terrestrial and marine environments and also act as corridors for free movement of aquatic organisms among various sub-environments. But unfortunately, extensive urbanisation and industrialisation consequent to the economic development in recent years have caused a worldwide increase in river input of geochemical constituents, especially nutrient elements of the coastal ecosystem leading to 'imposed eutrophication' incidences in many parts of the coastal areas. The situation is being complicated further by the massive discharge of toxic pollutants from point and non-point sources. Discharge of pollutants from urban, agricultural and industrial sources, indiscriminate mining, damming of rivers, inter-basin transfer of water, etc. have adversely affected the natural processes of these river systems. Moreover, riverine delivery of organic matter and nutrients has potential implications on the global carbon cycle, because remineralisation and the burial of carbon in coastal sediments influence the ocean’s ability to sequester atmospheric carbon dioxide. Moreover, changes to the global carbon cycle have captured the attention of the general public and researchers because of the possible climate changes due to increased emissions of greenhouse gases. However, the fate of organic matter to the coastal zone is challenging due to variable inputs from the adjacent continents, sorption-desorption phenomena’s (Hedges and Keil 1995) and the various factors controlling organic matter preservation (Wakeham and Canuel 2005).

1.2 Major sources of sedimentary organic matter

Photosynthetic fixation of inorganic carbon and nutrients into plant biomass is the primary source of sedimentary organic matter in aquatic systems (Lucas and Cloern 2002). Therefore, it is critical to have some basic understanding about the primary producer community in aquatic systems, in addition to the habitats and conditional constraints needed for growth. Inputs of dissolved organic matter, particulate organic matter and nutrients to sediments can occur from both coastal ocean and riverine end-members, which support both autotrophic and heterotrophic production in different aquatic regions. Resuspension and diffusive flux from sediments can also provide significant inputs of dissolved organic materials to support production, via benthic-pelagic coupling, particularly in shallow aquatic systems. The conversion of dissolved materials by heterotrophs and autotrophs is a crucial component of the “microbial loop” and it is critically linked to sedimentation processes such as, decomposition, aggregation and flocculation, which occur over different timescales in estuaries. Finally, the consumption and transformation of organic matter through metazoan and microbial (e.g., microbial loop) trophic levels are critical in the cycling of sedimentary organic matter (Wetzel et al. 1995).

Phytoplankton represents an important source of sedimentary organic matter to most of the aquatic regions. The dominant classes of phytoplankton include bacillariophyta (diatoms), cryptophyta (cryptomonads), chlorophyta (green algae), dinophyta (dinoflagellates), chrysophyta and cyanobacteria. Seasonal changes in phytoplankton abundance and the compositions are primarily controlled by changes in riverine inputs, nutrients, tidal variability, algal respiration, light availability and consumption by grazers (Boyer et al. 1993; Cloern 1996; O’Donohue and Dennison 1997; Thompson 1998; Lucas and Cloern 2002). Moreover, temperature has also been shown to influence phytoplankton growth in laboratory conditions (Eppley 1972; O’Donohue and Dennison 1997). Finally, it is well documented that river-derived nutrients are important in controlling phytoplankton abundance and composition (Oviatt et al. 1989; Hickey and Banas 2003; Riedel et al. 2003).

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; Sullivan and Moncreiff 1990; Pinckney and Zingmark 1993; de Jonge and Colijn 1994). Some benthic macroalgae commonly found in estuaries include, chlorophyta, phaeophyta and rhodophyta. The increase in anthropogenic loading of nutrients has resulted in numerous macroalgal blooms consisting primarily of the genera ulva, entermorpha, and gracilaria spp. (Rosenberg and Ramus 1984; Duarte 1995; Kamer et al. 2001). There is increasing evidence that in shallow environments, macroalgae and microphytobenthos are equally as important as phytoplankton in terms of total biomass and production (Cahoon 1999; Webster et al. 2002; Dalsgaard 2003). Even in the deepest coastal waters, it has been proved that the benthic algal production supports a significant fraction of the sedimentary organic matter on the shelf (Marinelli et al. 1998; Nelson et al. 1999). In addition to being a carbon source for benthic and pelagic organisms, microphytobenthos are an important sink for nutrients that are fluxing across the sediment-water interface produced from remineralized organic matter.

Aquatic sedimentary organic matter consists of a diverse range of allochthonous and autochthonous sources (Cauwet 2002; Findlay and Sinasbaugh 2003; Sinsabaugh and Findlay 2003). These major sources, primarily comprise of riverine inputs, autochthonous production from algal and vascular plant sources, benthic fluxes, groundwater inputs, and exchange with adjacent coastal systems. In estuaries, where the input from riverine sources is highly refractory and terrestrial dissolved organic matter dominates the bulk pool of organic matter (minor inputs from algal sources) conservative behaviour is more likely to occur (Kohler et al. 2003; Jaffe et al. 2004). Similarly, dissolved organic matter is associated with humic substance, a complex assemblage of organic molecules, which are derived from plants and soils (Hatcher et al. 2001), and it represents a large fraction of chromophoric dissolved organic matter (CDOM) in the aquatic systems (McKnight and Aiken 1998).

Lipids are one of the major biochemical classes present in all organisms, and it is defined operationally by its solubility in organic solvent. Lipid compounds include fatty acids, hydrocarbons, terpenoids, steroids and phospholipids which have biological functions such as, energy storage, structural components of cell membranes and important signalling molecules. Although lipids constitute only a small fraction of bulk organic matter (Meyers 2003), they have been widely used in many geochemical studies (Cranwell 1981; Koch et al. 2003; Burns et al. 2004; Muri et al. 2004). A wide variety of different lipid compounds have been found in aquatic sediments indicating to the diversity of biosynthetic pathways employed by aquatic organisms. Most of these compounds have distinctive structures and can be used as molecular markers for the source characterisation of sedimentary organic matter (Volkman 2006). The molecular distribution of lipid biomarker provides useful information about the source, diagenetic alteration, preservation and historical changes in organic matter as well as changes in trophic status (Canuel et al. 1995; Budge and Parrish 1998; Koch et al. 2003; Meyers 2003; Muriet al. 2004; Alfaro et al. 2006).

1.2.1 Anthropogenic sources of hydrocarbons

Although, naturally occurring biodegradable organic compounds are ubiquitous in aquatic systems, the influence of human-dominated terrestrial watersheds on receiving estuaries and coastal areas has considerably increased the loading of anthropogenic biodegradable organic compounds. In addition, the ever-increasing diversity of synthetic products results in the release of new compounds to these environments. In some cases, new organic compounds have no natural analog, which makes them difficult to transform due to the lack of expressing enzymes; in other cases, these compounds are similar enough to the other important biomolecules that they interfere with key biochemical processes. It is thus both the increased loads and the effects of these novel biodegradable organic chemicals that can cause significant alterations to habitats and communities. The highest loading is usually from petroleum products and their derivatives, of which more than 7 million $m^3 yr^{-1}$ enters aquatic environments through a number of sources (NRC 2003; Keller and Coleman 2011). Many of these compounds are common in estuarine and coastal systems, as they can seep out naturally. Moreover, their wide use in our modern society results in a large number of unintentional releases and intentional discharges of waste products that contain these compounds. Despite their natural occurrence, in many cases petroleum hydrocarbons are discharged at concentrations that are quite toxic to aquatic organisms, or in combinations that result in synergistic toxic effects. Moreover, the degradation products or metabolites of some of these compounds can also have significant toxicological implications. Furthermore, weathering and biodegradation frequently result in increased toxicity of these compounds (Davis et al. 1981; Middaugh et al. 1998; Shelton et al. 1999; Hamdoun et al. 2002). There are a number of derivative compounds included in this category that have many industrial and commercial uses, such as, aliphatic hydrocarbons (AHCs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and halogenated hydrocarbons. Many of these compounds enter the aquatic environment with sediments in storm water. Although, they are biodegradable, they may be only slightly bioavailable, and thus persist in the sedimentary fraction. Major classes of anthrpogenic organic compounds that may be released into the aquatic system from a variety of sources are given in Table 1.1.

Table 1.1 Major types of anthropogenic organic compounds discharged into the aquatic system (Keller and Coleman 2011)

Pollutant	Sources	Fate in environment	Potential toxicity
Petroleum	Natural seeps, accidental oil spillage, operational discharges	Low molecular weight compounds are biodegradable, others are more persistent	Absorption to tissues of biota
AHCs	Petroleum products and industrial fossil fuel emissions	Biodegradability, decreasing with increasing molecular weight	Moderate
Aromatics	Petroleum products, solvents, coal tar	Biodegradable	Relatively high
PAHs	Industrial, commercial and power generation	Very persistent	Low, except absorbed in sediments
PCBs	Industrial and commercial	Very persistent	Low, except absorbed in sediments
PPCPs	Domestic and health care	Degradable, but some compounds are more persistent	Moderate
PBDEs	Industrial and domestic	Persistent	Low, except absorbed in sediments
Pesticides	Agriculture, commercial and domestic	Variable degradability, from very high to very persistent	Variable
Phthalates	Industrial and commercial	Degradable	Moderate

AHCs- Aliphatic hydrocarbons; PAHs- Polycyclic aromatic hydrocarbons; PCBs- Polychlorinated biphenyls; PPCPs- Pharmaceuticals and personal care products; PBDEs- Polybrominated diphenyl ethers

Anthropogenic activities are known to have a wide range of potential effects on aquatic ecosystems, particularly from the point and non-point sources of pollution. Point sources discharge pollutant at specific locations, most of the time, through pipelines or sewers into the surface water. They originate from various sources such as urban and shipping, agriculture, the chemical industry, oil extraction and transport, commercial sources, wastewater treatment plants, pharmaceutical and other installations with a defined discharge point. For example, oil spills and dumping play a major role in point source pollution. Industrial point sources can contribute to pollution in the form of toxic chemicals and heavy metals. Pollutant discharged from chimneys is also regarded as a point source. Although, many hydrocarbons have limited volatility, and they occur at low concentrations in the atmosphere (Simcik et al. 1997; Mandalakis et al. 2002; Gioia et al. 2005; Di Filippo et al. 2007; Ding et al. 2007), even in Antarctica (Tanabe et al. 1983), far away from most sources. These compounds are likely to deposit mostly with dust and other particles present in the atmosphere, (increasing their loading on land surfaces) and during rain events, both rainwater and particles that wash off surfaces contribute to loading in storm water runoff (Schulz 2001; Orlando et al. 2003; Graves et al. 2004; Mitchell et al. 2005; Gasperi et al. 2006; Zhang et al. 2008). Generally, the presence of hydrocarbons in the aquatic environment has increased over the last 100 years; however, global concentrations may have stabilized due to air and water quality regulations (Fernandez et al. 2000).

Hydrocarbons usually present in the environment are often closely related to local and regional sources, although the remote area can be sites of hydrocarbon deposition through atmospheric processes and long range transport (Escartin and Porte 1999; Qin et al. 2013). These hydrocarbons can be released into the aquatic environment by accidental or operational spills produced during petroleum transportation or offshore exploration. On the other hand, biogenic hydrocarbons generate from short-term degradation of diagenetic precursors. For example, perylene, a polycyclic aromatic hydrocarbon frequently detected in coastal marine sediments, derives mainly from terrestrial precursors via diagenetic processes, but it could also be generated during pyrolitic processes (Baumard et al.1998; Guinan et al. 2001) or derived from aquatic organic matter and diatoms (Venkatesan 1988). In aquatic environments, hydrocarbon distribution is controlled by multiple and interdependent parameters. Some of these parameters are linked to intrinsic physicochemical properties of these compounds, mainly hydrophobicity, which controls their partition between dissolved and particulate phases. Others are related to the hydrological and geochemical characteristics of the environment, including water agitation and turbidity (Foster et al. 1987), sediment granulometry (Garrigues and Lamotte 1993) and particulate or dissolved organic matter content (Ankley et al. 1994).

Petroleum contains hundreds of organic compounds, since it is the alteration product of plants and other organisms underlying millions of years ago under anaerobic conditions. Usually, petroleum includes linear or branched alkanes, cycloalkanes, and aromatic hydrocarbons (e.g., benzene, toluene, xylenes and their derivatives). These hydrocarbons are further divided into paraffins, naphthenes, aromatics and asphaltics. The proportion of these compounds varies widely from region to region, and exist a significant gradient in composition, and hence, the presence of petroleum hydrocarbons in aquatic sediment indicates a wide range of compounds. However, the chemistry and physical properties of spilled oil change over time due to spreading, evaporation, dispersion, and emulsification (can modify oil properties) within several hours of spillage. For example, some oils can lose from 5% to 50% of light compounds via evaporation, and significantly increasing viscosity within hours to a few days (Mackay and Matsugu 1973; NRC 2003). Furthermore, water can be entrapped in the oil leading to the formation of water-in-oil emulsions, and oil can be broken into small globules, resulting in oil-in-water emulsions. Similarly, large volumes of brackish contaminated water are continuously released with oil and gas, in offshore platform operations (Durell et al. 2006), and this produced water contains significant amounts of the most soluble petroleum hydrocarbons (Utvik 1999; Wilson 2008). Oil-well operation is another potential release of petroleum hydrocarbons to the aquatic medium, estimated to be around 2% of petroleum wastes in the US (Reis 1992).

Although, many drilling fluids are most commonly water based and contain only clays and inorganic materials, some use mineral or synthetic oils. These oils are generally proprietary mixtures, which are mostly biodegradable hydrocarbons (Holdway 2002). Similarly, processed oil and gas are refined to produce many commercial products, including fuels (like natural gas, liquefied petroleum gas, gasoline or petrol, diesel, naphtha and kerosene), chemical intermediates (like benzene, ethylene, styrene, and polyols), solvents (eg., acetone, methyl-ethyl ketone, hexane, and ethyl acetate), and other compounds. Many of these are produced and used in large amounts, and are shipped around the world in bulk and large containers, therefore, an accidental or intentional releases still occur (Er-Raioui et al. 2009) results in the presence of these pollutants in the aquatic medium. Table 1.2 represents the major oil spills that have occurred in the world since 1976.

Table 1.2 Top major oil spills that have occurred in the world since 1967

Sl. no.	Location	Year	Spill size (million gallons)
1	Gulf War, Kuwait	1991	240
2	Mexican Gulf	2010	210
3	Bay of Campeche, Mexico	1979	140
4	Atlantic Empress, West Indies	1979	88
5	Fergana Valley, Uzbekistan	1992	88
6	Nowruz Oil Field, Persian Gulf	1983	80
7	ABT Summer, Off the coast of Angola	1991	80
8	Castillo De Bellver, Off Saldanha Bay, South Africa	1983	79
9	Amoco Cadiz, Off Brittany, France	1978	69
10	Haven, Genoa, Italy	1991	44
11	Odyssey Oil Spill, Nova Scotia, Canada	1988	43
12	Torrey Canyon, Scilly Isles, UK	1967	36
13	Prestige, Off Galicia, Spain	2002	19
14	Hebei Spirit, South Korea	2007	3

Reports from International Tanker Owners Pollution Federation Limited (ITOPF, http://www.itopf.com/knowledge-resources/data-statistics/statistics, accessed on October, 2015)

Many hydrocarbons that are produced from combustion processes become associated with aerosols at ambient temperatures. Although, the atmospheric transfer of hydrophobic organic hydrocarbon compounds has been shown to be important, still their pathway remains poorly documented (Bouloubassi and Saliot 1993). Aliphatic hydrocarbons (AHCs) and polycyclic aromatic hydrocarbons (PAHs) are two major classes of organic compounds, introduced into the aquatic environment through natural and anthropogenic sources (Zanardi et al. 1999). Fossil fuel combustion, coal gasification and liquification, petroleum cracking, waste incineration, and the production of coal tar pitch, coke, carbon black, and asphalt have all been shown to be possible sources of hydrocarbons (McVeety and Hites 1988; Montuori and Triassi 2012; Li et al. 2015). Due to their hydrophobic nature, and strong particulate oriented behaviour, they tend to adsorb onto particulate phase, making aquatic sediment a repository of these compounds (Xu et al. 2006), and hence bottom sediments act as a temporary or long-term pool of these contaminants (Pereira et al. 1996). The distribution and composition of hydrocarbons in surface sediments reflect the relative contributions from biogenic, petrogenic and pyrogenic sources (Hostettler et al. 1999). AHCs and PAHs in the sedimentary environment cause serious pollution to the aquatic ecosystems and directly influence the bottom-dwelling organisms that may affect wildlife and human health via the food chain (Zhang et al. 2009). Since, most of the AHCs are proved to be narcotic and irritant, and the majority of PAHs had strong toxicity, carcinogenicity, and mutagenicity (Yu et al. 2004), an understanding of the influence of these chemicals on sediment quality is essential for evaluating the overall status of the aquatic ecosystem.

AHCs (n-alkanes) are widely used to trace out the sources of organic matter in aquatic sediments, since many are source specific, and easily accumulated from biogenic and petrogenic sources (Gao and Chen 2008).

Biogenic hydrocarbons are known to be biosynthesised by a wide range of both marine and terrestrial plants. The difference in characteristic chain length of marine and terrestrial plants made the distribution of n-alkanes an effective tool for assessing sources of organic matter in terrestrial and marine sediments. For example, planktonic organisms generally produce a simple mixture of odd chain n- alkanes preferably C15, C17 and C19 (Gogou et al. 2000), while the abundance of C25, C27, C29, and C31 has been used as an indicator of terrestrially derived organic matter (Harji et al. 2008). The presence of unresolved complex mixture (UCM) and carbon preference index (CPI) value 1 or close to 1 indicate petroleum contamination (Ye et al. 2007).

Polycyclic aromatic hydrocarbons, a group of lipophilic organic pollutants that contain two or more fused aromatic rings, are introduced into the environment through biogenic, petrogenic, and pyrolytic processes (Montuori and Triassi 2012; Li et al. 2015). The majority of loading in urban environments is from products of incomplete combustion, particularly from burning diesel and other heavy fuels. Due to their low solubility and hydrophobic nature, PAHs in the aquatic environment rapidly associated with organic and inorganic suspended particles (Sun et al. 2009) and subsequently get deposited into the sediments. The stable molecular structure of PAHs amplifies their preservation in marine settings and hence, PAH analysis is a suitable paleo-environmental tool for recreating anthropogenic developments in diverse environments such as lakes, rivers, estuaries etc. (Chen and Chen 2011). Sources of PAHs can be distinguished by using a selection of isomeric ratios for PAHs (Baumard et al. 1998; Soclo et al. 2000; Yunker et al. 2002; Huang et al. 2012; He et al. 2014). For example, ratios of total methyl phenanthrenes to phenanthrene are used to identify PAHs as pyrolytic or petrogenic orgins (Laflamme and Hites 1979; Prahl and Carpenter 1983). Similarly, phenanthrene to anthracene (Ph/An), anthracene to phenanthrene + anthracene (An/An+ Ph), fluoranthene to pyrene (Flu/Py) and fluoranthene to fluoranthene + pyrene (Flu/Flu+Py) are also to classify the pollutant sources as either petrogenic or pyrolytic origin (Stortini et al. 2009) (detailed descripton in chapter 6).

1.3 Processes controlling the fate of sedimentary organic matter

The knowledge of the sources of organic matter and its preservation in sedimentary system, and factors controlling their distribution is important for understanding the contemporaneous global carbon cycle (Walsh et al. 1981). Usually, the type of organic material, heterotrophic processes, and the physical pathways by which these materials are distributed, control the fate of this material (Hedges et al. 1997; Cauwet 2002). In these transitional systems, a significant portion of organic matter sinks through the water column and is ultimately preserved in sediments (Budge and Parrish 1998). Thus, the sediments are dominant sites for organic matter breakdown and nutrient regeneration in which the sediment oxygen demand for organic matter degradation can induce hypoxic and anoxic conditions in bottom waters (Hedges et al. 1988; Prahl et al. 1994; Paerl et al.1998). The sediment oxygen demand and the quantities of nutrients regenerated are not only a simple function of total quantity of organic matter but also quality of organic matter. These processes are further complicated by seasonal variations in hydrology, which influence the organic input, dispersion and cycling of sedimentary organic matter in aquatic environments (Gordon and Goñi 2003). A better constraint on the sources of organic matter in marine sediments is needed to understand the processes responsible for its preservation (Calvert and Pedersen 1993; Hedges and Keil 1995; Meyers 1997; Keil et al. 1998). The major sources and the processes critical in controlling the fate of sedimentary organic matter in aquatic systems are represented in Fig. 1.1 (Sundquist 1993; Arthur 2000; Falkowski et al. 2000; Turner and Millward 2002).

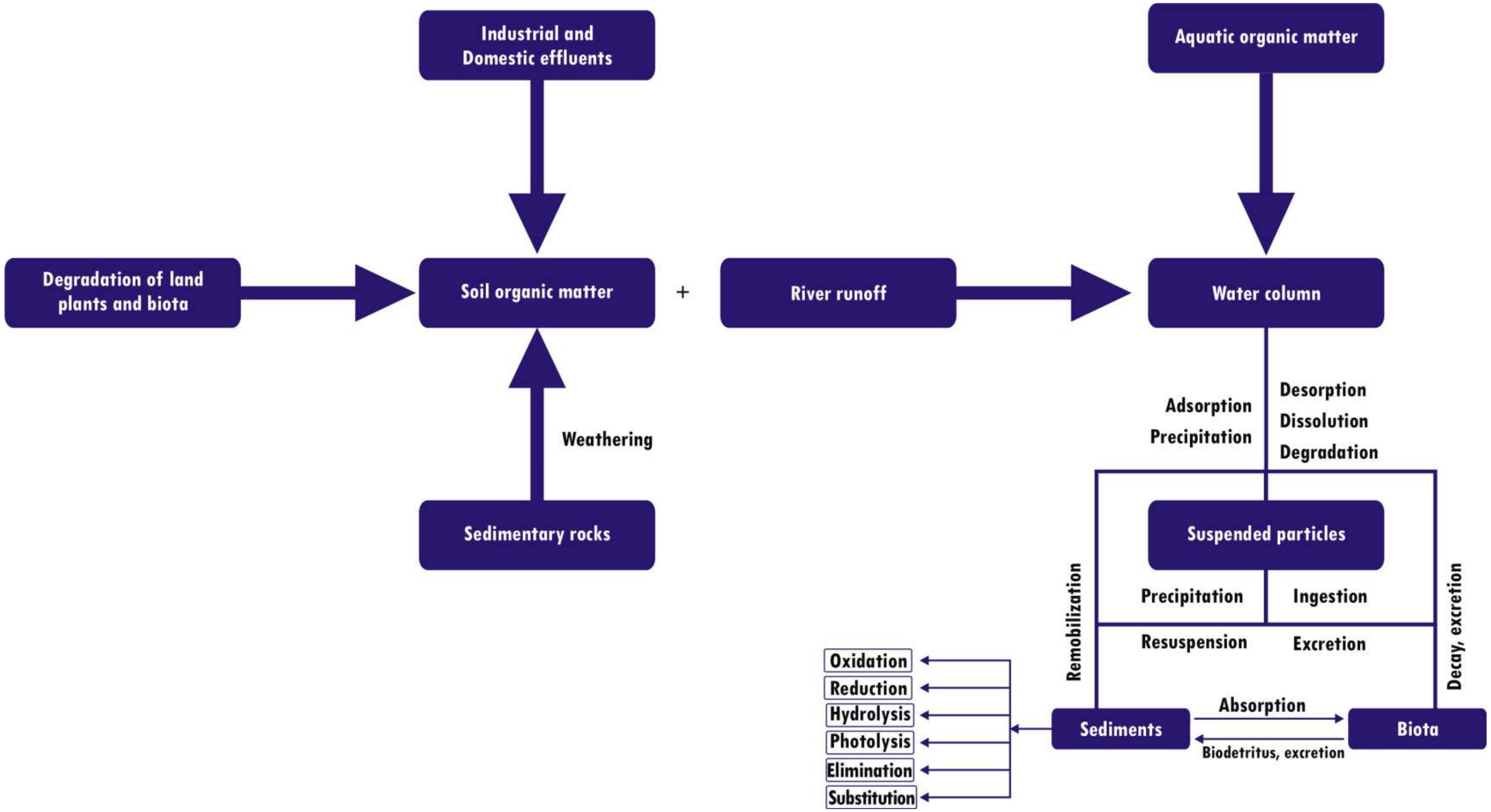

Fig 1.1 Schematic representation of major sources and the processes controlling the fate of sedimentary organic matter in aquatic systems

There are six main reaction mechanisms that can abiotically alter biodegradable organic compounds in the aqueous environment, viz. photolysis, oxidation, reduction, hydrolysis, elimination and substitution. Photo-oxidation provides another removal mechanism for terrestrially derived dissolved organic matter and potentially for particulate organic matter (Benner and Opsahl 2001). Direct photolysis unravels a number of organic compounds with an aromatic ring by trapping UV light at a specific wavelength (Bhatkhande et al. 2002; Keller and Coleman 2011). However, indirect photolysis, where photons create free radical species in water (eg., hydroxyl radicals) which then interact with the organic compounds, is more likely to occur. Oxidation in the water column requires a strong oxidant to begin the transformation of dissolved organic compounds to carbon dioxide, water and other products. Due to the relatively low solubility of oxygen in water, oxidation may actually be limited by its availability (Li et al. 1991; Shapiro and Vigalok 2008). Reduction usually occurs under anoxic conditions, which may frequently observe in aquatic systems, when a large volume of organic matter enters the system or is generated within it, then decays, and requires oxygen to decompose. There are many organic compounds that can incorporate a water molecule rapidly into their structure, which then destabilizes the compounds are unlikely to be significant sources of pollution, because they would be too unstable to pass intact through the various other processes mentioned above. Although some compounds are readily degraded abiotically, most transformations in the environment are significantly enhanced by the presence of the right enzymes, in many cases provided by microbes or fungi (Kagle et al. 2009; Sobiecka et al. 2009). The chemical reaction is generally the same as would occur abiotically (i.e., oxidation, reduction, hydrolysis, substitution and elimination), but the rate of reaction is significantly enhanced by having a suitable location (i.e., enzyme) to bring together the reactants. The energy barrier for the transformation is thus significantly reduced (Keller and Coleman 2011).

Remineralization is one process that may explain the lack of riverine flux of terrestrial organic matter to the global ocean reservoir (Opsahl and Benner 1997; Benner and Opsahl 2001). On continental margins, hydrodynamic sorting may regulate the distribution of organic matter along the shelf and slope. Fine sediment and less dense materials are transported downstream portion, while coarser sediment materials settle out in the lower reaches of the river and inner shelf sediments. The formation of "mobile muds" of benthic fluidized bed reactors, occurs at the muddy deltaic regions boundary (Aller 1998; Aller and Blair 2004), are dynamic reactors, where, reoxidation, mixing of fresh planktonic debris with refractory terrestrial materials and prolonged exposure to oxygen result in an efficient decomposition system independent from net sediment accumulation (Aller 1998; Aller and Blair 2004). Similarly, productivity, oxygen exposure time, sediment accumulation rate and organic matter composition can also influence organic matter preservation (Hedges and Keil 1995; Arzayus and Canuel 2005; Wakeham and Canuel 2005). Furthermore, granulometrically the fine grained (silty-clay) compositions of the surface sediments have a high surface area and small pore spaces that have been hypothesized to protect organic matter from microbial decay (Bianchi et al. 2002; Cotano and Villate 2006; Ramaswamy et al. 2008).

1.4 Toxicological significance of hydrocarbons

The effects of human intrusion on coastal systems have resulted in dramatic increases in the loadings of contaminants such as, trace metals, hydrophobic organic contaminants (HOCs) (e.g., hydrocarbons and chlorinated hydrocarbons), and nutrients (Wollast 1988; Schmidt and Ahring 1994: Jonsson 2000; Cloern 2001; Elmgren 2001). Inputs of these multiple stressors can interact to reduce, enhance, and/or mask the individual effects of each component (Breitburg et al. 1999). In the case of HOCs, these compounds tend to be lipophilic, allowing for bioaccumulation and biomagnification and the transfer of these contaminants up through the food web, via trophic interactions. The persistence of these contaminants, particularly in sediments, results in long-term impacts on recovery of benthic communities in aquatic systems (Elmgren et al. 1983). Among this, diesel fuel has been shown to be the most toxic contaminant because of its high polycyclic aromatic hydrocarbon content (Kennish 1991), and it includes highly carcinogenic compounds, such as, benzo[a]pyrene (Gelboin 1980; Denissenko et al. 1996). Moreover, it has been shown that diesel fuel contamination in sediments results in benthic diatom blooms due to reduced grazing pressure from *harpacticoid copepods* and from enhanced availability of dissolved inorganic nitrogen (Carman and Todaro 1996; Carman et al. 1996, 2000).

Petroleum is a complex mixture of many thousands of organic compounds, of which hydrocarbons predominate, with a high abundance of alkylated PAHs. Characteristically, crude oils contain significant concentrations of alkyl-substituted naphthalenes (mono- and di-methyl) and phenanthrenes (mono-and di-methyl). Petroleum hydrocarbons introduced to aquatic environment, whether in large-scale spills or low-volume chronic releases, are a major ecological problem and human concern worldwide. Main reasons for concern includes, the common use of petroleum and its products, spatial extent that spills and leaks can cover, the persistence of contamination, and the tendency for oil to adhere to environmental features and wildlife. For example, seabirds are a particularly well-studied group affected by marine petroleum pollution. Acute toxicity, such as, narcotic effects on organisms, is a function of water solubility, volatility, low molecular weight, and additive toxicity of the compounds composing petroleum hydrocarbons (Donkin et al. 1989). To summarize, the effects on wildlife include biosynthetic, energetic and reproductive disruption (Capuzzo et al. 1988). The impact of oil on development can be an useful indicator of petroleum effects, as it is easy to observe in a laboratory and ecologically crucial to the survival of species. Petroleum hydrocarbon can affect habitat as well, particularly biogenic structures. Tropical coral reefs near the surface and proximal to mangrove ecosystems are especially vulnerable to oil inputs due to continual exposures. Similarly, algal and mussel beds that exist intertidally subject to constant waves of contamination with the myriad creatures living in their microhabitats. Sub-tidal algal or biotic communities do not appear to be substantially affected by oil spills, as sinking weathered and flocculated oil is the primary mechanisms of exposure (Elmgren et al. 1983; NRC 2003). However, biotas near natural oil seeps subject to a less weathered, but a more biodegraded mixture of petroleum (Pearson and Rosenberg 1978). Seeping hydrocarbon and its degradation products and hypoxic conditions are toxic, but it tends to initiate a proximal food web. Moreover, the light carbon isotope composition of seep macrofauna indicates hydrocarbon metabolism establishes a local food web (Spies and Desmarais 1983; Bauer et al. 1990), which contains more abundant organisms than in surrounding areas (Spies and Davis 1979; Davis and Spies 1980; Steichen et al. 1996).

Recently another impact of petroleum hydrocarbon and other types of contaminants on aquatic organisms has been reported. Many low molecular weight and moderately hydrophobic hydrocarbons are prevented from entering or are shuttled out of cells actively by transmembrane proteins. These multixenobiotic-resistant (MXR) transporters act as the first line of defence in all living organisms, due to their position and broad substrate specificity (Epel 1998). However, when contaminants become concentrated and overwhelm transport proteins, the MXR process becomes inhibited. Studies have begun to document instances of this in estuarine and coastal systems, such as oils inhibiting sea urchins, and worms, PAHs inhibiting flounder and killifish, pesticides inhibiting mussels, a number of PPCPs such as fragrances inhibiting mussels and sea urchins, and pharmaceuticals inhibiting a great variety of organisms (Galgani et al. 1996; Bard 2000; Hamdoun et al. 2002; Luckenbach and Epel 2005).

PAHs in aquatic sediments have significant harmful effects on benthic communities (Wakeham et al. 1980; Bauer and Capone 1985a, b; Bunch 1987). In particular, low-energy environments, like salt marshes are susceptible to high accumulations of PAHs (Little 1987). Microphytobenthos are particularly susceptible to PAHs due to the high sediment-binding and residence-time properties of PAHs (Plante-Cuny et al. 1993; Carman et al. 1997). Estuarine coastal marshes in Louisiana (USA) have been exposed to high levels of petroleum-hydrocarbon contaminants due to the extensive offshore/nearshore drilling activities (Long 1992). Moreover, hydrocarbon additions to salt marsh sediments, collected from Louisiana, resulted in an increase in the abundance of hydrocarbon-degrading bacteria (Nyman 1999). In particular, bacterial degradation of PAHs, such as phenenthrene, was readily degraded in Louisiana marsh sediments. Similarly, the carcinogenic PAH, benz[*a*]anthracene, was found to be effectively metabolized by bacteria in sediments (Hinga et al. 1980). Based on the above observations, it is suggested that the bacterial community in this region is adapted to chronic exposure of petroleum hydrocarbons. In addition to the interactions between HOCs and microbial populations in sediments, there is evidence that contaminants affect the overall trophic structure of the benthic food web (Levinton and Bianchi 1981; Levinton et al. 1984). Associations of PAHs with particulate and dissolved organic matter can be altered by diagenetic transformations in sediments (Prahl and Carpenter 1983). While, PAHs have been shown to be highly concentrated in sediments, resulting in significant toxicological effects on the life history (Bridges et al. 1994) and immune systems (Tahir et al. 1993; Gunnarsson et al. 1999) of benthic organisms, macrobenthos can have significant effects on the fate and transport of HOCs.

1.5 Aim and scope of the present study

Cochin estuary form a complex system of shallow estuarine network, running parallel to the south west coast of India with an extensive system of narrow brackish water lagoons and backwaters. The major sources of fresh water into the estuary are, Periyar, a large river in the north, Muvattupuzhayar, a small river in the south and Chitrapuzha River. The major hydrographic variable in this backwater system is salinity. This highly productive tropical ecosystem acts as a nursery ground for many species including estuarine and marine fin fishes, molluscs and crustaceans. Moreover, the low lying swamps and tidal creeks dominated by thin patches of mangroves provide shelter to many important species. Cochin backwaters are exposed to increasing human interferences, and it receives a considerable quantity of pollutants from industrial units, domestic sewages, fishery industries and the Cochin sea port, which handles large quantities of petroleum products and industrial chemicals. Shipping activities and the discharge and loading of crude oil and its products and resultant localized oil spills introduce petroleum hydrocarbons into the harbour estuary. Hydrocarbon rich gaseous emissions from industries, road traffic and water transportation, eventually contaminate the inshore water and sediment through dry and wet deposition. The water and sediment quality of Cochin backwater is a matter of concern because of perceived impacts of the chemical pollutants on this ecosystem. The influence of industrial effluents on the general hydrography of Cochin backwater is high and it deteriorates the quality of water and sediment system, by loading with large quantities of pollutants, which often exceeds the carrying capacity of the aquatic system causing complete destruction of the biota. The lower reach of this river became part of National waterways, and is mainly used for transporting imported chemicals from Cochin port to industrial units located on the banks of the river.Therefore, in view of the above concerns, the broad aim of the present study was to establish water and sediment quality data and assess the extent of pollution of the inshore sediments from hydrocarbon contaminants, especially the environmental genotoxins, AHCs and PAHs. Considering the significance of the socio-economic and environmental scenario of the Chitrapuzha River basin, and in the overall development of Kerala, a detailed investigation on the sediment quality aspect of Chitrapuzha River has been attempted.

The present study aims:

1. To investigate the spatial and seasonal variations of organic matter in the surface sediments of Chitrapuzha River.
2 To assess the quality of organic matter using biochemical descriptors, and thereby identifying the benthic trophic status.
3 To investigate the sources of organic matter, its spatial and seasonal variations and to calculate the relative contribution of terrestrial derived organic matter using total organic carbon to total nitrogen (TOC/TN) ratios, $\delta^{13}C$ and $\delta\ ^{15}N$ signatures.
4 To characterise the sources of organic matter using fatty acids and aliphatic hydrocarbons (AHCs) in the sedimentary system, so as to discriminate the biogenic and anthropogenic contributions to organic matter.
5 To identify the possible sources of polycyclic aromatic hydrocarbons (PAHs) in surface sediments, and evaluate the contamination status and potential risk assessment of PAHs, using sediment quality guidelines and qualitative and quantitative indices based on PAHs.

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Chapter 2

MATERIALS AND METHODS

Contents

2.1 Description of the study area

Cochin backwater system (Latitude: 9° 40’ & 10° 12’ N and Longitude: 76° 10’ & 76° 30’ E) forms a complex, network of shallow brackish water (250 km^2) running parallel to the Kerala coast, is connected to the Arabian Sea through two permanent openings at Cochin and Azhikode. Six rivers, namely; Periyar, Muvattupuzha, Pamba, Manimala, Meenachil and Achencovil discharges about 20000 $x10^6$ m^3 of fresh water into the estuary annually (Srinivas 1999; Srinivas et al. 2003), and the variations in the river discharge induce a strong salinity gradient. Cochin backwaters forms a part of the Vembanad-Kol system, which is a protected wetland and one of the largest estuarine ecosystems in South Asia. Hydrography of these backwaters is controlled mainly by discharges from Periyar, Muvattupuzha and Chitrapuzha Rivers, and also by tidal action through the Cochin Barmouth. Cochin backwater 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 hydraulic barriers constructed on the southern limb of the backwater system at Thanneermukkam region, helps to prevent saline ingression into the upstream agricultural fields have imposed severe flow restrictions and increased sedimentation in the estuary (Menon et al. 2000). The study area, Chitrapuzha River system forms part of the Cochin backwaters as well as the National inland waterway No.111 (9°52' - 100 00'N; 76°15' - 76°25'E) (Fig 2.1). Chitrapuzha River hosts diverse aquatic organisms and several lower reaches have been converted into breeding pools, so as to increase fish production for commercial operation. Chitrapuzha River originates as a small stream from the upper reaches of high ranges in the eastern boundary of Kerala state, and passes through the Ambalamugal industrial area, and finally joins the southern arm of Cochin backwaters. A host of industries including a diesel power plant (BDPP), a fertilizer manufacturing unit (FACT), an organic chemical factory (HOCL), a petroleum refinery (BPCL) and a solid waste treatment plant is crowded in a small stretch of land by the side of Chitrapuzha River. The influence of industrial effluents on the general hydrography of Cochin backwater is high, and it deteriorates the quality of water and sediment by loading with large quantities of pollutants, which often exceeds the carrying capacity of the aquatic system, causing complete destruction of the biota. Complaints of massive fish kills and associated problems are common in this area. The quantity of effluent treated water discharged from these industries into Chitrapuzha River is estimated to be around 80 million litres per day (Rejomon et al. 2013). Moreover, shipping activities, discharge and loading of crude oil and resultant localized oil spills introduces petroleum hydrocarbons into the estuary. Hydrocarbon rich gaseous emissions from industries, road traffic and water transportation, eventually contaminate the inshore water and sediment through dry and wet deposition. The study area is under the profound influence of southwest monsoon accordingly, three seasons prevail viz. pre-monsoon (PRM; February-May) monsoon (MN; June-September) and post-monsoon (POM; October-January).

2.2 Sampling, storage and analytical methods

Based on specific geographical features, water flow regimes and the aforementioned anthropogenic activities, surface water and sediment samples were collected from five locations along Chitrapuzha River (Fig. 2.1). Three sediment samples were collected from each station, using a van Veen grab (0.042 m^2) during June 2012 (monsoon), April 2013 (pre-monsoon) and December 2013 (post-monsoon). Station 1 (S1) represents the far upstream portion of the river, and is beyond the influence of industrial pollution and has an average depth of 2.5 m. Stations 2 (S2), 3 (S3) and 4 (S4) constitute industrial zone, which are the recipients of effluent treated water discharge outlets of major industrial establishments. Station 2 (S2) is near the discharge outlet of petroleum refinery (BPCL) and petrochemical complex (HOCL), and has an average depth of 3 m. In addition to this, station 3 (S3) receives considerable quantities of effluents from fertilizer manufacturing unit (FACT). At station 4 (S4) the effluent ladened water mixes with water drained from agricultural runoff and leachates from the defunct solid waste treatment plant at Brahmapuram, and has a depth of 3.5 m. Station 5 (S5) is near Thevara ferry point, a tip of mainland from which a motor boat ferry service operates to adjacent islands, and it receives a considerable quantity of discharges from apartments on the mainland, as well as from the oil tanker berth, Cochin Port and Cochin Shipyard. The samples were packed in ice boxes for transportation to the laboratory, where it was stored in a deep freezer (-20^0C), till the analysis. All the analyses were carried out in triplicate and the average value was reported.

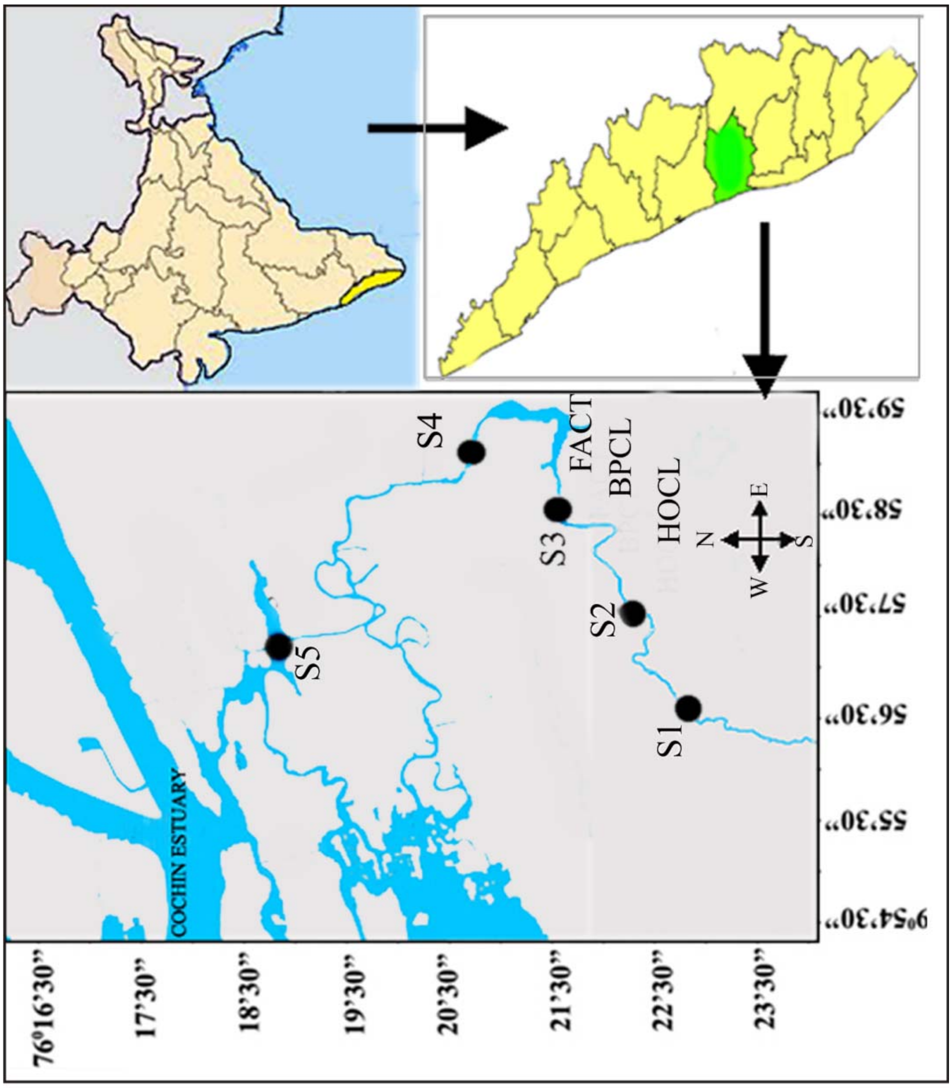

Fig. 2.1 Study area showing location of sampling sites

2.2.1 Laboratory procedure

All glassware for organic analysis was cleaned by ultra-sonic bath followed by heating at high temperature in the oven. All reagents used were of analytical grade and were checked and excluded for any possible contamination. Reagents and standard solutions were prepared with Milli-Q water. Calibration standards of fatty acids and hydrocarbons were purchased from Sigma-Aldrich (USA). Concentration of individual compounds was obtained by the external calibration method based on a four point calibration curve for individual compounds. Analyses of triplicate sediment sub samples indicated hydrocarbon reproducibility to be typically ± 7% and FAs reproducibility to be ± 9% of the mean concentration. Re-extracts yielded less than 2% of the original extracts both for hydrocarbons and fatty acids. The quantification limits were 0.06 μgg^{-1} for FAs and 0.02 ngg^{-1} for hydrocarbons. Data were acquired and processed with M.S Turbomass Version 5.4.2 and Total Chrom work station.

2.2.2 Analytical methodology

2.2.2.1 General hydrographic parameters

Surface water samples were collected in pre-cleaned polythene bottles using a clean bucket for the analysis of nutrients and other water quality variables. Sub sampling for the determination of pH and dissolved oxygen (DO) was done in situ. The samples were kept in ice boxes, and transported to the laboratory without any contamination. The analysis of nutrients was performed in the laboratory on the same day of sampling itself. General hydrographic parameters and nutrients of the surface waters were analysed using standard methods. pH of the surface water samples was measured in situ using a portable pH meter (Eutech- pH Tester 10). The 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). The concentration of nutrients (ammonia, nitrite, nitrate, silicate and phosphate) was estimated spectophotometrically (Grasshoff et al. 1983) using Genesys 10 UV Thermospectronic. 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 at a 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). 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). Determination of inorganic phosphate was based on the reaction of ortho phosphate ions with the acidified molybdate reagent to yield a phospho molybdate heteropoly acid, which is then reduced to a blue coloured compound (Grasshoff et al. 1999).

2.2.2.2 Sedimentary characteristics

Surface sediment samples were collected using a stainless steel van Veen grab (0.042 m^2) and stored in clean glass bottles and kept deep frozen till analyses. pH of the sediment samples was determined in situ using a portable pH meter (Eutech, pH Tester 10). Redox potential of the fresh wet sediment samples was measured by portable Eh meter (Eutech, ORP Tester 10), which was calibrated with Zobell solution (Brassard 1997). Sediment texture (sand, silt and clay) was determined by pipette analysis (Krumbein and Pettijohn 1938; Folk 1980), based on Stoke's law after removing the inorganic carbonates using 10% HCl and organic matter using 15% H_2O_2. A known weight of the wet sediment was dispersed overnight in sodium hexametaphosphate, 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 1980). Sediment samples were freeze-dried and finely powdered using agate mortar for further analyses. Total carbon, total nitrogen (TN) and total sulphur (TS) 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 detection limit for TOC was 0.06%.

2.2.2.3 Biochemical composition in surface sediments

Spectrophotometric methods were employed for the determination of biochemical constituents present in the sediment samples. 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. 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 protein, lipid and carbohydrate carbon equivalents was calculated as the biopolymeric carbon (BPC), and used as a reliable estimate of the labile fraction of organic matter (Fabiano et al. 1995). Analysis of chlorophyll and phaeopigments were carried out according to standard procedures (Lorenzen and Jeffrey 1980; APHA 1995). Pigments were extracted with 90% acetone (24 h in the dark at 4 °C). After centrifugation, the supernatant was used to determine chlorophyll pigments (chl-a, chl-b and chl-c) and was acidified with 0.1N HCl to estimate the concentration of phaeopigments. The phytopigment concentrations were defined as the sum of chl-a and pheo concentrations. The algal contribution to BPC (Cchl-a) was calculated as the percentage of chl-a to BPC concentrations, after converting chl-a concentration into carbon equivalents using a mean value of 40 mgC/mg chl-a (Pusceddu et al. 1999).

Stable isotope analysis $\delta^{13}C$ and $\delta^{15}N$ were carried out using Flash EA 1112 interfaced with IRMS (DELTA V PLUS, Thermo Electron Corporation) at the National institute of oceanography (NIO), Goa, India. Stable isotopic ratio values were reported in ‰ notation.

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

where, δ ‰ stands for $\delta^{13}C$ or $\delta^{15}N$, and R_{sample} and $R_{reference}$ are the isotopic ratios of the sample and the reference respectively. For carbon, the reference is PDB (Pee Dee Belemnite) and for N, it is air. All the analyses were carried out in triplicate and mean of the three measurements were reported.

2.2.2.4 Extraction of fatty acids (FAs) and hydrocarbons

The method described by Harvey (1994) was selected for the extraction of FAs, aliphatic hydrocarbons (AHCs) and polycyclic aromatic hydrocarbons (PAHs) (Fig. 2.2). Freeze dried sediment samples were extracted using soxhlet apparatus (Pelican Socsplus-SCS 08 R**)** for 72 hrs with a mixture of dichloromethane: methanol (2:1 ratio).The extracts were combined and evaporated to dryness using a rotary evaporator (Heidolph-RW-0525 G). The extracted residue was subjected to mild alkaline hydrolysis using 6% w/v KOH in methanol medium and refluxed for 4 hrs at 70^0C. The fraction containing neutral organic fraction was recovered with n-hexane, which was evaporated and kept for column chromatography. The remaining aqueous layer containing the fatty acid salts was acidified to pH 2; using 6M HCl and the fatty acid was extracted with dichloromethane (DCM). The polar lipid fraction containing FAs was evaporated to dryness using rotary evaporator and treated with 10 ml of 12% BF_3 in methanol (Sigma Aldrich) at 70^oC for 1hour, to form the fatty acid methyl esters (FAMEs). The FAMEs were subsequently partitioned from the reaction solution into10ml of hexane. The hexane layer was evaporated to dryness and the extract was then re-dissolved in n-hexane for gas chromatographic analysis. The neutral fraction obtained by mild alkaline hydrolysis of the total lipid extract was submitted to a column chromatography, for fractionation. The fractions containing AHCs were eluted with 50 ml n-hexane and the second fraction containing PAHs were eluted with 5 ml of hexane and 70 ml of methylene chloride- hexane (30:70), and dried under nitrogen.

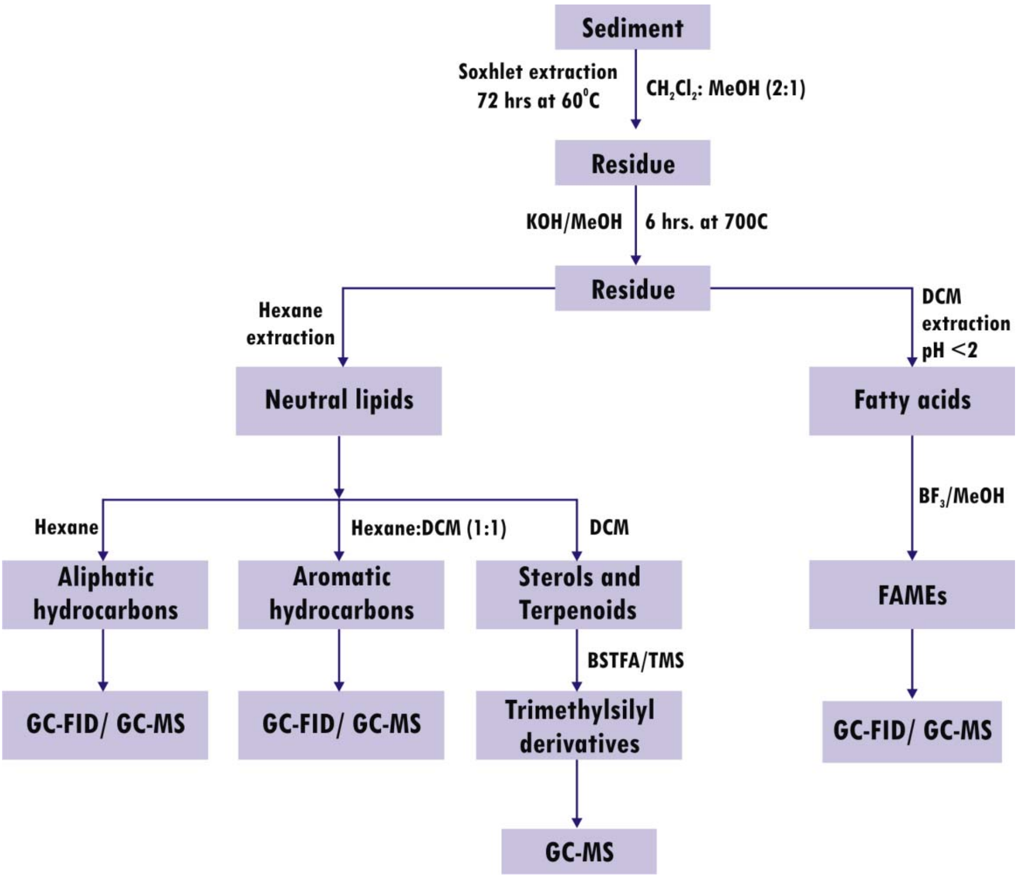

Fig. 2.2 Scheme of extraction for fatty acids, aliphatic hydrocarbons and polycyclic aromatic hydrocarbons (Harvey 1994)

2.2.2.5 Gas chromatographic analysis of FAs, AHCs and PAHs

The analysis of FAMEs were carried out by gas chromatography-mass spectrometry (GC-MS) using a Perkin Elmer Clarus GC 620, with MS detector equipped with a non-polar HP ultra-double-fused silica capillary column (30 m, 0.32mm id, 0.25µm film thickness). Operating conditions were as follows: ion source of electron voltage 70 eV kept at 200°C. Spectra were scanned from 50 to 600 m/z with a scan time of 1.50 s. Initially the temperature was increased from 50°C to 200°C at a rate of 2°C per minute and held at 200°C for 5 minutes. Then the temperature was again increased from 200°C to 280°C at a rate of 10°C per minute and held at 280°C for 10 minutes. The detector was held at 290°C, and helium was used as carrier gas. The full data acquisition was obtained with the use of MS turbo mass version 5.3.2. Quantification was achieved by calibration of FAMEs standards supplied by Sigma Aldrich (Fig. 2.3) (Supelco 37 Component FAME Mix, 18919-1AMP). Sample FAMEs were also injected under the above mentioned conditions and their concentrations were determined from the calibration plot.

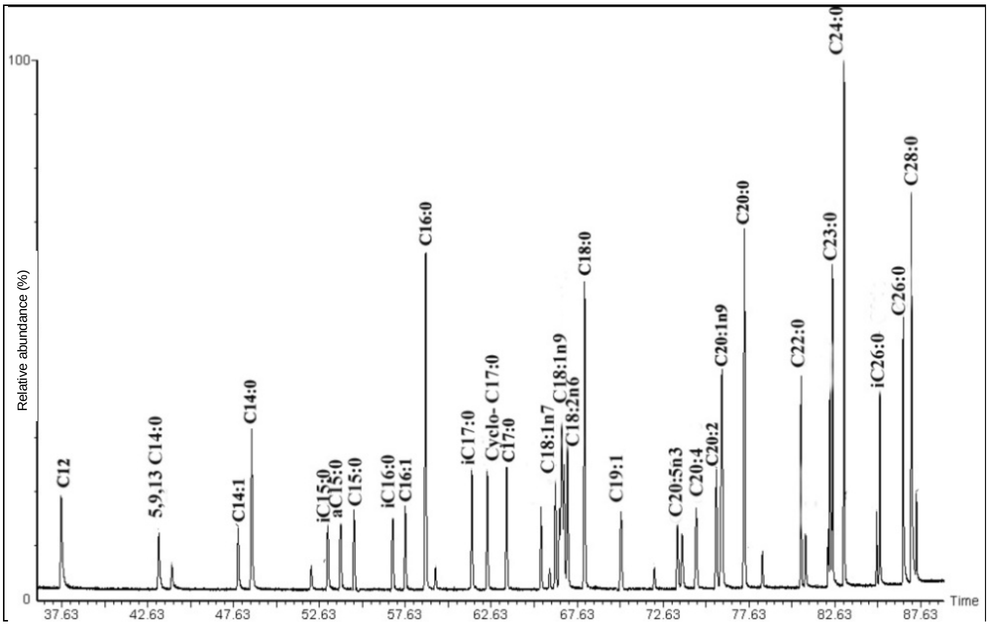

Fig. 2.3 Gas chromatogram of standard fatty acid methyl ester mixture

The hydrocarbon fraction eluted with n-hexane using a silica gel column was evaporated to 1ml under ultra high purity N_2 prior to concentration determination on a gas chromatograph equipped with flame ionisation detector (GC-FID), containing a DB-5 column (30m×0.25mm id., 0.25 µm film thickness). The oven temperature was held at 50°C for 5 minutes and then increased to 300°C at a rate of 3°C per minute and held for 5 minutes. The injector temperature was kept at 260°C and the detector temperature was maintained at 325°C. Nitrogen was used as carrier gas, with a flow rate of 2 ml/minute. Identification of individual compounds was achieved by comparison of GC retention times with those of standard compounds (Fig. 2.4).

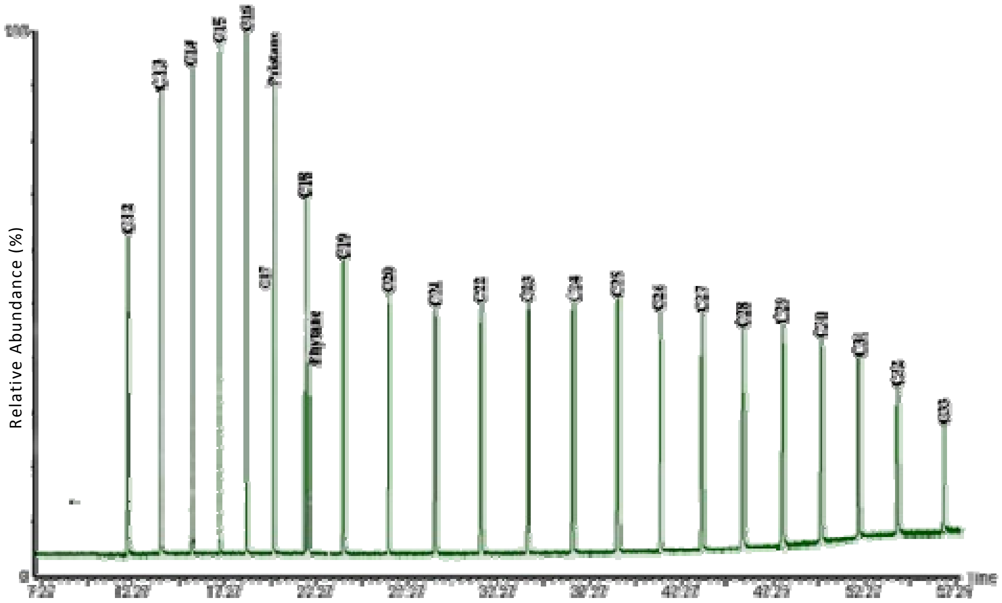

Fig. 2.4 Gas chromatogram of standard n-alkane mixture

Quantification was made based on the calibration with authentic standards (C_7-C_{40}) (Sigma Aldrich). The hopanes, eluted along with hydrocarbon fraction, were identified by scanning the mass spectra at m/z 191 and compared with mass fragmentation pattern of hopanes in NIST MS library (version: NIST MS Search 2.0) and that of published mass spectral data. For PAHs, the GC oven had an initial temperature of 70 ^{0}C (4 minutes hold) and was ramped at 8 ^{0}C/minute to 320 ^{0}C (8 minutes hold). The carrier gas was helium with a constant flow rate of 1.0 ml/minute. Concentration of individual compounds was obtained by the external calibration method based on a four point calibration curve for individual compounds (Fig. 2.5). (PAH mixture, Supelco, Sigma Aldrich). The relative standard deviation in measurements ranged from 5 to 10% (n = 3), and the averaged recoveries of the studied PAHs were 80 to 99%.

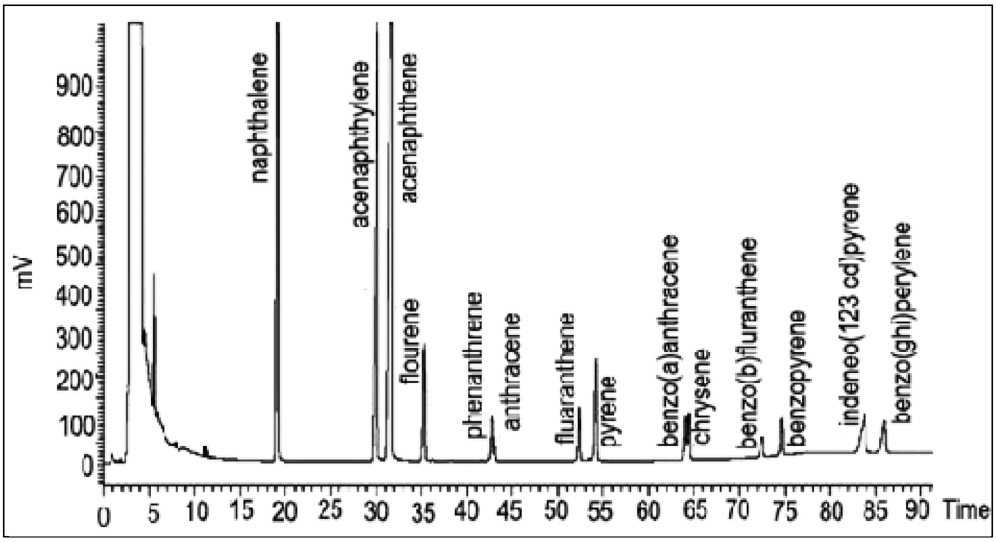

Fig. 2.5 Gas chromatogram of standard polycyclic aromatic hydrocarbon mixture

2.3 Statistical analysis

All statistical analysis was done using "statistical package for social sciences" (SPSS), version 13, to identify the sources of organic matter as well as the processes governing their distribution. Spatial and seasonal variations were assessed by the analysis of variance (ANOVA) with season and space (stations) as sources of variation. The log (x+1) transformation was used to obtain the normal distribution of the data set and normality was checked as per standard procedures (Webster 2001). Factor analysis was used to extract information from the most meaningful parameters that better describes the overall data, while afford data reduction with minimal loss of information (Katsaounos et al. 2007). In factor analysis, principal component analysis (PCA) and varimax rotation methods were used for extraction and deriving principal components (PCs) respectively. Factor loading was considered as significant, if they were >0.50. Similarly, cluster analysis (CA), a multivariate technique, whose primary purpose is to classify the object of the system into categories or clusters based on their similarities. Hierarchical clustering (HCA) is the most common approach in which clusters are formed sequentially, and it provides intuitive similarity relationships between any one sample and the entire data set, and is typically illustrated by a dendrogram/ tree diagram (Singh et al. 2004; Shrestha and Kazama 2007). In this study squared euclidean distance measurement and Ward's method were deployed.

Table 2.1 Seasonal and spatial variations of hydrographic parameters in the study area (n=3)

Seasons	Stations	pH	Salinity (psu)	DO (mg/L)	Silicate (µmol/l)	Nitrite (µmol/l)	Nitrate (µmol/l)	Ammonia (µmol/l)	Phosphate (µmol/l)
PRM	S1	6.88 ± 0.06	0	5.8 ± 0.4	32.4 ± 5.3	0.54 ± 0.15	1.24 ± 0.42	23.2 ± 4.1	6.3 ± 1.2
	S2	6.63 ± 0.07	0	2.9 ± 0.8	56.1 ± 6.4	16.8 ± 1.9	28.16 ± 2.6	148.7 ± 11.5	124.5 ± 12.8
	S3	6.25 ± 0.10	1.2 ± 0.8	3.3 ± 0.7	74.8 ± 9.2	14.6 ± 1.6	24.3 ± 3.7	135.8 ± 8.5	106.2 ± 11.4
	S4	6.32 ± 0.1	4.1 ± 1.6	3.6 ± 0.6	82.5 ± 7.6	10.4 ± 0.8	16.4 ± 3.9	128.9 ± 11.8	86.4 ± 12.3
	S5	6.9 ± 0.12	12.2 ± 2.9	4.7 ± 1.1	86.1 ± 4.3	0.86 ± 0.5	1.5 ± 0.5	68.3 ± 9.4	15.8 ± 5.2
MN	S1	6.95 ± 0.10	0	6.3 ± 0.4	49.3 ± 3.6	0.22 ± 0.1	0.86 ± 0.3	18.9 ± 3.4	4.8 ± 1.7
	S2	6.77 ± 0.08	0	3.2 ± 0.6	67.6 ± 5.1	12.8 ± 2.1	24.25 ± 3.6	121.3 ± 10.7	116 ± 8.4
	S3	6.65 ± 0.10	0	3.5 ± 0.4	54.3 ± 4.2	11.6 ± 1.5	19.1 ± 2.7	128.5 ± 9.2	102 ± 1o.7
	S4	6.6 ± 0.10	2.7 ± 1.6	4.1 ± 1.0	73.2 ± 6.8	8.1 ± 0.9	11.6 ± 1.6	109.2 ± 6.5	66.0 ± 7.8
	S5	7.1 ± 0.11	10.4 ± 3.7	4.9 ± 1.1	98.0 ± 3.3	0.28 ± 0.3	0.94 ± 0.6	41.3 ± 5.7	13.7 ± 3.2
POM	S1	6.85 ± 0.07	0	6.0 ± 0.6	41.7 ± 3.2	0.34 ± 0.13	1.0 ± 0.8	21.9 ± 2.6	6.05 ± 1.4
	S2	6.7 ± 0.12	0	3.1 ± 0.7	46.2 ± 4.8	17.3 ± 1.5	28.95 ± 4.2	133.5 ± 12.8	118.9 ± 14.3
	S3	6.34 ± 0.12	0.91 ± 1.2	3.1 ± 0.8	78.1 ± 3.6	16.1 ± 1.9	26.8 ± 3.7	122.9 ± 9.4	104.8 ± 6.7
	S4	6.45 ± 0.09	3.8 ± 2.6	3.9 ± 0.9	62.1 ± 3.1	8.4 ± 1.2	12.1 ± 2.2	121.0 ± 8.3	77.9 ± 9.3
	S5	7.05 ± 0.13	11.6 ± 4.3	4.5 ± 1.1	77.6 ± 5.9	0.44 ± 0.20	1.2 ± 0.8	49.7 ± 7.6	16.9 ± 4.2

PRM- Pre-monsoon; MN- Monsoon; POM-Post monsoon; DO- Dissolved oxygen

2.4 Results of general hydrographic parameters

The study of hydrographic parameters of the aquatic environment is of great importance to characterize the general features, distribution patterns and relative abundance of inorganic and organic pollutants. The knowledge of the various hydrographical parameters of Chitrapuzha River is of much significance, since it carries effluent water from a large number of industrial units, including a major fertilizer plant, a petroleum refinery, a petrochemical unit and a solid waste treatment plant. In addition to this, saline water intrusion associated with tides and the influx of fresh water brought in by the rivers, also play an important role in controlling the hydrographic variables in the study area. The general hydrographic parameters and nutrients in the water column have a direct control over the production, distribution and fate of sedimentary organic matter. Moreover, the fluctuations in physical, chemical and biological processes in estuarine salinity gradient have a considerable impact on the composition and distribution of sedimentary organic matter (Carreira et al. 2011; Costa et al. 2011). Therefore, a brief description of the distributions and concentrations of water quality variables is incorporated in this section.

The seasonal and spatial variations of different hydrographic parameters of surface waters are represented in Table 2.1 and Fig. 2.6. Terrestrial runoff associated with monsoon rainfall creates a remarkable seasonal variation in the hydrographic parameters in the Chitrapuzha River system. Present investigation recorded a pH variation from 6.25 (S3; PRM) to 7.10 (S5; MN) in surface water. Many of the life processes are dependent on the hydrogen ion concentration in the surrounding medium. The important factors, which influence the pH of the water column are photosynthetic activity, discharge of fresh water from the upper reaches of the river, salinity changes during salt water ingression and the discharge of effluent treated water from industrial units located on the banks of the river. Variation in pH due to chemical and other industrial discharges renders a stream unsuitable not only for recreational purposes, but also for the rearing of fish and other aquatic life (Webb 1982). Moreover, close monitoring of pH values enables to identify zones of pollution and other quality conditions of water (Clarks et al. 1977). The minimum pH value (6.25) recorded at S3 (Table 2.1) during PRM season, which indicated the slightly acidic nature of the aquatic system, may be due to the presence of acidic effluents from the nearby industrial units.

Salinity plays an important role in sustaining the aquatic life and the physical processes occurring in the aquatic system affect surface salinity. It has been accepted as an index of estuarine mixing processes and tidal effects (Renier and OXane 2004). Moreover, in estuaries, its distribution pattern mainly influenced by the mixing and diffusion phenomena (Uncles et al. 1983). Salinity is increased by evaporation processes, and reduced by the atmospheric run-off, increased river discharge and tidal intrusions, etc. A profound spatial variation of salinity was observed during the period of the survey, with declining values from estuarine mouth (S5) to riverine zone (S1) (Fig. 2.6). Observed salinity values ranged from not detectable to 12.2 psu for surface waters, and remarkably higher salinity was observed only at estuarine station S5. Salinity in the study area is mainly due to the intrusion of sea water through the Cochin barmouth, and station S5 is located more towards estuary, and therefore, prominent tidal activity can alter salinity in this region.

The observed values of dissolved oxygen (DO) content in the surface waters are given in Table 2.1. Wide fluctuation was exhibited by DO content and its concentrations ranged from 2.9 (S2; PRM) to 6.3 mg/l (S1; MN). Maximum DO content recorded at S1 during MN season, while minimum values recorded at industrial zone stations (S2, S3 and S4). Dissolved oxygen is an important parameter in water quality assessment, and it depends on temperature, salinity, turbulence of the water column and atmospheric pressure. The depletion of oxygen content in water leads to undesirable obnoxious odours under anaerobic conditions (Doudorof and Shumway 1970; Nelson 1978; Maya et al. 2011) and damage to aquatic life. Therefore, adequate amount of dissolved oxygen is essential for the survival of fish and other aquatic organisms. Moreover, in aquatic systems, oxygenation is the result of an imbalance between the process of photosynthesis, degradation of organic matter, re-aeration (Garnier et al. 1999), and physicochemical properties of water **(**Aston 1980). The decomposition of organic effluents and domestic sewage may reduce the DO to extremely low levels at S2, S3 and S4 (Pillai et al. 1975; Vijayan et al. 1976). Higher concentration of DO during monsoon season could be attributed to the high turbulence in river flow during the rainy season (Nair 1992).

The distribution of nutrients in aquatic systems is controlled by variations in physical, chemical and biological processes. These dynamic processes being different in each aquatic realm, a successful understanding is essential for the role of these systems as nutrient traps and filters. Among the dissolved nutrients, different forms of nitrogen (ammonia, nitrite and nitrate) were selected for the present study. Moreover, dissolved inorganic phosphorus was determined, in order to assess the phosphate loading in the water column. Concentrations of ammonia-nitrogen in water samples at all the stations during the survey are given in Table 2.1, and Fig. 2.6. The industrial zone (S2, S3 and S4) and estuarine station (S5) exhibited a sharp increase in ammonia content throughout the investigation period, without any definite seasonal pattern. The order of abundance of ammonia in the various stations followed the order: S2 > S3 > S4> S5 > S1. The upstream station displayed much lower concentration of ammonia compared to other sites, and it ranged from a higher value of 23.2 µmol/l to a low value of 18.9 µmol/l. However, in other stations the concentrations of ammonia varied from 41.3 (S5; MN) to 148.7 µmol/l (S2;

PRM). The elevated levels of ammonia in the industrial sites can be related to the increased rate of mineralization processes taking place there. Observations suggested the fact that oxygen is depleted in the water column due to the higher rate of organic matter degradation. The mineralization process releases assimilated nitrogen compounds, and NH_4^+ ion is the most preferred form since, the other oxidized compounds of nitrogen are highly unlikely to form in this oxygen-deficient environment. In highly anoxic sediments, pore water contains large amounts of NH_4^+ ions and diffusion of pore water NH_4^+ ions further contributes to the dissolved NH_4^+ in the overlying water. Moreover, the abundance of ammonia in this study area points out external input. A cluster of industries including a major fertilizer manufacturing factory, located on the banks of the river. Leachates from this industry may have contributed to the elevated level of NH_4^+ at this site.

Among the nutrients, nitrite and nitrate concentrations in surface water at station S1 and S5 corresponds to the normal values, and it ranged from 0.22 (S1; MN) to 0.86 µmol/l (S5; PRM) and 0.86 (S1; MN) to 1.5 µmol/l (S5; PRM) respectively. However, stations S2, S3 and S4 showed higher values of nutrient concentrations during the investigation period, irrespective of the seasonal changes. Maximum concentrations of nitrite (17.3 µmol/l) and nitrate (28.9 µmol/l) at S2 further supports our earlier conclusions that some effluent treated water rich in nitrogenous compounds added to this area from external sources. The concentration of silicate in surface water samples varied from 32.4 (S1) to 86.1 µmol/l (S5) during PRM, 49.3 (S1) to 98 µmol/l (S5) in MN, 41.7 (S1) to 78.1 µmol/l (S3) during POM seasons. Maximum concentration of silicate was recorded at estuarine station S5 during MN season and a minimum value was observed at S1 during PRM season. Usually, weathering processes and land run-off largely contribute to silicate concentration to the surface water (Liss and Spencer 1970; Manju et al. 2012). Decomposition of diatoms which associated with elevated temperatures also adds substantial proportions of silicate to the aquatic environment (Klarer and Hickman 1975; Happy-wood and Priddle 1984; Sushanth and Rajashekhar 2012).

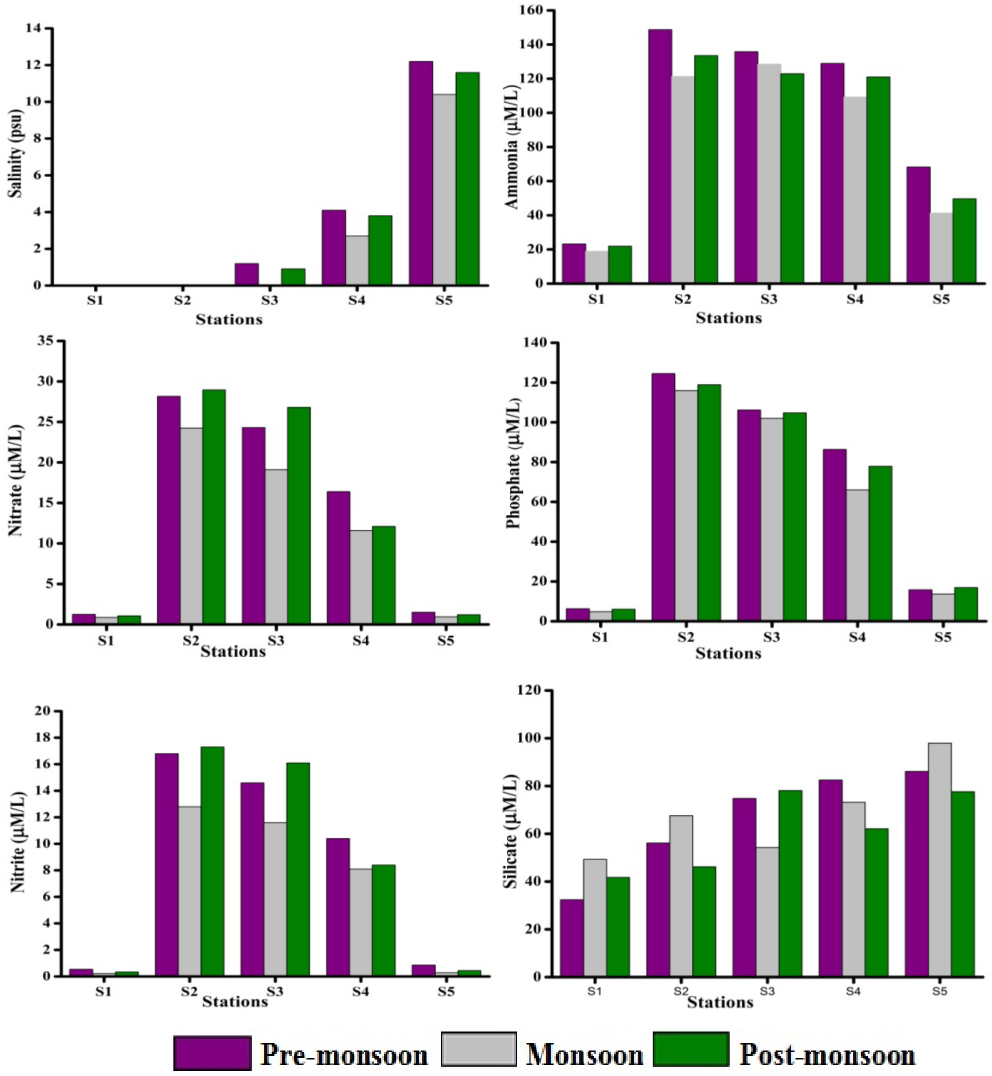

Fig. 2.6 Spatio-temporal variations of salinity and nutrients in the study area

The estimated inorganic phosphate concentrations ranged from 6.3 (S1) to 124.5 µmol/l (S2) in pre-monsoon, 4.8 (S1) to 116 µmol/l (S2) in monsoon and 6 (S1) to 119 µmol/l (S2) during post-monsoon seasons. Maximum phosphate concentration was recorded at S2 during PRM season, while, a minimum value recorded at S1 (4.8 µmol/l) during MN season. This observation is consistent with the previous studies that have reported higher concentrations of dissolved phosphate in Cochin backwaters during pre-monsoon period, which exceed the prescribed limit of water quality standards, but exhibited lower values during the monsoon season (Joseph 1974; Lakshmanan et al. 1982; Anirudhan 1988). The lower values during the monsoon season can be attributed to the high dilution effect and consequently higher rates of flushing of phosphate by fresh water from the river. Increased riverine discharges during monsoon period may result in low salinity, pH and high dissolved oxygen, favouring the removal of phosphorus from the overlying waters by the sedimentation of the particles. Very high concentrations of phosphate were observed at S2, S3 and S4 indicated the discharge of some effluent rich in phosphorus compounds from the adjacent fertilizer manufacturing unit.

In summary, anthropogenic activities have a remarkable influence on the quality conditions of hydrographic parameters in the study area (Siddiqui 2011; Clemente et al. 2011; Kiteresi et al. 2012). The stability of the aquatic system is influenced by salinity, nutrient enrichment and dynamics, physiological tolerance, predation and competition at the local level (Smith et al. 2003). Salinity exhibited its minimum during the monsoon season, due to fresh water runoff. In aquatic systems, oxygenation is the result of an imbalance between the process of photosynthesis, degradation of organic matter, recreation (Garnier et al. 1999), and physicochemical properties of water (Aston 1980). The anoxic conditions observed at various stations were attributed to the high concentration of organic matter and salinity variations. In the present study, nutrients exhibited higher concentration during pre-monsoon and lower content during monsoon. In aquatic region, nitrogen in a water body is determined by the balance between assimilation, mineralization, nitrification, denitrification and nitrogen fixation (Solanki et al. 2010). Stations S2, S3 and S4 receive a considerable amount of the sewages and industrial effluents, resulting to the reducing environment and elevated levels of nutrients. The highest concentration of silicate in surface water may be attributed to the weathering processes and land run-off events (Manju et al. 2012). Various chemical processes affect the diurnal variations of nitrogenous nutrients in the surface sediments of the study area. Nutrient distributions and variations in aquatic 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,nitrite and phosphate) within the study area indicated that the nutrient levels are controlled by some external inputs, while strong positive correlation between salinity and silicate ($r^2 = 0.71$, $p < 0.01$) indicated similar behaviour in the aquatic environment. From the interrelationships between nutrients, it could be inferred that they were derived from the similar sources.

Table 2.2 Pearson correlation coefficients for various hydrographic parameters in the study region (n=27)

	pH	Salinity	DO	Silicate	Nitrite	Nitrate	Ammonia	Phosphate
pH	1							
Salinity	0.42	1						
DO	0.64**	0.15	1					
Silicate	-0.08	0.71**	-0.29	1				
Nitrite	-0.74**	-0.51*	-0.91**	-0.01	1			
Nitrate	-0.69**	-0.52*	-0.90**	-0.02	0.99**	1		
Ammonia	-0.77**	-0.29	-0.95**	0.18	0.93**	0.90**	1	
Phosphate	-0.71**	-0.47	-0.93**	0.02	0.98**	0.98**	0.96**	1

DO - Disolved oxygen; Correlation significant at 0.01 level is given in ** and significant at 0.05 level is given in *

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Chapter 3

GENERAL GEOCHEMICAL CHARACTERISTICS

Contents

3.1 Introduction

Biochemical and physical processes play an important role in regulating the chemistry and biology of aquatic ecosystem (Bianchi et al. 1999; Hobbie 2000; Tesi et al. 2007; Yang and Leskovec 2015). Therefore, on a global basis, aquatic sediments are important sites for studying and evaluating fluxes, cycling and storage of organic compounds. The quantity and quality of organic matter preserved in aquatic sediment varies greatly, depending on the nature of material delivered to the sediment, as well as the, characteristics of the depositional environment. Biochemical processes taking place in aquatic ecosystem are highly complex due to the inputs from allochthonous, as well as, autochthonous sources into the sedimentary organic matter (Joseph et al. 2012). Riverine delivery of organic matter (OM) and nutrients has potential implications to the global carbon cycle, because, remineralisation and the burial of carbon in coastal sediments influence the ocean's ability to sequester atmospheric carbon dioxide. Moreover, changes to the global carbon cycle have captured the attention of the general public and researchers, because of possible climate changes due to increased emissions of greenhouse gases. However, the fate of OM in the sedimentary system is challenging due to variable inputs from the adjacent continents, sorption-desorption phenomena's, (Hedges and Keil 1999) and the various factors controlling OM preservation (Wakeham and Canuel 2005). The nature and extend of such forces coincided with biological processes are directly associated with remineralisation of sedimentary organic matter, which in turn has a significant impact on biogeochemical cycling of carbon, nitrogen and phosphorus (Burdige 2011). Therefore, the overall environmental conditions prevailing in any aquatic system cannot be explained by the mere application of geochemical parameters such as general sedimentary characteristics, phosphorus fractionation or heavy metal distribution studies, etc**.** Therefore, bulk sedimentary parameters are available for the evaluation of sources of OM and its fate within aquatic sediments, and it includes biochemical composition, elemental and stable isotopic ratios. Sources of organic matter and the significant processes involved in the transformation of organic matter have been reported in terms of bulk parameters such as, elemental ratios, isotopic compositions etc. (Tan et al. 1991; Thornton and Mc-Manus 1994; Mitchell et al. 1997; Andrews et al. 1998; Graham et al. 2001; Ramaswamy et al. 2008; Gireeshkumar et al. 2013; Trefry et al. 2014). Stable isotopic compositions (δ^{13}C and δ^{15}N) of various carbon inputs are usually different, making them as powerful tracers to differentiate between allochthonous as well as autochthonous organic carbon inputs (Middelburg et al. 1997; Bianchi et al. 2002; Zhang et al. 2007; Ramaswamy et al. 2008; Gireeshkumar et al. 2013).

3.1.1 Biochemical composition of sedimentary organic matter

Aquatic sediments consist of a complex combination of organic compounds, characterised by their large changeability in composition, distribution and reactivities (Middelburg and Herman 2007). Moreover, the quality of sedimentary organic matter largely depend on several factors, including source, composition and biochemical transformations**,** during their descent through the water column (Cowie and Hedges 1992). Sedimentary organic matter comprises, both labile and refractory organic compounds, whose abundance depends on degradation, heterotrophic utilisation, transformation, etc. (Viollier et al. 2003; Venturini et al. 2012). Usually, refractory organic materials are characterised by their lower degradation and higher preservation in sediments (Danovaro et al. 1994; Danovaro et al. 1999; Middleburg et al. 1999), while, labile organic matter (LOM) fraction consists of simple and/or combined organic biopolymer molecules such as proteins, carbohydrates and lipids, which are available for benthic organisms, and rapidly mineralised (Venturini et al. 2012). Among the bulk sedimentary parameters, biochemical composition has been commonly utilised to realize vital information on the origin and parameters governing the diagenetic fate of organic matter. Usually, labile organic compounds such as, proteins (PRT), carbohydrates (CHO), lipids (LPD) and chlorophyll pigments are considered as the best tools for the evaluation of the nature and quality of organic matter, which can provide clear insights into the biochemical characterisation of sedimentary environment in lakes, rivers, estuaries etc., (Colombo 1996; Dell' Anno et al. 2002; Pusceddu et al. 2009, 2011; Venturni et al. 2012). Biochemical characteristics serves not only as a useful methodology to establish the source of organic matter (Colombo et al. 1996), but also a valid tool to assess the nutritional quality of organic materials as available food for benthic consumers (Dell' Anno et al. 2000; Rossi et al. 2001; Cividanes et al. 2002; Rossi and Lardicci 2002; Joseph et al. 2008). Composition of OM in aquatic sediments has been documented as the major factor affecting metabolism 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 aquatic ecosystems (Cloern 2001; Renjith et al. 2013; Akhil et al. 2013). The portion of sedimentary OM which are more readily available to benthic consumers (labile fraction), has usually been assessed by estimation of the main biochemical classes of organic compounds (Fichez

1991; Danovaro et al. 1993; Fabiano et al. 1995; Dell'Anno et al. 2002). Total concentrations of proteins, carbohydrates and lipids in sediments are generally referred to as biochemical compositions and used as an effective tool for the assessment of the 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).

3.1.2 Bulk organic matter techniques

Sources of organic matter in aquatic sediments are characterized by a large variation in their composition, degradability, range of sources such as, phytoplankton and benthic microalgae to less degradable sources such as, mangrove detritus and terrigenous inputs (Bouillon et al. 2004). Due to this complex nature and multiple sources of organic matter (Jassby et al. 1993), a variety of parameters including elemental composition (TOC/TN ratio) and stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) have been used to assess the sources of organic matter (Andrews et al. 1998; Maksymowska et al. 2000; Naidu et al. 2000; Kerherve et al. 2001; Zimmerman and Canuel 2001). Therefore, along with biochemical compositions, bulk parameters like elemental compositions, $\delta^{13}C$ and $\delta^{15}N$ of the total organic matter were also used to characterize the sources of organic matter in aquatic sediment. The usefulness of these bulk parameters as source indicators is reliant on there being markedly different signatures between the different organic matter sources (Kennedy et al. 2004).

Application of stable isotope ratios and elemental composition is a common trend in biochemical studies. The basic principle involved in the application of stable isotopes in natural ecosystems is based on differences in the relative abundance of lighter isotopes from chemical rather than nuclear processes (Hoefs 1980). The most common stable isotopes used in biochemical studies are ^{18}O, ^{2}H, ^{13}C, ^{15}N, and ^{34}S. Photosynthesis is a vital process, where the chloroplast of green plants synthesizes sugars by using water and carbon dioxide in the presence of light. Most of the photosynthetic organisms include carbon into their biomass using the Calvin cycle or C_3 cycle, which discriminates against ^{13}C to produce a shift in $\delta^{13}C$ values of about -20‰ from the isotopic ratios of inorganic carbon sources. C_4 cycle or Hatch and Slack pathway is the other pathway of Calvin cycle taking place during the dark phase of photosynthesis, followed by many subtropical savannah grasses and sedges, leads to an isotopic shift of -7‰ from the inorganic carbon sources. Terrestrial organic matter produced by C_3 pathway has $\delta\ ^{13}C$ values ranged from -30 to -26‰, while, for C_4 pathway, it is varied from -16 to -9‰ (Pancost and Boot 2004). Similarly, the typical $\delta^{15}N$ values of terrestrial vascular plants ranged from -5 to 18‰ with an average value of ≈3 ‰, and marine organic matter ranged from 3 to 12‰, with a mean value of ≈6 ‰ (Wada and Hattori 1991; Muller and Voss 1999; Maksymowska et al. 2000). Similarly, total organic carbon to total nitrogen (TOC/TN) ratio has been used as a potentially useful indicator in elucidating the sources of sedimentary organic matter (Andrews et al. 1998; Muller and Voss 1999; Maksymowska et al. 2000), although, it does not give any evidence to the chemical features of the organic matter, due to the abundance of nitrogenous compounds, such as, humic materials. Generally, marine and terrestrial organic matter has TOC/TN values ≈5-8 and >15, respectively (Meyers 1997). However, post-depositional changes (Bordovskiy 1965; Prahl et al. 1980; Roman 1980), and the presences of significant fractions of inorganic nitrogen (adsorbed on clay minerals) can limit the usefulness of TOC/TN ratios, as source indicators of organic matter in aquatic sediments. Similarly, organic C, N and phosphorus (P) ratios (TOC/TN/TP) (Redfield ratio), have also been widely used to differentiate the origin of sedimentary organic matter, as a result of the distinctive TOC/TN/TP ratios of terrestrial and marine plants (Redfield et al. 1963; Ruttenberg and Goñi 1997).

Cochin backwaters are highly productive ecosystem, and its complex nature is attributed to permanent opening with the Arabian Sea and the inputs of significant quantities of OM and nutrients (Srinivas 2000). Large quantities of the sewages and other untreated and partially treated pollutants discharge into the Cochin backwater system, which causes significant toxic impact on the aquatic environment (Balachandran et al. 2005, 2008). Even though, information regarding the organic geochemical aspects of sediments in the Cochin estuarine system is available (Aneeshkumar and Sujatha 2012; Gireeshkumar et al. 2013; Renjith et al. 2013; Akhil et al. 2013), a long term monitoring and a detailed description of the sedimentary variables with regard to the Chitrapuzha River system is yet to be reported. Repeated regular assessment, coupled with seasonal changes is a prerequisite for evaluating the current health status of a deteriorating and vulnerable ecosystem. Therefore, this chapter intends to focus on the nature and quality of organic matter in the surface sediments of Chitrapuzha River, and attempts to unfold the sources of sedimentary organic matter by the application of biochemical compositions and bulk organic matter techniques. The information furnished in the present investigation will be helpful to future studies, as a reference data for formulating remedial environmental conservation strategies of this sensitive ecosystem.

3.2 Results and discussion

Surface sediment samples were collected from 5 locations along the Chitrapuzha River during monsoon (MN), pre-monsoon (PRM) and post-monsoon (POM) seasons. Station 1 (S1) represents the far upstream portion of the river, and is beyond the influence of industrial pollution. Stations 2 (S2), 3 (S3) and 4 (S4) constitutes industrial zones, which are the recipients of discharge outlets of major industrial units. Station 5 (S5) represents the downstream estuarine portion of the study area, and it receives a considerable amount of discharges from apartments on the mainland as well as from the oil tanker berth, Cochin Port and Cochin Shipyard (detailed description of the study area and sampling sites are described in chapter 2).

Table 3.1 General sedimentary characteristics of the surface sediments of Chitrapuzha River (n=27)

	Stations				
Parameters	**S1**	**S2**	**S3**	**S4**	**S5**
P^H	7.0 ± 0.21	6.81 ± 0.14	6.76 ± 0.20	6.76 ± 0.18	6.88 ± 0.12
Eh (mV)	-199.0 ± 3.6	-198.0 ± 4.2	-153.0 ± 2.3	-245.0 ± 2.0	-149.5 ± 1.7
Sand (%)	45.45 ± 0.79	21.46 ± 0.74	20.93 ± 0.45	17.18 ± 0.85	43.28 ± 1.3
Silt (%)	27.37 ± 0.62	54.98 ± 0.42	55.86 ± 0.71	58.53 ± 0.55	28.80 ± 0.36
Clay (%)	27.18 ± 0.22	23.56 ± 0.41	23.21 ± 0.82	24.29 ± 0.66	27.92 ± 0.73
TOC (%)	2.92 ± 0.21	7.42 ± 0.19	7.53 ± 0.33	8.9 ± 0.06	3.32 ± 0.18
TN (%)	0.19 ± 0.06	0.29 ± 0.07	0.33 ± 0.03	0.3 ± 0.07	0.18 ± 0.02
TS (%)	0.08 ± 0.03	3.84 ± 0.06	2.67 ± 0.1	0.77 ± 0.08	0.18 ± 0.06
TOC/TN	15.13 ± 0.06	25.49 ± 0.10	23.17 ± 0.08	29.69 ± 0.05	18.47 ± 0.03
TOC/TS	36.6 ± 0.12	1.93 ± 0.06	2.82 ± 0.11	11.56 ± 0.13	18.44 ± 0.11
$\delta\ ^{13}C$ (‰)	-26.05 ± 1.3	-27.43 ± 1.6	-27.31 ± 1.1	-27.94 ± 0.08	-27.66 ± 0.09
$\delta^{15}N$ (‰)	4.7 ± 0.05	1.66 ± 0.07	2.47 ± 0.10	3.68 ± 0.08	3.38 ± 0.15

TOC - Total organic carbon; TN - Total nitrogen; TS - Total sulphur

3.2.1 General sedimentary characteristics

General sedimentary characteristics of the surface sediments are given in Table 3.1. Sediment pH in the study area was slightly acidic in nature, except at S1, and the pH values ranged from 6.7 to 7.0 in surface sediments.

3.2.1.1 Redox potential (Eh)

Many chemical processes occurring in aquatic sediment depend on the redox potential or Eh of the sedimentary system. It is a measure of availability of electrons and their activity in the medium, and it controls the competing processes of electron donation (reduction) and acceptance (oxidation).

Positive values of the redox potential of the aqueous medium indicated an oxidising environment for chemical processes (aerobic processes), while, negative values in the range of -200 mV or less, a reducing environment for chemical processes (anaerobic processes), and the values between zero and -200 mV indicated the transition character of the medium. In the present study, Eh values fluctuated between -149 mV (S5) and -245 mV (S4) (Table 3.1), indicated the linkage of surface sediments to anaerobic conditions. An anaerobic reducing condition in an ecosystem may lead to microbial conversion of sulphates to sulphides, which may cause precipitation of metal ions such as mercury, cadmium, nickel etc. as their sulphides. Station S4 in the industrial zone was found to be more reducing in nature which coincides with high organic matter load, and this organic matter pool may lead to the existence of a reducing environment.

3.2.1.2 Texture

The average composition of sediment grain size in the study area is given in Table 3.1, and the seasonal and spatial variations are depicted in Fig. 3.1. Analysis of variance (ANOVA, Two-Factor without replication) showed that sediment texture did not have any significant seasonal variations, but exhibited spatial variations in these ecosystems. Texture analysis revealed that, sand and clay fractions dominated in the upstream (S1) and downstream (S5) stations, while, silt particles dominated in the industrial zone (S2, S3 and S4). The average sand content ranged from 17 (S4) to 45% (S1), and silt fraction varied from 27 (S1) to 59% (S4), while, clay particles varied from 23 (S3) to 28% (S5) in the surface sediments. Grain size analysis is a fundamental procedure in sedimentology and limnology and it gives some basic information on the sediment composition and depositional environment. Moreover, the sedimentary texture has a significant role in the physico-chemical processes as well as the species diversity of the depositional environment (Badarudeen et al. 1996; Ramaswamy et al. 2008; Gireeshkumar et al. 2013). The dependency of the physical properties of aquatic sediments on sediment grain size has previously described by Hamilton (1972) and Hamilton and Bachman (1982), and it is well documented that, a strong relationship exists between the sediment grain size and organic matter content, due to the greater absorption capacity of fine sediments with large surface areas (Meyers and Eadie 1993; Meyers and Lellier-Verge`s 1999). Therefore, the distribution of sediment grain size, is influential and important, when studying organic matter and geochemical signatures of coastal and marine sediments. In the present study, the silt fraction of sediment exhibited a strong positive correlation (Table 3.2) with TOC ($r^2 = 0.98$, $p < 0.01$), TS ($r^2 = 0.68$, $p < 0.01$), TN ($r^2 = 0.93$, $p < 0.01$), PRT ($r^2 = 0.84$, $p < 0.01$), CHO ($r^2 = 0.60$, $p < 0.05$), and LOM ($r^2 = 0.56$, $p < 0.05$), while sand exhibited a strong negative correlation ($r^2 = -0.99$, $p < 0.01$), substantiate the above observations.

The physical processes of transportation and deposition can also alter the grain sizes of surface sediments to a greater extent. Increased river runoff associated with high precipitation during the monsoon season (Menon et al. 2000), may result in higher sand content in the upstream and downstream stations (S1 and S5). Moreover, 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 (S1) of the study area. The percentage of clay varied from 22.5 (MN; S3) to 29% (PRM; S5) (Fig. 3.1) in surface sediments. Usually, clay acts as nucleation centres for flocculation and precipitation of dissolved and colloidal organic matter during estuarine mixing (Aston and Chester 1973; Sholkovitz 1976; Cotano and Villate 2006; Ramaswamy et al. 2008). Generally, the estuarine station S5 recorded higher values of clay content, while, the stations in the industrial zone recorded the highest percentage of silt. The percentage of clay was generally higher at all the stations during non-monsoon seasons (PRM and POM), compared to monsoon season, due to the settling of finer fractions of the sediment samples. On the contrary, the stations S2, S3 and S4 in the industrial zone showed slightly higher values of the silt fraction during monsoon season. This seasonal variation could be associated with the spillage of phosphogypsum, a waste product from the adjacent premises of the fertilizer manufacturing unit. In addition to this, the complex current pattern prevailing in the study area, and the dredging operations to facilitate water transportation of imported chemicals from the Cochn Port to the industrial units at ambalamugal industrial area, also influences the textural characteristics of the surface sediments of Chitrapuzha River.

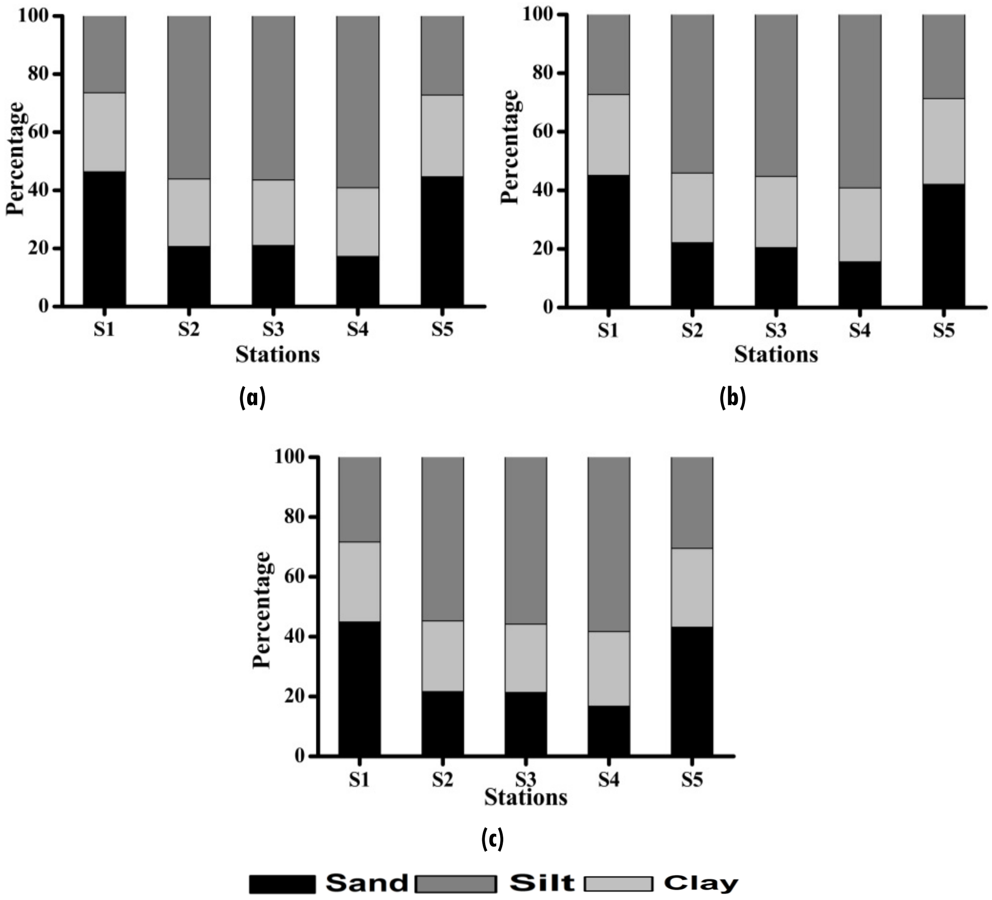


Fig. 3.1 Seasonal distribution of sand, silt and clay in the surface sediments of the study area, (a) monsoon (MN), (b) pre-monsoon (PRM) and (c) post-monsoon (POM)

Table 3.2 Pearson correlation coefficients for various sedimentary parameters in the study region (n=27)

	Sand	Silt	Clay	TOC	TS	TN	PRT	CHO	LPD	Chl-a	Chl-b	Chl-c	Phaeo	BPC	LOM	$\delta^{13}C$
Sand	1															
Silt	-0.99**	1														
Clay	-0.24	0.23	1													
TOC	-0.99**	0.98**	0.22	1												
TS	-0.65**	0.68**	0.15	0.60*	1											
TN	-0.92**	0.93**	0.40	0.91**	0.71**	1										
PRT	-0.85**	0.84**	0.22	0.83**	0.78**	0.76**	1									
CHO	-0.58*	0.60*	0.22	0.54*	0.83**	0.63*	0.69**	1								
LPD	0.66**	-0.64**	-0.05	-0.66**	-0.42	-0.52*	-0.79**	-0.16	1							
Chl-a	0.77**	-0.76**	-0.10	-0.78**	-0.49	-0.56*	-0.76**	-0.51*	0.49	1						
Chl-b	0.73**	-0.72**	-0.15	-0.76**	-0.27	-0.67**	-0.50	-0.52*	0.20	0.67**	1					
Chl-c	0.61*	-0.60*	-0.12	-0.66**	-0.02	-0.43	-0.30	-0.21	0.10	0.74**	0.81**	1				
Phaeo	0.73**	-0.72**	-0.11	-0.74**	-0.45	-0.56*	-0.63*	-0.582*	0.29	0.91**	0.72**	0.81**	1			
BPC	-0.26	0.29	0.23	0.23	0.58*	0.36	0.29	0.85**	0.33	-0.3	-0.44	-0.23	-0.47	1		
LOM	-0.53*	0.56*	0.26	0.50	0.78**	0.59*	0.62*	0.97**	-0.03	-0.54*	-0.55*	-0.27	-0.61*	0.92**	1	
$\delta^{13}C$	0.44	-0.42	-0.33	-0.45	-0.17	-0.41	-0.57*	-0.00	0.78**	0.25	0.14	-0.00	0.10	0.36	0.07	1

Correlation significant at 0.01 level is given in ** and significant at 0.05 level is given in *
TOC- Total organic carbon; TS- Total sulphur; TN- Total nitrogen; PRT- Total protein; CHO- Total carbohydrate; LPD- Total lipid; Chl-a-Chlorophyll-a; Chl-b- Chlorophyll-b; Chl-c-Chlorophyll-c; Pheo- Phaeophytin; BPC- Biopolymeric carbon; LOM- Labile organic matter

3.2.2 Bulk elemental and isotopic composition of the organic matter

3.2.2.1 Total organic carbon (TOC)

The spatial and seasonal distributions of TOC (Fig. 3.2) indicated that the sediments were rich in organic carbon, and the average values varied from 2.9 to 8.9% (Table 3.1), and recorded a maximum value at S4 during pre-monsoon season. However, it was observed that there is no significant seasonal difference in the TOC content of surface sediments in the Chitrapuzha River. TOC values obtained in the present study are comparatively slightly higher than that in the sediments of the northern parts of the Cochin estuary (Balachandran et al. 2005; Martin et al. 2010). The observed TOC content in the study area was influenced by the in situ primary production, addition of terrestrial materials, deposition rate and textural characteristics of the surface sediments. Fig 3.2 revealed that stations in the industrial zone (S2, S3 and S4) recorded higher TOC contents, while, the upstream and downstream stations (S1 and S5), which are sand dominated, exhibited lower TOC concentrations. This is probably associated with the combined effect of river inputs, coupled with the discharge of organic wastes from different industrial, agricultural and aquaculture sectors leads to a large scale accumulation of organic matter in the surface sediments. Moreover, the efficiency of sedimentary systems in trapping the suspended material from the water column depends largely on a range of factors, such as, particle size, salinity, tidal pumping and the areal extent of the intertidal zone (Wolanski 1995). The fine grained sediments can provide enough surface area for the adsorsption and retention of organic matter. Therefore, granulometrically, the fine grained (silty-clay) compositions of surface sediments in the industrial zone (Fig. 3.2), facilitate trapping of organic matter and its accumulation (Cotano and Villate 2006; Ramaswamy et al. 2008); whereas, the inability of sandy sediment to trap fine particles resulted in lower TOC values at stations S1 and S5. Furthermore, correlation analysis confirmed the ganulometric dependency of TOC concentrations, and exhibited a strong significant positive correlation (Table 3.2) with silt ($r^2 = 0.98$, $p < 0.01$) and an inverse relationship with that of sand ($r^2 = -0.99$, $p < 0.01$), implies its size dependent scavenging nature (Muraleedharan Nair and Ramachandran 2002).

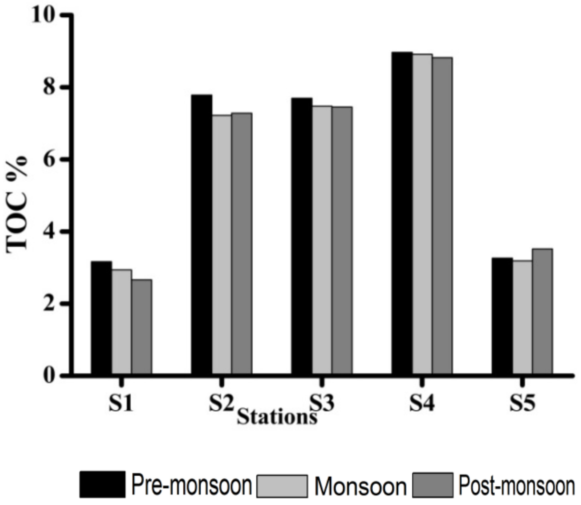


Fig. 3.2 Seasonal and spatial variations of TOC in the surface sediments of the study area

3.2.2.2 Total nitrogen (TN) and total sulphur (TS)

Concentrations of total nitrogen (TN) content were relatively lower in surface sediments of Chitrapuzha River, and it ranged from 0.18 to 0.33% (Table 3.1), and exhibited comparatively higher values in the stations of industrial zone (S2, S3 and S4) during the post-monsoon season except at station S1 (Fig. 3.3). TN varied significantly among the sampling sites, but it did not display any seasonal differences. Nitrogen is a key nutrient element which governs the functions of aquatic ecosystems by limiting the biological growth and it is capable of driving eutrophication (Montagna et al. 2002; Gruber and Galloway 2008). The distribution of nitrogen content in aquatic systems is controlled by variations in physical, chemical and biological processes. These dynamic processes being different in different aquatic realm, a successful understanding is essential to identify the role of these systems as nitrogen traps and filters. Biological nitrogen fixation, the conversion of atmospheric N_2 to NH_4^+, is an important source of sedimentary nitrogen in the aquatic environment. Organic nitrogen mineralisation in sediments can be a significant source of ammonia-N to the overlying water column. Nitrification and denitrification are often tightly coupled near the oxic-anoxic boundary of the sediment with little loss of fixed nitrogen to the overlying water column (Thamdrup and Dalsgaard 2008). Usually, the total nitrogen contents can be used as an indicative of contribution of aquatic flora in marine sediments (González- Vila et al. 2003). Most of the stations in the study area recorded a consistent trend in concentration of TN during the study period. Relatively high levels of TN recorded at the industrial sites (Table 3.1), might be due to the direct input of nitrogenous compounds from external sources, mainly from the industrial run-off and domestic sewages (Purvaja and Ramesh 2000; Subramanian 2004). A cluster of industries including a major fertilizer unit, located on the banks of the Chitrapuzha River can act acts as a point source of nitrogen content in the surface sediments. This observation was in good agreement with our earlier views with regard to the elevated levels of nutrient concentrations in the industrial sites (chapter 2). Low levels of nitrogen content at S1 and S5 may be due to the variations in the land discharge, and also by the sandy nature of the surface sediments with poor retention of nitrogen contents. Moreover, lesser nitrification rate and degradation of organic nitrogen to inorganic forms can also be resulted in lower levels of nitrogen content (Bala Krishna Prasad and Ramanathan 2008).

Total sulphur (TS) varied from 0.08 to 3.84%, and recorded its higher values during the pre-monsoon season at S2 (Fig. 3.3). Strong positive correlation between TN, TOC ($r^2 = 0.91$, $p < 0.01$) and TS ($r^2 = 0.71$, $p < 0.01$) (Table 3.2), point towards a common origin and the similar behaviour in the aquatic environment.

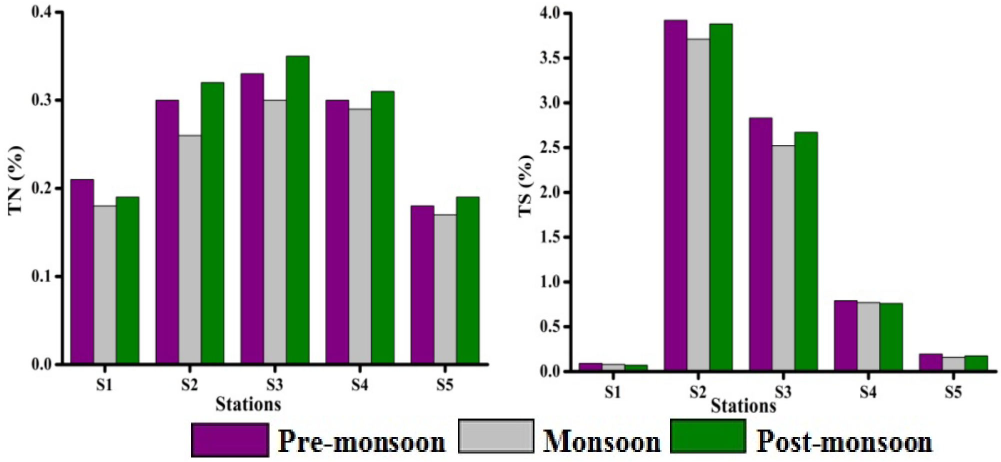


Fig. 3.3 Spatio temporal variations of TN and TS in the surface sediments

The composition of sedimentary organic matter in aquatic systems mainly depends on the in situ production, allochthonous inputs, interaction of organic matter with mineral particles and oxygen availability. Moreover, sources of organic matter in the sedimentary environment are influenced by different pathways, including marine algae and terrestrial plants (Volkman et al. 2000). Due to this complex nature and multiple sources of organic matter (Jassby et al. 1993), bulk geochemical proxies such as total organic carbon to total nitrogen (TOC/TN) and total carbon to total sulphur (TOC/TS) ratios have been commonly used to distinguish the sources of organic matter - autochthonous or marine versus allochthonous or terrestrial (Canuel et al. 1995; Andrews et al. 1998; Maksymowska et al. 2000; Naidu et al. 2000; Kerherve et al. 2001; Zimmerman and Canuel 2001; Perdue and Koprivnjak 2007). For example, TOC/TN ratios have been used as an effective tool to trace out the sources of organic matter, based on the fact that marine and terrestrial derived organic materials have a distinct TOC/TN values. Terrestrial plants are characterized by their low protein content and abundance of cellulose, display high TOC/TN ratios (≥20) (Meyers and Ishiwatari 1993; Filley et al. 2001). While, algae and phytoplankton display, low TOC/TN ratios (4 to12) due to their high protein content and the absence of cellulose (Meyers and Ishiwatari 1993; Meyers 1994; Filley et al. 2001). In the present study, TOC/TN ratios ranged from 15 to 30, 16 to 31, and 14 to 28 in pre-monsoon, monsoon and post-monsoon seasons respectively (Fig. 3.4). Intermediate values of TOC/TN ratios recorded in the surface sediments signalled a combined input of both autochthonous and terrestrial organic matter sources. (Verma and Subramanian 2002; Muri et al. 2004; Gireeshkumar et al. 2013). The observed results are consistent with the previous studies as reported by Liu et al. (2006) and Gao et al. (2012), that demonstrated the decomposition processes such as autolysis, leaching and microbial remineralisation, significantly alter the TOC/TN ratios in surface sediments. The TOC/TN values of sedimentary organic matter are expected to increase or decrease during sediment diagenesis by the selective degradation of organic matter components (Meyers et al. 1996; Liu et al. 2006). Increase in TOC/TN values at S2, S3 and S4 (Fig. 3.4) indicated the accumulation of a higher proportion of terrestrially derived organic matter; whereas, lower TOC/TN at S1 and S5 indicate the higher portion of algal derived organic materials (Guilizzoni et al. 1996). However, the selective degradation of organic matter components and the presence of inorganic N fraction could alter the TOC/TN ratios (Meyers et al. 1996; Muller 1997; Talbot 2001; Liu et al. 2006). The TOC/TN ratios tend to be increase more due to the rapid degradation of proteinaceous organic matter than non-nitrogen organic components, while it tends to decrease due to the release of CO_2 and CH_4 as the degradation products (Gao et al. 2012). Hence, the organic matter degradation processes may influence the distribution of TOC/TN ratios to a great extent, and can lead to misinterpretation of source of organic matter in marine sediments.

The amount of metabolizable organic matter available to support sulphate reducing bacteria usually increases with the total amount of organic matter in the sediment water interface. As a result, the sedimentary pyrite sulphide content is positively correlated with the non metabolized organic matter content (Akhil et al. 2013). Therefore, the ratio of total organic carbon to total sulphur (TOC/TS) in sediments will provide the nature of sedimentary environment in the aquatic ecosystem. The TOC/TS ratios ranged from 1.99 (S2) to 35 (S1), 1.95 (S2) to 37 (S1), and 1.88 (S2) to 38 in pre-monsoon, monsoon and post-monsoon seasons respectively (Fig. 3.4). According to Raiswell et al. (1987), a qualitative evaluation of the redox status of the sedimentary environment can be achieved using TOC/TS values. Under anoxic conditions, dissolved sulphate is reduced to hydrogen sulphide gas, and that will react with iron minerals to form iron sulphides and resulted a qualitative redox status of the environment under deposition (Akhil et al. 2013). Usually, TOC/TS ratio > 5 has been categorised as oxic sediment with oxygenated bottom water, TOC/TS = 1.5 to 5 indicates sediments deposited under periodic anoxia, while, TOC/TS <1.5 reflects anoxic sediments with anoxic water (Raiswell et al. 1987; Hedges and Keil 1995; Niffy Benny 2009; Akhil et al. 2013). In the present study, average values of TOC/TS ranged from 1.9 to 36.6 (Table 3.1), and thus sediments in the study region can be included predominantly in the oxic/periodic anoxic category, during the entire sampling periods (Hedges and Keil 1995).

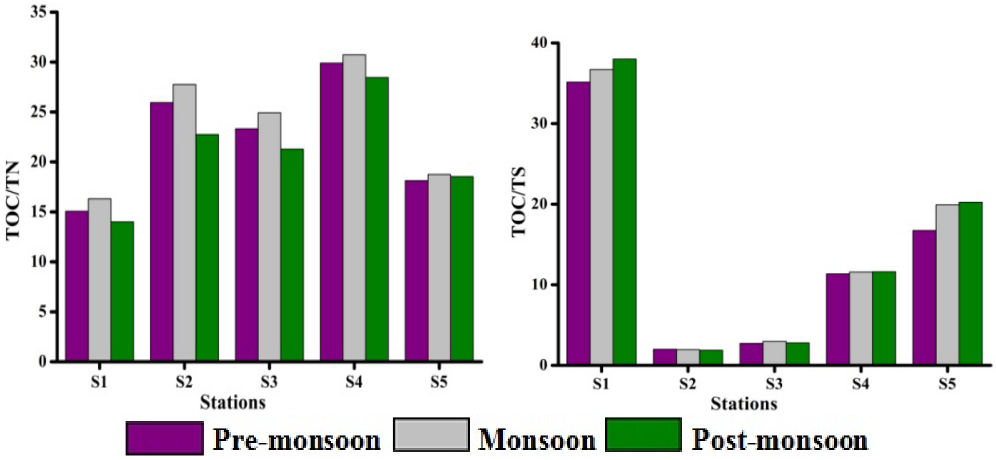


Fig. 3.4 Variations of TOC/ TN and TOC/TS in the surface sediments of the study area

3.2.2.3 $\delta^{13}C$ and $\delta^{15}N$

Spatial and seasonal variations in $\delta^{13}C$ and $\delta^{15}N$ in the surface sediments are presented in Fig. 3.5, and it ranged from -27.1 to -27.9‰ in pre-monsoon, -24.3 to -27.4‰ in monsoon and -26.7 to -28.4‰ in post-monsoon seasons. Comparatively, more enriched values were obtained at station S1, while, more depleted values were recorded at station S4. Generally, more enriched values in the low organic carbon sites, and more depleted values in organic rich sediments were observed. The $\delta^{15}N$ values in the surface sediment varied from 1.46 to 4.85‰, 1.67 to 4.68‰ and 1.84 to 4.71‰ in pre-monsoon, monsoon and post-monsoon seasons respectively. Stable carbon isotopic signatures ($\delta^{13}C$) have been usually employed for the source characterisation of organic matter in aquatic sediments (Andrews et al. 1998; Liu et al. 2006; Zhang et al. 2009), to distinguish between allochthonous and autochthonous organic carbon inputs (Middelburg et al. 1997; Bianchi et al. 2002). Terrestrial organic carbon sources transported to the coastal zone fall in two categories, C_3 and C_4 plant derived matter, each with a distinct and non-overlapping $\delta^{13}C$ values. Usually, terrestrial organic matter produced by C_3 pathway have $\delta^{13}C$ values ranged from -30 to -26‰, while C_4 pathway exhibited values from -16 to -9‰ (Pancost and Boot 2004). Organic matter produced by marine phytoplankton has $\delta^{13}C$ values between -22 and -18‰ (Cifuentes et al. 1998). However, the isotopic source signal can become complicated in coastal areas, which receive considerable amount of organic matter from algae, and both C_3 and C_4 vascular plants (Fry et al. 1977). In these areas, addition of TOC/TN ratios to $\delta^{13}C$ determinations allows better discrimination of organic matter sources. These combinations of elemental and carbon isotopic values can be used to distinguish sources of organic matter in sediments and in settling particles. The stable carbon isotope ratios recorded in the present investigation was comparable with those obtained from other aquatic systems in the world (Andrews et al. 1998; Graham et al. 2001; Liu et al. 2006; Zhang et al. 2009). The distributional characteristics of $\delta^{13}C$ values indicated major inputs of terrestrial higher plant debris to sedimentary organic matter in Chitrapuzha River.

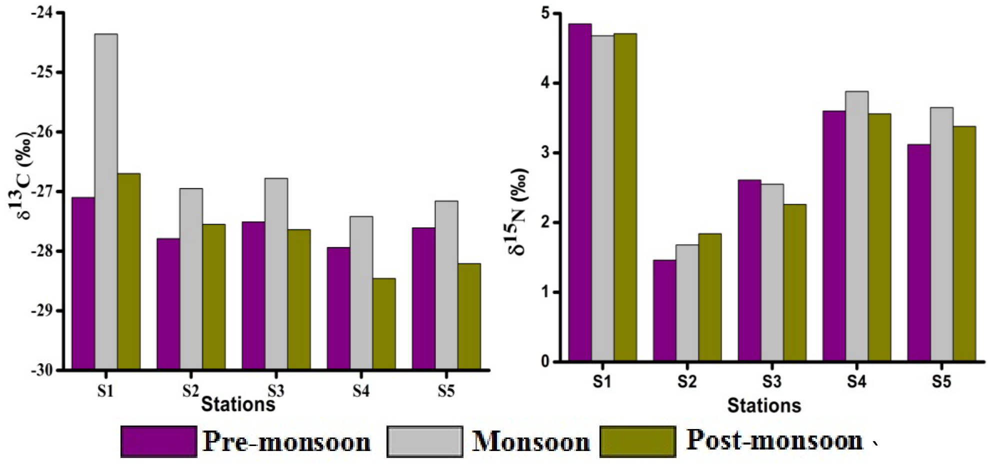


Fig. 3.5 Variations of δ^{13}C and δ^{15}N in the surface sediments of the study area

The potential sources of organic matter in surface sediments of Chitrapuzha River include terrestrial detritus, in situ primary production and industrial and domestic sewages. However, it has been reported in the literature that sewage has no significant influence on the isotopic source signals (Liu et al. 2006), and the observed values of δ^{13}C were similar to that of C3 plants, which averages -27‰ (Gearing et al. 1984; Rao et al. 1994; Machiwa 2000; McKee et al. 2002; Fourqurean and Schrlau 2003). Moreover, Chitrapuzha River originates as a small stream from the upper reaches of high ranges in the eastern boundary of Kerala state and passes through the Ambalamugal industrial area, and finally joins the southern arm of Cochin backwaters, and a large amount of terrestrial organic matter is transported from the catchment area of river to the Cochin backwater system (Thottathil et al. 2008). All the above observations led to the assumption that significant amount of organic matter were derived mainly from the terrigenous inputs in the surface sediments. Schultze and Calder (1976) proposed a quantitative method for estimating the terrestrial derived organic matter using two end member δ^{13}C values, i.e. marine and terrestrial as -20.5 and -30 % respectively (Jia and Peng 2003; Hu et al. 2006;

Liu et al. 2006; Zhang et al. 2009). The terrestrial organic matter (T_rOM) was calculated using the equation:

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

The contribution of T_rOM in surface sediments of Chitrapuzha River ranged from 41 to 84% (Fig. 3.6) and possesses significant spatial variations, and exhibited an increasing trend from upper reaches to the downstream stations.

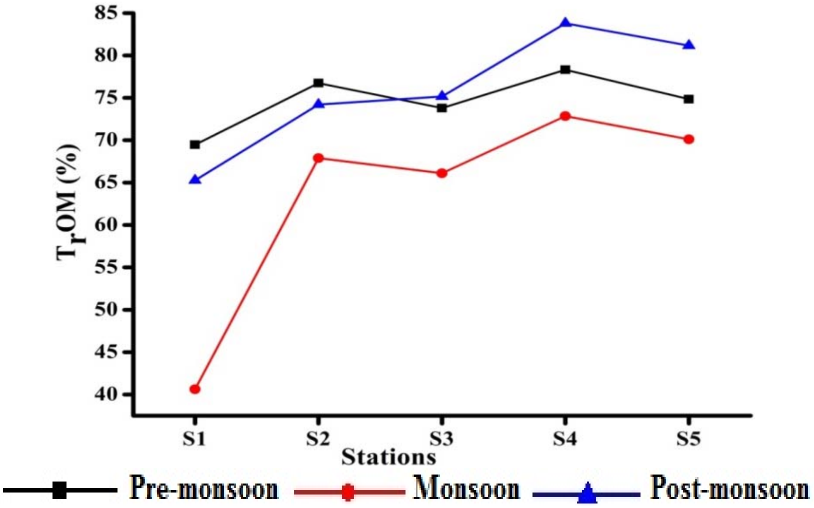


Fig. 3.6 Variations of terrestrial organic matter (T_rOM) in the surface sediments

The average $\delta^{15}N$ values in the surface sediment varied from 1.66 to 4.75‰ and recorded its higher values in pre-monsoon season at S1 (Fig. 3.5), and did not display any spatial or seasonal variations. Usually, marine organic matter has an average $\delta^{15}N$ values of 5 to 7‰ (Lamb et al. 2006; Gao et al. 2012), while, organic matter derived from nitrogen fixing land plants are around zero, whereas plants using soil nitrogen compounds have positive $\delta^{15}N$ values (Gaye-Haake et al. 2005). It has been reported that the transformation and recycling of dissolved nitrogen compounds can cause isotopic fractionation of nitrogen (Wu et al. 2003; Gao et al. 2012). Moreover, the organic matter diagenesis in surface sediments tends to increase the $\delta^{15}N$ values (Liu et al. 2006). It has also been reported that the nitrogen isotopic enrichment is related to heterotrophic microorganisms, and the $\delta^{15}N$ values of microbial reworked terrestrial organic matter increased from -4 to 9‰ (Caraco et al. 1998). The observed trends in the $\delta^{15}N$ values indicate that the sediment decomposition processes have considerable effects on nitrogen isotopic composition in the surface sediments of Chitrapuzha River. The presences of marine phytoplankton like *Trichodesmium* fix dissolved molecular nitrogen in sediments, and exhibited $\delta^{15}N$ values close to zero (Altabet 1996; Ramaswamy et al. 2008). The occurrence of *Trichodesmium* in the lower reaches of Cochin backwaters and the adjacent coastal waters are reported by various authors (Gopinathan et al. 1974; Verma and Agarwal 2000; Krishnan et al. 2007; Ashadevi et al. 2010) and its presence may result in the low $\delta^{15}N$ values in sediments of the study area.

3.2.3 Biochemical composition of sedimentary organic matter

Spatial and seasonal distributions of biochemical components in the surface sediments of the study area are presented in Fig. 3.7, and the average values are shown in Table 3.3. Total protein (PRT) concentrations ranged from 5.05 to 10.6 mg/g (7.75 ± 2.1), 5.64 to 11.05 (8.4 ± 2.06) mg/g and 5.84 and 11.36 (8.9 ± 2.15) mg/g during monsoon, post-monsoon and pre-monsoon seasons respectively. Higher PRT concentrations were observed during the pre-monsoon season, when compared with the other two seasons. Carbohydrate (CHO) concentrations in the sediments ranged from 4.9 to 9.94 mg/g (7.24 ± 1.87), 5.74 to 9.96 mg/g (7.8 ± 1.42) and 5.76 to 10.23 mg/g (7.75 ± 1.70) during the monsoon, post-monsoon and pre-monsoon seasons respectively. Lipid (LPD) concentrations in the sediments ranged from 3.55 to 8.25 mg/g (4.85 ± 1.95), 2.87 to 6.84 mg/g (4.27 ± 1.5) and 1.94 to 7.18 mgg^{-1} (3.71 ± 2.01) during the monsoon, post-monsoon and pre-monsoon seasons respectively. Biopolymeric carbon (BPC) concentrations in the sediments ranged from 8.5 to 11.75 mg/g (10.34 ± 1.48), 8.0 to 12.0 mg/g (10.48 ± 1.44) and 8.71 to 11.86 mg/g (10.25 ± 1.22) during the monsoon, post-monsoon and pre-monsoon seasons respectively (Fig. 3.12).

Table 3.3 Average concentrations of biochemical components in the surface sediments of Chitrapuzha River (n=27)

	Stations				
Parameters	**S1**	**S2**	**S3**	**S4**	**S5**
PRT (mg/g)	5.51 ± 0.41	11.0 ± 0.31	8.75 ± 0.53	9.51 ± 1.02	7.10 ± 0.89
CHO (mg/g)	7.25 ± 0.50	9.93 ± 0.41	8.03 ± 0.63	7.34 ± 0.99	5.48 ± 0.47
LPD (mg/g)	7.42 ± 0.74	3.41 ± 0.38	3.81± 0.46	3.08 ± 0.79	3.68 ± 0.94
PRT/CHO	0.77 ± 0.11	1.1 ± 0.04	1.09 ± 0.02	1.31± 0.12	1.29 ± 0.05
LPD/ CHO	1.02 ± 0.07	0.34 ± 0.05	0.48 ± 0.09	0.43 ± 0.16	0.69 ± 0.24
BPC (mg C/g)	11.17 ± 0.52	11.92 ± 0.07	10.36 ± 0.34	9.90 ± 0.75	8.43 ± 0.32
LOM (mg/g)	20.18 ± 0.66	24.34 ± 0.48	20.59 ± 0.31	19.92 ± 0.88	16.26 ± 1.1
Chl- a (μg/g)	12.98 ± 0.84	5.8 ± 0.80	11.31 ± 1.28	5.61 ± 1.19	12.68 ± 1.36
Chl- b (μg/g)	3.28 ± 0.23	2.59 ± 0.42	3.11 ± 0.45	0.89 ± 0.25	5.61 ± 0.78
Chl- c (μg/g)	2.47 ± 0.60	2.24 ± 0.27	2.53 ± 0.61	0.48 ± 0.24	3.46 ± 0.64
Phaeo (μg/g)	18.85 ± 1.77	10.41 ± 1.82	17.29 ± 0.99	9.39 ± 1.20	22.11 ± 5.5
Chl-a/phaeo	0.69 ± 0.05	0.56 ± 0.08	0.68 ± 0.10	0.60 ± 0.15	0.59 ± 0.10

The average value of CHO concentrations varied from 5.48 to 9.93 mg/g in surface sediments, and recorded its maximum value at S2 (Table 3.3). Organic detritus in aquatic sediments has been recognized for its influence on the biogeochemical cycles, and its importance as a benthic food resource (Mann and Lazier 1991; Bianchi and Bauer 2011). The biochemical composition of sedimentary organic matter is the result of dynamic equilibrium between external input, autochthonous production and heterotrophic utilisation. The quality and quantity of organic matter in sediments can be assumed to be one of the major factors influencing the community structure and metabolism of benthos. Carbohydrates represent the largest fraction of photosynthetically assimilated carbon and the most abundant constituents of vascular plants (Martins et al. 2015). In the present investigation, among the biochemical components, carbohydrates were the dominant class in labile organic compounds. Decay of floating plants in the aquatic medium greatly contributes to the comparatively higher concentrations of sedimentary carbohydrates. Moreover, increased levels of CHO in the sediments could 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). In the present study, comparatively higher concentration of CHO was recorded at stations S2 and S3, implying greater contribution of vascular plant debris to sedimentary OM (Cowie and Hedges 1984). While, the dominance of CHO over PRT at upstream station (S1) pointed out the input of terrestrially derived OM, and also inferred to the detrital-heterotrophic nature of the study area (Danovaro 1996; Renjith et al. 2013). Carbohydrate showed a positive correlation with silt, TOC ($r^2 = 0.54$, $p < 0.05$), TN ($r^2 = 0.63$, $p < 0.05$), and TS ($r^2 = 0.83$, $p < 0.01$) (Table 3.2). The association between CHO and BPC states its dominant contribution to BPC ($r^2 = 0.85$, $p < 0.01$).

Protein concentrations found in the present study were comparable to those reported in other aquatic systems (Dell'Anno et al. 2002; Cotano and Villate 2006; Venturini et al. 2012), and the average concentration ranged from 5.5 to 11.0 mg/g, with its maximum value at S2 during pre-monsoon season (Table 3.3 and Fig. 3.7). Proteins in sediments constitute a significant portion of the labile organic matter, derived from a wide range of sources including autochthonous and allochthonous inputs. A number of industrial units including fish peeling and processing units, a solid waste treatment plant and a dairy unit located adjacent to the Chitrapuzha River, discharge bulk quantities of waste materials into the study area, contributing OM enriched with protein, which ultimately adsorbed in the surface sediment (Vasudevan 2000; Balasubramanian et al. 2012). The average value of LPD concentration varied from 3.08 to 7.4 mg/g in surface sediments, and recorded its maximum value at S1 (Table 3.3). The biochemical composition of sedimentary organic matter in this station exhibited a dominance of LPD followed by CHO and PRT. Compared to other seasons, the monsoon season displayed higher content of LPD in most of the stations except slight variations at S3 and S4 (Fig. 3.7). Lipids constitute a major fraction of dissolved and particulate organic matter in aquatic ecosystems (Borsheim et al. 1999; Burdige et al. 2000), and the elevated level of total lipid reflects the productive nature of the aquatic environment (Gremare et al. 1997; Akhil et al. 2013). Lipids in sediments are derived not only from aquatic biota, but also from wax of higher plants and are abundant in eutrophic systems than in oligotrophic systems (Gremare et al. 1997). Like proteins, it also indicates the productivity of the system (Gremare et al. 1997; Akhil et al. 2013). Furthermore, the prominent levels of lipids in the sediments pointed towards the greater availability of labile organic matter compounds (Cartes et al. 2002; Gremare et al. 2002). The incursion of surplus quantities of allochthonous OM into the Cochin backwaters has already been reported (Balachandran et al. 2003; Babu et al. 2006; Thottathil et al. 2008; Martin et al. 2010).

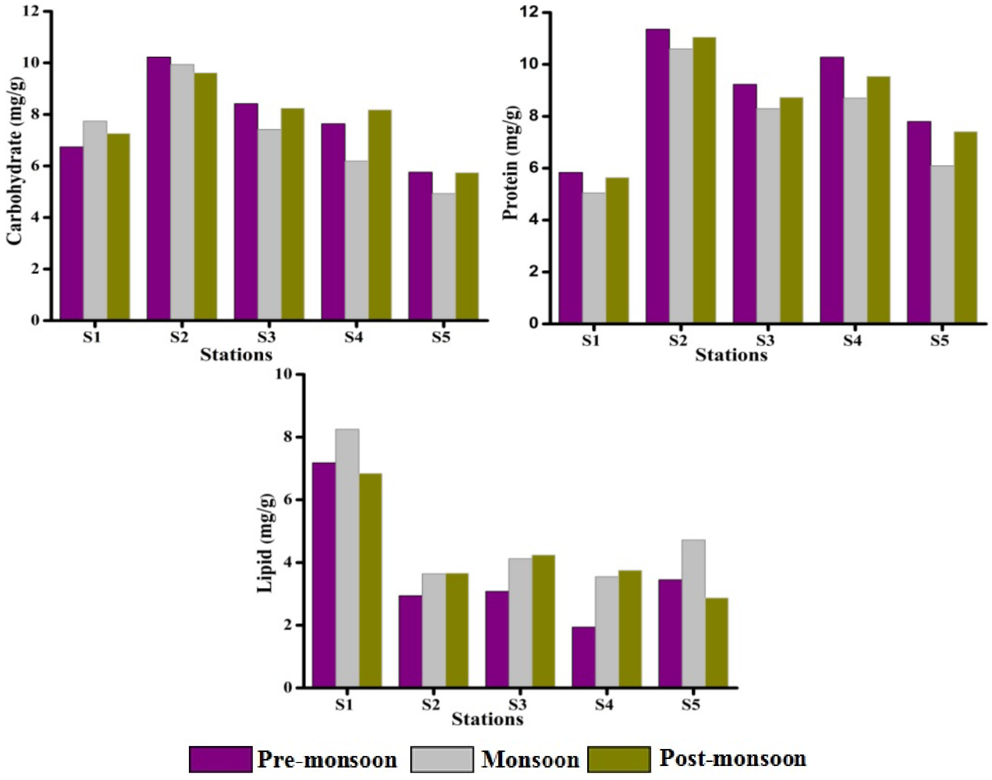


Fig. 3.7 Seasonal and spatial variations of carbohydrate, protein and lipid in the surface sediments of the study area

The abundance and ratios of biologically important elements in the biogeochemical cycles provide fundamental information on OM sources and cycling in aquatic systems. Usually, PRT/CHO ratio has been used as an index to assess the status of the biochemical degradation processes in sedimentary environment (Galois et al. 2000), and to differentiate between the fresh and aged organic materials (Danovaro et al. 1993; Cividanes et al. 2002). PRT/CHO ratio >1 indicates a major fraction of sedimentary OM consists of freshly produced labile organic matter (Pusceddu et al. 2000), while PRT/CHO ratio <1 suggest the predominance of aged organic matter in surface sediments (Danovaro et al. 1993; Pusceddu et al. 2000), may be due to the reduced availability of organic matter for consumers (Pusceddu et al. 2005, 2009). Strong positive correlation between BPC and labile organic matter (LOM) ($r^2 = 0.92$, $p < 0.01$), substantiate this observation (Table 3.2). The observed values of PRT/CHO ratios >1 (Fig. 3.8) in majority of the stations indicated freshly deposited detritus in sediments, while, upstream station S1 exhibited the predominance of aged organic matter. Similarly, lipid to carbohydrate ratio (LPD/CHO) has been used as an index to explain the energetic quality of the organic matter in the surface sediments (Gremare et al. 1997; Fabiano and Pusceddu 1998; Gremare et al. 2002). LPD/CHO ratios in the surface sediments of the study area ranged from 0.29 (S2) to 1.07 (S1), 0.37 (S2) to 1.07 (S1) and 0.38 to 0.94 in pre-monsoon, monsoon and post-monsoon seasons respectively (Fig. 3.8). Comparatively, lower values were observed in the industrial zone (S2, S3 and S4) and estuarine region (S5), in turn reflects the presence of lower nutritive quality of the organic content in sediment during the sampling periods, which may be associated with industrial and land runoff (Jacob et al. 2008, 2009).

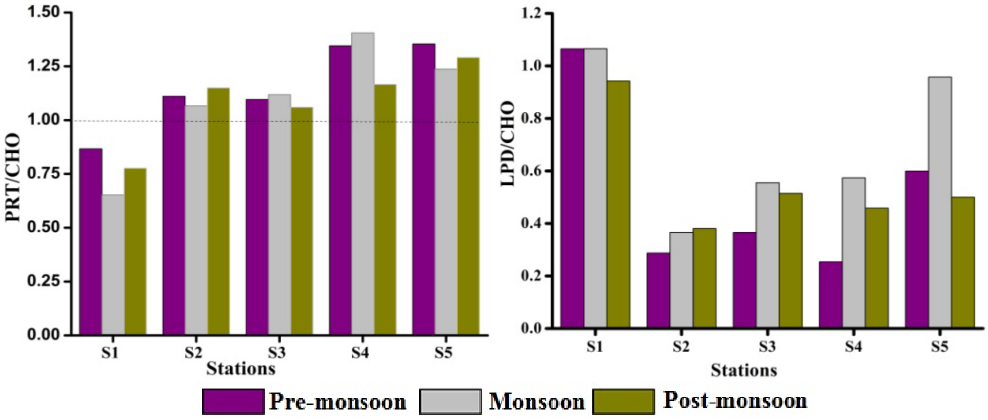
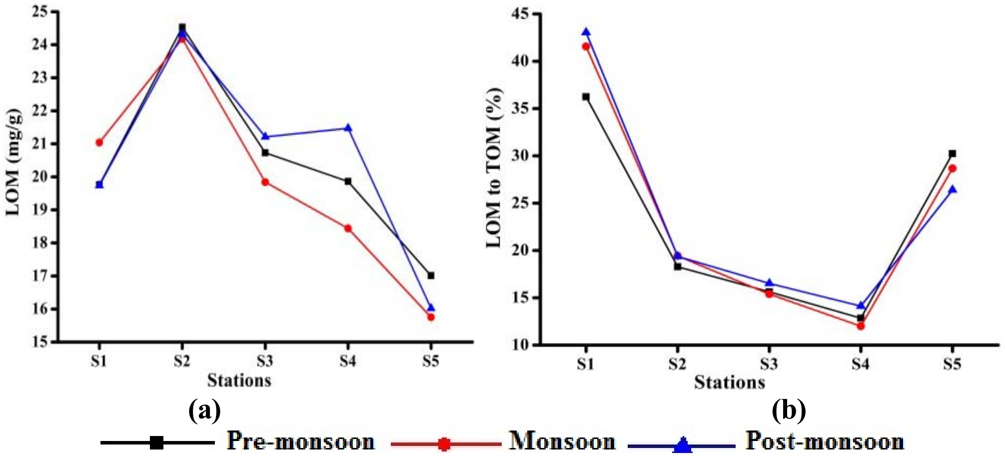


Fig. 3.8 Variations of PRT/CHO and LPD/CHO in the surface sediments of Chitrapuzha River

Evaluation of the nature of organic matter, whether it is labile or refractory, is an inevitable part of organic geochemical studies, and that can be achieved by the use of biochemical compositions. Labile organic matter (LOM) can be defined as the sum of all proteins, carbohydrates and lipids (Danovaro et al. 1993; Cividanes and Souza 2003), and it represents the easily assimilable portion of organic matter available for aquatic organisms including benthos. The labile organic matter content in surface sediments recorded a variation from 17 to 24.5 mg/g in pre-monsoon, 15.7 to 24 mg/g in monsoon and 16 to 24.3 mg/g in post-monsoon seasons (Fig. 3.9a), and its contribution to TOM varied from 12 (S4, MN) to 43% (S1, POM) (Fig. 3.9b). The upstream and downstream stations S1and S5 indicated higher contributions of LOM to TOM, and the elevated levels established enhanced productivity, coupled with external supply of terrigenous materials. Similar observations were reported from the surface sediments of the Cochin estuarine system, and it ranged from 9 to 31 % (Joseph et al. 2008), which pointed out the fact that a significant fraction of TOM represents refractory materials. The biopolymeric carbon (BPC) fraction of sedimentary organic matter, calculated by summing of protein, carbohydrate and lipid carbon, has been reported as the fraction of total organic carbon potentially available to the benthic consumers (Pusceddu et al. 2009). The average values of BPC concentrations in the surface sediments varied from 8.4 (S5) to 11.9 mg C/g (S2) (Table 3.3), and exhibited slight variations, which might be attributed to the changes in organic matter deposition associated with the strong river discharge from the upper reaches of the Chitrapuzha River.



(a) (b)

Fig. 3.9 Distribution of LOM (a) and percentage contribution of LOM to TOM (b) in the surface sediments

3.2.4 Chlorophyll pigments in sedimentary organic matter

Spatial and seasonal variations in chlorophyll-a (chl-a), chlorophyll-b (chl-b), chlorophyll-c (chl-c) and phaeophytin (pheo) are depicted in Fig. 3.10. Chl-a concentration in the surface sediments ranged from 5.09 to 13.06 µgg^{-1} (10.01 ± 3.85), 4.76 to 12.1 µgg^{-1} (8.6 ± 3.51) and 5.87 to 13.99 µgg^{-1} (10.41 ± 3.79) in pre-monsoon, monsoon and post-monsoon seasons respectively (Fig. 3.10a). Maximum chl-a concentration was recorded at S5 during the post-monsoon season, while the lowest concentration was observed at S4 during the monsoon season. Chl-b concentration ranged from 0.97 to 5.86 µgg^{-1} in pre-monsoon, 0.6 to 6.24 µgg^{-1} in monsoon and 1.1 to 4.73 µgg^{-1} in post-monsoon seasons (Fig.

3.10b). Chl-c displayed, comparatively lower concentrations in the study region, and it ranged from 0.73 to 3.99 μgg^{-1} in pre-monsoon, 0.24 to 2.74 μgg^{-1} in monsoon and 0.48 to 3.65 μgg^{-1} in post-monsoon seasons (Fig. 3.10c). Pheo concentration in the surface sediments ranged from 8.28 to 24.12 μgg^{-1} (16.32 ± 6.82), 8.7 to 17.24 μgg^{-1} (13.73 ± 3.79) and 9.21 to 26.4 μgg^{-1}(16.78 ± 6.58) in pre-monsoon, monsoon and post-monsoon seasons respectively (Fig. 3.10d). Maximum pheo concentration was recorded at S5 during post-monsoon season, while the lowest value recorded at S4 during pre-monsoon season.

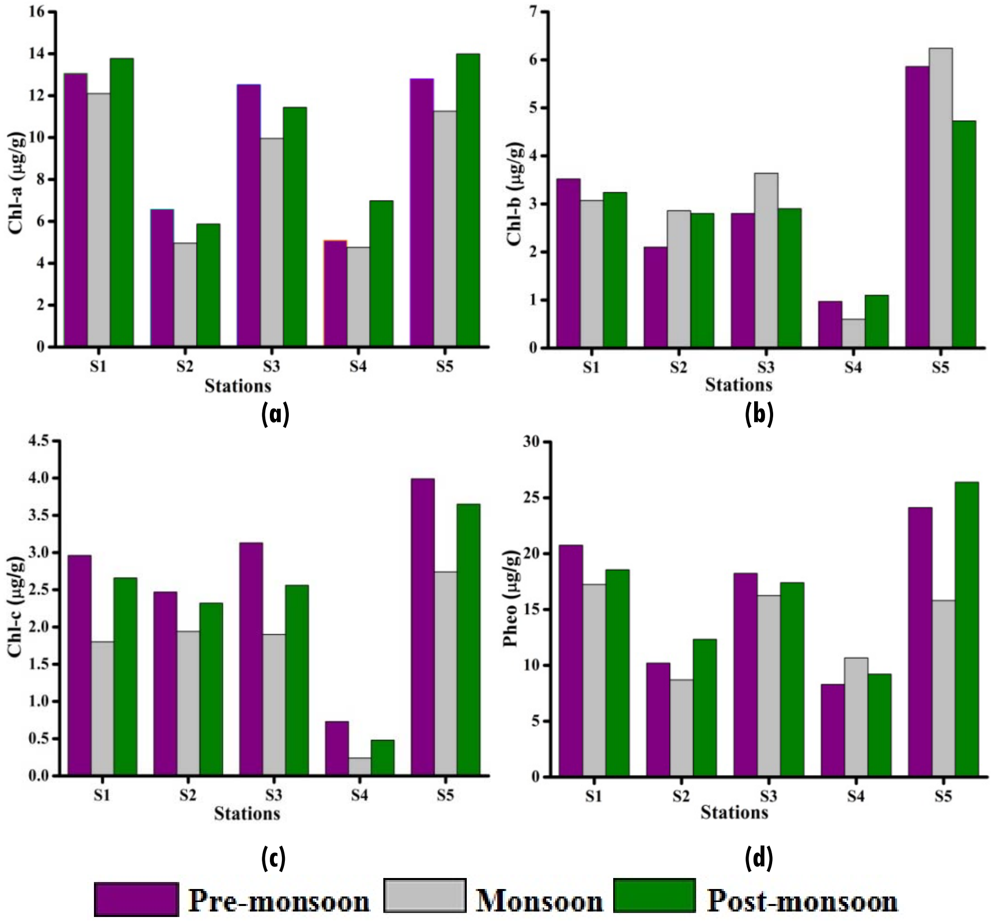


Fig. 3.10 Spatial and seasonal variations of (a) chlorophyll-a, (b) chlorophyll-b, (c) chlorophyll-c and (d) phaeophytin in the surface sediments of Chitrapuzha River

Phytopigment concentrations can be used as a descriptor of the trophic state and productivity of most estuarine and shallow coastal systems (Lucas et al. 2000). Significantly higher levels of chloropigments exhibited by the sediments indicated the possibility of higher autochthonous production in the study area. From the results, it was found that chl-a concentration displayed highly significant spatial variations (Fig. 3.10a) in the study region but lack significant seasonal differences. Comparatively higher chlorophyll concentrations were observed during non monsoon seasons, while lower values were observed in monsoon season. The dominance of chl-b and chl- c at some stations might be due to the species diversity (an increase in the groups of diatoms and dinoflagellates). However, chl-b, chl-c and pheo concentrations did not show any seasonal variations, but exhibited differences among the sampling sites. The observed values of chl-a/pheo ratio in the present investigation ranged from 0.45 (S4) to 0.79 (S3) (Fig. 3.11) and the ratio <1 in the surface sediments indicated the prevalence of detritus in sediments (Josefson and Conley 1997; Hagy et al. 2005). Moreover, the ratio of chl-a/pheo content can be utilised as an indicator of the fraction of metabolically active autotrophic component (Plante-Cuny and Bodoy 1987). Lower values of chl-a/pheo can be the result of increased turbidity, chemical contamination or any other factor affecting photosynthetic potential (Bhadauria et al. 1992; Porter et al. 1999). On the other hand, the prevalence of phaeopigments might be recognized to high turbidity, chemical contamination or other factors affecting photosynthetic potential of the primary producers in the surface sediments (Dell' Anno et al. 2002). The phyto pigments chl-a, chl-b, chl-c and phaeo exhibit mutual coherency (Table 3.2), due to their similar nature in physiological processes. Chl-a shows a strong association with phaeo than other phyto pigments, thereby explaining its sensitivity towards pheophytinization ($r^2 = 0.91$, $p < 0.01$) (Bowe 2002).

Significant correlation is obtained between chl-a and chl-b (Table 3.2) (r^2 = 0.67, $p < 0.01$) indicating the presence of green algae in the surface sediments (Kowalewska 2005). On the other hand, phyto pigment chl-a is negatively correlated with the biochemical components of organic matter, mainly CHO (r^2 = 0.51, $p < 0.05$) and PRT (r^2 = 0.76, $p < 0.01$) (Table 3.2). It indicates a minor contribution of microphyto benthic populations and their detritus to the bulk sedimentary organic matter (Cotano andVillate 2006).

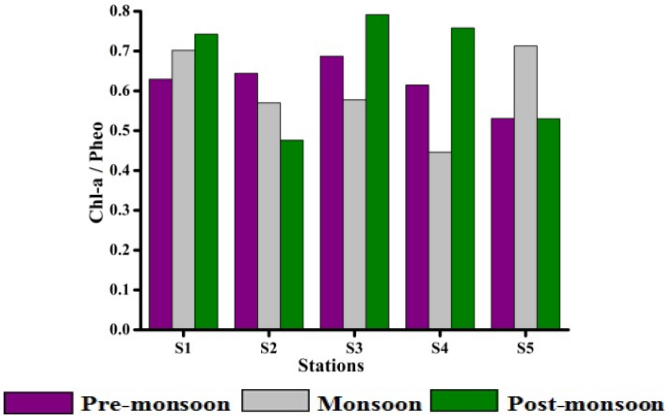


Fig. 3.11 Spatio-temporal variations of chl- a/pheo ratios in the surface sediments

3.2.5 Benthic trophic structure of the study area

Trophic status, a useful means of classifying the aquatic environment, and describing the aquatic processes, in terms of the productivity of the system. The main factors that regulate the trophic state includes, rate of nutrient supply (bedrock geology of the watershed, soils and vegetation), climate (amount of sunlight, temperature and hydrology) and morphometry (depth, volume and surface area and watershed to aquatic surface area ratio) (Cloern 2001; Coelho et al. 2007). However, many authors reported that the evaluations of these predictive and responsive variables are inadequate to provide better insights into the trophic status of aquatic ecosystems (Izzo et al. 1997; Cognetti 2001; Dell'Anno et al. 2002; Pusceddu et al. 2011). The assessment of trophic state in aquatic environments is crucial in understanding food web linkages, as well as, biogeochemical characteristics of the study area. The lower reaches of Chitrapuzha River are a part of the Cochin estuarine system, and possess positive net ecosystem productivity (Qasim 2003). But, as the consequence of terrestrial organic matter inputs, a seasonal shift in net pelagic production to heterotrophic conditions has been created (Thottathil et al. 2008). Basic studies on the assessment of trophic status have been based on the evaluation of physical, chemical and biological measurement of water column variables, such as turbidity, inorganic nutrients and chlorophyll content (Zurlini 1996; Cloern 2001; Coelho et al. 2007). However, in shallow ecosystems like Chitrapuzha River, there is a significant contribution of chl-a from microphytobenthose and macroalgae to benthic primary production (Dell'Anno et al. 2002), thereby creating divergences in the assessment of trophic status (Conde et al. 1999; Pusceddu et al. 1999). Moreover, indiscriminate and unscientific application of fertilisers, industrial inputs and domestic sewages introduces bulk quantities of nutrients into Chitrapuzha River, which have affected the food web structure and alterations in trophic status.

Dell'Anno et al. (2002) proposed a new approach for the assessment of trophic status, based on the threshold levels of PRT and CHO values (hypertophic (H): PRT>4 and CHO > 7 mgg^{-1}, eutrophic (E): PRT 1.5 to 4 mgg^{-1} and CHO = 5 to 7 mgg^{-1}, meso-oligotrophic (MO): PRT<1.5 mgg^{-1} and CHO <5 mgg^{-1}). Moreover, BPC and algal contribution to BPC (Cchl-a) level, put forward by Pusceddu et al. 2011 (eutrophic- BPC >3 mgg^{-1} and algal contribution to BPC <12%) are also used in this study. Seasonal and spatial variations of BPC and Cchl-a in the surface sediments are given in Fig. 3.12. Based on the above mentioned approach, the benthic trophic classifications of the stations are presented in Table 3.4. As per these criteria, all the stations in the study area are classified as hypertrophic using PRT threshold levels, while CHO concentrations reflect eutrophic conditions in the estuarine station S5. Moreover, both BPC and an algal contribution to BPC reflected eutrophic conditions prevailing in the study area. From the above observation, it is clear that, different biochemical descriptors inferred different trophic conditions for the study area, hence a detailed examination is needed for assessing the applicability of these biochemical descriptors in the tropical estuarine and coastal systems. However, high BPC and PRT values and lower algal contribution to BPC indicate a high degree of benthic eutrophication in Chitrapizha River.

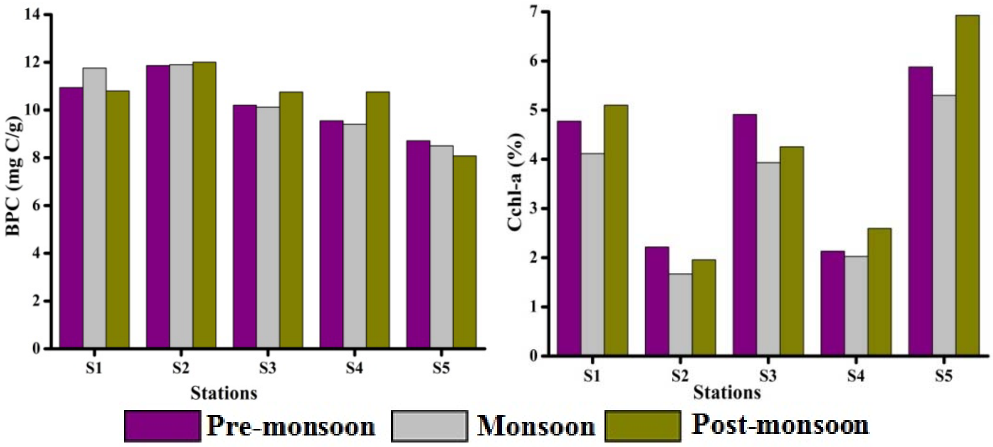


Fig. 3.12 Seasonal and spatial variations of BPC and algal contribution to BPC pool (Cchl-a) in the surface sediments

Table 3.4 Benthic trophic classifications of sampling sites in the study area

Stations	PRT (mg/g)	Tropic status*	CHO (mg/g)	Tropic status*	BPC (mg C/g)	Cchl-a (%)	Tropic status**
S1	5.51	H	7.25	H	11.17	4.66	E
S2	11.0	H	9.93	H	11.92	1.95	E
S3	8.75	H	8.03	H	10.36	4.37	E
S4	9.51	H	7.34	H	9.90	2.25	E
S5	7.10	H	5.48	E	8.43	6.03	E

PRT- Proteins; CHO- Carbohydrates; BPC- Biopolymeric Carbon; Cchl-a- Algal contribution to BPC; H- Hypertophic: E- Eutrophic; * Dell'Anno et al. 2002; ** Pusceddu et al. 2011

3.3 Conclusions

General sedimentary analysis revealed the predominance of sand and clay fractions in the upstream and downstream stations, while, silt particles dominated in stations of industrial zone. The percentage of clay was generally higher at all the stations during non-monsoon seasons, compared to monsoon season, due to the settling of finer fractions of the sediment samples. On the other hand, the stations in the industrial zone recorded slightly higher values of the silt fraction during monsoon season may be associated with the spillage of phosphogypsum, a waste product from the adjacent premises of the fertilizer manufacturing unit. The results of organic carbon content and biochemical components in the surface sediments of Chitrapuzha River exhibited comparable concentrations to those reported in highly industrialised and urbanised eutrophic systems. TOC and TN concentrations strongly depend on the granulometric composition of surface sediments in the study region. TOC values obtained in the present study are comparatively higher than that in the sediments of the northern parts of the Cochin backwater system. The input of organic matter derived from rivers, coupled with the discharge of organic wastes from different industrial, agricultural and aquaculture sectors leads to a large scale accumulation of organic matter in the surface sediments. Concentrations of total nitrogen were relatively lower in the sediments of Chitrapuzha River, and intermediate values of TOC/TN ratios signalled a combined input of both autochthonous and terrestrial organic matter sources.

The distributional characteristics of $\delta^{13}C$ values indicated major inputs of terrestrial higher plant debris to sedimentary organic matter, while, $\delta^{15}N$ values indicated decomposition processes in sediments have a considerable effect on the isotopic compositions. The distribution features of terrestrial organic matter exhibited significant spatial differences with higher contribution in the lower reaches of the study area. The observed values of PRT/CHO ratios >1 in the majority of the stations indicated freshly deposited detritus, while, the upstream station exhibited the predominance of aged organic matter in surface sediments. Similarly, comparatively lower LPD/CHO ratios observed in the industrial zone and estuarine region reflects the lower nutritive quality of organic content during the sampling periods. The threshold levels of PRT, BPC and algal contribution to BPC suggests the eutrophic conditions prevailing in the study area. The application of biochemical compositions and bulk indices like elemental composition and isotopic ratios are useful techniques for the evaluation of total quality, benthic trophic structure and relative contribution of marine and terrestrial derived organic matter in surface sediments. However, when more detailed information on sources of organic matter is required, molecular source indicators i.e., biomarkers can be applied.

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Chapter 4

FATTY ACID COMPOSITION AS BIOMARKERS

Contents

4.1 Introduction

Sedimentary systems are complex environments with a broad spectrum of sub-environments such as, terrestrial, littoral and benthic compartments that contribute to organic matter and which have different reactivity in biogeochemical processes (Bianchi and Canuel 2011). A significant portion of organic matter sinks through the water column and ultimately gets stored in sediments (Hu et al. 2006; Gireeshkumar et al. 2015; Pruski et al. 2015), by the interaction of a series of physical, chemical and biological processes (Liu et al. 2006). The knowledge of the source, distribution, reactivity and transformations of organic matter in aquatic sediments are important for the understanding of carbon biogeochemistry, and the extent of anthropogenic impacts on these valuable ecosystems (Zhang et al. 2009; Al-Timari et al. 2015). However, understanding these processes is a difficult task due to complex and multiple sources of organic matter, as well as the difference in reactivity and the spatial variability in different processes that occurring in aquatic sediments (Bianchi and Canuel 2011; Carreira et al. 2011). The sources of organic matter in aquatic systems are often diverse, as they receive from both autochthonous and allochthonous sources (Carreira et al. 2015) including phytoplankton (Meyers 1997), algae (Meziane and Tsuchiya 2000), bacteria (Dale 1974), terrestrial vegetation (Tam et al. 1990; Meyers 1997; Mfilinge et al. 2005), and anthropogenic sources, such as, waste-water treatment and industrial effluents, and aquaculture (Requejo et al. 1986; Tam et al. 1998; Graham et al. 2001). Due to this reason, a variety of approaches, including elemental analysis (C/N ratios), carbon and nitrogen stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) and biomarkers have been widely used to study the source, fate and transformations of organic matter in aquatic systems (Hedges et al. 1988; Canuel et al. 1997; Zimmerman and Canuel 2001; Mead et al. 2005). The bulk elemental and isotopic techniques are based on the assignments of end member values to terrestrial and marine organic matter (Schultze and Calder 1976), but have its limitations. For example, terrestrial organic matter has more negative $\delta^{13}C$ values (about -27‰) and high C/N ratios (>12), while, marine derived organic matter have $\delta^{13}C$ values around -20‰ and lower C/N ratios (6 to 8) (Xing et al. 2011). The variations in $\delta^{13}C$ values of marine organic matter can be large and C_4 plants exhibit more $\delta^{13}C$ values (-12‰), which may lead to significant errors in determining the provenance of organic matter in sediments (Barros et al. 2010). Due to the aforementioned limitations, when more detailed information on sources of organic matter is required, molecular source indicators, i.e., biomarkers can be applied (Wakeham et al. 1984; Mead et al. 2005; Alfaro et al. 2006; Zhang et al. 2014; Carreira et al. 2015). The distribution of these biomarkers in sediments ultimately provides useful information about the source, diagenetic alteration, preservation and historical changes in organic matter, as well as, changes in trophic status (Alfaro et al. 2006). Recently lipid biomarker proxies such as n-alkanes, fatty acids (FAs) and sterols were used more effectively to assess the sources of sedimentary organic matter in coastal areas, estuaries, rivers and lakes (Jaffe et al. 2001; Bianchi et al. 2002; Mead et al. 2005; Mfilinge et al. 2005; Zhang et al. 2014; Carreira et al. 2015). The relative contributions of fatty acids in aquatic environments can reveal the origins of organic materials found in the surface sediments (Carrie et al. 1998), and used as a specific biomarker for plants and micro-organisms (Leveille et al. 1997).

4.1.1 Fatty acids as biomarkers

The term applied to biomarkers has been defined by Meyers (2003) as “compounds that characterize certain biotic sources that retain their source information after burial in sediments, even after some alteration”. The biomarker is defined as "a molecule whose carbon skeleton can unambiguously be linked to that of a known biological precursor compound" (Killops and Killops 2005). These organic molecules with a high degree of structural complexity are particularly informative, and thus suitable for studying geochemical characteristics, because they provide the possibility of relating a certain product to a specific precursor. Despite the various biological and abiotic alterations of the biomolecules in the sediments, some compounds retain their basic skeletal structures, and can be used as characteristic molecular markers (Peters et al. 2006; Parrish 2013). The presences of sedimentary biomarkers have been reported in the rocks back to the precambrian (Brocks et al. 2005). Alkanes, fatty acids, amino acids, carbohydrates, terpenoids etc. were identified in a number of studies to address the source of organic matter in aquatic sediments (Volkman et al. 2007; Freese et al. 2008; Hu et al. 2009; He et al. 2010; Yang et al. 2010; Aneeshkumar and Sujatha 2012; Hu et al. 2012; Hu et al. 2013), and its diagenetic transformations in the environment (Ding and Sun 2005; Chen et al. 2008; Carreira et al. 2011).

Fatty acids are mono carboxylic acids with long aliphatic chains that are either saturated or unsaturated, with carbon numbers usually ranging from C_{12} to C_{36}. They constitute the main part of lipids in living organisms, and predominantly even numbers of carbon atoms due to their enzymatic biosynthesis, as they are formed from acetyl (C_2) units of glucose. The myriad structure of FAs (Sargent et al. 1987), unique distributions in various organisms and distinct structural features, make them as an effective biomarker tool (Kedrowski et al. 2009; Bianchi and Canuel 2011). The relative abundances of individual fatty acids are useful in evaluating the relevant importance of inputs of bacteria, microalgae, marine fauna and higher plants (Tolosa et al. 2004; Kanthilatha et al. 2014). The chain length of fatty acids encountered in sediments is largely used as an indicator of this type of sources. However, more specific information on the organic matter sources is obtained by the unsaturation and branching degree of fatty acids found in sediments (Killops and Killops 2005).

Fatty acids are broadly classified into saturated fatty acids (SAFAs), unsaturated fatty acids and branched chain fatty acids (BFAs), based on their structure. Saturated fatty acids are commonly straight chains with even carbon number, represented by the general formula CH_3 $(CH_2)n$ COOH (Table 4.1). They are named from the saturated hydrocarbon with the same number of carbon atoms as alkanoic acid. For example, the fatty acid with 18 carbon atoms is termed as octadecanoic acid (stearic acid) and denoted as $C_{18:0}$. An unsaturated fatty acid contains one or more double bonds in the fatty acid chain, and if it contains one double bond, the fatty acid is monounsaturated (MUFAs) and polyunsaturated (PUFAs), if it contains more than one double bond (Table 4.2). A simple notation indicating its carbon chain length, the number of double bonds and their positions in the carbon chain represent the unsaturated fatty acids. For example, oleic acid can be represented by $C_{18:1\omega9,}$ where 18 is the number of carbon atoms, the number of double bonds (1) is given after the colon, and the number following ω is the position of the double bond from the opposite end of the acid group. Branched-chain fatty acids are normally saturated and the branch is a methyl-group, methoxy or a hydroxy substitution. They have usually either an iso or anteiso structure. Iso-methyl branched fatty acids have the branch point on the penultimate carbon, while, anteiso-methyl-branched fatty acids have the branch point on the ante-penultimate carbon atom.

Table 4.1 Most common saturated fatty acids

Lipid numbers	Common name	Systematic name	Structural formula
$C_{12:0}$	Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$
$C_{13:0}$	Tridecylic acid	Tridecanoic acid	$CH_3(CH_2)_{11}COOH$
$C_{14:0}$	Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$
$C_{15:0}$	Pentadecylic acid	Pentadecanoic acid	$CH_3(CH_2)_{13}COOH$
$C_{16:0}$	Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$
$C_{17:0}$	Margaric acid	Heptadecanoic acid	$CH_3(CH_2)_{15}COOH$
$C_{18:0}$	Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$
$C_{19:0}$	Nonadecylic acid	Nonadecanoic acid	$CH_3(CH_2)_{17}COOH$
$C_{20:0}$	Arachidic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$
$C_{21:0}$	Heneicosylic acid	Heneicosanoic acid	$CH_3(CH_2)_{19}COOH$
$C_{22:0}$	Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$
$C_{23:0}$	Tricosylic acid	Tricosanoic acid	$CH_3(CH_2)_{21}COOH$
$C_{24:0}$	Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$
$C_{25:0}$	Pentacosylic acid	Pentacosanoic acid	$CH_3(CH_2)_{23}COOH$
$C_{26:0}$	Cerotic acid	Hexacosanoic acid	$CH_3(CH_2)_{24}COOH$
$C_{27:0}$	Heptacosylic acid	Heptacosanoic acid	$CH_3(CH_2)_{25}COOH$
$C_{28:0}$	Montanic acid	Octacosanoic acid	$CH_3(CH_2)_{26}COOH$
$C_{29:0}$	Nonacosylic acid	Nonacosanoic acid	$CH_3(CH_2)_{27}COOH$
$C_{30:0}$	Melissic acid	Triacontanoic acid	$CH_3(CH_2)_{28}COOH$
$C_{31:0}$	Henatriacontylic acid	Henatriacontanoic acid	$CH_3(CH_2)_{29}COOH$
$C_{32:0}$	Lacceroic acid	Dotriacontanoic acid	$CH_3(CH_2)_{30}COOH$

Table 4.2 Most common unsaturated fatty acids

Common name	Designation	Structural formula
Myristoleic acid	$C_{14:1\omega5}$	$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$
Palmitoleic acid	$C_{16:1\omega7}$	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Oleic acid	$C_{18:1\omega9}$	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic acid	$C_{18:2\omega6}$	$CH_3(CH_2)_4CH=CHCH_2CH=CH\ (CH_2)_7COOH$
α-Linolenic acid	$C_{18:3\omega3}$	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7\ COOH$
Arachidonic acid	$C_{20:4\omega6}$	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CH\ CH_2CH=CH(CH_2)_3COOH$
Eicosapentanoic acid	$C_{20:5\omega3}$	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2$ $CH=CHCH_2CH=CH(CH_2)_3COOH$
Erucic acid	$C_{22:1\omega9}$	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$
Docosahexaenoic acid	$C_{22:6\omega3}$	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH\ CH_2CH=CHCH_2CH=CH$ $CH_2CH=\ CH(CH_2)_2COOH$

The relative abundances of individual fatty acids are useful in evaluating the relevant importance of inputs (Table 4.3) from bacteria, micro algae, marine fauna and continental higher plants (Tolosa et al. 2004; Oliveira et al. 2013). Generally speaking, the short-chain homologues ($n \leq C_{20}$) are widely found in multiple sources, including plankton, photosynthesis bacteria, and aquatic plants (Meyers and Ishiwatari 1993; Philip 2003; Eerkens 2005). For example, fatty acids with distributions $C_{14:0}$, $C_{16:0}$, and $C_{16:1}$ are abundant in phytoplankton, while, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ are frequently predominant in zooplankton. Zooplankton generally contributes in greater proportion to the SAFAs, $C_{16:0}$ and $C_{18:0}$ as compared to phytoplankton (Muri et al. 2004; Marchand et al. 2005). Diatoms, like zooplankton, are also rich in fatty acids with distribution of $C_{14:0}$, $C_{16:0}$ and $C_{16:1}$, but present low composition in homologues C_{18} fatty acids. On the other hand, fatty acids $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{18:4}$ predominant in dinoflagellates and freshwater algae (Azevedo et al. 2003). Sources of terrestrial vegetation, bacterial and algal production can be identified by the presence of long chain fatty acids ($>C_{24}$), odd short chain or branched fatty acids ($<C_{18}$) and

even short chain non-branched fatty acids ($<C_{18}$) respectively. During the burial, short chain fatty acids undergo degradation diagenetically faster than long chain fatty acids (Haddad et al. 1992; Eerkens 2005; Kanthilatha et al. 2014). Saturated fatty acid (SAFAs) $C_{14:0}$ is present in phytoplankton particularly in diatoms (Reitan et al. 1994) and act as a diatom marker, while, $C_{16:0}$ and $C_{18:0}$ are ubiquitous in the marine environment, and can be used as a measure of total community biomass (Parkes 1983). Branched fatty acids (BFAs) with odd number of chain carbon atoms (iso- and anteiso- C_{13}-C_{17}) are generally present in several bacteria, allowing their use as bacterial biomarkers (Bodineau et al.1998; Palomo and Canuel 2010; Bianchi and Canuel 2011; Zhang et al. 2015). The fact of branching fatty acids being found in low concentrations in other organisms makes them useful indicators for bacterial contribution in sediments (Thoumelin 1997). Compounds generally attributed to bacterial sources (branched fatty acids iso- and anteiso- $C_{15:0}$, $C_{17:0}$ and $C_{18:1\omega7}$) are lost or present in small amounts in sediments due to the bacterial decomposition process which occurs when particles cross the water column (Wakeham 1995; Zegouagh et al. 2000; Marchand et al. 2005). Moreover, the sum of odd numbered carbon ($C_{15:0}$ and $C_{17:0}$) and branched-chain (iso- and anteiso-) fatty acids was regarded as bacterial derived fatty acids (BAFAs), since, bacterial communities synthesize them. Similarly, hydroxy fatty acids are widely distributed in nature and their occurrence correlates with environments enriched in bacteria (Parker et al. 1982; Tyagi et al. 2015). Fatty acids with carbon atoms $\geq C_{22}$ are synthesized generally by vascular plants (Volkman et al. 1980), and are considered as a higher plant marker in sediments (Scribe et al. 1991; Colombo et al. 1996; Carrie et al. 1998). Terrestrial plants are considered as the main source of long chain fatty acids (LCFAs; $C_{22} – C_{28}$), with strong even order predominance (Shi et al. 2001; Zimmerman and Cannel 2001; Hu et al. 2005).

Moreover, LCFAs have been found in high concentrations in mangrove leaves (Wannigama et a1. 1981; Meziane and Tsuchiya 2000, 2002). Furthermore, the ratio of long chain to shorter chain lipids ($C_{24:0}/C_{16:0}$) can be used to assess the relative contribution of allochthonous vs. autochthonous components (Meyers et al. 1984), while the ratios of $C_{16:1}/C_{16:0}$ and $\sum C_{16}/\sum C_{18}$ are used to distinguish between diatom and dinoflagellate dominance in the sedimentary ecosystem (Birgel et al. 2004; Al-Timari et al. 2015).

Table 4.3 Fatty acids used as biomarkers and their sources in aquatic sediments

Fatty acids	Source	References
$C_{14:1}$	Cyanobacteria	Caudales and Wells 1996
$C_{15} + C_{17}$ Branched FAs	Bacteria	Parkes and Taylor 1983; Kaneda 1991
Σ $C_{13} + C_{17}$ Odd FAs	Bacteria	Kaneda 1991
Σ C_{18} Polyunsaturated FAs	Algae / plants	Volkman et al. 1989
Σ C_{20} Polyunsaturated FAs	Labile algal organic matter	Shaws and Johns 1985
Σ Short chain even FAs C_{12}-C_{16}	Marine algae / bacteria	Meyers 1997
Σ Long chain even FAs C_{24}-C_{30}	Higher plants	Nichols et al. 1982
$C_{24:1}$	Zooplankton	Joseph et al. 2012
$C_{22:6\omega3}$	Dinoflagellate	Nichols et al. 1984
$C_{14:0}$, $C_{16:1\omega7}$ and $C_{16:1\omega5}$	Diatoms	Reitan et al. 1994; Berge et al. 1995
Cy-$C_{15:1}$	Clostridia	Vestal and White 1989
$C_{16:1}$	Algae	Reitan et al. 1994
Cy-$C_{17:0}$	Iron-oxidising bacteria	Kneif et al. 2003
$C_{16:0}$ and $C_{18:0}$	Plankton	Parkes 1987
$C_{16:1\omega5}$, $C_{16:1\omega7}$ and $C_{16:1\omega9}$	Diatoms	Berge et al. 1995; Suzuki and Matsuyama 1995
$C_{18:1\omega9}$	Brown algae	Jamieson and Reid 1972; Johns et al.1979
$C_{18:2\omega6}$, $C_{18:3\omega6}$ and $C_{18:3\omega3}$	Green algae	Volkman et al. 1989; Dunstan et al. 1992; Zhukova and Aizdaicher 1995

Unsaturated fatty acids are usually linked with algae. For example, MUFA, $C_{24:1}$ has been attributed to a zooplankton origin (Wakeham 1995), while, $C_{20:1}$ are commonly found in marine animals such as zooplankton and fish (Ota et al. 1995; Albers et al. 1996). Moreover, MUFAs such as $C_{16:1\omega5}$, $C_{16:1\omega7}$, and $C_{16:1\omega9}$ are also used as signals of diatom derived organic matter, (Carrie et al.

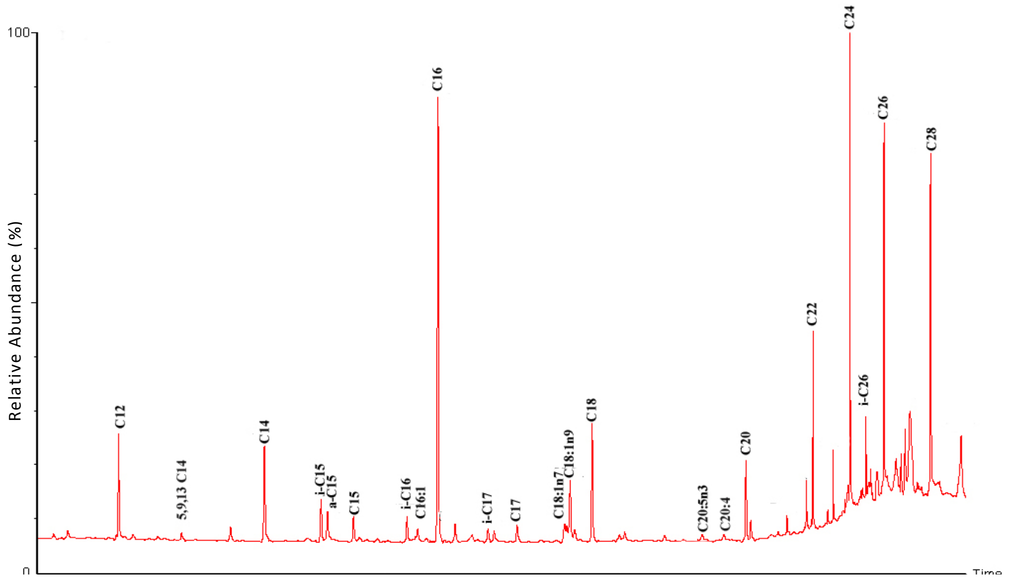
1998; Joseph et al. 2012; Pruski et al. 2015) while, $C_{18:1\ \omega9}$ has been reported as a biomarker for brown algae (Jamieson and Reid 1972; Johns et al. 1979). PUFAs are important structural components of cells that confer membrane fluidity and selective permeability (Hyne et al. 2009). PUFAs are labile in nature (Smith et al. 1983), and due to this nature, its presence may indicate recent detritus inputs (Shaw and Johns 1985; Carrie et al. 1998; Dunn et al. 2008; Sushchik et al. 2013). PUFAs are normally linked with phytoplankton. For example, $C_{20:5\omega3}$, $C_{16:2\omega7}$, $C_{12:2\omega7}$ and $C_{20:4\omega6}$ are used as diatom markers in marine environments (Volkman et al. 1989; Pond et al. 1998), whereas, the abundance of $C_{22:6\omega3}$ in sediments usually indicates dinoflagellate origin (Viso and Marty 1993; Parrish et al. 2000). The presence of $C_{18:2\omega6}$, $C_{18:3\omega3}$ and $C_{18:3\omega6}$ indicates markers of green algae (Napolitano et al. 1997; Meziane and Tsuchiya 2000; Bachok et al. 2009; Joseph et al. 2012) while, $C_{20:5\omega3}$ and $C_{16:4\omega3}$ are typical diatom markers (Colombo et al. 1996; Dai et al. 2009; Pruski et al. 2015).

Chitrapuzha River system is subjected to increasing human interferences, and it receives a considerable amount of organic matter from, both autochthonous and allochthonous sources, including phytoplankton, algae, bacteria, terrestrial vegetation, and anthropogenic sources, such as waste treatment plant, industrial effluents, domestic sewages, fishery industries and the Cochin sea port, which handles large quantities of petroleum products and industrial chemicals. Numerous industrial units, including a diesel power plant, a fertilizer manufacturing unit, an organic chemical factory and a petroleum refinery are crowded along a small stretch of land by the side of Chitrapuzha River. The quantity of effluent treated water discharged from these industries into Chitrpuzha River is estimated to be around 80 million litres per day (Rejomon et al. 2013). The influence of industrial effluents on the general hydrography of Chitrapuzha

River is highly significant, and it deteriorates the quality of water and sediment by loading with large quantities of organic matter, which often exceeds the carrying capacity of the aquatic system, causing complete destruction of the biota. Therefore, the water and sediment quality of Chitrapuzha River is a matter of concern because of perceived impacts of the chemical pollutants on the ecosystem. Although, many data sets are available with regard to heavy metals and nutrients (Jose 1999; Joseph and Chacko 2006) in the surface sediments of the Chitrapuzha River system, studies pertaining to the source characterization of organic matter using fatty acid biomarkers, still remain not reported. Therefore, the present study was planned to generate a preliminary data on the distribution levels of FAs, and their probable sources, so as to discriminate the biogenic contributions of various organic matter in the surface sediments of Chitrapuzha River.

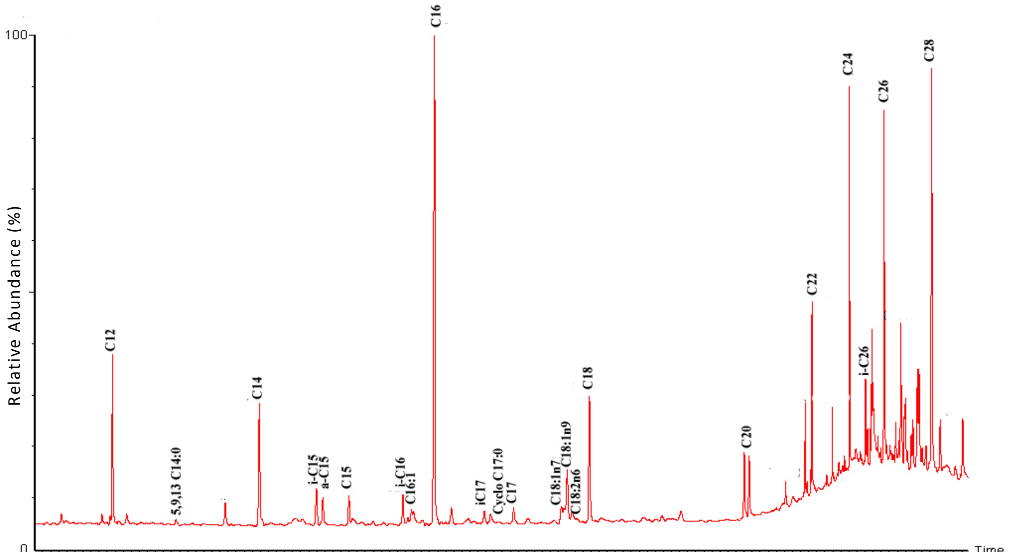
4.2 Results and discussion

Surface sediment samples were collected from 5 locations along the Chitrapuzha River system during monsoon (MN), pre-monsoon (PRM) and post-monsoon (POM) seasons**.** Station 1 (S1) represents the far upstream portion of the river, and is beyond the influence of industrial pollution. Stations 2 (S2), 3 (S3) and 4 (S4) constitutes industrial zones, which are the recipients of discharge outlets of major industrial units. Station 5 (S5) represents the downstream estuarine portion of the study area, and it receives a considerable amount of discharges from apartments on the mainland as well as from the oil tanker berth, Cochin Port and Cochin Shipyard (detailed description of the study area and sampling sites are available in chapter 2). Fatty acids were extracted from the surface sediments from each sampling site of Chitrapuzha River, as fatty acid methyl esters (FAMEs). Fatty acids ranging from C_{12} to C_{28} were identified (Table 4.4 and Fig. 4.1), representing a spectrum of organic matter inputs into the surface sediments. Identified fatty acids included SAFAs, MUFAs, PUFAs and BFAs. SAFAs were broadly distributed throughout the study area, and confirmed the greatest contribution of fatty acids (63 to 83.6%) to the overall FAMEs pool (Table 4.5). The spatial and seasonal variations of total fatty acids (TFAs) in the study area are presented in Fig. 4.2, and the total concentration ranged from 129 (S5) to 358 μgg^{-1} (S3) in monsoon, 162 (S5) to 390 μgg^{-1} (S3) in pre-monsoon and 152 (S5) to 403 μgg^{-1} (S3) in post-monsoon seasons. There is a wide spatial and seasonal variations in total concentrations of fatty acids from upstream station S1 to the downstream station S5, and displays some notable trends.

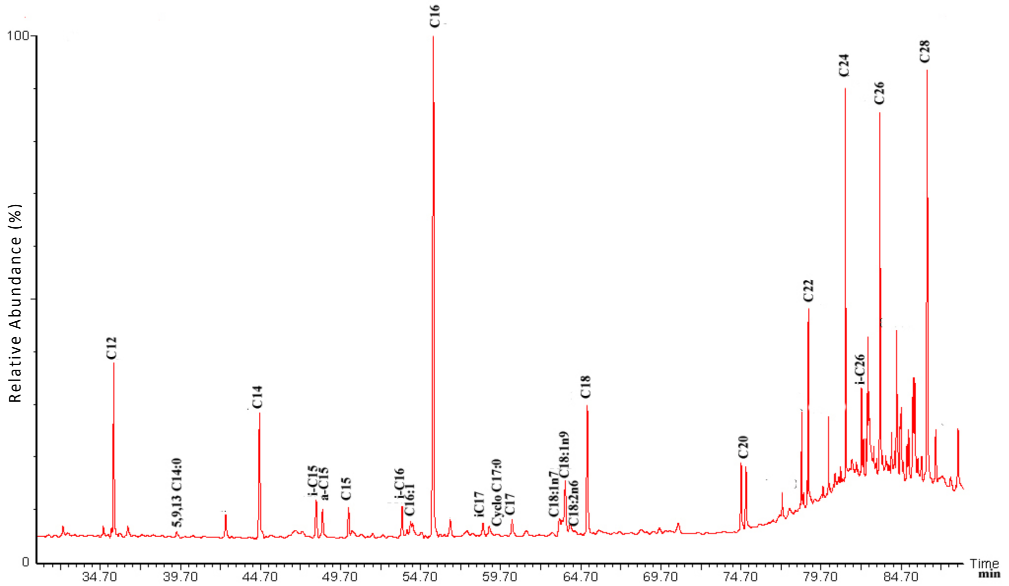


(S1)

Fig. 4.1 Gas chromatograms of the methyl esters (FAMEs) of the fatty acids extracted from the sediments of the study area (continue…)

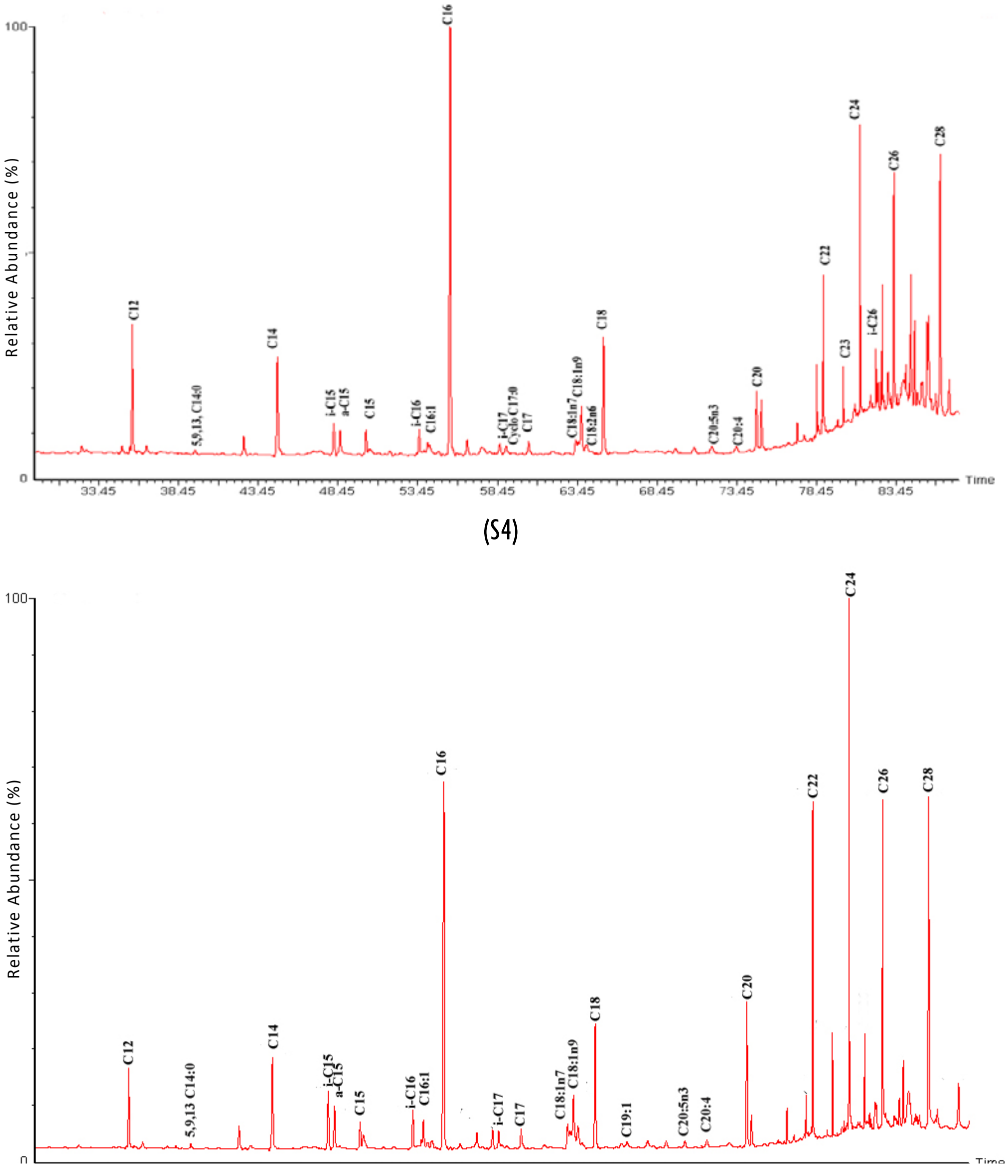


(S2)



(S3)

Fig. 4.1 Gas chromatograms of the methyl esters (FAMEs) of the fatty acids extracted from the sediments of the study area (continue----)



(S4)

(S5)

Fig. 4.1 Gas chromatograms of the methyl esters (FAMEs) of the fatty acids extracted from the sediments of the study area

Table 4.4 Average concentrations of fatty acids (μgg^{-1}) in the surface sediments of the study area (n=27)

Fatty acids (μgg^{-1})	Stations				
	S1	S2	S3	S4	S5
$C_{12:0}$	1.64 ± 0.46	7.7 ± 1.6	10.0 ± 0.45	9.7 ± 0.61	5.3 ± 1.63
$C_{14:0}$	2.19 ± 0.56	23.8 ± 2.3	23.0 ± 13.6	11.0 ± 1.6	13.2 ± 2.11
5,9,13-$C_{14:0}$	0.33 ± 0.08	8.8 ± 0.63	22.0 ± 2.6	3.1 ± 1.02	5.34 ± 2.83
i$C_{15:0}$	0.71 ± 0.24	19.63 ± 1.49	21.52 ± 1.7	13.29 ± 1.44	11.96 ± 2.04
a$C_{15:0}$	1.22 ± 0.12	9.8 ± 0.43	15.0 ±1.17	7.37 ± 0.85	5.28 ± 1.07
$C_{15:0}$	0.54 ± 0.09	13.85 ± 0.56	10.23 ± 1.5	2.94 ± 1.36	4.25 ± 1.21
$C_{16:0}$	96.98 ± 4.6	97.63 ± 2.30	100.2 ± 4.87	40.21 ± 4.9	50.02 ± 5.3
$C_{16:1}$	14.95 ± 1.47	15.11 ± 1.54	13.33 ± 0.95	4.78 ± 0.54	9.87 ± 1.46
i$C_{16:0}$	0.59 ± 0.04	7.85 ± 1.39	11.39 ± 1.06	1.68 ± 0.64	2.56 ± 1.20
$C_{17:0}$	7.46 ± 1.12	7.53 ± 0.92	6.4 ± 0.93	.80 ± 0.64	2.79 ± 0.82
i$C_{17:0}$	0.18 ± 0.03	3.25 ± 0.13	8.10 ± 0.5	1.87 ± 0.23	3.32 ± 0.81
Cy-$C_{17:0}$	Nd	2.51 ± 0.50	Nd	0.75 ± 0.27	Nd
$C_{18:0}$	2.15 ± 0.39	25.44 ± 1.52	24.8 ± 2.2	17.8 ± 2.07	11.7 ± 1.6
$C_{18:1\omega7}$	1.40 ± 0.28	9.9 ± 0.69	16.55 ± 0.86	1.55 ± 0.43	5.0 ± 1.4
$C_{18:1\omega9}$	1.11 ± 0.13	12.05 ± 0.30	20.30 ± 2.4	2.86 ± 1.03	6.4 ± 0.9
$C_{18:2\omega6}$	Nd	4.67 ± 0.98	0.30 ± 0.12	1.76 ± 0.13	Nd
$C_{19:1}$	Nd	Nd	Nd	Nd	0.68 ± 0.21
$C_{20:0}$	7.72 ± 0.96	16.73 ± 0.49	10.16 ± 1.24	2.65 ± 0.18	1.45 ± 0.26
$C_{20:4}$	0.28 ± 0.04	Nd	Nd	0.87 ± 0.1	1.39 ± 0.17
$C_{20:5}$	0.38 ± 0.08	Nd	0.60 ± 0.55	0.89 ± 0.47	0.72 ± 0.2
$C_{22:0}$	13.99 ± 0.67	15.31 ± 0.79	15.98 ± 0.67	10.38 ± 2.1	1.54 ± 0.20
$C_{23:0}$	Nd	Nd	Nd	1.64 ± 0.43	Nd
$C_{24:0}$	11.50 ± 0.95	21.09 ± 3.04	19.88 ± 2.1	7.76 ± 4.1	4.27 ± 4.4
$C_{26:0}$	1.71 ± 0.65	15.0 ± 2.68	18.02 ± 0.53	6.38 ± 0.78	0.89 ± 0.48
i$C_{26:0}$	8.66 ± 0.99	16.49 ± 0.27	14.73 ± 2.7	9.92 ± 1.66	Nd
$C_{28:0}$	5.64 ± 1.04	7.41 ± 0.33	9.06 ± 1.6	8.8 ± 2.17	Nd

Nd- Not detectable

Table 4.5 Percentage contributions of various fatty acid groupings to the overall FAMEs pool

Fatty acids (%)	Stations				
	S1	S2	S3	S4	S5
SAFAs	83.6	69.5	63.3	70.5	64.4
MUFAs	9.6	10.3	12.8	5.4	14.8
PUFAs	0.4	1.3	0.2	2.0	1.3
BFAs	6.4	18.9	23.7	22.1	19.2
BAFAs	10.9	24.8	27.9	25.8	24.4
LCFAs	18.1	16.3	16.1	19.4	4.5
C_{14}	1.2	6.6	5.9	6.4	8.9
C_{15}	1.21	6.58	5.86	6.39	8.90
C_{16}	53.48	27.00	25.59	23.41	33.81
$C_{16:1}$	8.25	4.18	3.41	2.78	6.67
C_{18}	1.18	7.04	6.34	10.35	7.89
C_{22}	7.72	4.24	4.08	6.04	1.04
C_{24}	6.34	5.83	5.08	4.52	2.89
$C_{18:1\omega7}$	0.77	2.74	4.23	0.90	3.38
$C_{18:1\omega9}$	0.61	3.33	5.18	1.67	4.33

SAFAs -Satuated fatty acids; MUFAs - Monounsaturated fatty acids; PUFAs -Ployunsaturated fatty acids; BFAs - Branched fatty acids; BAFAs - Bacterial fatty acids; LCFAs - Long chain fatty acids

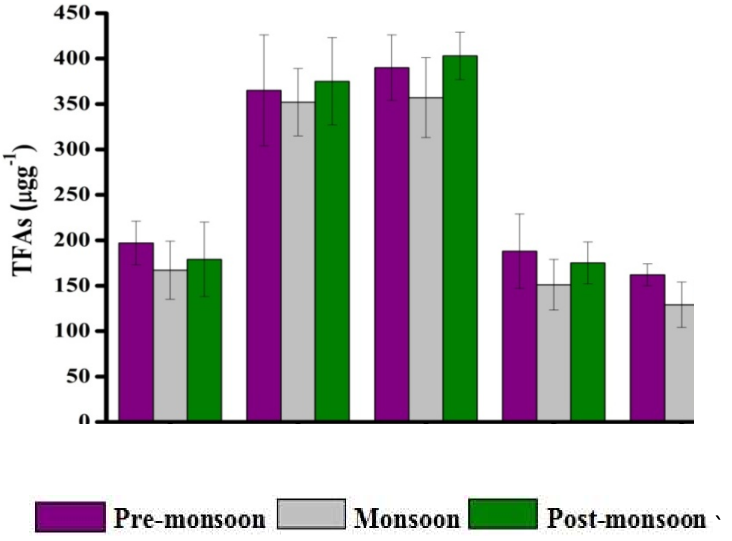


Fig. 4.2 Spatial and seasonal distributions of total fatty acids (TFAs) in the surface sediments of the study area

The station S1, the far upstream portion of the river, beyond the influence of industrial pollution, exhibited the predominance of FAs $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{20:0}$, $C_{22:0}$, $C_{24:0}$ and $iC_{26:0}$ (Table 4.4), with an average total concentration of 181 μgg^{-1} dry weight (Table 4.6). Among the 22 individual fatty acids detected, $C_{16:0}$ recorded maximum concentration (97 μgg^{-1}), which contributed 53% (Table 4.5) of total fatty acids, with substantial contributions of $C_{16:1}$ (8%), $C_{22:0}$ (7.8%) and $C_{24:0}$ (6%). SAFAs contributed 84% of total fatty acid pool, while BFAs constituted 6.4% and MUFAs and PUFAs contributed 9.6 and 0.4% respectively. The MUFA, $C_{16:1}$ was the second most abundant (14.95 μgg^{-1}) fatty acid, which accounted for 8% of TFAs, while, LCFAs and BAFAs contributed 18 and 11% respectively. Stations S2, S3 and S4 constitute industrial zones, which are the recipients of effluents from major industrial units, clearly exhibited the dominance of fatty acid concentrations. A total of 22 fatty acids were characterized, at station S2 with an average concentration of 361 μgg^{-1} dry weights. SAFAs contributed 70% of total fatty acid pool, followed by BFAs (18.9%) while, MUFAs and PUFAs contributed 10.0 and 1.3% respectively (Table 4.5). The SAFAs $C_{16:0}$ and $C_{18:0}$ were dominant in this region, contributing 27 and 7% of TFAs respectively. Among MUFAs, $C_{16:1}$ was the most abundant fatty acid (15 μgg^{-1}), which accounted for 4% of TFAs, followed by $C_{18:1\omega9}$ (3.3%) and $C_{18:1\omega7}$ (2.7%). The PUFA, $C_{18:2\omega6}$ identified at this station, contributed to approximately 1.3% of TFAs. LCFA and BAFA contributed 16% and 25% respectively. The highest concentration of TFAs (392 μgg^{-1}) was obtained at S3. Among the 22 individual fatty acids detected, $C_{16:0}$, $C_{18:0}$ and $C_{14:0}$ were the dominant classes, which accounted 25.6, 6 and 5.9% respectively. SAFAs contributed 63% of total fatty acid pool, followed by BFAs (24%), while, MUFAs and PUFAs contributed 13 and 0.2% respectively. Among the MUFAs, $C_{18:1\omega9}$ was the most abundant (20.3 μgg^{-1}) with a significant amount of

$C_{18:1\omega7}$ (16.6 µgg^{-1}) and $C_{16:1}$ (13.3 µgg^{-1}). Comparatively higher concentrations of LCFAs (63 µgg^{-1}), BAFAs (109 µgg^{-1}) and iC_{15} (21.5 µgg^{-1}) were reported at this station. Station S4 revealed comparatively low fatty acid concentrations (172 µgg^{1}) compared to other upstream stations S1, S2 and S3. SAFAs contributed 70.5% of total fatty acid pool, while, BFAs constituted 22.0% and MUFAs and PUFAs contributed 5 and 2% respectively. Among SAFAs, $C_{16:0}$ is dominated (40.0 µgg^{1}) at this station, followed by $C_{18:0}$ (18 µgg^{1}) and $iC_{15:0}$ (13 µgg^{1}). Among MUFAs, $C_{16:1}$ and $C_{18:1\omega9}$ were identified in the surface sediments. LCFAs contributed 19% and BAFA constituted 26% of the total FAMEs pool. The lowest total fatty acid concentration (148 µgg^{-1}) was observed at S5. Among the 21 individual fatty acids detected, $C_{16:0}$, $C_{14:0,}$ $C_{15:0}$ and $iC_{15:0}$ were dominated. SAFAs contributed 64% and BFAs constituted 19%, while, very low concentrations of LCFAs were recorded (6.7 µgg^{1}) which accounted 4.5% of the total fatty acid pool. However, compared to other stations, higher concentrations of MUFAs (15%) were recorded at S5, with substantial contributions of BAFAs (24%).

SAFAs were the major fraction at all stations contributing 63 (S3) to 84% (S1), followed by BAFAs, which contributed 11 (S1) to 28% (S3) to the total fatty acid pool. SAFAs, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$, $C_{24:0}$ and $C_{26:0}$ were detected at all the stations, among this $C_{16:0}$ is dominated with significant amounts of $C_{14:0}$, $C_{15:0}$, $C_{18:0}$, $C_{20:0}$ and $C_{22:0}$. Station S2 showed maximum SAFAs concentrations (251 µgg^{-1}), while, station S5 displayed minimum concentrations (95 µgg^{-1}). BFAs constituted about 6 to 24% (Table 4.5) in the study area with maximum concentrations at S3 and a minimum content at S1. The total BFAs concentrations were in the range 11.69 to 92.75 µgg^{-1}. Among the BFAs, 5, 9, 13-$C_{14:0}$, $iC_{15:0}$, $aC_{15:0}$, $iC_{16:0}$ and $iC_{17:0}$ were detected at all the stations, while, iC_{14} and aC_{17} were not recognized in the surface sediments. MUFAs constituted about 5 to 15% (Table 4.5) of total fatty acids and it ranged from 9 to 50 µgg^{-1} in surface sediments. PUFAs constituted only a small portion of total fatty acids (0.2 to 2%) (Table 4.5), which ranged from 0.66 to 4.67 µgg^{-1}. Bacterial fatty acids (BAFAs) ranged from 19.6 (S1) to 109.3 µgg^{-1} (S3), while, LCFAs constituted 4.5 to 19% of total fatty acids and it ranged from 7 to 63 µgg^{-1} in surface sediments.

Table 4.6 Fatty acid indices in the surface sediments of the study area of Chitrapuzha River (n=27)

Fatty acids (µgg^{-1})	Stations				
	S1	S2	S3	S4	S5
TFAs	181.33 ± 14.8	361.35 ± 11.5	391.57 ± 23.4	171.76 ± 18.6	147.93 ± 16.8
SAFAs	151.53 ± 11.2	251.44 ± 9.0	247.73 ± 15.4	121.08 ± 11.6	95.33 ± 7.2
MUFAs	17.46 ± 1.8	37.07 ± 3.8	50.18 ± 4.2	9.19 ± 1.9	21.94 ± 4.0
PUFAs	0.66 ± 0.12	4.67 ± 1.15	0.90 ± 0.68	3.52 ± 0.47	2.11 ± 0.20
BFAs	11.69 ± 1.75	68.30 ± 4.6	92.75 ± 9.5	38.00 ± 5.6	28.45 ± 5.8
BAFAs	19.69 ± 3.2	89.68 ± 2.8	109.38 ± 11.9	44.39 ± 7.6	36.17 ± 7.9
SCFAs	101.32 ± 6.6	146.88 ± 10.2	148.0 ± 11.7	68.98 ± 8.6	74.86 ± 9.0
LCFAs	32.85 ± 2.3	58.81 ± 2.2	62.94 ± 3.6	33.32 ± 2.03	6.70 ± 4.9
C_{24}/C_{16}	0.12 ± 0.06	0.22 ± 0.10	0.20 ± 0.09	0.19 ± 0.07	0.09 ± 0.08
$C_{16:1}/C_{16:0}$	0.15 ± 0.10	0.15 ± 0.07	0.13 ± 0.09	0.12 ± 0.10	0.20 ± 0.06
SCFA/LCFA	3.08 ± 0.40	2.50 ± 0.36	2.35 ± 0.24	2.07 ± 0.36	11.18 ± 1.3
$\Sigma C_{16}/\sum C_{18}$	24.16 ± 2.6	2.32 ± 0.09	2.02 ± 0.08	1.95 ± 0.04	2.71 ± 0.10
$\sum C_{18}/\sum C_{16}$	1.10 ± 0.04	1.31 ± 0.11	1.32 ± 0.12	1.28 ± 0.06	1.25 ± 0.12

TFAs- Total fatty acids; SAFAs- Saturated fatty acids; MUFAs-Monounsaturated fatty acids; PUFAs- Polyunsaturated fatty aids; BFAs-Branched fatty acids; BAFAs- Bacterial fatty acids; SCFAs- Short chain fatty acids; LCFAs- Long chain fatty acids

The distribution profile, total fatty acid concentrations (TFAs) and selected FAs ratios are given in Table 4.6. Analysis of variance (ANOVA) registered significant spatial and seasonal variations ($p<0.01$) of FAs with maximum concentrations near to the industrial areas (S2 and S3) during post-monsoon seasons, while, minimum value recorded at the estuarine station S5 during monsoon season (Fig. 4.2). This high average value could be attributed to its proximity to the industrial units, which discharge 80 million litres of effluent treated water per day (Rejomon et al. 2013), possibly in response to increased inputs of organic matter from industrial run-off events. The diversity in the origin of aquatic organic matter depends not only on the biological and geographical contexts, but also on the socio-economic environment through the effects of urbanisation, industrialisation and regional development (Galois et al. 2000). Furthermore, the sediment texture has a significant influence on the physico-chemical process as well as the species diversity of the depositional environment (Badarudeen et al. 1996; Hyun et al. 2002; Dunn et al. 2008; Ramaswamy et al. 2008; Gireeshkumar et al. 2013; Al-Timari et al. 2015). The dependency of the physical properties of aquatic sediments on sediment grain size has previously been described by Hamilton (1972) and Hamilton and Bachman (1982). A strong relationship exist between the sediment grain size and organic matter content, due to the greater absorption capacity of fine sediments with large surface areas (Meyers and Eadie 1993; Meyers and Lellier-Verge`s 1999). As a result, the distribution of sediment grain size is influential and important, when studying organic matter and geochemical signatures of coastal and marine sediments. Therefore, granulometrically, the fine grained (silty-clay) compositions of the surface sediments in the industrial zones (chapter 3) enhances the organic matter accumulation, leading to a higher TFAs content (Cotano and Villate 2006; Ramaswamy et al. 2008; Al-Timari et al. 2015). While, the lowest concentration of fatty acids at estuarine station S5 is likely due to the larger distance from the industrial units, and thus the anthropogenic organic matter sources. Moreover, the out welling of organic matter as a result of high tidal flushing (Ratnayake et al. 2005), and the dredging operations in this area to facilitate water transportation, may further reduce the fatty acid level. Dredging is a continuous process necessary to maintain or improve/extend navigable depths in coastal areas. Sediment granulometry showed predominantly silt and clay prior to dredging, which changed to sand with the onset of dredging (Lohrer and Wetz 2003; Nayar et al. 2004). Finer fractions of sediments get resuspended during a dredging event, and are dispersed spatially as the result of tides and water movements. Lohrer and Wetz (2003) suggested that these fine-grained sediments (silt -clay) remained suspended in the water column for a long time and contained greater concentrations of soluble regenerated nutrients and contaminants. Once the lighter and finer sediments were resuspended in the water column, the coarser and heavier sandy sediments settled quickly on the bottom substratum (Lohrer and Wetz 2003), explaining the relatively higher levels of sand in the sediments at this station (chapter 3), and thus lower concentrations of organic matter (Zimmerman et al. 2003; Dunn et al. 2008). From an environmental perspective, natural processes such as waves and currents are of much less concern than dredging.

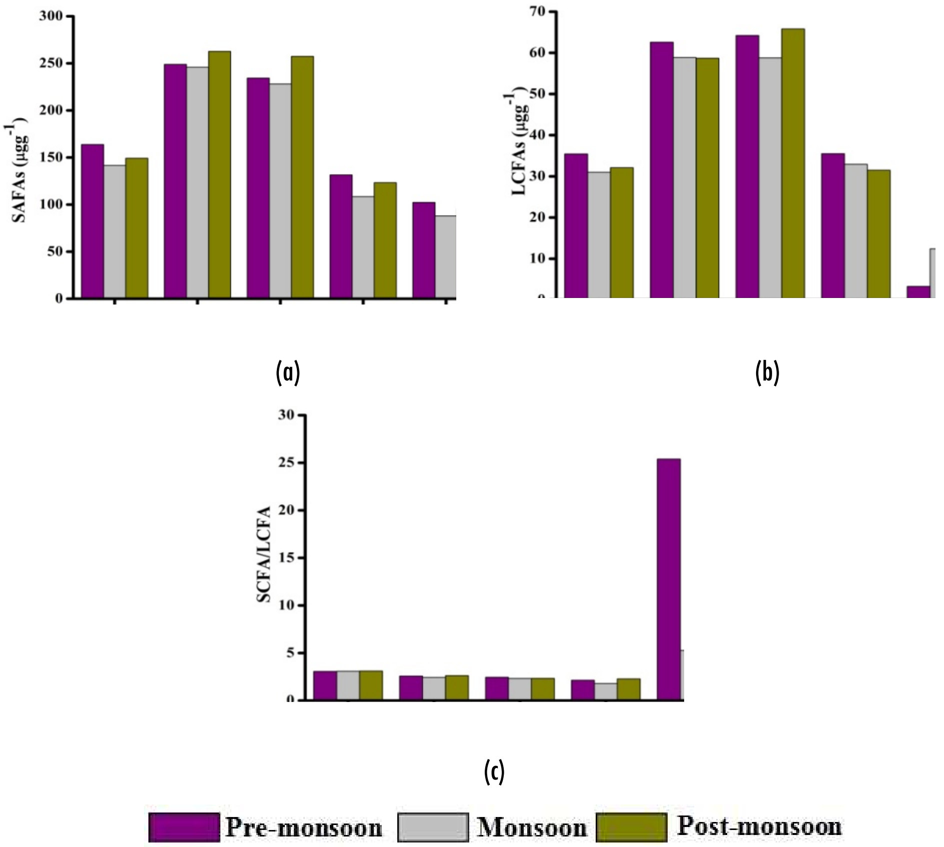
The fatty acid molecular distributions in the surface sediments of Chitrapuzha River suggested different sources of organic matter in the sedimentary system. The identified fatty acids included SAFAs, BFAs, MUFAs, PUFAs, long chain fatty acids (LCFAs; $C_{22:0}$, $C_{24:0}$, $C_{26:0}$ and $C_{28:0}$) and bacterial derived fatty acids (BAFAs).

4.2.1 Saturated fatty acids (SAFAs)

The distribution profile of fatty acids in the surface sediments of Chitrapuzha River showed the predominance of SAFAs, which accounted 63.3 (S3) to 83.6% (S1) of the total fatty acid pool (Table 4.5), and it varied from 102 (S5) to 249 μgg^{-1} (S2) in pre-monsoon, 88 (S2) to 246 μgg^{-1} (S5) in monsoon and 96 (S5) to 263 µgg^{-1} (S2) in post-monsoon seasons (Fig. 4. 3a). Station S2 displayed maximum SAFA concentration, while S5 showed minimum concentration. Among the individual fatty acids detected, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0,}$ $C_{17:0}$ and $C_{18:0}$ were dominated in the surface sediments, and $C_{16:0}$ (palmitic acid) was the most abundant fatty acid, which contributed 23 to 53% (Table 4.5) of the total fatty acid pool. These fatty acids are ubiquitous in organisms of a wide range of genera (Alfaro et al. 2006; Dunn et al. 2008). $C_{14:0}$ fatty acids (myristic acid) are usually present in phytoplankton, especially in diatoms (Reitan et al. 1994), and to a lesser extend in dinoflagellates (Napolitano et al. 1997). $C_{16:0}$ is a major component of phytoplankton, while, zooplanktons exhibit elevated amounts of $C_{18:0}$ fatty acids (stearic acid). High contributions of SAFAs are indicative of older partially degraded materials in the surface sediments of the study region (Reitan et al. 1994) and to a lesser extend in dinoflagellates (Napolitano et al. 1997). The marine phytoplankton and settling particles in the marine environments exhibit characteristic fatty acid composition in the range of C_{14} to C_{22} (Claustre et al. 1989; Reemtsma et al. 1990; Colombo et al. 1996). The ratio $\sum C_{16}/\sum C_{18}$ is considered to be an indicator of benthic phytoplankton (Parrish et al. 2000) and this ratio was relatively high at station S1 (24), and varied 2 to 24 in the surface sediments (Table 4.6), which supports the significant contribution of diatom organic matter. Similarly, the ratio of the sum of all fatty acids having 18 carbon numbers to sum of fatty acids having 16 carbon numbers ($\sum C_{18}/\sum C_{16}$) was projected as a diatom marker (Parrish et al. 2000). Relatively high concentrations of these fatty acids were obtained in the study area, and the ratios ranged from 1.10 to 1.32 (Table 4.6).

Long chain fatty acids C_{22} to C_{28} were identified in the surface sediments, with a strong, even order predominance of carbon atoms. LCFAs,

$C_{22:0}$, $C_{24:0}$, $C_{26:0}$ and $C_{28:0}$ were detected at all stations, while odd chain LCFAs were not recognized in the surface sediments. Long chain fatty acids are generally associated with the waxy leaf coatings of terrestrial plants, and thus considered as an indication of higher plant inputs (Shi et al. 2001; Zimmerman and Cannel 2001; Hu et al. 2005). Usually, LCFAs are found to be more refractory than saturated fatty acids from autochthonous planktonic sources, and it contributed 4 to 19 % (Table 4.5) of total fatty acids, and exhibited maximum concentration at S3 (66 μgg^{-1}) during post-monsoon season, while the minimum was recorded at S5 (3 μgg^{-1}) during pre-monsoon season (Fig. 4. 3b). Compared to other stations S2 and S3 recorded higher concentrations of $C_{24:0}$ (21.0 μgg^{-1}) and $C_{26:0}$ (18 μgg^{-1}). Moreover, the ratios of longer chain to shorter chain lipids ($C_{24:0}/C_{16:0}$) can be used to assess the relative contribution of allochthonous vs. autochthonous components of organic matter (Meyers 1997). The observed ratios (Table 4.6) ranged from 0.09 (S5) to 0.22 (S2), indicated that Chitrapuzha river system receives only minor amount of organic matter from higher plant inputs. Furthermore, the ratio of even numbered short chain fatty acids (SCFAs; $C_{14:0} + C_{16:0} + C_{18:0}$) to even numbered LCFAs ($C_{22:0} + C_{24:0} + C_{26:0} + C_{28:0}$) was used to differentiate between terrestrial and aquatic organic matter sources within the sediments (Meyers 1997; Jaffe et al. 2001; Waterson 2005). SCFAs/LCFAs ratios in the study area demonstrated a high degree of variability, ranging from 2 (S4) to 25 (S5) (Fig. 4.3c). The higher values at S5 indicated dominant input of labile autochthonous (marine) OM, while lower concentrations at other stations might be an indication of reduced inputs of autochthonous OM (Dunn et al. 2008).



(a)

(b)

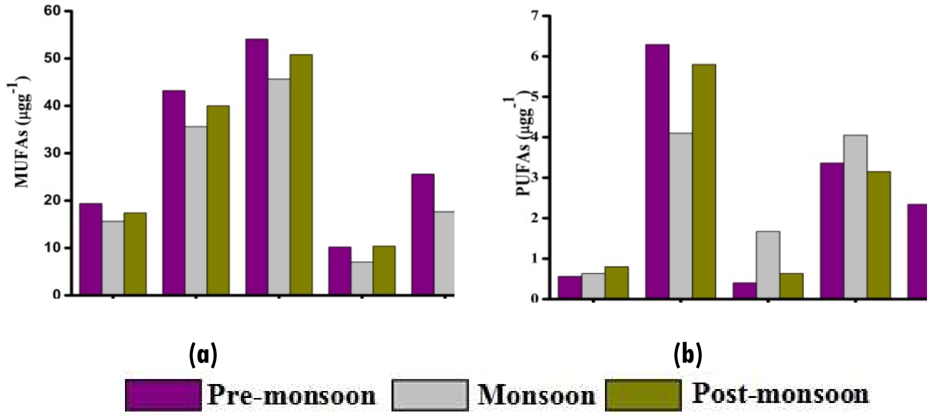
(c)

Fig. 4.3 Spatial and seasonal variations of (a) SAFAs, (b) LCFAs and (c) SCFA/LCFA in the surface sediments of the study area

4.2.2 Monounsaturated fatty acids (MUFAs)

Spatial and seasonal variations of MUFAs are depicted in Fig. 4. 4a. MUFAs constituted 5 (S4) to 14% (S5) (Table 4.5) of total fatty acid pool, and it ranged from 10 (S4) to 54 μgg^{-1} (S3) in pre-monsoon season, 7 (S4) to 46 μgg^{-1} (S3) in monsoon season and 10 (S4) to 51 μgg^{-1} (S3) in post-monsoon season. MUFAs are generally aerobic prokaryote indicators in aquatic sediments, and $C_{17:1}$ is a typical biomarker of desulfobulbus-type bacteria in marine sediments (Taylor and Parkes 1985). Generally, increase in concentrations of BFAs, and MUFAs are indicative of significant amount of bacterial production in aquatic sediments (Gillan and Hogg 1984). Among MUFAs, $C_{16:1}$, $C_{18:1}\omega_7$ and $C_{18:1}\omega_9$

were detected at all stations, while, $C_{19:1}$ was identified only at station S5. The most abundant MUFA, $C_{16:1}$ accounted for 2.8 to 8% (Table 4.5) of total identified fatty acids, and it is common in marine algal species (Reitan et al. 1994; Berge et al. 1995). Additionally, two more MUFAs $C_{18:1\omega7}$ and $C_{18:1\omega9}$ were identified in the surface sediments, and vaccenic acid ($C_{18:1\omega7}$) is a well-known bacterial marker found in both aerobic and anaerobic bacteria, and it ranged from 1.4 (S1) to 16.6 µgg^{-1} (S3). Moreover, various studies reported that $C_{18:1\omega9}$ is produced by bacteria from corresponding cis isomer under conditions of stress (Kieft et al. 1994; Fang et al. 2004; Harji et al. 2010), and higher concentration observed at S3 might be due to the influence of municipal sewages and leachates from the defunct waste treatment plant, proximity to this area. Similarly, the ratio $C_{16:1}/C_{16:0}$ is usually used to distinguish between diatoms and dinoflagellates, and values >1.6 have been used as an evidence for diatom-derived fatty acids in different aquatic systems (Budge and Parrish 1998; Hu et al. 2005; Joseph et al. 2012). In the present study, the ratios were well below 1 (Table 4.6), might be due to the higher susceptibility of unsaturated fatty acids to the biological and chemical degradation during sedimentation (Birgel et al. 2004; Hu et al. 2005; Joseph et al. 2012).



(a) (b)

Fig. 4.4 Spatial and seasonal variations of (a) MUFAs and (b) PUFAs in surface sediments of the study area

4.2.3 Polyunsaturated fatty acids (PUFAs)

Spatial and seasonal variations of PUFAs are depicted in Fig. 4. 4b. In the present study, PUFAs ($C_{18:2\omega6}$, $C_{20:4}$ and $C_{20:5}$) constituted only a meager portion of the total fatty acid pool (0.2 to 2%) (Table 4.5), and it ranged from 0.4 (S3) to 6.29 μgg^{-1} (S2), 0.63 (S1) to 4.05 μgg^{-1} (S2) and 0.80 (S1) to 5.8 μgg^{-1} (S2) in pre-monsoon, monsoon and post-monsoon seasons respectively. PUFAs are usually linked with phytoplankton marker. For example, $C_{20:5\omega3}$ and $C_{16:4\omega1}$ are related to diatoms (Colombo et al. 1996), while $C_{18:2\omega6}$, $C_{18:3\omega3}$ and $C_{18:3\omega6}$ have been used as markers of green macro algae (Meziane and Tsuchiya 2000). The lack of PUFAs within the river sediments is not a direct indication of the absence of phytoplankton sourced organic matter, but as a result of the lability of PUFAs, which are subject to rapid losses by bacterial degradation or by zooplankton grazing (Alfaro et al. 2006; Hu et al. 2006). Thus, PUFAs associated with phytoplankton may not be preserved in their original amounts, which may explain the absence of several diagnostic PUFAs in the surface sediments (Carrie et al. 1998). However, the prevalence of PUFAs within the study area confirmed the delivery of labile autochthonous organic matter to the surface sediments, however, did not show any significant contributions to the overall FAMEs pool, nor they frequently observed throughout the study area. Moreover, the low levels of PUFAs in the surface sediments indicate the effective bacterial recycling of algal fatty acids during the whole settling and depositing process. In estuarine biogeochemical cycles, phytoplankton-derived organic matter is subjected to rapid modification than terrestrially derived organic matter by bacterial degradation (Hu et al. 2006). Algal derived organic matter is rapidly assimilated and degraded, as evident from the lack of polyunsaturated fatty acids, even in samples where algal contributions would be expected. This reflects not only the microbial processes occurring in surface sediments, but also the effects of grazing fauna such as crabs (Guest and Connolly 2004; Guest et al. 2006). Several studies in various aquatic sediments already established the rapid assimilation of microalgal derived fatty acids in surface sediments (Volkman 2005; Hu et al. 2006; Dunn et al. 2008).

4.2.4 Bacterial fatty acids (BAFAs)

The spatial and seasonal variations of BAFAs is depicted in Fig. 4.5. BAFAs contributed 11 (S1) to 28% (S3) of total fatty acid pool (Table 4.5), and it ranged from 23 (S1) to 120 μgg^{-1} (S3) in pre-monsoon, 17 (S1) to 96 μgg^{-1} (S3) in monsoon and 19 (S1) to 112 μgg^{-1} (S3) in post-monsoon seasons. The maximum concentration was observed at S3 during pre-monsoon season, while the minimum value obtained at S1 during monsoon. Usually, odd chain and branched chain fatty acids such as iso- and anteiso-homologue of C_{15} and C_{17} carbon atoms are commonly produced by bacteria, and can be used as bacterial biomarkers (Volkman et al. 1980; Goosens et al. 1989; Haddad et al. 1992; Meyers and Ishiwatari 1993; Rajendran et al. 1997; Palomo and Canuel 2010; Bianchi and Canuel 2011; Zhang et al. 2015). Bacteria occur in all sedimentary environments, including polar, deep-sea, anoxic-basin, intertidal, and lacustrine sediments (Gong and Hollander 1997; Dunn et al. 2008). Microbial-mediated degradation processes appear to have a significant effect on the composition of sedimentary organic matter. The fatty acid composition of microorganisms is known to vary in response to temperature changes (Sushchik et al. 2003; Teoh et al. 2004), irradiance conditions (Brown et al. 1996) or with growth phase (Brown et al. 1996; Gugger et al. 2002), but in most cases the changes are limited to variations in their relative abundance.

Moreover, the fatty acid composition is closely linked with bacterial respiratory types (aerobic or anaerobic), and the concentrations of these biomarkers are considerably increased below the oxic/anoxic interface (Wakeham and Beier 1991; Ding and Sun 2005). Aquatic microorganisms are generally classified into four distinct functional groups, specifically, micro-eukaryotes (PUFAs), aerobic prokaryotes (MUFAs), gram-positive and other anaerobic bacteria (saturated and branched fatty acids in the range of C_{14} to C_{16}), and sulphate reducing bacteria and other anaerobic bacteria (saturated and branched fatty acids in the range of C_{16} to C_{19}), based on their fatty acid composition (Findlay and Dobb 1993). Branched fatty acids, although not specific to any particular organism, are present in significant quantities in broad chemo type of gram-negative bacteria specifically $C_{16:1\omega7}$, C_y-$C_{17:0}$, $C_{18:1\omega7}$, and Cy-$C_{19:0}$ (Perry et al. 1979). Saturated and branched fatty acids in the range of C_{14} to C_{16} obtained in the surface sediments indicated the presence of gram reducing and other anaerobic bacteria in the study area.

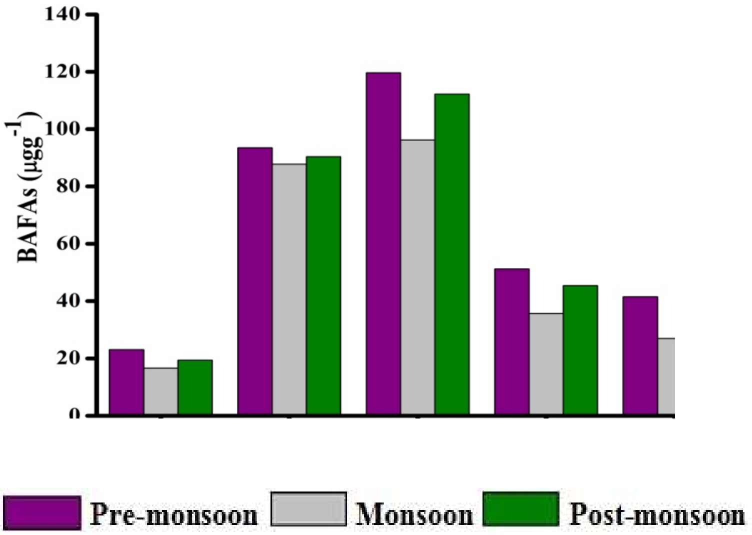


Fig.4.5 Spatial and seasonal variations of bacterial fatty acids (BAFAs)

The abundance of odd chain and branched chain fatty acids and lower concentrations of PUFAs in the study area establish high bacterial reworking of organic matter (Joseph et al. 2012; Oyo Ita and Oyo Ita 2012). This is because, bacteria not only contribute their own metabolic constituents, but also, alter the signature of organic matter through microbial mediated diagenetic transformation reactions. Less reactive forms of fatty acids (eg., SAFAs) become more dominant and more reactive forms (unsaturated FAs) are utilized by microbes and by deposit-feeding animals or in heavily worked sediment, only the most resistant forms of the FAs remain. Unsaturated fatty acids (MUFAs + PUFAs) are more susceptible to attract in sediments than SAFAs (Meyers and Ishiwatari 1993). They are rapidly degraded by microbial attack (Cranwell et al. 1987), as the existence of carbon-carbon double bond obviously facilitates microbial utilization and enhances their degradability, resulting lower values of MUFAs and PUFAs in sediments. The branched fatty acids $iC_{15:0}$, $aC_{15:0}$, $iC_{16:0}$ and $iC_{17:0}$ detected in the surface sediments are well-known biomarkers of gram-positive bacteria, gram-negative anaerobes and sulphate reducing bacteria (Wakeham et al. 1984; Rütters et al. 2002; Harvey et al. 2006; Dunn et al. 2008; Widenfalk et al. 2008; Fu et al. 2015). Concentrations of $iC_{15:0}$, $aC_{15:0,}$ $iC_{16:0}$ and $iC_{17:0}$ ranged from 0.71 to 21.5 , 1.22 to 15, 0.59 to 11.39 and 0.18 to 8.1 μgg^{-1} (Table 4.4) respectively, and recorded the maximum at S3. The occurrence of cyclopropyl fatty acids can be associated with cell age, nutrient deprivation and/or anaerobiosis (Kieft et al. 1994). Fatty acids derived from cyclo-propane ($C_{17:0,}$ and $C_{19:0}$) are typical for anaerobic organisms (Dowling et al. 1986). Moreover, *desulfobacter spp* showed high levels of cyclopropyl fatty acids, including two isomers of both methylene hexadecanoic (Cy-$C_{17:0}$) and methylene heptadecanoic (Cy-$C_{18:0}$) acids (Dowling et al. 1986). The presence of Cy-$C_{17:0}$ was detected at S2 and S4, and the concentrations ranged from 0.75 (S4) to 2.51 (S2) μgg^{-1}, while, Cy-$C_{18:0}$ was not observed in the surface sediments. A comparison of the present investigation with major studies involving fatty acid distribution in the aquatic sediments around the world is presented in Table 4.7.

Table 4.7 Concentration of total fatty acids in aquatic sediments - a comparison with other studies conducted on elsewhere

Sl. no	Location	Total fatty acids (μgg^{-1})	References
1	Chitrapuzha, South India	148 - 392	Present study
2	Cochin Estuary	23 - 441	Gireeshkumar et al. 2015
3	South Mid-Atlantic Ridge	7 - 30	Huang et al. 2015
4	Arcachon Bay, France	35 - 186	Rontani et al. 2014
5	Northern South China Sea	2 - 24	Xu et al. 2014
6	Mangrove sediments, Cochin	37 - 95	Joseph et al. 2012
7	Rodrigo de Freitas Lagoon, Brazil	20 - 66	Stefens et al. 2007
8	Chilean coastal region	20 - 203	Niggemann and Schubert 2006
9	Florida Bay	5 - 21	Xu et al. 2006
10	Matapuri Estuary	295 - 325	Alfaro et al. 2006
11	Pearl River Estuary	1 - 42	Hu et al. 2006
12	Oura Bay Estuary	223	Mfilinge et al. 2005
13	Gulf of Papua	20 - 2215	Burns et al. 2004
14	Western Pacific Ocean	13 - 184	Yamanaka and Sakata 2004
15	Okinawan Estuary, Japan	89 - 614	Meziane and Tsuchiya 2002
16	Santa Monica Basin	6 - 40	Pearson et al. 2001
17	Estuaries of south Florida	7 - 223	Jaffe et al. 1996

4.2.5 Distributional characteristics of fatty acids

Molecular distributions of fatty acids indicated several sources of organic matter in the surface sediments of Chitrapuzha River. From the individual fatty acid compositions, three different source signals could be well distinguished, viz. algal, bacterial and higher plant signals.

4.2.5.1 Algal inputs

Unsaturated fatty acids are usually associated with algae (Volkman et al. 1989; Carrie et al. 1998), and the presence of PUFAs in sediments have been used to indicate fresh algal organic matter sources (Canuel and Martens 1993), and its existence established the inputs of labile autochthonous organic matter to the surface sediments of Chitrapuzha River. It also implies that the fatty acid diagenesis during particle settling through the water column is not intense, allowing the deposition of labile fatty acids. Sinking time of organic matter is short in shallow aquatic ecosystems, resulting in a relatively less decomposition of organic matter in the water column. As a consequence a better preservation of organic matter occurs during their faster burial. After the burial, degradation of unsaturated fatty acids might also be low due to the anoxic nature of the sediments. However, changes in redox condition significantly affect the lability of specific organic compounds. Under anoxic conditions, bacteria do not efficiently remineralize lipid compounds, results in their preservation (Sun et al. 2002). Chlorophyta contain a high abundance of C_{18} PUFAs, especially with the positional isomers ω3 and ω6 (Volkman et al. 1989; Dunstan et al. 1992; Zhukova and Aizdaicher1995), and the presence of $C_{18:2\omega6}$ at S2, S3 and S4 indicated markers of green algae (Dunstan et al. 1992; Napolitano et al. 1995; Meziane and Tsuchiya 2000; Palomo and Canuel 2010; Bianchi and Canuel 2011**)**. $C_{16:1}$ (MUFAs) fatty acids were detected in appreciable levels at all the stations, and these fatty acids are relatively common in marine algal species (Reitan et al. 1994; Berge et al. 1995; Fagervold et al. 2014; Mrozik et al. 2014). The SAFAs $C_{16:0}$ and $C_{18:0}$ are the major contributors to the total fatty acid pool, and derived from marine, bacterial as well as terrestrial sources. However, $C_{16:0,}$ and $C_{18:0}$ are less source-specific than mono and polyunsaturated fatty acids, since, these fatty acids can also be formed from biochemical reduction of unsaturated fatty acids, which limit their biomarker potential (Birgel et al. 2004). Therefore, it can be inferred that calculation of marine fatty acids could not give the correct prediction as phytoplankton or algal contribution. Phytoplankton generally contain more $C_{16:0}$ (Wakeham 1995), whereas zooplankton contain more $C_{18:0}$ (Sargent 1976). Therefore, the ratio $\sum C_{16}/\sum C_{18}$ is considered to be an indicator of benthic phytoplankton (Parrish et al. 2000; Birgel et al. 2004; Palomo and Canuel 2010). The ratio was relatively high (24) at station S1 (Table 4. 6), supports the significant contribution of diatom organic matter. The saturated fatty acid $C_{14:0}$ was present at all stations, and it is reliably derived from primary production, on account of its high abundance in diatoms (Volkman et al. 1989; Viso and Marty 1993; Nahon et al. 2010). Diatoms are a major group of eukaryotic microalgae, and are one of the most common types of phytoplankton. Microalgae, a major source of fatty acids in most sedimentary environment is epiphytic and grow on the aerial roots of the trees and on the sediments. They include green (Chlorophyta), brown (Phaeophyceae), red (Rhodophyta) and blue-green algae (Cyanophyta).

4.2.5.2 Higher plant source signals

Long chain saturated fatty acids (LCFAs; $\geq C_{22}$) are generally linked with the waxy leaf coatings of higher plants, and thus considered as an indication of higher plant inputs (Boon and Duincveld 1996; Canue1 and Martens 1996; Colomho et al. 1996; Meyers 1997; Carrie et al. 1998; Hu et al. 2006; Wang et al. 2013; Pruski et al. 2015). Moreover, long chain fatty acids also found in higher concentrations in mangrove leaves (Wannigama et a1. 1981; Meziane and Tsuchiya 2000, 2002). Usually, the long chain fatty acids derived from terrigenous sources are more refractory than the shorter chained fatty acids from autochthonous planktonic sources (Camacho Ibar et al. 2003). Four higher plant biomarkers $C_{22:0}$, $C_{24:0,}$ $C_{26:0}$ and $C_{28:0}$ were characterised in this study, and the total concentration ranged from 3.2 to 64 μgg^{-1} (pre-monsoon), 12 to 59 μgg^{-1} (monsoon), and 4.4 to 66 μgg^{-1} (post-monsoon) seasons with its maxmum at S3 during pre-monsoon (Fig. 4.3 b). Additionally, ratio of fatty acids representing terrestrial and aquatic organic matter was used to discriminate organic matter sources within the surface sediments. The SCFAs/LCFAs ratio is a measure of even- numbered short chain fatty acids ($C_{14:0}$ + $C_{16:0}$ + $C_{18:0}$) to even numbered

LCFAs ($C_{22:0}$ + $C_{24:0}$ + $C_{26:0}$ + $C_{28:0}$), indicator of aquatic vs. terrestrial organic matter sources (Meyers 1997; Jaffe et al. 2001; Waterson 2005). SCFAs/LCFAs ratios within the surface sediments demonstrated a high degree of variability, (Fig. 4.3c) and increased ratios indicated dominant inputs of labile autochthonous (marine) sourced organic matter, occurred predominantly within the estuarine sediments (S5), while, lower values at other stations indicated reduced inputs of autochthonous organic matter within the riverine sediments.

4.2.5.3 Bacterial signals

Fatty acids are usually used as biomarkers to identify the bacterial sources, and fate of organic matter in aquatic environments due to their structural diversity and high biological specificity (Harvey 1994; Gong and Hollander 1997; Dunn et al. 2008). Usually, odd chain and branched chain fatty acids such as iso- and anteiso-homologue of C_{15} and C_{17} carbon atoms are commonly produced by bacteria, and can be used as bacterial biomarkers (Volkman et al. 1980; Goosens et al. 1989; Haddad et al. 1992; Meyers and Ishiwatari 1993; Rajendran et al. 1997; Palomo and Canuel 2010; Bianchi and Canuel 2011; Zhang et al. 2015). In the present study, BAFAs contributed 11 to 28% of total fatty acid pool (Table 4.5), and the maximum concentration was observed at S3 during pre-monsoon season, while the minimum value obtained at S1 during monsoon (Fig. 4.5). The presence of odd chain fatty acids ($C_{15:0}$, $C_{17:0,}$ $C_{19:1}$ and $C_{23:0}$) and branched chain fatty acids ($iC_{15:0}$, $aC_{15:0}$, $iC_{16:0,}$ $iC_{17:0}$ and $iC_{26:0}$) established high bacterial reworking of organic matter in the surface sediments of Chitrapuzha River (Joseph et al. 2012). Among this, $C_{15:0}$ and $C_{17:0}$ are reported to be produced by both aerobic and anaerobic bacteria (Parish et al. 2000), and the detection in relatively low levels of iso- and anteiso- $C_{15:0}$ and $C_{17:0}$ FAs, as well as, the absence of β-hydroxy FAs, most likely are a reflection of the oxic nature of the sediment and sensitivity of *gram negative* bacteria to photo-degradation (a result of increased light penetration through the shallow water column) (Michele et al. 1996). The branched fatty acids $iC_{15:0}$, $aC_{15:0}$, $iC_{16:0}$ and $iC_{17:0}$ detected in the surface sediments, are well-known biomarkers of *gram-positive* bacteria, *gram-negative* anaerobes and *sulphate reducing* bacteria (Wakeham et al. 1984; Rütters et al. 2002; Harvey et al. 2006; Widenfalk et al. 2008; Dunn et al. 2008; Mrozik et al. 2014). Moreover, the distribution of saturated and branched fatty acids in the range of C_{14} to C_{16} indicated the presence of gram reducing and other anaerobic bacteria in the surface sediments (Widenfalk et al. 2008; Fu et al. 2015**)**. Additionally, the existence of MUFAs can also give an indication of bacterial population in the surface sediments. The presence of $C_{18:1\omega7}$ in surface sediment is linked to *sulphur* bacteria (Grimalt et al. 1992; Mrozik et al. 2014; Pruski et al. 2015), type II methane-oxidizers (Naganuma et al. 1996), *gram-negative* bacteria, *cyanobacteria* and diatoms **(**Wilkinson and Ratledge 1988; Dijkman et al. 2010). Finally, the occurrence of cyclopropyl fatty acids (Cy-$C_{17:0}$) at S2 and S4 can be associated with cell age, nutrient deprivation, and/or anaerobiosis (Kieft et al. 1994), produced when microorganisms move from the logarithmic to the stationary phase of growth (Poerschmann et al. 2005). Moreover, *Desulfobacter spp* showed high levels of cyclopropyl fatty acids, including methylene hexadecanoic (Cy-$C_{17:0}$) fatty acids (Dowling et al. 1986).

4.2.6 Principal component analysis (PCA)

PCA is used as an explorative tool to investigate the number of components needed to explain the variance in the observed data, and to examine the pattern of relative concentrations of various fatty acids. Varimax rotated factor analysis was carried out in the present study and the SPSS (version- 13) computer software was used. The Varimax rotation provided the correlation between the variables and the factors (Table 4.8)**.** Factor loading was considered as significant, if they were >0.50. PCA converted the contribution of variables into two or three principal components (PCs) and facilitated identified possible sources. This method is based on the comparison of the relative differences between contents of individual components in different samples and has an advantage of revealing changes in minor components, which might play a significant role in the identification of the state of degradation (Niggemann and Schubert 2006). Four principal components were found in PCA results, accounted for 38.95, 27.19, 21.33 and 9.18% of the total variance (95.65), respectively. PC1 accounts for the major proportion of the total data variance, while, second and following PCs progressively explain smaller amounts of data variations. PC1 was characterized by high loading of FAs including SAFAs ($C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{18:0}$ and $C_{26:0}$), BFAs ($iC_{15:0,}$ $aC_{15:0}$, $iC_{16:0}$, $iC_{17:0}$ and 5,9,13 $C_{14:0}$), LCFA ($C_{26:0}$), MUFAs ($C_{18:1\omega7}$ and $C_{18:1\omega9}$), total organic carbon (TOC) (value from chapter 3) and fine grained sediments (silt) (value from chapter 3), indicating the terrigenous signature and geochemical accumulation of organic matter with respect to the grain size in the surface sediments. The negative loading of sand (value from chapter 3) content is attributed to the lack of accumulation of organic matter in this sedimentary portion. Generally speaking, the short-chain homologues ($n \leq C_{20}$) are widely found in multiple sources, including plankton, photosynthesis bacteria, and aquatic plants (Meyers and Ishiwatari 1993; Philip 2003). For example, $C_{14:0}$ is abundant in phytoplankton, while, $C_{18:0}$ has been frequently predominant in zooplankton. The zooplankton generally contributes in greater proportion to the saturated fatty acids (SAFAs) $C_{16:0}$ and $C_{18:0}$, as compared to phytoplankton (Volkman et al. 1980; Muri et al . 2004; Marchand et al. 2005). Fatty acids such as iso and anteiso fatty acids and MUFAs are biomarkers for bacteria, which is attributed to colonization of bacterial communities. Moreover, meaningful loading of LCFAs ($C_{26:0}$) indicated terrestrial inputs of organic matter into the surface sediments.

Table. 4.8 PCA analysis of fatty acid

Variables	PC1	PC2	PC3	PC4
5,9,13$C_{14:0}$	0.968	0.102	0.177	-
$C_{14:0}$	0.963	-	-	0.248
a$C_{15:0}$	0.962	0.212	-	0.126
i$C_{17:0}$	0.955	-	-	-0.217
i$C_{15:0}$	0.953	-	-0.19	0.226
$C_{18:1\omega9}$	0.936	-	0.336	-
$C_{18:0}$	0.93	0.14	-0.174	0.288
$C_{15:0}$	0.901	-	0.264	0.325
i$C_{16:0}$	0.899	0.207	0.356	-
$C_{12:0}$	0.862	0.206	-0.391	0.173
$C_{18:1\omega7}$	0.858		0.501	-
Silt	0.651	0.64	-0.304	0.263
TOC	0.631	0.607	-0.378	0.283
$C_{19:1}$	0.119	-0.976		-0.112
$C_{28:0}$	-	0.968		0.152
$C_{22:0}$	-	0.953	0.266	-
i$C_{26:0}$	-	0.952	0.18	0.204
$C_{24:0}$	0.185	0.729	0.454	0.109
$C_{20:4}$	-0.212	-0.728	-0.641	-
$C_{26:0}$	0.645	0.724	-	0.132
Clay	-0.608	-0.713	-	-0.1
Sand	-0.571	-0.629	0.431	-0.284
$C_{16:1}$	-	-	0.993	-
$C_{17:0}$	-	0.321	0.92	-
$C_{16:0}$	-	0.397	0.909	-
$C_{23:0}$	-	0.278	-0.905	0.206
$C_{20:0}$	0.162	0.647	0.696	0.244
$C_{20:5}$	-	-0.184	-0.679	-0.571
Cy-$C_{17:0}$	0.241	0.275	-	0.919
$C_{18:2\omega6}$	0.319	0.382	-	0.845
% of variance	38.95	27.19	21.33	9.17

PC2 explained 27.19% of the total variance contributed by SAFAs ($C_{20:0}$, $C_{22:0}$, $C_{24:0}$, $C_{26:0}$ and $C_{28:0}$), BFAs (i$C_{26:0}$), LCFAs ($C_{22:0}$, $C_{24:0}$, $C_{26:0}$ and $C_{28:0}$), TOC and silt. Traditionally, LCFAs are assigned to terrestrial inputs; however, they are also produced in situ bacterial reworking (Gong and

Hollander 1997). Furthermore, Naraoka and Ishiwatari (2000) observed that LCFAs in sediments from the open Ocean derived mainly from marine inputs rather than terrestrial sources. PC3 contributed 21.33% of the total variance and showed positive loadings of odd chain fatty acid ($C_{17:0}$), and MUFAs ($C_{16:1}$ and $C_{18:1\omega7}$). Bacteria contain the most distinct fatty acid compositions of all marine taxa, with high proportions of C_{13} to C_{21} odd-numbered fatty acids (Claustre et al. 1989). The MUFA $C_{16:1}$ is common in marine algal species (Reitan et al. 1994; Berge et al. 1995), while, vaccenic acid ($C_{18:1\omega7}$) is a well-known bacterial marker found in both aerobic and anaerobic bacteria. Consequently, PC3 was defined as a bacterial derived component. PC4 accounted 9.1% of the total variance correlated with bacterial and algal input by the presence of Cy-$C_{17:0}$ and $C_{18:1\omega6}$ respectively.

4.3 Conclusions

Fatty acids were extracted from the surface sediments of Chitrapuzha River during pre-monsoon, monsoon and post-monsoon seasons. Fatty acids ranging from C_{12} to C_{28} were identified, representing a spectrum of organic matter inputs into the surface sediments. Identified fatty acids include SAFAs, MUFAs, PUFAs, BFAs, LCFAs and BAFAs. SAFAs were broadly distributed throughout the study area, and confirmed the greatest contribution to the overall FAs pool. The total fatty acid concentration levels ranged from 129 to 358 μgg^{-1} (monsoon), 162 to 390 μgg^{-1} (pre-monsoon) and 152 to 403 μgg^{-1} (post-monsoon). Analysis of variance (ANOVA) registered a significant spatial and seasonal variation of FAs with maximum concentrations near to the industrial areas (S2 and S3) during post-monsoon season, while, minimum value recorded at the estuarine station S5 during monsoon. This high average value could be attributed to its proximity to the industrial units, possibly in response to increased inputs of organic matter from industrial run-off events. The lower concentration of fatty acids at estuarine station S5 is likely due to the larger distance from the industrial units, and thus the anthropogenic organic matter sources. Moreover, the out welling of organic matter as a result of high tidal flushing as well as the dredging operations in this area to facilitate water transportation may further reduce the fatty acid level.

Among the individual saturated fatty acids, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0,}$ $C_{17:0}$ and $C_{18:0}$ were dominated, and $C_{16:0}$ (palmitic acid) was the most abundant fatty acid, which contributed 23 to 53% of total fatty acids. LCFAs identified in the surface sediments, with a strong, even order predominance of carbon atoms, contributed 5 to 18% of total fatty acids, with a maximum concentration at S3 during post-monsoon season and a minimum value at S5 during pre-monsoon season. MUFAs constituted 5 to 15% of total fatty acid pool, with maximum concentrations at S3 during pre-monsoon season, while the minimum value recorded at S4 during monsoon season. PUFAs constituted only a meager portion of total fatty acids, and it ranged from 0.66 to 4.67 μgg^{1}. The abundance of odd chain and branched chain fatty acids and lower concentrations of PUFAs in the study area established high bacterial reworking of organic matter in the surface sediments. Municipal sewages and the effluents from the adjacent industries in the study area influenced the distribution of bacterial fatty acids. From the fatty acids biomarker analysis, it is deduced that major inputs of organic matter in the surface sediments derived from phytoplankton, bacteria and terrestrial organic matter. The variations in the sedimentary organic matter sources and the diagenentic pathways due to the environmental settings could also be well documented through this biomarker approach. However, it is difficult to discriminate between the natural and anthropogenic inputs of organic matter through fatty acid biomarker alone. Hence, other lipid biomarkers such as n-alkanes, sterols and terpenoids along with the fatty acids could be more useful to distinguish the various organic matter sources.

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Chapter 5

ALIPHATIC HYDROCARBONS

Contents

5.1 Introduction

Aquatic sediments consist of a complex mixture of organic compounds, derived from a range of sources, including autochthonous inputs of planktonic and benthic primary productivity, along with allochthonous inputs such as continental, river runoff and anthropogenic sources (Thornton and McManus 1994; Graham et al. 2001; Carreira et al. 2015). The organic matter pool entering the aquatic sediment has potentially modified by several pathways such as, photolytic reactions (Amon and Benner 1996; Miller and Moran 1997), heterotrophic degradation (McCallister et al. 2004), respiration (Findlay et al. 1998; Moran et al. 1999), flocculation (Sholkovitz et al. 1978; Fox 1983) and sorptive fractionation etc. (Aufdenkampe et al. 2001). Variations in productivity as well as, fluctuations in delivery, make it difficult to resolve processes contributing to the storage of organic matter in sedimentary environment. Moreover, the differential reactivity of individual organic compounds to various modification processes produce a diverse range of end products causing further complexities to organic matter sources. Therefore, the overall environmental conditions existing in any sedimentary system cannot be explained by the application of geochemical parameters such as, general sedimentary characteristics and single biomarker compound like fatty acid, but need multi-proxy approaches. In order to get more specific and understandable knowledge on the sources and diagenetic processes of organic matter in aquatic sediments, lipid compounds such as n-alkanes, sterols and terpenoids can be effectively used (Jaffe et al. 2001; Bianchi et al. 2002; Mead et al. 2005). Among these, the geochemical approach of hydrocarbon is appropriate since, it differs in terms of their residence time, stability, transport mechanisms, etc. according to their physicochemical properties and sources (Pancost and Boot 2004; Zhang et al. 2014).

Aliphatic hydrocarbons (AHCs) constitute a major class of organic compounds, introduced into the aquatic environment through natural and anthropogenic sources (Zanardi et al. 1999). Due to their hydrophobic nature and strong particulate oriented behaviour, they tend to adsorb onto particulate phase, making aquatic sediment a repository of these compounds (Karickhoff 1984; Hartmann et al. 2004; Xu et al. 2007), and hence bottom sediments act as a temporary or long-term pool of these contaminants (Budzinski et al. 1997; Pereira et al. 1999, Krauss et al. 2000). These compounds exhibit distinct well defined sources, such as, terrestrial plant waxes, marine phytoplankton, bacteria, biomass combustion, as well as, other anthropogenic inputs (Meyers 2003; Maioli et al. 2010). Aliphatic hydrocarbons exhibit both low chemical reactivity and bioavailability for microorganisms, due to their lack of functional groups and low water solubility. However, some microorganisms such as bacteria, fungi and yeast have the metabolic capacity to utilise these components as a source of carbon and energy for their growth (Berthe-Corti and Fetzner 2002; Van Beilen et al. 2003). It has also been reported that some bacterial species are able to degrade these hydrocarbons, and play a pivotal role in the removal of sedimentary organic matter from the polluted environment (Head et al. 2006; Yakimov et al. 2007; Singh et al. 2012). Therefore, an assessment of aliphatic hydrocarbons in aquatic sediments is widely used as a powerful methodology to distinguish between different organic carbon inputs, because of their stability in the natural environment (Hu et al. 2009; Tarozo et al. 2010; Silva et al. 2012; He et al. 2014). For example, the AHCs in core sediment have been used for source characterization and the reconstruction of the historical records of environmental impact studies (Hostettler et al. 1999; Wu et al. 2001; Hu et al. 2011). Similarly, marine sediments have been recognized as an important reservoir of organic matter including hydrocarbons from a diversity of sources (Killops and Killops 1993; Yamamoto et al. 2003; Silva et al. 2012), they are often employed to recreate records of marine paleoenvironments and paleoclimates (Meyers 1997). Moreover, the distribution and composition of hydrocarbons in surface sediments reflect the relative contributions from biogenic, petrogenic and pyrogenic sources (Yunker et al. 1993; Lipiatou and Albaiges 1994; Lipiatou et al. 1997; Hostettler et al. 1999; Apostolopoulou et al. 2015). AHCs present in the sedimentary environment will cause serious pollution to the aquatic ecosystems, and directly influences the bottom-dwelling organisms, that may affect wildlife and human health via the food chain (Zhang et al. 2009). Since, most of the AHCs are proved to be narcotic and irritant, an understanding of the trend of these chemicals on sediment quality is essential for evaluating the overall status of the aquatic ecosystem.

Aliphatic hydrocarbons are widely used to trace out the sources of organic matter in aquatic sediments, since many are source specific and easily accumulated from biogenic and petrogenic sources (Yunker et al. 1993; Hostettler et al. 1999; Wu et al. 2001; Gao and Chen 2008). The straight chain hydrocarbons (n-alkanes) are usually regarded as molecular markers. Generally, n-alkanes in nonsiliceous species of phytoplankton and macrophytes are C15, C17 and C19 (Youngblood and Blumer 1973), whereas terrestrial plants contain mainly C29, C31 and C33 with high carbon preference index (CPI) values (Reddy et al. 2000; Xing et al. 2011). Biogenic hydrocarbons are known to be biosynthesised by a wide range of both marine and terrestrial plants. The difference in characteristic chain lengths of marine and terrestrial plants made the distribution of n-alkane as an effective tool for assessing sources of organic matter in terrestrial and marine sediments. For example, AHCs in the range of C14-C22 derived from diatoms (Elias et al. 2000; Carr et al. 2014), whereas bacteria displays predominance of n-alkanes C18 and C20 (Han and Calvin 1969). Planktonic organisms generally produce a simple mixture of odd chain n-alkanes preferably C15, C17 and C19 (Gogou et al. 2000), while the abundance of C25, C27, C29 and C31 has been used as an indicator of terrestrially derived organic matter (Zhao et al. 2003; Harji et al. 2008). Generally, shorter chain n-alkanes (C14 to C24) are largely derived from algal or phytoplankton sources (Meyers and Ishiwatari 1993) while, freshwater aquatic and marine mactophytes have a dominant mid chain length C23 to C27 hydrocarbons. On the other hand, slightly longer mid chain n-alkanes predominant in mangrove leaves (Mead et al. 2005). Sediment containing higher concentrations of unresolved complex mixture (UCM) and carbon preference index (CPI) value 1 or close to 1 indicates petroleum residues (Pearson and Eglinton 2000; Wang and Fingas 2003; Ye et al. 2007). Similarly, n-alkane indices such as, average chain length (ACL), short chain to long chain n-alkane ratio (SC/LC), total n-alkanes to C16, C17/pristane and C18/phytane are also used to delineate the sources of n-alkanes in aquatic sediments (Bouloubassi et al. 2001; Harji et al. 2008; Xing et al. 2011; Huang et al. 2013).

The isoprenoid alkanes, pristane (Pr; 2,6,10,14-tetramethylpentadecane) and phytane (Ph; 2,6,10,14-tetramethyl-hexadecane) (Fig. 5.1) are products of geological alteration of phytol and other isoprenoidyl natural products, and are not primary constituents of most terrestrial biota (Li et al. 1995; Gao et al. 2007; Harji et al. 2008). These hydrocarbons commonly occur in petroleum (Readman et al. 2002) and have been used to assess the microbial degradation of petroleum residues in soils and sediments (Wu et al. 2001; Zhu et al. 2005). However, in a marine environment, zooplankton and other higher marine animals can be contributors of pristine, while pytane is not only a normal component of oil, but can also be synthesised by the methanogenic and photosynthetic bacteria (Steinhauer and Boehm 1992; Sakata et al. 1997). The more complex molecular structure of isoprenoids, compared with linear alkanes results in higher resistance to degradation (Zhu et al. 2005). Therefore, Pr/Ph, n-C17/Pr and n-C18/Ph ratios have been used to describe the origin and the degree of deterioration of the hydrocarbons. For example, Pr/Ph value is higher than 1 for uncontaminated sediments (Chiou et al. 1998) while, the ratio lower than 1 indicated, traces of oil contamination in sediments. The important hydrocarbons used for the source characterization of sedimentary organic matter in aquatic system are mentioned in Table 5.1.

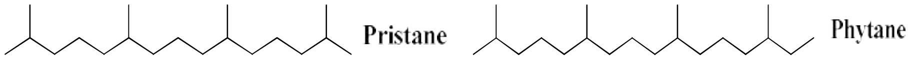


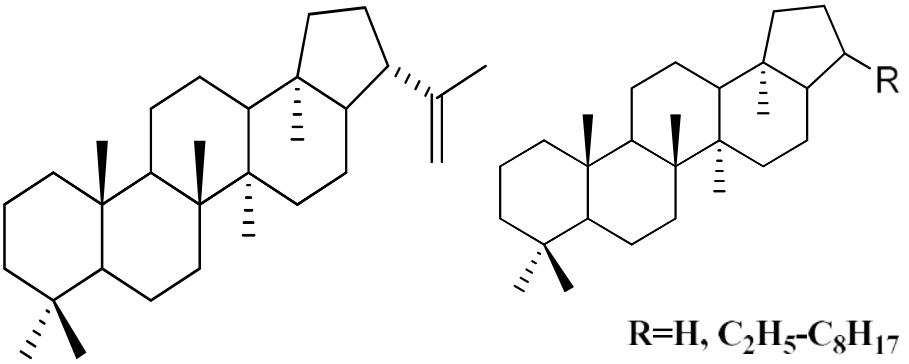
Fig. 5.1 Structure of pristane and phytane

Table 5.1 Hydrocarbons used for the source characterization in the aquatic environment

Component	Sources	References
C15, C17 and C19	Algae	Sikes et al. 2009
C21, C23 and C25	Aquatic macrophytes	Mead et al. 2005
C25,C27, C29, and C31 predominance	Terrestrial plants	Zhao et al. 2003; Harji et al. 2008
C20 to C40	Petroleum	Peters et al. 2005
Unresolved complex mixture (UCM)	Petroleum	Peters et al. 2005
C14 to C22	Diatoms	Elias et al. 2000
Branched isoprenoids C20, C25 and C30	Diatoms	Damste et al. 1999
Long chain n- alkanes	Micro algae	Volkman et al. 1992
Pristane	Zooplankton and erosion of sedimentary rocks	Meyers 2003
Phytane	Methanogenic and photosynthetic bacteria	Risatti et al.1984
Steranes	Microbial dehydration of sterols	Wakeham and Lee1993
Gammacerane	Crude oil	Rohmer et al. 1984; Prahl 1985
Diploptene	Methylotrophic bacteria and cyanobacteria	Rohmer et al. 1984; Prahl 1985

Cyclic alkanes are known to be less susceptible to bacterial degradation than other aliphatic hydrocarbons, and can be used as biological markers.

Among the cyclic alkanes, hopanes and steranes are considered to be of great importance due to their characteristic distributions of structural and stereochemical isomers in oils and sedimentary organic matter (Seifert and Moldowan 1980; Tissot and Welte 1984). The major precursors for hopanes includes bacteriohopanetetrol and related bacteriohopanes, and its stereoisomers exist as different α/β and/or R/S configurations that have different thermodynamic stability. For example, 17β (H), 21β (H) carbon skeleton occurs in living organisms and in ancient shales which have experienced little thermal stress (Seifert and Moldowan 1980). During sedimentation, the thermodynamically unstable biogenic 17β (H), 21β (H) hopane isomers are gradually replaced by the geologically stable 17α (H), 21β (H) hopane isomers, which do not occur in living systems (Lichtfouse and Rullkotter 1994; Damste et al. 1995). Hopanes with the 17α (H), 21β (H) configuration in the range of C27 to C35 carbon atoms are characteristic of petroleum because of their greater thermodynamic stability compared with other epimeric series (Mackenzie et al.1980). Gammacerane is distinguished as a well-known marker for crude oil derived from lacustrine sources while, there are several sources of diploptene including, methylotrophic bacteria and cyanobacteria (Rohmer et al. 1984; Prahl 1985). Moreover, the erosion of soil-derived bacterial organic matter can be considered as the major source of diploptene into the surface sediments. The general structure of hopane and diploptene are depicted in Fig. 5.2.



$R=H, C_2H_5-C_8H_{17}$

Diploptene.

Hopane

Fig. 5.2 General structure of hopane and diploptene

Cochin backwaters are highly productive ecosystem, and its complex nature is attributed to permanent opening with the Arabian Sea and the inputs of significant quantities of organic matter and nutrients (Srinivas 1999). The study area, the Chitrapuzha River system forms part of the Cochin backwaters, as well as the National Inland waterway No.111. Chitrapuzha River hosts diverse aquatic organisms and many areas have been converted into breeding pools, so as to increase fish production for commercial exploitation. The influence of industrial effluents on the general hydrography of Chitrapuzha River is high, and it deteriorates the quality of water and sediment by loading with large quantities of pollutants which often exceeds the carrying capacity of the aquatic system, causing complete destruction of the biota. Large quantities of sewages and other untreated and partially treated pollutant discharge into the Chitrapuzha River, which causes significant toxic impact on the aquatic environment. Moreover, shipping activities and the discharge and loading of crude oil and its products, and resultant localized oil spills introduces petroleum hydrocarbons into the harbour estuary. Hydrocarbon rich gaseous emissions from industries, road traffic and water transportation, eventually contaminate the inshore water and sediment through dry and wet deposition Therefore, the water and sediment quality of Chitrapuzha River is a matter of concern because of perceived impacts of the chemical pollutants on the ecosystem. Very few reports are available on the distribution of heavy metals and fatty acid biomarkers (Joseph and Jacob 2006; Sanil Kumar and Nair 2015) in surface sediments of Chitrapuzha River, but no scientific studies concerning hydrocarbon contamination, with special reference on source characterization. Therefore, this chapter intends to explore the spatial and seasonal distributions, and the possible sources of AHCs in surface sediments of Chitrapuzha River. The information furnished in the present investigations will be helpful to future studies, as a reference data for formulating remedial environmental conservation strategies of this sensitive ecosystem.

5.2. Results and discussion

Surface sediment samples were collected from 5 locations along the Chitrapuzha River during monsoon (MN), pre-monsoon (PRM) and post-monsoon (POM) seasons. Station 1 (S1) represents the far upstream portion of the river, and is beyond the influence of industrial pollution. Stations 2 (S2), 3 (S3) and 4 (S4) constitutes industrial zones, which are the recipients of discharge outlets of major industrial units. Station 5 (S5) represents the downstream estuarine portion of the study area, and it receives a considerable amount of discharges from apartments on the mainland as well as from the oil tanker berth, Cochin Port and Cochin Shipyard (detailed description of the study area and sampling sites are described in chapter 2). Aliphatic hydrocarbons, extracted from the surface sediments of the Chitrapuzha River (Harvey 1994), indicated the presence of n-alkanes ranging from C12 to C33 (Fig. 5.3 a, b, c, d, e). The average concentrations of individual n-alkanes are given in Table 5.2, and the total concentrations (Σn-alkanes) ranged from 7754 (S5) to 41173 ngg^{-1} (S2). The maximum n-alkane concentration was recorded at station S2 while, minimum values were observed at S5. The isoprenoid alkanes, pristane and phytane ranged from 16 to 698 ngg^{-1} and 29 to 705 ngg^{-1} respectively. Detection of hopanes was carried out by monitoring ions at m/z=191. The identified hopanes (Table 5.3) in sediment samples include 18α-22,29,30-trisnorhopane (Ts), 17α-22,29,30-trisnorhopane (Tm), 17β(H)-22,29,30-trinorhopene (Te), 17α(H)21β(H)-30-norhopane (C29αβ),17β(H)21α(H)-30-norhopane (C29 βα), 17α(H)21β(H)-hopane (C30αβ), 17β(H),21α(H)-hopane (C30βα), 17α(H),21β(H)-homohopane (C31αβ22R), 17α(H),21β(H)-homohopane (C31αβ22S), 17α(H),21β(H)-bishomohopane (C32αβ22R), 17α(H),21β(H)-bishomohopane (C32αβ 22S), gammacerane (G) and A'-Neogammacer-22(30)-ene or Hop-22(29)-ene or diploptene (NG).

Table 5.2. Average concentration of n-alkanes (ngg^{-1}) in the surface sediments of Chitrapuzha (n=27)

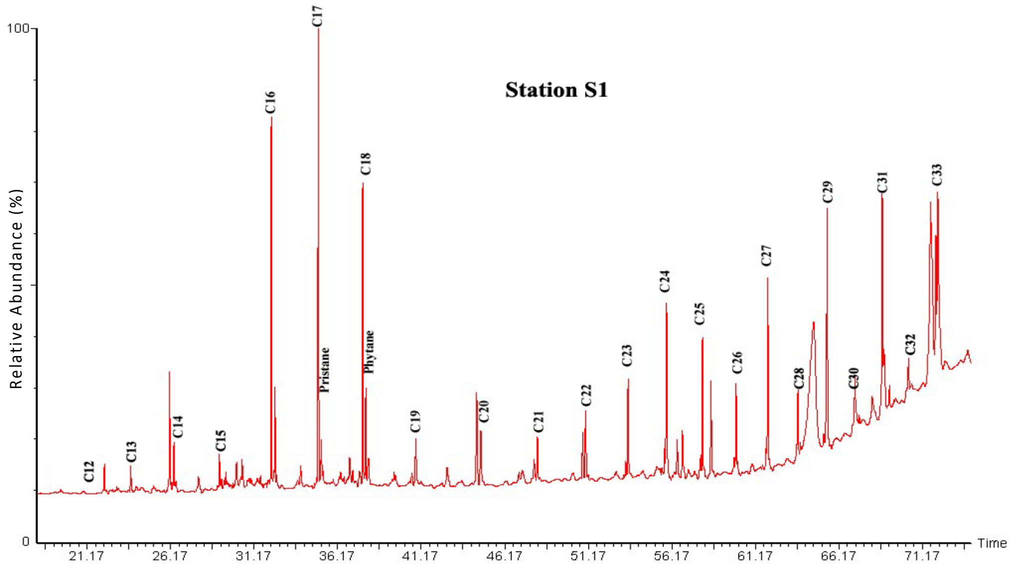
AHCs (ngg^{-1})	Stations				
	S1	S2	S3	S4	S5
C12	588 ± 62	2120 ± 113	1845 ± 128	1268 ± 111	256 ± 7
C13	980 ± 63	1873 ± 113	1697 ± 243	1029 ± 113	355 ± 6
C14	1519 ± 62	2450 ± 109	2313 ± 128	1772 ± 115	267 ±17
C15	64 ± 11	1400 ± 112	742 ± 127	458 ± 112	91 ± 55
C16	1048 ± 60	2777 ± 111	1091 ± 127	1515 ± 113	183 ± 7
C17	58 ± 40	2436 ± 254	1744 ± 128	1531 ± 115	80 ± 6
C18	933 ± 61	1821 ± 112	1832 ± 128	794 ± 115	32 ± 6
C19	43 ± 6	1863 ± 114	1867 ± 128	1432 ± 115	48 ± 8
C20	1388 ± 58	1726 ± 115	1715 ± 127	786 ± 114	294 ± 4
C21	1358 ± 60	1888 ± 113	1489 ± 127	1348 ± 114	167 ± 12
C22	1213 ± 63	2404 ± 264	2148 ± 128	1029 ± 114	298 ± 15
C23	980 ± 62	1388 ± 113	1710 ± 128	938 ± 112	213 ± 18
C24	1011 ± 62	1761 ± 112	1788 ± 130	715 ± 113	407 ± 28
C25	959 ± 61	1453 ± 113	1103 ± 126	786 ± 114	434 ± 14
C26	803 ± 71	1145 ± 116	1164 ± 129	255 ± 31	512 ± 8
C27	659 ± 60	1764 ± 114	1166 ± 129	902 ± 115	645 ± 15
C28	378 ± 108	1204 ± 159	1313 ± 126	496 ± 114	611 ± 16
C29	708 ± 62	2759 ± 112	2554 ± 126	1786±114	703±15
C30	578 ± 63	1329 ± 112	1607 ± 127	521 ± 113	411 ± 13
C31	761 ± 60	3091 ± 121	2962 ± 129	1722 ± 113	857 ± 27
C32	471 ± 22	1320 ± 113	1466 ± 125	850 ± 115	317 ± 23
C33	1200 ± 24	1203 ± 112	1512 ± 128	892 ± 116	573 ± 10
Pristane	698 ± 66	677 ± 68	675 ± 65	358 ± 36	16 ± 6
Phytane	368 ± 32	691 ± 55	705 ± 63	251 ± 22	29 ± 11
∑n-alkane	17702 ± 425	41173 ± 568	36828 ± 510	22825 ± 448	7754 ± 224

∑n- alkane; C12 to C33

Table 5.3 Hopanes and hopenes identified from the surface sediments

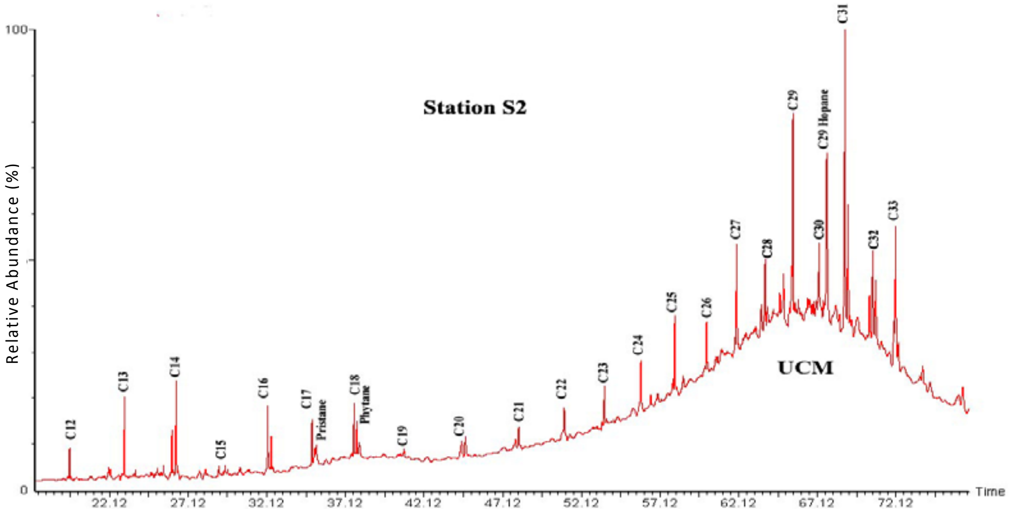
Sl. No.	Hopane	Symbol
1	18α-22,29,30-trisnorhopane	Ts
2	17α-22,29,30-trisnorhopane	Tm
3	17β (H) -22,29,30-trinorhopene	Te
4	17α(H),21β(H)-30-norhopane	C29αβ
5	17β(H),21α(H)-30-norhopane	C29 βα
6	17β(H),21α(H)-hopane	C30βα
7	17α(H),21β(H)-hopane	C30αβ
8	17α(H),21β(H)-homohopane	C31αβ 22R
9	17α(H),21β(H)-homohopane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

The station S1, the far upstream portion of the river, beyond the influence of industrial pollution, exhibited the predominance of n-alkanes C14, C16, C20, C21, C22, C24 and C33. Among the individual n-alkanes detected, C14 recorded maximum concentration (1519 ngg^{-1}) while C19 exhibited minimum value (43 ngg^{-1}). Stations S2, S3 and S4 constitute industrial zones, which are the recipients of effluents from major industrial units, clearly showed the dominance of n-alkane concentration. At station S2, maximum concentrations of Σn-alkanes were reported, with an average value of 41173 ngg^{-1}. The predominance of C12, C14, C16, C17, C22, C29 and C31 was observed, with a maximum concentration of C31 (3091 ngg^{-1}), while, C26 recorded minimum concentration. Noteworthily, substantial contributions of short chain n-alkanes especially C12, C13, C15 and C19 were also reported at this station. Station S3 was characterised by 22 individual n-alkanes with maximum concentration of 36828 ngg^{-1}. The highest concentration of n-alkane was recorded by C31 (2962 ngg^{-1}) while, minimum value exhibited by C15 (742 ngg^{-1}). Both pristane and phytane recorded higher values at this station. Station S4, the downstream portion of the industrial zones, receives a considerable amount of leachates from nearby solid waste treatment plant, recorded with an average concentration of 22825 ngg^{-1}. The n-alkanes C14, C29 and C31 predominated at S4 followed by considerable concentrations of short chain n-alkanes C12, C13, C14, C16, C17 and C19. The lowest concentration of Σn-alkane (7754 ngg^{-1}) was reported at station S5, which is a mixing zone of fresh water from Chitrapuzha River and the saline water from Cochin estuary. Comparatively, lower concentrations of short chain n-alkanes and long chain n-alknes were recorded at this station. Moreover, both pristane (16 ngg^{-1}) and phytane (29 ngg^{-1}) exhibited lower concentrations at this station.

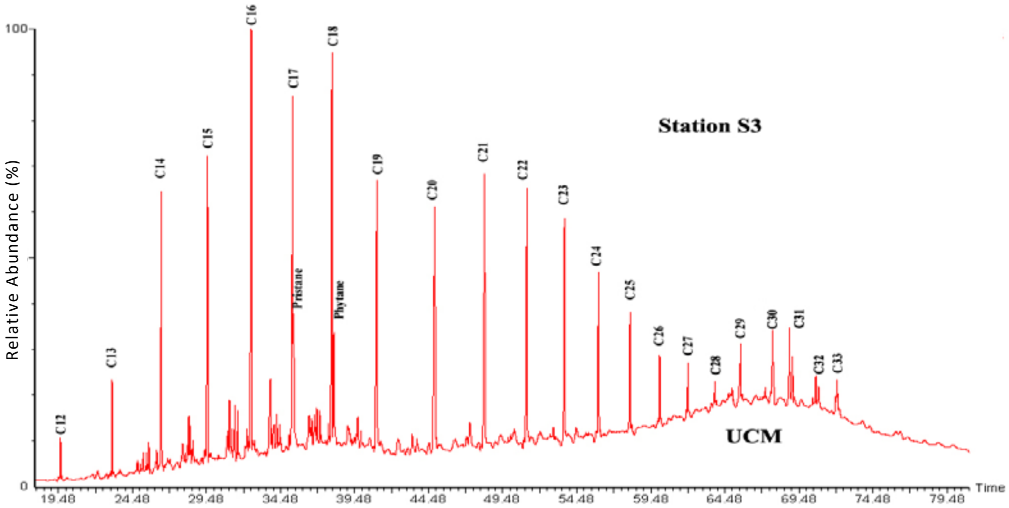


(a)

Fig. 5.3 (a, b, c, d, e) Total ion chromatograms of n-alkane fractions at different stations of the study area (continue...)

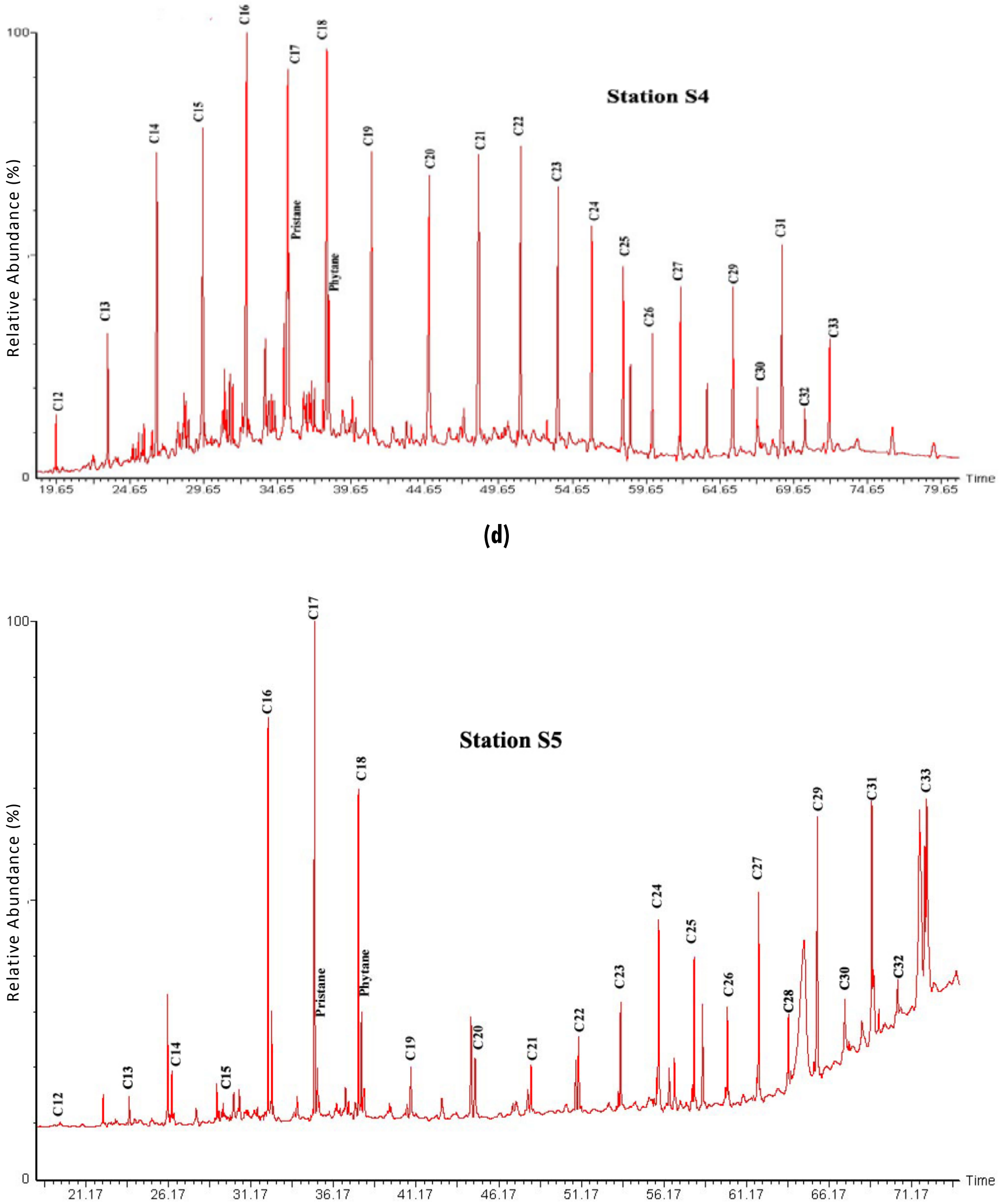


(b)



(c)

Fig. 5.3 (a, b, c, d, e) Total ion chromatograms of n-alkane fractions at different stations of the study area (continue…)



(d)

(e)

Fig. 5.3 (a, b, c, d, e) Total ion chromatograms of n-alkane fractions at different stations of the study area

To distinguish probable sources of hydrocarbons, several n-alkane indices such as carbon preference index (CPI) average chain length (ACL), short chain/long chain ratio (SC/LC), low molecular weight to high molecular weight (LMW/HMW), terrigenous/aquatic ratio (TAR), pristane/phytane (pr/ph), C17/pristane (C17/pr), C18/phytane (C18/ph), and C31 to C19 (C31/C19) were also calculated in addition to absolute concentrations (Table 5.4). The range of C17/pr was from 0.08 to 5.5 while, C18/ph ranged between 1.11 and 3.6. The estimated values of pristane to phytane ratios varied from 0.58 to 1.91. CPI values, both short chains (CPI^a) and long chains (CPI^b) were calculated to distinguish between the biogenic and petrogenic sources of organic matter. The CPI^a results varied from 0.79 to 1.28 while, CPI^b fluctuated between 1.50 and 2.50. The ratio of short chain carbon to long chain carbon (SC/LC) varied from 0.36 to 1.32 and the terrigenous to aquatic ratio (TAR) ranged from 1.29 to 12.56 while, almost consistent ratios of ACL values (29.0) were reported in the surface sediments.

Table 5.4 Alkane isomeric ratios calculated from the surface sediments of Chitrapuzha River (n=27)

Alkane indices	Stations				
	S1	S2	S3	S4	S5
pr/ph	1.91 ± 0.26	0.98 ± 0.3	0.96 ± 0.16	1.51 ± 0.44	0.58 ± 0.37
nC17/pr	0.08 ± 0.03	3.67 ± 0.27	2.61 ± 0.30	4.55 ± 0.46	5.52 ± 0.51
nC18/ph	2.56 ± 0.38	2.66 ± 0.55	2.63 ±0.31	3.6 ± 0.43	1.11 ± 0.21
TAR	12.56 ± 0.84	1. .34 ± 0.28	1.52 ± 0.77	1.29 ± 0.27	10.37 ± 1.05
SC/LC	1.08 ± 0.15	1.24 ± 0.62	1.01 ± 0.06	1.32 ± 0.18	0.36 ± 0.09
ACL	29.27 ± 2.1	29.16 ± 1.9	29.56 ± 2.4	29.34 ± 0.66	29.30 ± 0.53
CPI^a	0.79 ± 0.25	1.03 ± 0.09	1.02 ± 0.05	1.28 ± 0.06	1.16 ± 0.13
CPI^b	1.64 ± 0.16	1.73 ± 0.05	1.50 ± 0.02	2.50 ± 0.18	1.52 ± 0.02
LMW/HMW	0.60 ± 0.01	0.81 ± 0.02	0.68 ± 0.01	0.87 ± 0.02	0.26 ± 0.01
C31/C19	18.1 ± 3.9	1.66 ± 0.04	1.59 ± 0.04	1.2 ± 0.02	18.1 ± 2.3

Pr- pristane; Ph- phytane; TAR- terrigenous/aquatic ratio; SC/LC- short chain/long chain ACL- average chain length; CPI^a - carbon preference index for short chains; CPI^b - carbon preference index for long chains; LMW- low molecular weight; HMW- high molecular weight

5.2.1 Aliphatic hydrocarbons

The distribution profile, total hydrocarbon concentrations (Σn-alkanes) and selected n-alkanes ratios are given in Table 5.2 and Table 5.4. Analysis of variance (ANOVA) registered a significant spatial and seasonal variations ($p<0.01$) of n-alkanes with maximum concentrations near to the industrial areas (S2, S3 and S4) during pre-monsoon season while, minimum value recorded at estuarine station S5 during monsoon (Fig. 5.4). This high average value could be attributed to its proximity to the industrial units, which discharge 80 million litres of effluents per day (Rejomon et al. 2013). Furthermore, the sediment texture has a significant role in the physico-chemical process as well as the species diversity of the depositional environment (Badarudeen et al. 1996; Ramaswamy et al. 2008; Gireeshkumar et al. 2013). Granulometrically, the fine grained (silty-clay) compositions of the surface sediments in the industrial zones (detailed in chapter 3**)** were found to be the dominant factor influencing the hydrocarbon accumulation (Cotano and Villate 2006; Ramaswamy et al. 2008). The lower concentrations of Σn-alkanes in the other two sites with the lowest at S5 are likely due to the larger distance from the industrial units and thus the anthropogenic hydrocarbon sources. Furthermore, the dredging process in this area to facilitate water transportation may further reduce the hydrocarbon level. Dredging is a continuous process necessary to maintain or improve/extend navigable depths in coastal areas. Sediment granulometry showed predominantly silt and clay prior to dredging, which changed to sand with the onset of dredging (Nayar et al. 2004). Finer fractions of sediments get resuspended during a dredging event, and are dispersed spatially as the result of tides and water movements.

Lohrer and Wetz (2003) suggested that these fine-grained sediments (silt - clay) remained suspended in the water column for a long time and contained greater concentrations of soluble regenerated nutrients and contaminants. Once the lighter and finer sediments were resuspended in the water column, the coarser and heavier sandy sediments settled quickly on the bottom substratum (Lohrer and Wetz 2003), explaining the relatively higher levels of sand in the sediments at this station (explained in chapter 3), and thus lower concentrations of hydrocarbons in sediments (Zimmerman et al. 2003), a few months after the commencement of dredging operation. From an environmental perspective, natural processes such as waves and currents are of much less concern than dredging. Actually, pollutants are not necessarily permanently immobilized in dredged sediments, and hydrocarbons remobilization from sediments due to bioturbation and resuspension may result in the loss of sedimentary hydrocarbons. Moreover, dredging results in the removal of bottom sediments which are possibly contaminated can also alter the sedimentation rate as well as the chronological deposition of sediments.

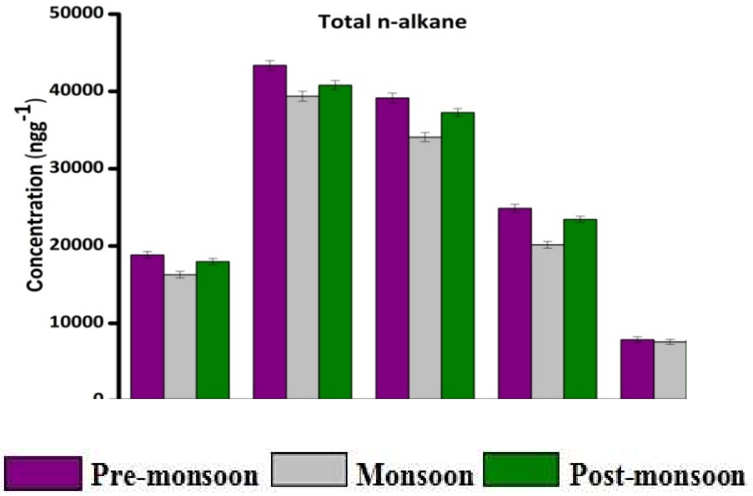


Fig.5.4 Seasonal and spatial variations of n-alkanes in the study area

The n-alkanes distribution profiles in the surface sediments of Chitrapuzha River can be regarded as a combination of two distinct sources i.e., biogenic and petrogenic origin. The results showed an even over odd predominance in the n-alkane homologous at station S1, and it is quite different from compositional patterns of n-alkanes observed in the marine and freshwater sediments. This indicates the deposition conditions and biological sources of n-alkanes, including plankton, a microbial reworking of algal detritus and recent diagnosis of fatty acids and other lipid materials in the surface sediments (Hu et al. 2013). Meanwhile, the higher concentrations of even number carbon chains at the industrial zones (S2, S3 and S4) compared to the other two stations (S1 and S5) can be mainly attributed to the direct biogenic contribution from algae, bacteria, fungi and yeast species (Nishimura and Baker 1986; Grimalt and Albaiges 1987; Zalmon et al. 2013) as well as the direct influence from petroleum-derived inputs (Harji et al. 2008; Hu et al. 2012). There is a strong possibility of direct petroleum inputs causing the n-alkane even predominance in and around the study region, especially at S2, S3 and S4, while the chance for such oil spillage is limited towards the upstream station S1. Thus, even low molecular weight predominance of n-alkanes in sediments can be due to the inputs of both natural and anthropogenic organic matter which have differed in their relative proportions from station S1 to S5 (Ovalle et al. 2013).

The fate of individual hydrocarbon in aquatic environment has usually determined by their physico-chemical properties and inputs from different sources (Tolosa et al. 2004). The short chain *n*-alkanes (C12 to C21) are usually derived from algae and bacteria, while, higher plants are considered as the major inputs of long chain n-alkanes (C22 to C33) (Ficken et al. 2007). Moreover, it has been well established that n-alkanes with different carbon chain length have different biotic sources (De Souza et al. 2011). For example, the predominance of short chain, odd alkanes such as C15, C17 and C19 obtained in the surface sediments, pointing to aquatic sources of organic matter (Jaffe et al. 2001) particularly, C17 is considered to be a marker for algae and photosynthetic bacteria (Meyers 2003). Additionally, cyanobacteria, red, green and brown algae also produce short chain, odd n-alkanes (Clark and Blumer 1967; Gogou et al. 2000). The long chain, odd n-alknes like C25, C27, C29 and C31 indicate terrestrial higher plant debris (Rielley et al. 1991; Pearson and Eglinton 2000; Zhao et al. 2003; Jeng and Huh 2008). While, short chain even number n-alkanes in the range of C12 to C22 originate from diatoms, bacteria, algae, and fungi (Grimalt and Albaigés 1987; Elias et al. 2000). The predominance of even to odd carbon in the short chain homologues was observed in aquatic sediments with various depositional conditions and biological sources, including plankton (Nishimura and Baker 1986; Grimalt and Albaiges 1987; Elias et al. 2000; Volkman et al. 1998; Ekpo et al. 2005; Harji et al. 2008). The microbial reworking of algal detritus and recent biogenesis of fatty acids and other lipid materials (Hu et al. 2013) also contribute to the predominance of even to odd carbon in the short chain homologues. Furthermore, the even n-alkanes predominance is also reported to be produced by the reduction of fatty acids under anoxic environments (Dastillung and Corbet 1978). The strong odd (∑Odd n-alkanes) to even carbon predominance of n-alkanes in the study area except at S1 revealed the prominent terrigeneous contribution from higher vascular plant wax (Aboul-Kassim and Simoneit 1996; Hu et al. 2013). Since, odd number carbon chains tend to dominate in biological materials, whereas petrogenic (fossil carbon) materials have a high prevalence of even numbered carbons (∑ Even n-alkanes). Therefore, a clear indication of strong biogenic and petrogenic sources of n-alkane inputs demonstrated at industrial zone stations S2, S3 and S4.

To distinguish probable sources of hydrocarbons, several n-alkane indices such as CPI, average chain length (ACL), short chain/long chain ratio (SC/LC), terrigenous/aquatic ratio (TAR), UCM, C17/pristane and C18/phytane were also used in addition to absolute concentrations.

5.2.2 Carbon preference index (CPI)

CPI differentiates n-alkane sources, and thus providing supportive evidence for the incorporation of different hydrocarbon sources, and has been utilized as a potential chemotaxonomic tool (Hong et al. 1995; Guo and Fang 2012). The n-alkane distribution exhibits high odd over even predominance for long-chain (C24-C32), which characterizes hydrocarbons from vascular plants versus those in petroleum and bacterial sources (Gearing et al. 1976; Cranwell 1982). CPI for n-alkane is defined as a ratio of the summation of the odd carbon number homologues over a range, to the summation of even carbon number homologues over the same range (Elias et al. 2000; Gao et al. 2007). In organic geochemistry, CPI values are used to indicate the degree of diagenesis of straight-chain geolipids, and are a measure of the original biological chain length specificity (Meyers and Ishiwatari 1993). CPI value 1 or close to 1 indicates the presence of petroleum sources, whereas CPI values ranging from 4 to 10 have been recorded as the terrestrial plant sources (Pearson and Eglinton 2000; Wang and Fingas 2003). However, Johnson and Calder (1973) found that CPI values near one indicate bacterial activity, while vascular plants have values ranged from 3 to 40 (Wang et al. 2006). In the present study, the CPI values were calculated according to Harji et al. (2008) and the CPI^a (full range n-alkanes) and CPI^b (higher chain n-alkanes) were in the range 0.79 to 1.28 and 1.50 to 2.50 respectively (Table 5.4), and the values close to 1 at S2 and S3 indicated significant contributions from oil-derived hydrocarbons, while biogenic sources dominated at S1 (Wang and Fingas

2003). This observation further supports the above conclusion that the n-alkanes in the study area were mostly derived from biogenic and/or petroleum sources with substantial contribution from terrestrial inputs. The spatial and seasonal variations of CPI values are depicted in Fig. 5.5 and it indicated comparatively higher values at S4 during monsoon season.

$$\mathbf{CPI^a = \sum Odd\ C13–C33/ \sum Even\ C12–C32}$$

$$\mathbf{CPI^b = \sum Odd\ C23–C33/ \sum Even\ C24–C32}$$

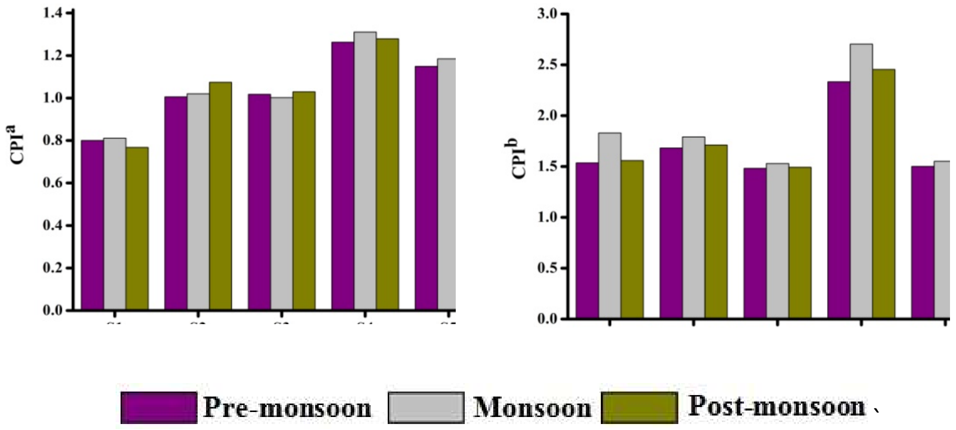


Fig. 5.5 CPI index for full range (CPI^a) and higher chain n-alkanes (CPI^b)

5.2.3. Terrigenous/aquatic ratio (TAR)

The n-alkane index TAR (Bourbonniere and Meyers 1996), is the ratio between the total concentrations of n-alkanes C27+C29+C31 and C15+C17+C19, is used to evaluate the importance of terrigenous verses aquatic inputs. The predominance of C27, C29 and C31 is characteristics of terrestrial higher plant debris (Eglinton and Hamilton 1967; Rielley et al. 1991) while, the prevalence of C15, C17 and C19 indicates aquatic sources (Volkman et al. 1992; Jaffe et al. 2001). Whereas, the total content of n-alkane C15+C17+C19 is a typical marker of marine organic matter (Xing et al. 2011) and usually plankton produces a mixture of hydrocarbons dominated by a short chain, odd carbon C15, C17 and C19 (Goutx and Saliot 1980; Gogou et al. 2000). Therefore, TAR value is used to determine the changes in the relative contributions of organic matter from land and aquatic flora (Meyers 1997). The TAR values were calculated using the equation

$$\mathbf{TAR = C27 + C29 + C31 / C15 + C17 + C19}$$

In the present study, TAR values ranged from 1.3 to 15.8 (Fig. 5.6), and the higher TAR values at S1 and S5 indicated the preferential preservation of terrestrially derived organic matter due to the local dominant fluvial contribution (Rielley et al. 1991). On the other hand, lower TAR values at the industrial stations are possibly due to higher bacterial activity or a petroleum contribution of short chain (<C20) n-alkanes, as suggested by the CPI values. Therefore, contamination of petroleum effluents rich in low molecular weight hydrocarbons (e.g. diesel oil) may influence the lower TAR values at stations S2, S3 and S4.

Moreover, another n- alkane index, the ratio of low molecular weight (≤C20) to high molecular weight hydrocarbons (≥C21) (LMW/HMW), was used to assess anthropogenic and biogenic sources of sedimentary hydrocarbons. According to the literature, LMW to HMW ratios less than 1 usually corresponds to n-alkanes produced by biogenic sources like higher plants, marine animals, and sedimentary bacteria and, LMW/HMW ratios close to 1 suggest n-alkanes that are mainly from petroleum and plankton sources, while values greater than 2 indicated fresh oil in sediments (Wang et al. 2006; Gao et al. 2007). The calculated LMW/HMW values ranged from 0.25 (S5 during pre-monsoon) to 0.88 (S4 during monsoon) (Fig. 5.6) pointed towards the biogenic and petrogenic sources of hydrocarbons in the surface sediments. Furthermore, the sources of hydrocarbons from terrestrial or marine environment can also be calculated by the ratio C31 to C19, based on the assumption that C31 correspond to terrestrial input sources while, C19 represents the marine inputs (Sarkari et al. 2012). The values obtained in the present study ranged from 1.2 to 18.0 (Table 5.4), and the higher values of C31/C19 at the upstream (S1) and downstream stations (S5) exhibited higher terrestrial inputs while, marine inputs dominated at the stations in industrial zone.

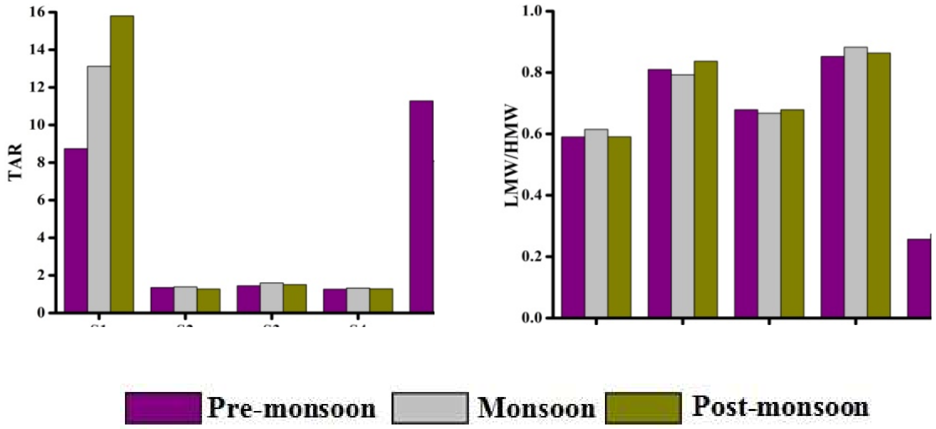


Fig. 5.6 Seasonal and spatial variations of TAR and LMW/HMW values

5.2.4. Unresolved complex mixture (UCM)

The existence of petroleum contamination in the study area was further confirmed by the presence of UCM, a mixture of structurally complex isomers and homologues of branched and cyclic hydrocarbons, that cannot be resolved by capillary columns, usually observed as a ‘hump’ in the chromatogram (Hostettler et al. 1989; Hong et al. 1995) of sediment samples (Fig. 5.3 b, c). Generally, sedimentary hydrocarbons consist of a series of resolved compounds mainly n-alkanes, branched alkanes and unresolved components. UCM indicates the anthropogenic inputs of petroleum hydrocarbons and it is used to distinguish between biogenic (aquatic or terrestrial) and petrogenic sources of hydrocarbons (Bouloubassi et al. 2001). Usually, UCM appeared as a broad bimodal hump in the range of C14 to C36 centered on C18-C19 and C29-C30. This bimodal hump may correspond to light and heavy weathered petroleum residues, respectively (Jacquot et al. 1996). Moreover, UCM magnitude is related to the degree of anthropogenic (petroleum) contribution (Mazurek and Simoneit 1984; Commendatore et al. 2000) and it is shown clearly in the chromatograms of sediment samples from the industrial zone stations S2 and S3, further confirmed the contamination from petroleum sources (Commendatore et al. 2000). In contrast, UCM was absent at upstream station S1 (reference station) and S5 (estuarine station) (Fig.5.3 a,e), indicating the absence of petrogenic inputs, and suggesting a biogenic prevalence of sedimentary hydrocarbons.

5.2.5. Average chain length (ACL)

The average chain length (ACL) is an additional tool for studying the petrogenic hydrocarbons in marine sediments and also to compare the relative amounts of vascular plants and mature fossil hydrocarbons. The distribution of ACL has been related to the geographical distribution of fluvial and eolian inputs and it describes the weight average number of carbon atoms per molecule based on the abundance of the odd-numbered higher plant-derived alkanes (Poynter and Eglinton 1990; Boot et al. 2006). ACL may vary among plant species, signifying that taxonomy exert the major control on n-alkane distributions. For example, leaf lipids derived from grasslands have an average longer chain lengths than do leaf lipids from plants in forests (Eglinton and Hamilton 1967; Cranwell 1982; Seki et al. 2010). It has been suggested that plants produce longer-chain compounds in warmer climates and a positive correlation between ACL in marine sediments and estimated sea-surface temperatures proposes that ACLs could serve as a proxy for continental temperature (Rinna et al. 2002).

$$ACL = 25 \times C25 + 27 \times C27 + 29 \times C29 + 31 \times C31 + 33 \times C33/C25 + C27 + C29 + C31 + C33$$

ACL values obtained in the present study have no significant spatial seasonal variations, and reported to be around 29 (Table 5.4). The ACL could be lowered if there is an addition of petro-genic hydrocarbons to sediments containing biogenic hydrocarbons alone. However, higher TAR values and elevated ACL at S1 and S5, further indicated the preferential preservation of terrestrial derived organic matter over planktonic organic matter in surface sediments (Meyers et al. 1984; Volkman et al. 1987).

5.2.6. Pristane and phytane

The isoprenoid alkanes, pristane (Pr) and phytane (Ph), were detected in the study region, and the values ranged from 16 to 698 ngg^{-1} and 29 to 705 ngg^{-1} respectively (Table 5.2). The geological formation of pristane and phytane strongly depends on the depositional conditions existing in the sedimentary environment. For example, under anoxic conditions, the cleavage of phytyl side chain of chlorophyll-a to yield phytol, which undergoes reduction to give phytane as the final product. While, oxic environment promotes the conversion of phytol to pristane by the oxidation of pytol to phytenic acid followed by decarboxylation and reduction. These hydrocarbons are usually present in petroleum (Readman et al. 2002) and have been used to assess the microbial degradation of petroleum residues in soils and sediments (Wu et al. 2001; Zhu et al. 2005). Therefore, Pr to Ph, C17 to Pr and C18 to Ph ratios have been used to characterize the sources and the degree of deterioration of hydrocarbons in sediments. Phytane is usually absent in the uncontaminated sediments while, pristane can be derived from zooplanktons and some other marine animals (Volkman et al. 1992). The spatial and seasonal variations of pristane and phytane in the study region are depicted in Fig 5.7 (a,b). The highest concentrations of pristane and phytane were recorded in the surface sediment during pre-monsoon season except at estuarine region S5 (Fig. 5.7 a, b), and Pr/Ph value close to 1 at S2 (0.98) and S3 (0.96) implying an anthropogenic inputs of petroleum hydrocarbons (Gao and Chen 2008), likely due to the influence of effluents from the adjacent petroleum refinery. While, Pr/Ph ratio >1, at S1 and S4, indicating the predominance of biogenic sources of hydrocarbons (Steinhauer and Boehm 1992). The relatively low values of C17/Pr at S1 and C18/Ph at S5 (Table 5.4) are likely due to an early stage of biodegradation, and also by the preferential depletion of n-alkanes prior to isoprenoids (Jacquot et al. 1996). However, higher values of C17/Pr and C18/Ph at other stations indicated that microbial degradation processes are generally limited. A comparison of the present investigation with important studies involving n-alkane distribution in various aquatic environments is given in Table 5.5

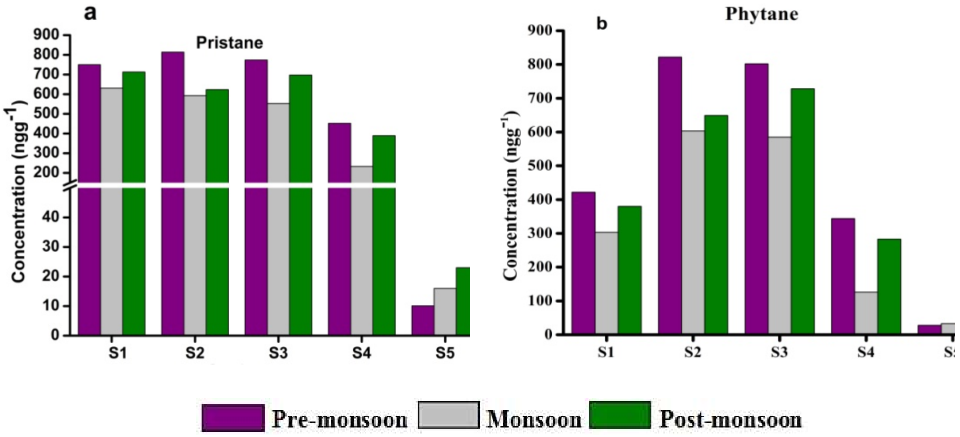


Fig.5.7 Spatial and seasonal variations of (a) Pristane and (b) Phytane

Table 5.5 Comparison of the present study with similar studies in the world

Sl. No.	Study Area	n-Alkane (ng g^{-1})	References
1	Chitrapuzha, South India	7754-41173	Present study
2	Cochin estuary, India	6033-43233	Gireesh kumar et al. 2015
3	Hecate Strait ,Canada	350-1880	Yunker et al. 2014
4	Visakhapatnam Harbour, India	200- 31000	Punyu et al. 2013
5	Mundau-Manguaba estuary, Brazil	2780-13950	Silva et al. 2012
6	Sergipe River, Brazil	9900- 30800	Lima et al. 2012
7	Coastal marine sediments, China	120-1680	Liu et al. 2012
8	Mundau-Manguaba estuary, Brazil	390- 43380	Maioli et al. 2010
9	Hauraki Gulf, New Zealand	326000- 819000	Sikes et al. 2009
10	Mandovi estuary, India	800- 3200	Harji et al. 2008
11	Marmugoa Harbour, India	1600- 10700	Harji et al. 2008
12	Gulf of, France	7800-180000	Mille et al. 2007
13	Pearl River estuary, US	3430- 8460	Gao et al. 2007
14	Jiaozhou Bay, China	540- 8120	Wang et al. 2006
15	Patos lagoon estuary, Brazil	200- 7500	Medeiros et al. 2005
16	Sao Sebastiao, Brazil	30- 4770	Medeiros and Bicego 2004
17	Fraser River Basin, Canada	1600- 20600	Yunker and Macdonald 2003
18	Changijiang estuary, China	2200-11820	Bouloubassi et al. 2001
19	Santos and Cananeia, SP, Brazil	1050- 4290	Nishigima et al. 2001
20	Cretan Sea, Mediterranean Sea	562—5697	Gogou et al. 2000

5.2.7. Hopanoids

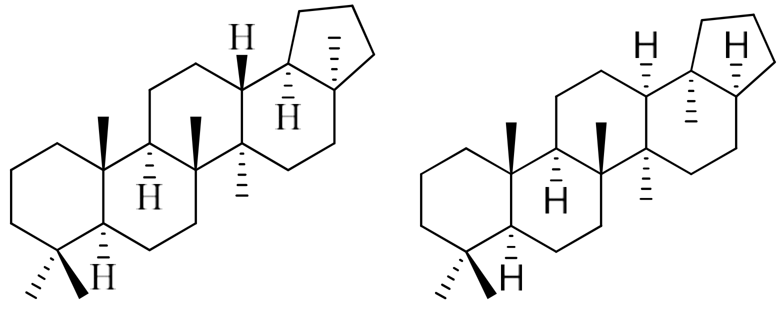
Hopanoids, an important class of specific biological markers, mainly derived from bacteriohopanepolyols, undergoes net reduction to hopanes during diagenesis. They have wide application in geochemical studies of petroleum sources, rocks and oils, and have the potential to provide more convincing evidences for petroleum contamination (Bouloubossi et al. 2001; Hu et al. 2013). Hopanes detected from sedimentary rock records, where their taxonomic and/or physiological signals are conserved over billions of years (Pearson and Rusch 2009). Naturally occurring hopanoids display stereochemistry 17β (H), 21β (H), which is thermodynamically unstable, and hence, the diagenesis and catagenesis can be resulted in the 17α (H), 21β (H) hopanes and 17β (H), 21α (H) moretanes (Ourisson et al. 1987**;** Philp 1985). Among the four possible hopane stereoisomers, namely 17β (H), 21β (H) (living organism), 17α (H), 21α (H) (not present in fossil fuels), 17β (H), 21α (H) (pyrolyzates-also called moretanes), 17α(H),21β(H) (natural fossil fuels), the last two are the most stable ones (Seifert and Moldowan 1980). The surface sediments of Chitrapuzha River recorded the hopane distribution ranging from C26 to C34 and comprised a series of 17α (H), 21β (H) compounds with less prominent 17β (H), 21α (H) hopanes. A total of 13 hopanes were recognized in the surface sediments of Chitrapuzha River (Table 5.6), among this 17α- 22, 29, 30-trisnorhopane (Tm) and 17β (H) 22, 29, 30-trinorhopene (Te), 17α (H), 21β (H)-30-norhopane (29 αβ), 17α (H), 21β (H)-homohopane (C31αβ 22S), 17α (H), 21β (H)-homohopane (C31αβ 22R) and diploptene were widespread. Diploptene, a pentacyclic triterpene identified in the surface sediments, can be related to cyanobacteria and methylotrophic bacteria, Moreover, the erosion of soil-derived bacterial organic matter can be considered as the major source of diploptene into the surface sediments with substantial contribution of methane oxidising bacteria. Fig. 5.8 depicts the structure of different hopanes identified in the surface sediments.

Table 5.6 Hopanes and hopenes identified from the surface sediments

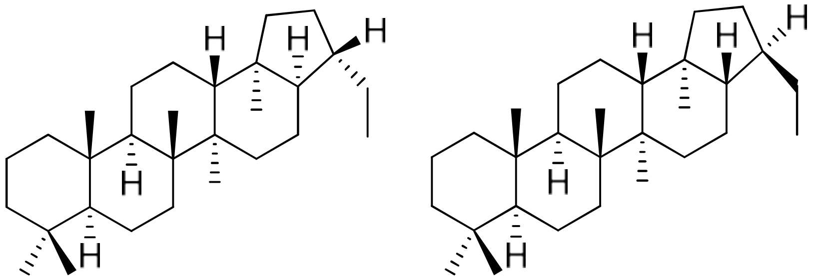
Sl. No	Hopane	Stations				
		S1	S2	S3	S4	S5
1	Ts	*	*	*	*	*
2	Tm	-	*	*	*	*
3	Te	*	*	*	-	*
4	C29αβ	*	*	*	*	*
5	C29βα	*	*	*	*	*
6	C30βα	-	*	*	*	-
7	C30αβ	-	*	*	*	*
8	C31αβ 22R	*	*	-	*	-
9	C31αβ 22S	*	*	*	*	*
10	C32αβ 22R	*	-	*	*	-
11	C32αβ 22S	*	*	*	*	*
12	G	-	-	*	*	*
13	NG	*	-	*	*	-

*detected, - not detected

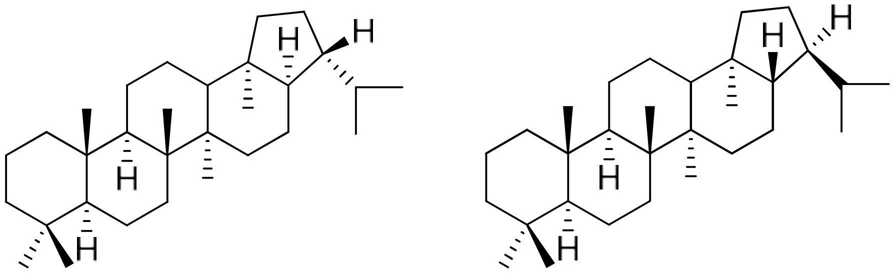
Ts-18α 22, 29, 30-trisnorhopane, Tm- 17α 22, 29, 30-trisnorhopane, Te- 17β (H) -22, 29, 30-trinorhopene C29αβ- 17α (H), 21β (H)-30-norhopane, C29 βα- 17β (H), 21α (H)-30-norhopane, C30βα- 17β (H), 21α (H)-hopane, C30αβ- 17α (H), 21β (H)-hopane, C31αβ 22R- 17α (H), 21β (H)-homohopane, C31αβ 22S-17α (H),21β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



18α (H)-22, 29, 30- Trisnorhopane (Ts) **17α (H)-22, 29, 30-Trisnorhopane (Tm)**



17α (H), 21β (H)-30- Norhopane (29αβ) **17β (H), 21 α (H)-30-Norhopane (29βα)**



17α (H), 21β (H) - hopane (30αβ) **17β (H), 21α (H) - hopane (30βα)**

Fig. 5.8 Structure of hopanes identified in the study area

Gammacerane, initially thought to be an indicator of hypersalinity, is now recognised to be resulting from tetrahymanol (Fig. 5.9), which is formed in abundance under the conditions of high bacterial productivity within stratified water columns (Damste et al. 1999). It is distinguished as an indicator for crude oil derived from lacustrine sources (Peters et al. 2005), and its presence in the surface sediments of study area confirmed the biogenic and petrogenic sources of hydrocarbons. Further, detailed investigation has to be conducted to recognize the extent of petroleum contamination in this area.

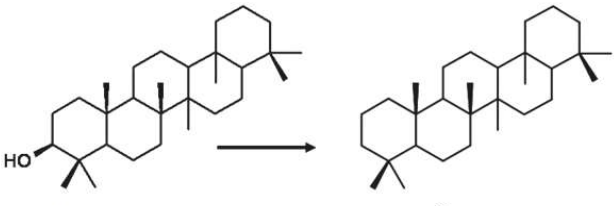


Fig. 5.9 Conversion of tetrahymanol to gammacerane

5.3 Statistical analysis

The statistical significance of the observed spatial variations of n-alkanes in surface sediments was checked using Analysis of variance (ANOVA-two way without replication). Pearson correlation analysis and factor analysis (multivariate tools) were done using 'Statistical Package for Social Sciences (SPSS), version-13 to identify the source of organic matter as well as the process governing their distribution. Factor loading was considered significant if they were >0.50. In factor analysis, principal component analysis (PCA) and varimax rotation methods were used for extraction and deriving principal components (PC) respectively.

5.3.1 Analysis of variance (ANOVA)

ANOVA registered significant spatial variations ($p< <0.01$) of n-alkanes with maximum concentrations (Σn-alkane) near to the industrial areas (S2, S3 and S4).

5.3.2 Pearson correlation analysis

Correlation analysis (Table 5.7) revealed that most of the n-alkanes exhibited a positive correlation with silt and negative correlation with sand signifying the fact that the distributions of n-alkanes are mainly controlled by fine grained fractions. The negative loading of sand content is attributed to the lack of accumulation of organic matter in this sedimentary portion.

Table 5.7 Correlation analysis of n-alkanes (n=27)

	Sand	Clay	Silt	TOC	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33
nd	1																									
ay	0.91*	1																								
ilt	-0.99**	-0.94*	1																							
OC	-0.99**	-0.94*	0.99**	1																						
12	-0.80	-0.94*	0.86	0.83	1																					
13	-0.64	-0.87	0.72	0.68	0.96**	1																				
14	-0.69	-0.92*	0.76	0.74	0.93*	0.96**	1																			
15	-0.69	-0.83	0.76	0.73	0.94*	0.88*	0.81	1																		
16	-0.59	-0.80	0.65	0.65	0.82	0.79	0.82	0.88*	1																	
17	-0.89*	-0.95*	0.93*	0.91*	0.97**	0.86	0.85	0.94*	0.82	1																
18	-0.57	-0.83	0.66	0.61	0.92*	0.99**	0.95**	0.82	0.73	0.80	1															
19	-0.92	-0.95	0.96	0.93*	0.96**	0.86	0.85	0.88*	0.72	0.98**	0.81	1														
20	-0.35	-0.68	0.45	0.40	0.79	0.92*	0.90**	0.68	0.67	0.62	0.95*	0.62	1													
21	-0.58	-0.86	0.65	0.65	0.85	0.90*	0.97**	0.75	0.87	0.76	0.89*	0.73	0.89**	1												
22	-0.56	-0.81	0.65	0.60	0.91*	0.98**	0.94**	0.80	0.70	0.78	0.99**	0.80	0.96**	0.88*	1											
23	-0.61	-0.84	0.69	0.65	0.88*	0.95**	0.95**	0.72	0.64	0.76	0.97	0.81	0.93**	0.88*	0.98**	1										
24	-0.48	-0.74	0.58	0.52	0.89*	0.97**	0.89**	0.81	0.69	0.75	0.98**	0.76	0.95**	0.82	0.98**	0.94*	1									
25	-0.47	-0.78	0.57	0.54	0.88*	0.95*	0.92**	0.86	0.89*	0.78	0.93*	0.72	0.93**	0.93*	0.92*	0.86	0.92*	1								
26	-0.09	-0.39	0.20	0.12	0.64	0.78	0.63	0.62	0.45	0.45	0.82	0.46	0.86	0.57	0.83	0.75	0.90*	0.77	1							
27	-0.63	-0.80	0.70	0.63	0.92*	0.88*	0.80	0.99**	0.90*	0.91*	0.82	0.84	0.71	0.76	0.80	0.72	0.83	0.89*	0.66	1						
28	-0.50	-0.65	0.59	0.51	0.85	0.87	0.72	0.83	0.55	0.76	0.87	0.78	0.75	0.58	0.86	0.8	0.91	0.76	0.86	0.83	1					
29	-0.83	-0.92*	0.89*	0.85	0.98**	0.92*	0.87	0.94*	0.76	0.97**	0.88*	0.98**	0.71	0.76	0.86	0.84	0.85	0.81	0.60	0.91*	0.87	1	.			
30	-0.54	-0.72	0.63	0.55	0.88*	0.92*	0.81	0.79	0.55	0.77	0.93*	0.81	0.84	0.68	0.94*	0.90*	0.96**	0.80	0.87	0.79	0.97**	0.88*	1			
31	-0.76	-0.8	0.83	0.78	0.97**	0.92*	0.84	0.93*	0.72	0.94*	0.89*	0.95*	0.73	0.72	0.88*	0.84	0.88*	0.81	0.68	0.91*	0.93*	0.99**	0.92	1		
32	-0.79	-0.90*	0.85	0.81	0.97**	0.94*	0.89**	0.88*	0.68	0.93*	0.92*	0.96**	0.77	0.77	0.92*	0.91*	0.90*	0.81	0.67	0.85	0.90*	0.98**	0.93	0.98**	1	

Correlation significant at 0.01 level is given in ** and significant at 0.05 level is given in *.

The highly significant correlation of LMW n-alkanes and even mid chain n-alkanes reveals the biochemical degradation pathway operating in the surface sediments. Correlation between n-alkanes in the range C12 to C26, indicating the biogenic contribution of organic matter and microbial derived organic matter as the major source. Moreover, from the interrelationships between individual n-alkanes, it could be inferred that they were originated from the same source. A relatively good correlation existed among most of the low molecular weight n-alkanes with high molecular weight n-alkanes indicated that n-alkanes in the study area had presumably undergone similar environmental processes or transport mechanism and could be related to both biogenic and petrogenic orgin (Nishimura and Baker 1986; Ekpo et al. 2005).

5.3.3 Principal component analysis

Principal component analysis (PCA), a multivariate statistical approach, widely used to distinguish natural and anthropogenic inputs, was applied to explore the origin and geochemical factors influencing n-alkane distribution in sediments. Varimax rotated factor analysis was carried out in the present study and the SPSS (version- 13) computer software was used. The Varimax rotation gives the correlation between the variables and the factors (Table 5.8). Factor loading was considered as significant, if they were >0.50.

Table 5.8 PCA analysis of n-alkanes

Variables	Component		
	1	2	3
Sand	-0.96	-0.09	-0.17
Clay	-0.80	-0.27	-0.51
Silt	0.94	0.18	0.23
TOC	0.95	0.09	0.25
C12	0.70	0.54	0.44
C13	0.49	0.64	0.58
C14	0.53	0.44	0.71
C15	0.67	0.52	0.36
C16	0.55	0.20	0.67
C17	0.85	0.38	0.33
C18	0.41	0.68	0.59
C19	0.85	0.43	0.27
C20	.015	0.65	0.73
C21	0.42	0.31	0.85
C22	0.38	0.70	0.58
C23	0.41	0.62	0.60
C24	0.32	0.78	0.52
C25	0.35	0.54	0.72
C26	-0.04	0.92	0.36
C27	0.61	0.54	0.39
C28	0.42	0.88	0.15
C29	0.76	0.56	0.30
C30	0.41	0.85	0.29
C31	0.69	0.66	0.25
C32	0.68	0.64	0.32
C33	0.20	0.66	0.63
% variance	81.53 %	10.81%	4.00%

PCA was employed on data sets including n-alkanes, TOC and sediment grain size components. PCA indicated three principal components (PC1, PC2 and PC3) accounting for 81.53%, 10.81% and 4.0% (Rotated component matrix given in Table 5.8) of the total variance respectively. PCA results focus on the first two principal components, which explained 92% of total cumulative variance. PC1 displayed a high positive loading on the variables of n-alkanes C12, C15, C16, C17, C19, C29, C31 and C32, TOC and fine grained sediments (silt) indicating the terrigenous signature and geochemical accumulation of organic matter with respect to the grain size. PC1 was characterized by high loadings of odd number n-alkanes including C15, C17, C19, C27, C29 and C31. In general, odd number carbon chains tend to dominate in biological materials, whereas petrogenic (fossil carbon) materials have a high prevalence of even numbered carbons. Therefore, PC1 is considered as a biogenic component. PC2 is distinguished by highly significant loading for n-alkanes in the range C18 to C33 except C19 and C21, indicating the contribution of biogenic organic matter and petrogenic derived organic matter as the main components. The predominance of odd numbered n-alkanes with C27 and C31 is a characteristic of terrestrial higher plant debris (Rielley et al. 1991) while, C15 and C17 indicate aquatic sources (Volkman et al. 1992; Jaffe et al. 2001). Meanwhile, significant loading of n-alkanes with even carbon predominance (C12 to C22) in the sediments was derived mainly from the biogenic contribution of algae, bacteria and fungi (Nishimura and Baker 1986; Grimalt and Albaiges 1987), and also from petroleum-derived inputs (Ekpo et al. 2005; Harji et al. 2008). Consequently, PC2 was defined as a biogenic and petrogenic component. PC3 accounted only 4.0% of the total variance and recorded positive loadings of n-alkanes C14, C16, C20, C21 and C25. Thus, PCA analysis clearly established the biogenic and petrogenic contributions of organic matter in the surface sediments.

5.4 Conclusions

The distribution and sources of organic matter in surface sediments of Chitrapuzha River were investigated for the first time using n-akane markers. The predominance of short chain and long chain odd carbon n-alkanes in the study region indicates, autochthonous and allochthonous inputs of organic matter. Among the individual n-alkanes detected, C31 exhibited maximum concentration, while, C18 recorded minimum value. A significant spatial and seasonal variation of n-alkanes was observed with maximum concentrations near to the industrial areas (S2, S3 and S4) during pre-monsoon, while minimum value recorded at S5 during monsoon. This high average value could be attributed to its close proximity to the industrial units, which discharge 80 million litres of effluents per day. Furthermore, the sediment texture has a significant role on the physico-chemical process as well as the species diversity of the depositional environment. The higher TAR values and elevated ACL at the upstream and the downstream portion of study area revealed the preferential preservation of terrestrial derived organic matter over planktonic organic matter. The biochemical degradation pathway operating in surface sediments causes substantial alterations to the planktonic organic matter in surface sediments. Meanwhile, the higher concentrations of even number carbon chains at the industrial zones compared to other two stations (S1 and S5) can be mainly attributed to the direct biogenic contribution from algae, bacteria, fungi and yeast species as well as the direct influence from petroleum-derived inputs. The calculated n-alkane isomeric ratios like CPI, TAR, SC/LC and Pr/Ph indicates, both natural (i.e., biogenic) and anthropogenic (petrogenic) sources of organic matter in surface sediments. The existence of petroleum contamination in the study area was confirmed by the presence of UCM appeared in the sediment samples. CPI values based on short-chain alkanes confirming the significant inputs of organic matter from microorganisms and petroleum sources. This was further confirmed by the presence of hopanes in the surface sediments. Among the hopanes, the 17α (H), 21β (H)-hopanes, that ranged from C27 to C34 were detected. This includes, 17α-22, 29, 30-trisnorhopane (Tm), 17β (H)–22, 29, 30-trinorhopene (Te), 17α (H), 21β (H)-30-norhopane (29 αβ), 17α (H),21β (H)-homohopane (C31αβ 22S), 17α (H),21β (H)-homohopane (C31αβ 22R) and diploptene. The principal component analysis indicated terrestrial, bacterial as well as petroleum inputs as the major source of organic matter in the surface sediments. Thus, n-alkane study was successful to discriminate biogenic and anthropogenic contributions of various organic matter present in the surface sediments of Chitrapuzha River.

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Chapter 6

POLYCYCLIC AROMATIC HYDROCARBONS

Contents

6.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of lipophilic organic pollutants, contain two or more fused aromatic rings, in a linear, angular or cluster arrangement (Montuori and Triassi 2012; Li et al 2015). Naphthalene is the simplest example of a PAH, and it occurs in oil, coal, and tar deposits, and is produced as byproducts of fuel burnings (fossil fuel or biomass). PAHs are potentially carcinogenic, mutagenic, and teratogenic and are widely exist in the environment as a persistent organic pollutant (Juhasz and Naidu 2000; Wilcke 2000; Dugay et al. 2002; Meudec et al. 2006; Man et al. 2013; Havelcova et al. 2014). Rapid industrialization and economic developments in the world inevitably contribute to contamination by various pollutants, including PAHs. But in recent years, man-made sources have substantially increased the flux of many organic pollutants in the marine environment via the atmosphere, surface runoff and direct discharges (Wang et al 2015). Due to their low solubility, biochemical persistence and hydrophobicity (Tao et al. 2006; Sun et al. 2009), PAHs in the aquatic environment rapidly associated with organic and inorganic suspended particles

(Sun et al. 2009), and subsequently get deposited into the sedimentary environment (Barakat et al. 2013). The organic carbon content and sedimentary (soil) texture were estimated to be the most significant parameters controlling the environmental availability of PAHs (Murphy et al. 1990; Weissenfels et al. 1992; Conte et al. 2001). The stable molecular structure of PAHs amplifies their preservation in marine settings, and hence, PAH analysis is a suitable paleo-environmental tool for recreating anthropogenic developments in diverse environments such as lakes, rivers, estuaries, etc. (Benlahcen et al. 1997; Yunker and Mcdonald 2003; Christensen and Bzdusek 2005; Cavalcante et al. 2009; Qiu et al. 2009; Choudhary and Routh 2010). It has been reported that 90% of PAHs are strongly fixed and stored in sediments (Wild and Jones 1995; Aichner et al. 2013) and can be absorbed by plants and potentially transferred into animals and humans via the food chain (Froehner et al. 2011). The distribution of PAHs in the sedimentary systems has attracted the attention of environmental chemists and scientific community for many years. European Union (EU) and U.S. Environmental Protection Agency (USEPA) have listed 16 kinds of PAHs as priority pollutants (Mastral and Callen 2000; Barakat et al. 2013) and have divided them into carcinogenic and non-carcinogenic groups.

6.1.1 Source, transport and fate of PAHs in aquatic environments

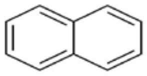
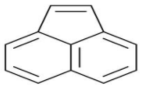
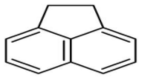
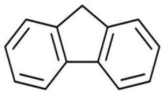
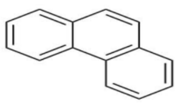
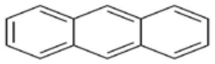
Polycyclic aromatic hydrocarbons are global environmental contaminants, and they have been found in a variety of media, including, soil, sediment, water and surface dust etc. (Wang et al. 2011; Xing et al. 2011; Khairy and Lohmann 2013). The main primary source of PAHs in the environment is the diagenetic transformation of organic material into fossil fuels. They are usually produced by the incomplete combustion or pyrolysis of organic materials. Natural crude oil and coal deposits contain significant amounts of PAHs, arising from chemical conversion of natural product molecules, such as steroids, to aromatic hydrocarbons. However, various anthropogenic activities including industrial activities, such as tar and coal gas processing activities, oil refinery operations and incomplete combustion of fossil fuels etc. will increases the load of PAHs in the aquatic environment (Mastral and Callen 2000). Consequently, soils at the industrial sites (e.g., petroleum fields, petrochemical plants, and natural oil gas plants) had become a great pool for depositing PAHs and other organic pollutants (Wild and Jones 1995; Khodadoust et al. 2000; Viglianti et al. 2006). PAHs have low water solubilities, high thermal stabilities, and high octanol-water distribution coefficients. Some studies have reported that PAHs are concentrated in larger soil particles (Wang et al. 2001; Ahrens and Depree 2004; Oen et al. 2006; Yang et al. 2008; Araghi et al. 2014). However, Maruya et al. (1996) have found PAHs concentrated in smaller soil particles such as clay and silt. The adsorption of PAHs onto soil particles could reduce their mobility and bioavailability, thus slowing down their biodegradation, transport and transformation. In air, PAHs are present in the gaseous phase or sorbed to particulates. PAHs having two to three rings are predominantly found in the vapour phase, those with four rings exist both in the vapour and particulate phase, and those with five or more rings exist predominantly in the particle phase (Jones et al. 1992; Qin et al. 2013). In surface water, PAHs can undergo volatilization, photolysis, oxidation, biodegradation, bind to suspended particles or sediments (adsorption) or accumulate in aquatic organisms (with bioconcentration factors often in the 10-10,000 range) (ATSDR 1995; Sigman et al. 1998).

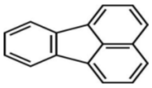
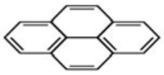
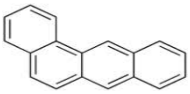
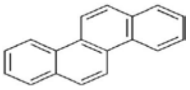
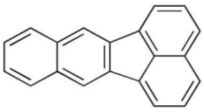
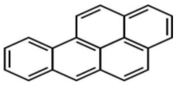
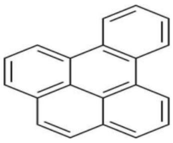
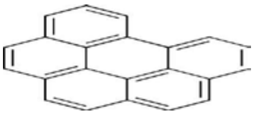
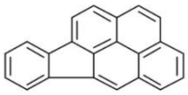
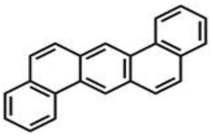
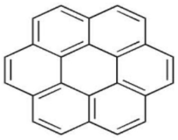
The diverse sources of PAHs can also be differentiated by the composition of the hydrocarbon assemblages. Different sources of PAHs (pyrolytic, petroleum and diagenetic hydrocarbons) give rise to different characteristic PAH patterns, therefore, it is possible to estimate the major processes that generate these compounds (Soclo et al. 2000). For example, petroleum is a complex mixture of many thousands of organic compounds, of which hydrocarbons predominate, and is characterized by a high abundance of alkylated PAHs. The composition of crude oil from different sources varies widely depending on factors such as, source materials, conditions of pressures and temperatures during formation, the structure and chemical composition of source rocks and conditions in the reservoirs. Characteristically, crude oils contain significant concentrations of alkyl-substituted naphthalenes (Mono- and di-methyl) and phenanthrenes (mono-and di-methyl). Similarly, coal comprises of complex macromolecular materials of variable compositions, and the pyrolysis of coal produces a large number of complex PAHs and heteroaromatic compounds. However, it has been identified that the primary products of coal liquefaction are PAHs, (Later et al. 1986) including 3, 4 and 5-ring structures, such as phenanthrenes, f1uorenes and benzo[a]pyrene. In addition to this, the yield and molecular weight distribution of PAHs formed by pyrolysis depend on several factors in which temperature has a significant influence. In general, high pyrolysis temperature favours the formation of unsubstituted over alkyl-substituted PAHs (Youngblood and Blumer 1975; Blumer 1976). As pyrolysis temperature decreases, the ratio of alkyl-substituted to unsubstituted PAHs increases. As a result, crude oils have a predominance of alkyl substituted over the parent PAHs. Consequently, to a certain extent, it is possible to infer the sources of PAHs assemblages of the distribution of alkyl substituted homologues, particularly differentiating between freshly spilled petroleum or crude oil and incomplete combustion sources. Finally, very few PAHs may have a diagenetic origin. For example, the indirect biosynthesis of PAHs by the reduction of polycyclic quinone pigments has been reported (Aizenshtat 1973; Wakeham et al. 1980). Most frequently detected PAH, perylene can be derived from a biogenic precursor via short-term diagenetic processes. Moreover, it has been hypothesized that, perylene is formed in anaerobic sediments through reduction of perylene quinones of biological origin (Orr and Grady 1967; Aizenshtat 1973). Reducing sediments, which receives significant amounts of terrestrial organic matter, contain relatively high amounts of perylene. Perylene could also be derived from aquatic material or diatoms, and during combustion processes (Venkatesan 1988).

6.1.2 Major PAH compounds

Although, the health effects of individual PAHs are not exactly alike, the following 18 PAHs (Table 6.1) are considered as a group in this profile issued by the Agency for Toxic Substances and Disease Registry (ATSDR).

Table 6.1 Major PAHs with their molecular weights and structural identity

IUPAC Nomenclature	Molecular Weight	Structural Identity
Naphthalene (Nap)	128.29	
Acenaphthylene (Acy)	152.21	
Acenaphthene (Ace)	154.21	
Fluorene (Fl)	166.23	
Phenanthrene (Ph)	178.24	
Anthracene (An)	178.24	

Fluoranthene (Flu)	202.26	
Pyrene (Py)	202.26	
Benzo(a)anthracene (BaA)	228.30	
Chrysene (Chr)	228.30	
Benzo(b)fluoranthene (BbF)	252.32	
Benzo(k)fluoranthene (BkF)	252.32	
Benzo(a)pyrene (BaP)	252.32	
Benzo(e)pyrene (BeP)	252.32	
Benzo(ghi)perylene (BgP)	276.34	
Inden(1,2,3cd)pyrene (InP)	276.34	
Dibenzo(ah)anthracene (DbA)	278.36	
Coronene (Co)	300.36	

IUPAC- International Union of Pure and Applied Chemistry

Polycyclic aromatic hydrocarbons are commonly classified into one of two groups based on their molecular structures. Low molecular weight (LMW) hydrocarbons have three or fewer aromatic rings, while high molecular weight (HMW) PAHs have four or more rings (Law et al. 2002). LMW PAHs include naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), anthracene (An), phenanthrene (Ph) and fluorene (Fl) while, HMW PAHs comprise four to seven rings such as, fluoranthene (Flu), pyrene (Py), benzo(a)anthrecene (BaA), chrysene (Chr), benzo(a)pyrene (BaP), benzo(e)pyrene (BeP), benzo (b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3 cd)pyrene (InP), benzo(ghi)perylene (BgP), dibenzo(ah)anthracene (DbA) and coronene (Co). The differences in the structure and size of individual PAHs result in substantial variations in the physical and chemical properties of these substances. Moreover, the properties and the environmental fate of PAHs depend on the number of rings and molecular weight. For example, LMW PAHs are less persistent, highly volatile, slightly soluble in water and less carcinogenic, but toxic to fish and other aquatic organisms, since they accumulate in their tissues (bioaccumulation), and are able to move up in the food chain (biomagnification) and adversely affect humans upon consumption (Law et al. 2002). While, HMW PAHs persist less volatile and more resistant to oxidation and are more insoluble, when alkyl substituent groups are attached to one or more rings. The substitution of an alkyl or chlorine group to PAHs changes the fate and characteristics, making them more reactive and potentially more toxic than the parent PAHs (Irwin 1997).

6.1.3 Toxicity of PAHs

Polycyclic aromatic hydrocarbons are known to readily accumulate in bottom sediments, and could be released to the water column from natural (e.g., bioturbation) and anthropogenic processes (e.g., dredging), resulting in potential adverse health effects to aquatic organisms, including coral reefs (Daskalakis and Oconnor 1995; Long et al.1995; Argese et al. 1997; Ross and De Lorenzo 1997). Some of the adverse health effects associated with these contaminants are toxic to the kidney, nervous and reproductive systems, as well as endocrine disruption and mutations (Nirmala et al. 1999; Ketata et al. 2007; Liu et al. 2008; Brar et al. 2010). In addition, PAHs are known to bioaccumulation in edible aquatic organisms (e.g., fish), thus, represent a health risk to top predators, including humans (Fox et al. 1991; Renzoni et al. 1998; Huang et al. 2006; Díez et al. 2009). They are generally well absorbed in the body, highly soluble in fats and may bind irreversibly to lipophilic sites in the cell, disrupting cellular processes, or metabolites and causing chronic adverse effects. For example, LMW PAHs such as, naphthalene, anthracene and phenanthrene usually cause acute toxicity while, HMW PAHs tend to cause chronic toxicity, attributed to their lower aqueous solubility and slower penetration through cell walls (Neff 1979). The ability of PAHs to bind onto biological membranes interferes with vital physiological processes, and thus disrupts neurotransmission, muscle contraction and osmoregulation etc., (Neff 1979; Lotufo 1997; Wirth et al. 1998). In addition, PAHs interferes with the normal activity of membrane associated enzymes (Stegeman and Teal 1973). A number of metabolic processes compete to produce a variety of different metabolites. Phase I reactions add one or more hydroxyl groups to the parent core and phase II reactions attach highly water-soluble groups in the PAH molecule (Neff 1979; Hellou 1996). Phase I reactions are controlled by enzymes epoxide hydrolase and a subset of cytochrome P-450 mixed-function oxidases called aryl hydrocarbon hydroxylase (AHH) (Law et al. 1997; Aas et al. 2000). Major metabolites of PAHs, due to their greater reactivity and solubility are able to cause acute toxicity to aquatic organisms, often relatively more potent than the parent compound (Boese et al.1999; Choi and Oris 2000).

Electrophilic metabolites are primarily attributed with causing chronic toxic effects in the form of mutagenesis, carcinogenesis and teratogenesis, when they bind to cellular macromolecules (Rossi and Anderson 1976; Chandler et al. 1997). Variations in the tumorigenicity of PAH compounds are due to the differences in the location of the metabolic modifications and the activities of the intermediate metabolites formed. Some of the metabolites formed are diol epoxides, and these diol epoxides are in turn converted into carbonium ions (Sigman et al. 1998). Carbonium ions can react with DNA and proteins to form adducts, and induces genotoxic damage. It is these alkylating agents that are thought to be the primary carcinogens, acting as initiators. Initiation is the first step in the development of cancer (Neff 1979). The enzymes required for the conversion of parent PAH compounds into the reactive diol epoxides are found mainly in the liver, but also in the lungs, skin basal cell layer, intestinal mucosa and other tissues. Genotoxic effects of some PAHs have been repeatedly demonstrated both in-vivo tests in rodents and in - vitro tests using mammalian (including human) cell lines, as well as in prokaryotes (Yuan et al. 1999). A genotoxic event is postulated as a required step in the carcinogenicity process and may play a role in some forms of developmental toxicity. Adverse respiratory effects, including acute and subacute inflammation, and fibrosis, have been demonstrated experimentally. With BaP, severe and long-lasting hyperplasia and metaplasia were observed as precancerous lesions and are consistent with the general assertion that one of the main targets of PAH toxicity is the respiratory tract (Vogelbein et al. 1990).

Polycyclic aromatic hydrocarbons, found in the environment are of great concern due to their carcinogenic properties that are harmful to human and ecosystem health (Yunker et al. 1996; Dai et al. 2011;Wang et al 2015). The ability of some PAHs to cause cancer in mammals and/or aquatic animals has long been recognized. The International Agency for Research on Cancer (IARC) has classified several PAHs into probable (2A) or possible (2B) human carcinogens (IARC 1987). Among those PAHs compounds, the seven ones of BaA, Chr, BbF, BkF, BaP, InP and DbA were considered potentially carcinogenic (IARC 1987). Moreover, different epidemiological studies have demonstrated a correlation between PAHs exposure and cancer incidence for various human tissues (Brender et al. 2003; Brody and Rudel 2003). Strong evidence of a carcinogenic effect of PAHs on the skin, bladder and scrotum has been reported (Everall et al. 1978; Boffetta et al. 1997). Workers in several industries with appreciable exposure to PAHs have also been shown to be at risk of lung cancer (IARC 1993; Nadon et al. 1995; Mastrangelo et al. 1996**.** Moreover, studies in the United States have suggested high rates of lung cancer and an increased risk of brain cancer among people living near petrochemical plants (Gottlieb et al. 1982; Olin et al. 1987). Studies conducted in Taiwan have reported an excess rate for liver and lung cancer (Yang et al. 1998; Yang et al. 1999) and an excess of deaths from bone, brain, and bladder cancer in young adults associated with residence near petrochemical industries (Pan et al. 1994). One of the best known PAHs, BaP is classified by the International Agency for Research on Cancer (IARC) as carcinogenic to human health (IARC 2010), and can induce cancer in the epithelial tissues, such as, the skin, lungs, stomach, and digestive tract (Xing et al. 2010). USEPA and others have developed a relative potency estimate approach for PAHs (USEPA 1993). By using this approach, the cancer potency of the other carcinogenic PAHs can be estimated based on their relative potency to benzo(a) pyrene. The toxicity equivalence factors (based on carcinogenicity) calculated for PAHs are given in Table 6.2. Yet, occupational exposure limit for total-PAHs has not been established because of the complexity of PAHs in their chemical composition.

Table 6.2 Carcinogenic action of PAHs (Kanaki 2005)

PAHs	Indicator of carcinogenesis [1]	Total estimation [2]	EPA classification (USEPA 1994) [3]	Toxicity equivalence factor (TEFi; USEPA 1993)
Acy	-	-	D	0.001
An	I	2C	D	0.01
BaA	S	2A	B2	0.1
BbF	S	2B	B2	0.1
BkF	S	2B	B2	0.1
BgP	I	2C	D	0.01
BaP	S	2A	B2	1.0
BeP	I	2C	-	-
Chr	L	2C	B2	0.01
Flu	I	2C	D	0.001
Fl	I	2C	D	0.001
InP	S	2B	B2	0.1
Ph	I	2C	D	0.001
Py	I	2C	D	0.001
DbA	S	2A	B2	5

[1] No adequate data for humans, for animals: I- insufficient data; L- limited data; S-sufficient data;
[2] Carcinogen for humans; 2A- probable carcinogen for humans; 2B- possible carcinogen for humans; 2C- not classified regarding carcinogenicity for humans; [3] D- not classifiable as to human carcinogenicity; B2- probable human carcinogen

Although, the toxic potency of PAHs to the aquatic systems is well established, as it is reported through various studies across the world (Viguri et al. 2002; Oen et al. 2006; Chen and Chen 2011; Barakat et al. 2013; Acquavita et al. 2014), their monitoring in Indian River systems is totally lacking. It is therefore of immediate concern to generate baseline information on the availability, distribution and origin of this class of organic pollutants in Indian River systems, so that appropriate improvement measures can be taken. The study area, the Chitrapuzha River system is subjected to increasing human interferences and it receives a considerable amount of pollutants from industrial units, domestic sewages, fishery industries and the Cochin sea port, which handles large quantities of petroleum products and industrial chemicals. Numerous industrial units, including a diesel power plant, a fertilizer manufacturing unit, an organic chemical factory and a petroleum refinery, are crowded along a small stretch of land by the side of Chitrapuzha River. The influence of industrial effluents on the general hydrography of Chitrapuzha River is high, and it deteriorates the quality of water and sediment by loading with large quantities of pollutants, which often exceeds the carrying capacity of the aquatic system, causing complete destruction of the biota. Moreover, shipping activities and the discharge and loading of crude oil and its products, and resultant localized oil spills introduce petroleum hydrocarbons into the harbour estuary. Hydrocarbon rich gaseous emissions from industries, road traffic and water transportation, eventually contaminate the inshore water and sediment through dry and wet deposition. Therefore, the water and sediment quality of Chitrapuzha River is a matter of concern because of perceived impacts of the chemical pollutants on the ecosystem. Though, references are available on the distribution of heavy metals and nutrient profiles in the Chitrapuzha river system (Jose 1999; Joseph and Chacko 2006), information on the biogeochemistry of organic matter with special emphasis on source characterization are not yet attempted. Therefore, the present study was planned to generate a preliminary data on the concentration of PAHs and their probable sources in the sedimentary environment of Chitrapuzha River. The information furnished in the present investigations will be helpful to future studies, as a reference data for formulating the strategic impetus to the pollution abatement programme of the local government.

6.2 Result and discussion

Surface sediment samples were collected from 5 locations along the Chitrapuzha River during monsoon (MN), pre-monsoon (PRM) and post-monsoon (POM) seasons**.** Station 1 (S1) represents the far upstream portion of the river, and is beyond the influence of industrial pollution. Stations 2 (S2), 3 (S3) and 4 (S4) constitutes industrial zones, which are the recipients of discharge outlets of major industrial units. Station 5 (S5) represents the downstream estuarine portion of the study area, and it receives a considerable amount of discharges from apartments on the mainland as well as from the oil tanker berth, Cochin Port and Cochin Shipyard (detailed description of the study area and sampling sites are described in chapter 2). A total of 14 PAHs (Nap- 2 ring to BgP- 6 ring), including LMW PAHs and HMW PAHs were identified from the surface sediments of Chitrapuzha River. Concentration of PAHs in sediment samples are given in Table 6.3 and the results are given as mean value of triplicate analysis for each composite sample and each sampling site on dry weight basis. The gas chromatogram of PAHs extracted from the surface sediments of the study area are presented in Appendix 1.1. The ΣPAHs refers to the sum of identifying PAHs components in the investigated samples. The ΣPAH values ranged from 4906 ng/g at S1 during monsoon to 33735 ng/g at S4 during pre-monsoon season (Table 6.3). LMW PAHs such as Nap, Ace, Acy, An, Ph and Fl, and HMW PAHs such as Flu, Py, BaA, Chr, BaP, BbF, InP and BgP were identified in the study area. The highest concentrations were obtained from station S4, followed closely by station S5, while, the lowest concentration was obtained from station S1. Relatively higher contamination levels were indicated at the industrial zones (S3 and S4) and estuarine region

(S5). The spatial and seasonal variations of ΣPAHs in the study area are presented in Fig. 6.1, and the total concentration ranged from 4906 (S1) to 31688 ng/g (S4) in monsoon, 5188 (S1) to 33735 (S4) ng/g in pre-monsoon and 5045 (S1) to 32962 (S4) ng/g in post-monsoon seasons.

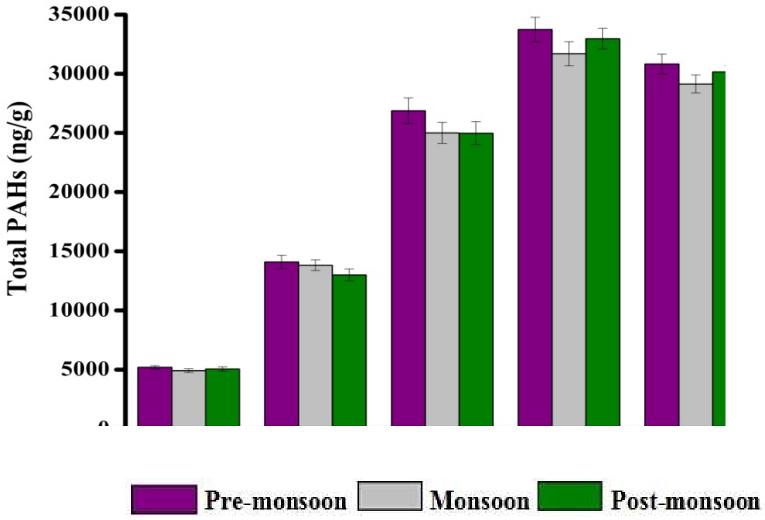


Fig. 6.1 Seasonal and spatial variations of ΣPAHs in the study area

The upstream station S1 recorded comparatively low ΣPAH concentration compared to other stations, with an average value 5046 ng/g. Among the 13 individual PAHs detected, Ace, Acy, Ph, Flu, BaP and BbF were dominated and Flu (818 ng/g) was the most abundant PAH, which contributed 16% of total PAHs followed by Ace (816 ng/g) and BaP (812 ng/g). While, BgP (8 ng/g), Nap (121 ng/g) and An (121 ng/g) exhibited minimum values, with substantial contributions of Ph (614 ng/g) and BbF (710 ng/g). Stations S2, S3 and S4, in the industrial zones revealed the dominance of ΣPAHs concentration. A total of 12 PAHs was detected at S2 with an average concentration of 13637 ng/g dry weights. The predominance of Ace, Acy, Ph, Flu, Py, BbF and BaP was observed with a maximum concentration of BaP (2350 ng/g), which accounted for 17% of total PAHs. The LMW PAHs contributed 48% of total PAHs pool, with a maximum concentration of Ph (1992 ng/g) followed by Acy (1637 ng/g) and Ace (1612 ng/g). Station S3 was characterized by a total of 12 individual PAHs, with an average concentration 25616 ng/g. The highest concentration was recorded by Ph (6497 ng/g) while, the minimum value exhibited by An (454 ng/g). LMW PAHs contributed 54% of total PAHs pool, with substantial contribution of Ace (2084 ng/g) and Fl (2126 ng/g). A total of 14 individual PAHs was identified at station S4, and the highest concentration of ΣPAHs was recorded (32795 ng/g). Among the individual PAHs detected, Ph, BaP and Py were the dominant classes, which constituted 19%, 12% and 9% of total PAHs respectively. LMW PAHs Nap, Ace, Acy, Fl and Ph predominated at S4, followed by considerable concentrations of HMW PAHs including Flu, Pyr, BgP and BaP. The higher-ringed PAH compounds, such as InP and BgP recorded 42 ng/g and 2365 ng/g respectively. The estimated concentration ΣPAHs at station S5 is comparable to the industrial station S4, with an average value of 30035 ng/g. This station is characterized by a high abundance of both LMW PAHs and HMW PAHs including Ace, Fl, Flu, Py, BaA, Chr, BbF, BaP, InP and BgP. LMW PAHs contributed 47% of total PAHs pool, with a maximum concentration of Ph (6252 ng/g) followed by Fl (2508 ng/g) and Nap (2040 ng/g). Among the HMW PAHs, BaP (3462 ng/g) was dominated, followed by Py (3000 ng/g) and Flu (2438 ng/g).

Table 6.3 Concentrations of individual PAHs (ng/g dry wt.) in the surface sediments of Chitrapuzha River (n=9)

ations	Seasons	Nap	Acy	Ace	Fl	An	Ph	Flu	Py	BaA	Chr	BbF	BkF	BaP	InP	BgP	DbA	ΣPAHs
S1	Pre-monsoon	132 ± 26	422 ± 18	828 ± 33	136 ± 19	136 ± 14	628 ± 33	830 ± 42	154 ± 17	194 ± 23	178 ± 14	724 ± 48	ND	826 ± 33	ND	ND	ND	5188.0
	Monsoon	109 ± 19	40 ± 12	804 ± 27	113 ± 21	108 ± 21	598 ± 16	806 ± 37	131 ± 22	170 ± 22	152 ± 18	696 ± 33	ND	799 ±25	ND	24 ± 12	ND	4906.0
	Post-monsoon	121 ± 32	414 ± 43	81 ± 16	125 ± 18	124 ± 31	616 ± 24	818 ± 48	142 ± 16	182 ± 31	165 ± 11	710 ± 61	ND	812 ± 33	ND	ND	ND	5045.0
	Average	120.6	412.6	816.0	124.6	120.6	614.0	818.0	142.3	182.0	165.0	710.0	ND	812.33	ND	8.0	ND	5046.3
S2	Pre-monsoon	493 ± 48	1899 ± 112	1624 ± 120	604 ± 31	279 ± 26	2041 ± 120	1310 ± 65	1422 ± 33	385 ± 26	415 ± 44	1088 ± 121	ND	2536 ± 142	ND	ND	ND	14096.0
	Monsoon	477 ± 36	1812 ± 97	1612 ± 86	592 ± 54	264 ± 19	2024 ± 88	1294 ± 51	1406 ± 18	369 ± 41	391 ± 18	1070 ± 52	ND	2500 ± 98	ND	ND	ND	13811.0
	Post-monsoon	484 ± 22	1200 ± 66	1600 ± 88	600 ± 44	252 ± 31	1912 ± 77	1320 ± 44	1420 ± 26	366 ± 53	384 ± 12	1452 ± 28	ND	2014 ± 67	ND	ND	ND	13004.0
	Average	484.6	1637.0	1612.0	598.6	265.0	1992.3	1308.0	1416.0	373.3	396.6	1203.3	ND	2350.0	ND	ND	ND	13637.0
S3	Pre-monsoon	1612 ± 102	1203 ± 18	2212 ± 52	2132 ± 61	509 ± 27	7012 ± 124	2698 ± 115	2804 ±62	1302 ± 45	1602 ± 102	1369 ± 71	ND	2418 ± 102	ND	ND	ND	26873.0
	Monsoon	1412 ± 72	1264 ± 31	2031 ± 24	2045 ± 41	439 ± 12	6251 ± 154	2173 ± 69	2734 ± 112	1226 ± 78	1518 ± 81	1482 ± 68	ND	2424 ± 80	ND	ND	ND	24999.0
	Post-monsoon	1469 ± 33	1125 ± 26	2010 ± 38	2200 ± 28	414 ± 31	6227 ± 210	2148 ± 43	2710 ± 126	1203 ± 42	1495 ± 22	1650 ± 82	ND	2325 ± 77	ND	ND	ND	24976.0
	Average	1497.6	1197.3	2084.3	2125.6	454.0	6496.6	2339.6	2749.3	1243.6	1538.3	1500.3	ND	2389.0	ND	ND	ND	25616.0
S4	Pre-monsoon	2011 ± 104	1316 ± 55	2205 ± 42	2710 ± 60	483 ± 22	6310 ± 122	2640 ± 71	3014 ± 22	1941 ± 66	2157 ± 102	2206 ± 33	ND	4162 ± 91	125 ± 23	2455 ± 39	ND	33735.0
	Monsoon	1981 ± 66	1284 ± 81	2132 ± 61	2640 ± 71	422 ± 31	6238 ± 105	2710 ± 76	2307 ± 61	1808 ± 81	2095±58	2161 ± 128	ND	3989 ± 79	ND	1921 ± 66	ND	31688.0
	Post-monsoon	1968 ± 82	1305 ± 28	2125 ± 52	2652 ± 43	432 ± 16	6250 ± 126	2450 ± 52	3010 ± 48	1800 ± 53	2100 ± 46	2150 ± 88	ND	4000 ± 58	ND	2720 ± 81	ND	32962.0
	Average	1986.6	1301.6	2154.0	2667.3	445.6	6266.0	2600.0	2777.0	1849.6	2117.3	2172.3	ND	4050.3	41.6	2365.3	ND	32795.0
S5	Pre-monsoon	2122 ± 65	1123 ± 48	2013 ± 106	2503 ± 58	506 ± 23	6322±132	2494 ± 60	3055 ± 116	1803 ± 67	2034±42	978 ± 96	ND	3724 ± 63	221 ± 45	1928 ± 61	ND	30826.0
	Monsoon	1983 ± 71	1071 ± 64	1802 ± 112	2496 ± 88	408 ± 48	6223±103	2397 ± 103	2961 ± 87	1705 ± 72	1992±71	862 ± 73	ND	3012 ± 88	ND	2215 ± 108	ND	29127.0
	Post-monsoon	2015 ± 81	1123 ± 43	1725 ± 124	2525 ± 39	396 0 ±61	6211±76	2422 ± 63	2985 ± 61	1660 ± 55	1964±32	1034 ± 62	ND	3652 ±58	ND	2440 ±71	ND	30152.0
	Average	2040.0	1105.6	1846.6	2508.0	436.6	6252.0	2437.6	3000.3	1722.6	1996.6	958.0	ND	3462.6	73.6	2194.3	ND	30035.0

ND- Not detectable

Table 6.4 Characteristic values of molecular indices for different origins of PAHs (n=27)

Molecular indices	Origin		Stations					
	Pyrolytic	Petrogenic	S1	S2	S3	S4	S5	References
LMW/HMW	<1	>1	0.78 ± 0.18	0.94 ± 0.15	1.18 ± 0.22	0.82 ± 0.14	0.90 ± 0.06	Tam et al. 2001
$\sum$Comb/$\sum$PAHs	<1	>1	0.56 ± 0.02	0.52 ± 0.04	0.46 ± 0.06	0.55 ± 0.04	0.53 ± 0.03	Crnkovic et al. 2007
Ph/An	<10	>10	5.14 ± 0.54	7.53 ± 0.62	14.35 ± 1.1	14.13 ± 0.88	14.07 ± 1.6	Soclo 1986; Baumard et al. 1998
An/ An+Ph	> 0.1	< 0.1	0.16 ± 0.02	0.12± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	Gogou et al.1998; Maioli et al. 2011
Flu/Py	>1	< 1	5.77 ± 0.85	0.89 ± 0.23	0.85 ± 0.31	0.87 ± 0.18	0.81 ± 0.44	Sicre et al. 1987; Baumard et al.1998
Flu/Flu+Ph	>0.1	<0.1	0.57 ± 0.04	0.40 ± 0.04	0.27 ± 0.03	0.29 ± 0.14	0.28 ± 0.06	da Luz et al. 2010
Flu/Flu+Py	>0.5	<0.5	0.85 ± 0.23	0.48 ± 0.08	0.46 ± 0.07	0.45 ± 0.33	0.45 ± 0.29	Budzinski et al. 1997; Gogou et al. 1998
Chr/BaA	<1	>1	0.90 ± 0.04	1.06 ± 0.31	1.24 ± 0.04	1.15 ± 0.64	1.16 ± 0.29	Soclo et al. 2000
InP/BgP+InP	> 0.2	< 0.2	ND	ND	ND	0.05 ± 0.04	0.1 ± 0.04	Yunker et al. 2002

ND- Not detectable

An overview of the results of the concentration levels of PAHs in the surface sediments of the Chitrapuzha River system is summarized in Table 6.3 and in Fig. 6.1, and it indicated the current sediment contaminant status of the study area. Higher concentrations of ΣPAHs were reported in the sediments from the industrial zones (S4), followed by the estuarine region (S5) during pre-monsoon, while the minimum value was recorded at S1 during the monsoon season (Fig. 6.1). The observed ΣPAHs values were 3-6 times higher at the industrial sites and estuarine station, as compared to the rural upstream site S1. This high average value could be attributed to the combined effect of industrial inputs, coupled with the discharges from apartments on the mainland, oil tanker berth, Cochin Port and Cochin Shipyard, close to this area. In general, ΣPAHs levels showed a rising trend from the upstream station (S1) to the downstream (S4) region of the study area, and then a slight decline at estuarine station S5. This is attributed to the biodegradation and desorption of low molecular weight PAHs during transport and deposition from the upper reaches to the lower reaches (Mai et al. 2002), and also by the out welling of organic matter as a result of high flushing (Ratnayake et al. 2005). Moreover, the sandy texture of the sediments observed in this area (chapter 3**)** has a significant role in the physico-chemical process as well as the species diversity of the depositional environment (Badarudeen et al. 1996; Ramaswamy et al. 2008; Gireeshkumar et al. 2013). In addition to this, the dredging operations in this area, to facilitate water transportation to adjacent islands may further reduce the hydrocarbon level. Sediment is a composite material consisting of inorganic components, mineral particulates and organic matter at various stages of decomposition. However, the fate of organic matter to the sedimentary system is challenging due to variable inputs from the adjacent continents, sorption-desorption phenomena (Hedges and Keil 1999) and the various factors controlling organic matter preservation (Wakeham and Canuel 2005). The nature and extent of such forces coincided with biological processes are directly associated with remineralisation of sedimentary organic matter which in turn has significant impact on the distribution of organic matter. Dredging is a continuous process necessary to maintain or improve/extend navigable depths in coastal areas**.** Sediment granulometry revealed that grain size of sediments in the estuary were predominantly silt and clay prior to dredging, which changed to sand with the onset of dredging (Nayar et al. 2004). Finer fractions of sediments get resuspended during a dredging event and are dispersed spatially as a result of tides and water movements. Lohrer and Wetz (2003) suggested that the fine-grained sediments (silt and clay) remained suspended in the water column for a long time and contained greater concentrations of soluble regenerated nutrients and contaminants. Once the lighter and finer sediments were resuspended in the water column, the coarser and heavier sand dominated fractions settled quickly on the bottom substratum (Lohrer and Wetz 2003), explaining the relatively higher levels of sand in the sediments (Zimmerman et al. 2003) and thus lower concentrations of nutrients and organic carbon in sediments after a few months of the commencement of dredging operation. From an environmental perspective, natural processes such as waves and currents are of much less concern than dredging. Actually, pollutants are not necessarily permanently immobilized in dredged sediments, and hydrocarbons remobilization from sediments due to bioturbation and resuspension may result in the loss of sedimentary organic matter resulting lower concentration of PAHs at station S5. Whereas, low levels of PAHs with pyrogenic signature at S1 indicated that the upper reaches of Chitrapuzha River were beyond the influence of industrial (petroleum) pollution, likely due to the larger distance from the industrial units, and thus less anthropogenic hydrocarbon sources. Moreover, the sediment texture characterized by the predominance of sand fraction at S1 (chapter 3), has a significant role in the PAHs diversity of the depositional environment (Ramaswamy et al. 2008). The concentration estimated in the present study exhibited a considerable spatial gradient, and the PAH level follows the order: S1 < S2 < S3 < S5 < S4 (Fig. 6.1). This spatial variability is enhanced mainly because of many localized inputs along the banks of Chitrapuzha River and the higher concentrations of ΣPAHs at S4 and S5 indicated the influence of industrial effluents in the surface sediments, as these sites are located immediately after the discharge outlets of major industrial units, including a diesel power plant, Cochin port and Cochin shipyard.

The seasonal trend of ΣPAHs in the study area was not consistent from pre-monsoon to post-monsoon seasons at different sites (Fig. 6.1). The average concentrations of ΣPAHs at all the sites revealed highest concentrations during pre-monsoon period and lowest values during monsoon, except slight variations at stations S2 and S3. This unique seasonal variation could be attributed to the changes in the discharge pattern of the river as except in the monsoon season, the river is stagnant. In monsoon, the water flow remains maximum, which is as high as up to 80% of the annual flow. The high flow rate of river water and rainfall in monsoon season has a good dilution impact on the river flow. This leads to the decrease in total concentrations of micro pollutants like PAHs during monsoon season (Doong and Lin 2004). Though, various sources collectively contribute to the PAH pollution, during pre-monsoon season, the average water flow rate decreased drastically, and the major sources of organic pollutants are industrial effluents, runoff from garbage dumps, deposed particulate matter and chemicals and, carbonized oil spills from the localized inputs that get its way into the aquatic environment in huge quantity. Moreover, most of the industrial units located on the banks of Chitrapuzha River under annual shutdown and maintenance activities during the month of April and May (pre-monsoon), as a result the industrial runoff heavily contaminated with significant contribution of pollutant load, further enhances the PAH levels in the surface sediments. Understandably, this could be the main reason for higher PAH concentrations at the industrial zones and estuarine region during pre-monsoon season.

A comparison with earlier studies across the world revealed that PAH concentrations reported in the surface sediments of Chitrapuzha River are on the higher side (Table 6. 5). The total PAH concentrations in sediments from the study area are slightly more than the levels found in San Francisco Bay, USA (Pereira et al. 1996) and the Yamuna River, Delhi, India (Agarwal et al. 2006). Although, the concentration levels of PAHs in the present study are two to three orders higher than the levels in many other rivers reported (Table 6.4), they are roughly two orders lower than those detected in the river of Tianjin area, China (Shi et al. 2005).

Table 6.5 PAHs concentrations (ng/g) in sediments at different locations around the world

Sl. No.	Location	ΣPAHs (ng/g)	n*	References
1	Chitrapuzha, South India	4906 - 33735	16	Present study
2	Don River, Russia	14 - 529	19	Sazykin et al. 2015
3	Yangtze Estuary, China	129 - 308	16	Ying et al. 2015
4	Yangpu Bay, China	1583 - 5701	16	Li et al. 2015
5	Songhua River, China	68 - 654	16	Zhao et al. 2014
6	HormuzganPersian Gulf	765 - 1898	15	Mirza et al. 2014
7	Manzala Lake, Egypt	246 - 9910	39	Barakat et al. 2013
8	Monastir Bay, Tunisia	26 - 577	17	Nouira et al. 2013
9	Yangtze estuary, China	77 - 2937	16	Wang et al. 2012
10	Liaodong Bay, China	145 - 292	16	Hu et al. 2011
11	Kaohsiung Harbor, Taiwan	472 - 16201	17	Chen and Chen 2011
12	South Carolina, USA	64 - 26288	16	Weinstein et al. 2010
13	Yellow River Estuary, China	11 - 252	16	Hui et al. 2009
14	James Ross Island, Antarctica	1 - 205	16	Klanovaa et al. 2008
15	Mersey Estuary, U.K	626 - 766	16	Vane et al. 2007
16	Naples harbour, Italy	9 - 31774	16	Sprovieri et al. 2007
17	Marine environment, Korea	9 - 18500	16	Yim et al. 2007
18	Yamuna river, India	4502 - 23527	16	Agarwal et al. 2006
19	Norwegian Harbor, Norway	2000 - 76000	16	Oen et al. 2006
20	Rivers in Tianjin, China	787 - 1943000	16	Shi et al. 2005
21	Gomati River, India	208 - 3365	16	Malik et al. 2004
22	Santander Bay, Northern Spain	20 - 25,800	15	Viguri et al. 2002
23	Izmit Bay, Turkey	2500 - 25000	14	Tolun et al. 2001
24	San Francisco Bay, USA	2653 -2 7680	17	Pereira et al.1996

n*; number of PAHs analyzed in each study

The composition and distribution of PAHs in the environment are influenced by their production sources. The production processes have linked to certain characteristic PAH patterns, namely pyrolytic and petrogenic or diagenetic processes (Soclo et al. 2000). Based on the number of rings in the PAHs, the 14 PAHs identified in the study area can be divided into three groups: (2+3) ring, 4-ring and (5+6) ring components, representing low, medium and high-molecular weight PAHs, respectively (Amoako et al. 2011). A ternary plot showing the PAHs abundance categorized by the number of rings are shown in Fig. 6.2 (2-ring PAHs include Nap; 3-ring PAHs include Acy, Ace, Fl, Ph and An; 4-ring PAHs include Flu, Py, BaA and Chr; 5-ring PAHs include BbF, BkF, BaP and DbA; 6-ring PAHs include InP and BgP). The PAH composition pattern is helpful to track the contaminant sources, and illustrate the fate and transport of PAHs in a multimedia environment, since different sources have distinct PAHs patterns (Gigliotti et al. 2002). For example, anthropogenic PAHs are formed mainly via two mechanisms: incomplete combustion of fossil fuels and discharge of petroleum-related materials. In general, the petroleum-derived residues contain relatively higher concentrations of 2-3 ring PAH compounds such as Nap, Ph and An (Tolosa et al. 1996). Moreover, the composition of PAHs varies depending on the combustion temperature. At low to moderate temperature (Lake et al. 1979), or as from the combustion of coal (Laflamme and Hites 1978), low molecular weight parent PAH compounds are abundant. At high temperature, such as at the vehicle emissions, the high molecular weight parent PAH compounds are dominant (Lee et al. 1977). Therefore, on account of the anthropogenic source, the LMW PAHs have both petrogenic and combustion (low temperature pyrolysis) sources, whereas the high molecular parent PAHs are predominantly pyrogenic in nature (Pies et al. 2008; Wang et al. 2010). However, some studies reported that Fl, Ph, Nap and An are related to the emission of coal and biomass combustion (Dong and Lee 2009; Singh et al. 2013). Contrary to typical individual PAHs composition in sediment of other polluted areas, higher proportion of LMW PAHs (2-4 ring) predominated in the surface sediments of Chitrapuzha River, which reflects the presence of significant petrogenic sources and low-temperature pyrogenic processes (Mai et al. 2002). More than 70% of the total concentration was represented by PAHs with 2-4 rings. Similar observations were reported by Malik et al. (2004) and Shi et al. (2005), with regard to the dominance of 2-4 ring PAHs in sediments from river of Tianjin area, China and Gomti River, India respectively. However, abundance of high molecular weight PAHs in surface sediment samples has been reported by Zhang et al. (2004). Among the LMW PAHs, Ph was the most dominant compound in most of the stations studied, accounting for about 15 to 25% of total PAHs followed by Fl and Ace. Therefore, the predominance of LMW PAHs indicated either a local source of PAHs or a relatively recent introduction of these pollutants into the surface sediments (chemicals), since, small-size PAHs are more labile, and are expected to degrade faster than HMW PAHs (Fernandes et al. 1997; Simo et al. 1997; Macias-Zamora et al. 2002).

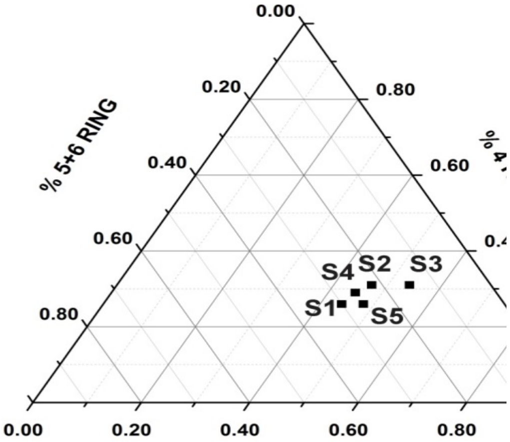


Fig. 6.2 Ternary plot showing PAH compositions in the sediments from the study area

The processes controlling the PAH level in aquatic sediments are complex, especially due to their water solubility, the composition of sediment such as organic carbon and clay contents (Yang et al. 1998), combustion temperature of organic material during PAH formation, photo-oxidation, transport mechanisms, weathering, dilution effect, biodegradation and natural formation. Moreover, identifying the origin of the contaminants in the aquatic environment is complicated by the possible coexistence of several different sources, e.g. various pyrolytic and petrogenic sources and early diagenesis. Therefore, in a situation of chronic contamination, an average distribution pattern can provide indications on sources of the PAHs. Such indicators include various molecular indices based on PAH variability such as the ratio of concentrations of LMW and HMW PAHs. The presence of HMW (4 to 6- rings) and LMW (2 to 3 rings) PAHs have been associated with sources of PAHs related to fuel-combustion (pyrolytic) and crude oil (petrogenic) contamination, respectively (Tam et al. 2001; Lee et al. 2005; Yan et al. 2009). Therefore, a ratio of LMW/HMW >1 suggests a petrogenic origin of pollution; while for a value <1, the PAHs origin is pyrolytic (Yan et al. 2009; Abdollahi et al. 2013). In the present study, the contribution of LMW PAHs ranged from 44 to 54% of the total PAHs in surface sediments, and the LMW/HMW values varied from 0.78 (S1) to 1.18 (S3) (Table 6.4), indicated both pyrogenic and petrogenic sources of hydrocarbons in surface sediments. In contrast, the value slightly greater than 1 at station S3, suggesting a petrogenic source of PAHs. As mentioned earlier, PAHs have significant variation in their composition with regard to different combustion sources, and the characteristic spectrum of PAHs could be used as a fingerprint to identify their sources (Khalili et al. 1995). Usually, the composition of PAHs in urban and recreational soils is characterized with HMW PAHs, and considered as a combustion origin product (Khalili et al. 1995; Budzinski et al. 1997). Therefore, ratio of major combustion-specific PAH compounds (PAHcomb, including Flu, Py, BaA, Chr, BbF, BkF, BaP, Inp and BgP) to the total concentration of 14 EPA- PAHs (ΣComb/ΣPAHs) also indicates that the sources of PAHs are combustion of fossil fuels (Crnkovic et al. 2007). The average ratio of ΣComb/ΣPAHs in the surface sediments was 0.52 ± 0.04 (Table 6.4), further indicated, combustion activities (mostly affected the PAHs in examined soils) also have a greater influences on the distribution profile of PAHs in the study area.

A further attempt to elucidate the source-processes and sources of PAHs in surface sediment was accomplished by examining a selection of molecular indices for PAHs (Baumard et al. 1998; Soclo et al. 2000; Yunker et al. 2002; Huang et al. 2012; He et al. 2014) (Table 6.4). The molecular indices considered for the source characterization of PAHs included, the mean ratios of the concentrations of phenanthrene to anthracene (Ph/An), anthracene to phenanthrene + anthracene (An/An+Ph), fluoranthene to pyrene (Flu/Py,) fluoranthene to fluoranthene + pyrene (Flu/Flu+Py), fluoranthene against fluoranthene + phenanthrene (Flu/Flu+Ph), chrysene to benzo[a]anthracene (chr/BaA), and indeno (1,2,3 cd) pyrene against indeno (1,2,3 cd) pyrene + benzo (ghi) perylene (InP/InP+BgP). These indices of individual PAH compounds are based on peculiarities in PAH composition and distribution pattern as a function of the emission sources (Gschwend and Hites 1981; Colombo et al. 1989), and thus, it is possible to classify the pollutant sources as either petrogenic or pyrolytic origin (Stortini et al. 2009). Molecular indices used for the source characterization and their characteristic values are tabulated in Table 6.4. The ratio between Ph and An was extensively used to infer the nature of PAH pollution in sediments (Gschwend and Hites 1981; Sicre et al. 1987; Budzinski et al. 1997; Soclo et al. 2000; Magi et al. 2002). Ph is thermodynamically more stable than An, and its prevalence over An supports petrogenesis for PAHs in surface sediments. Indeed, petroleum usually exhibits a quite high Ph/Ant ratio. Similarly, Flu is less thermodynamically stable than Py; they are often associated with each other in natural matrices, and a predominance of Flu over Py is characteristic of a pyrolytic process, while in petroleum-derived PAHs, pyrene is more abundant than fluoranthene (Soclo et al. 2000). Generally, a Ph/An ratio <10 and a Flu/Py ratio >1 are characteristics of pyrolytic origin (Qiao et al. 2006), while the values >10 and <1 are representative of petroleum-derived PAHs (Cardellicchio et al. 2007). The calculated values of Ph/An and Flu/Py ratios ranged from 5.14 to 14.35 and 0.81 to 5.8 respectively, and exhibited prominent contributions from the petrogenic origin of PAHs at stations S3, S4 and S5, while, pyrogenic inputs predominant at S1. Sediment samples with Ph/An <10 and Fl/Pyr <1 were characteristic of a mixed pattern of pyrogenic and petrogenic contamination, indicated at S2. In order to provide a good estimate of PAH sources, Budzinski et al. (1997) proposed the use of a plot of combined ratios between Ph/An vs Flu/Py and defined two zones from the plot: one is characteristic of a petrogenic origin and the other, a pyrogenic origin. The cross plot obtained in the present study (Fig. 6.3) proposed a mixed pattern of pyrogenic and petrogenic in nature, with predominance of petrogenic inputs in the surface sediments of Chitrapuzha River.

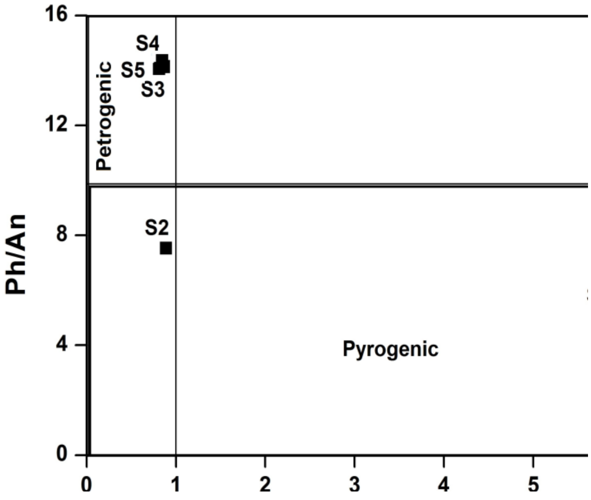


Fig. 6.3 Cross plots of Ph/An ratio against Flu/Py ratio for the surface sediments of the study area

The possible major sources of combustion derived PAH include fossil fuels (gasoline, crude oil and coal) and biomass (wood and grasses) (Singh et al. 2013). Therefore, recent studies showed that Flu/(Flu+Py) ratio less than <0.4 is consistent with petroleum derived PAH input; values between 0.4 to 0.5, mainly from the combustion of petroleum sources, while the ratio higher than 0.5 implies combustion of grass, wood or coal (Liu et al. 2009; Hu et al. 2010). The estimated values of Flu/(Flu+Py) ranged from 0.45 to 0.85, and the values from 0.4 to 0.5 in the industrial zones (S2, S3 and S4) and estuarine region (S5) (Table 6.4) gives an indication of major levels of PAHs possibly derived from the direct input of unburned fossil fuels/petroleum (kerosene, shale oil etc.). While, the ratio greater than 0.50 at S1, established the prevalence of pyrogenic PAHs derived from biomass (wood) combustion. Moreover, the isomeric ratio Flu/Flu+Ph (<0.1 petrogenic and >0.1 pyrogenic) ranged from 0.27 to 0.57 further demonstrated the pyrogenic signature of PAH compounds (da Luz et al. 2010) in the surface sediments.

Further discrimination of different sources of PAHs is achieved using diagnostic ratios such as Chr/BaA, InP/BgP+InP and An/An + Ph, which can identify the possible sources of PAHs (Yunker and Macdonald 2003). The ratio Chr/BaA >1.0 indicated petrogenic sources of hydrocarbons while, the value <1.0 directed to pyrolytic origin (Qiao et al. 2006). The detected values ranged from 0.90 to 1.2, confirming the suspected inputs of petrogenic PAHs in the surface sediments, except at S1. Similarly, the ratio InP/BgP+InP <0.2 point out petroleum origin of PAHs, while, values varied from 0.2 to 0.5 specify liquid fossil fuel combustion and the values >0.5 suggest grass, wood and coal combustion (Yunker et al. 2002). In the present study, the detectable concentrations of InP and BgP were recorded from downstream stations S4 and S5, and the ratios (0.05 ± 0.04 and 0.1 ± 0.04) further confirmed the petroleum contamination. The ratios of Ant/An+Ph >0.1 represented combustion processes, whereas lower values represented petroleum contamination (Maioli et al. 2011). The values above 0.1 at stations S1 and S2, and the values below 0.1 at stations S3, S4 and S5 indicated pyrolytic and petrogenic sources are responsible for the prevailing input of PAHs in the sediments at the respective stations. In summary, the PAHs detected in the study area, predominantly from pyrolytic sources at S1, while, petrogenic inputs are the major sources at all other stations. Although, distinct sources can be inferred from PAH isomer pair ratios, it is well documented that in urban areas PAHs can originate from a variety of sources and be transported by different pathways (Oros and Ross 2004; Qin et al. 2013). The wide ranges of the PAH isomer pair ratios found in river sediments established the fact that the PAH source signature could be altered by biological (e.g., bacterial degradation), chemical (e.g., oxidation and reduction), and physical (e.g., air mass mixing and sediment re-suspension) processes during transport and after deposition into sediments.

6.3 Statistical analysis

6.3.1 Principal component analysis (PCA)

PCA method, which is widely used to distinguish natural and anthropogenic inputs, was applied to explore the origin and geochemical factors influencing PAH distributions in surface sediments. Table 6.6 indicated three principal components (PC1, PC2 and PC3) accounting for 62.68 %, 20.69% and 6.70 % of the total variance, respectively. PCA loading scores higher than 0.50 were considered, and PC1 was characterized by higher loadings of both LMW PAHs and HMW PAHs including Nap, Ace, Fl, Ph, An, Flu, Py, BaA, Chr, BaP and BgP. Moreover, BbF and InP also scored a significant eigen value. In general, the heavier PAHs are the result of combustion or pyrolitic processes and are usually absent in petrogenic sources (Wang et al. 1999). Flu and Py are characteristic markers for pyrolysis or incomplete combustion while, diesel engine emission predominantly contains Ph, Flu and py (Wang et al. 2009). BbF is linked with fossil fuels combustion (Kavouras et al. 2001), while, BaP and InP are associated with traffic emission, whereas, BaA appears to be the main tracer with respect to gasoline (Wang et al. 2009). The presence of a meaningful BgP loading led to interpret this component has been known as a tracer of auto emissions **(**Harrison et al. 1996). Consequently, PC1 was defined as a petrogenic and pyrolitic fuel combustion component. PC2 presented its maximum positive loadings for PAHs such as Ace and Acy in sediments, which are abundant in petrogenic sources, mainly caused by petroleum spills like crude oil and fuel oil **(**Ye et al. 2007), and interpreted as the petrogenic source of PAHs. Thus, PCA analysis clearly established the petrogenic and pyrolytic origin of PAHs in the surface sediments of Chitrapuzha River.

Table 6.6 Varimax-rotated component matrix obtained from PCA

Compound	PC1	PC2	PC3
Nap	**0.991**	0.079	-0.053
Acy	0.319	**0.608**	**0.618**
Ace	**0.823**	**0.520**	0.121
Fl	**0.972**	0.164	-0.099
Ph	**0.928**	0.233	-0.108
An	**0.914**	0.287	0.056
Flu	**0.951**	0.238	-0.063
Py	**0.941**	0.279	0.033
BaA	**0.988**	0.069	-0.084
Chr	**0.982**	0.096	-0.107
BbF	**0.539**	**0.717**	-0.127
BaP	**0.899**	0.233	0.231
InP	**0.520**	-0.395	0.298
BgP	**0.830**	-0.204	-0.005
TOC	0.250	**0.932**	0.109
SAND	-0.256	0.126	**0.854**
CLAY	0.091	-0.965	-0.082
SILT	0.171	**0.960**	0.154
% Variance	62.68	20.69	6.70

6.3.2 Hierarchical cluster analysis (HCA)

HCA is a multivariate technique, whose primary purpose is to identify the homogeneous groups of individual PAHs occurring in the sedimentary system. Hierarchical clustering is the most common approach in which clusters are formed sequentially. It provides intuitive similarity relationships between any one sample and the entire data set, and is typically illustrated by a dendrogram/ tree diagram (Shrestha and Kazama 2007). The most similar objects are grouped first, and these initial groups are merged according to their similarities. The result of HCA is indicated in the hierarchical dendrograms (Fig. 6. 4), which distinguished the 14 individual PAHs into two statistically significant clusters, cluster-1 and cluster-2. The first group included the high-molecular-weight PAHs with four to six rings (Chr, BaA, BgP, BbF and InP) and some three-ring PAHs like Nap, Acy and An. The first major group included three subgroups. The first subgroup contained Nap, BaA, BgP and Chr, all of them, including 4 rings, with the exception of Nap. These PAHs are usually detected in pyrogenic sources, e.g., combustion of coal, wood, vehicle fuel and waste tire (Wang et al. 2009). The second (Acy and BbF) and third subgroups (An and Inp) included the low molecular weight PAHs and HMW PAHs, reveals the influence of anthropogenic and natural activities on sediment contamination (Yunker and Macdonald 2003). The second major group contained Ace, Flu, Fl, Pyr and BaP. The LMW PAHs (Ace and Fl) with three rings were mainly from oil spills, volatilization, or combustion of petroleum while, HMW PAHs such as Flu, Pyr and BaP typically released during combustion of coal, natural gas and vehicle exhaust of gasoline and diesel (Mastral and Callen 2000). From the above statistical data, PAHs in the study area could be attributed to the mixed sources of petroleum combustion and biomass combustion. Moreover, the oil spill, volatilization or combustion of petroleum contributes to the major sources of PAHs in the surface sediments of the industrial zones and estuarine region (Hu et al. 2010).

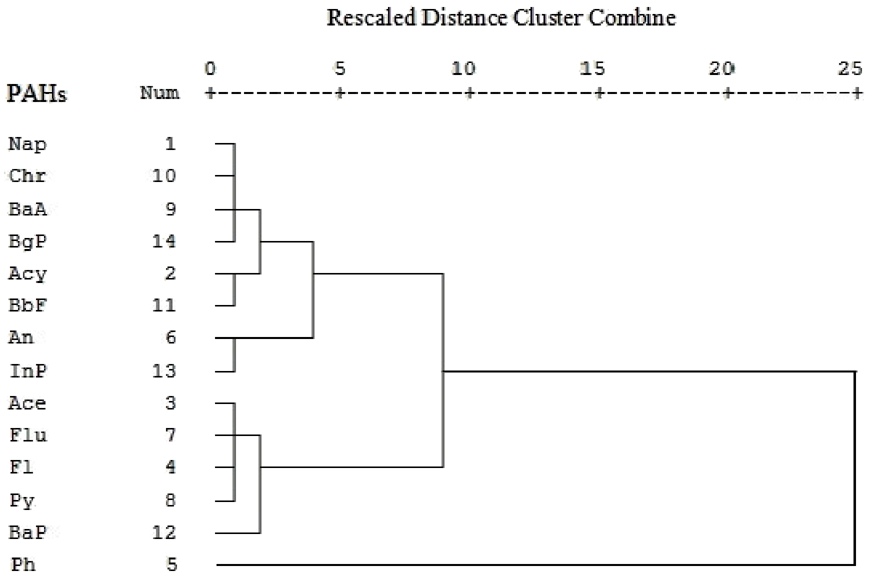


Fig. 6.4 Hierachical cluster analysis (HCA) result

6.4 Toxicological significance of PAHs in surface sediments

Cochin Backwaters in India are part of the Vembanad-Kol system, which is a protected wetland and one of the largest estuarine ecosystems in South Asia. This well known biodiverse wetland ecosystem is under the threat of severe ecological degradation due to massive reclamation (Gopalan et al. 1983), increased industrialization and urbanization (Menon et al. 2000; Qasim 2003). Anthropogenic activities are known to have a wide range of potential effects on these ecosystems, particularly from point and non-point sources of pollution. Generally, the presence of PAHs in the aquatic environment has increased over the last 100 years; however, global concentrations may have stabilized due to air and water quality regulations (Fernandez et al. 2000). PAH concentrations in the environment are often closely related to local and regional sources, although remote area can be sites of PAHs deposition through atmospheric processes and long range transport (Escartin and Porte 1999; Qin et al. 2013). These PAHs can be released in aquatic environment by accidental or operational spills produced during petroleum transportation or off-shore exploration. On the other hand, biogenic PAHs generate from short-term degradation of diagenetic precursors. For example, perylene, frequently detected in coastal marine sediments, derives mainly from terrestrial precursors via diagenetic processes, but it could also be generated during pyrolitic processes (Baumard et al.1998; Guinan et al. 2001) or derive from aquatic organic matter and diatoms (Venkatesan 1988). In aquatic environments, PAH distribution is controlled by multiple and inter-dependent parameters. Some of these parameters are linked to intrinsic physicochemical properties of these compounds, mainly hydrophobicity which controls their partition between dissolved and particulate phases. Others are related to the hydrological and geochemical characteristics of the environment including water agitation and turbidity (Foster et al. 1987), sediment granulometry (Garrigues and Lamotte 1993) and particulate or dissolved organic matter content (Ankley et al. 1994).

The release of pollutants into the aquatic environments is a major human concern worldwide. Because of their potentially hazardous properties, persistence and prevalence in the environments, the efforts have been made to reduce PAH emission in many countries. For example, 16 of PAHs have been listed as priority control pollutants by the Environmental Protection Agency of the USA (USEPA 1993). These contaminants are known to readily accumulate in bottom sediments, which serve as a repository of pollutants. Sediment contaminants could be released to the overlying water column from natural and anthropogenic processes, resulting in potential adverse health effects to aquatic organisms, including reproductive toxicity, photo-induced toxicity, mutagenicity and carcinogenicity (Long et al. 1995; Argese et al. 1997; Ross and DeLorenzo 1997). Moreover, PAHs are known to bioaccumulate in edible aquatic organisms including fish, and thus, represent a health risk to its top predators, including humans (Fox et al. 1991; Renzoni et al. 1998; Huang et al. 2006; Díez et al. 2009). They are usually well absorbed in the body, and may bind irreversibly to lipophilic sites in the cell, disrupting cellular processes, or metabolites and causing chronic adverse effects. For example, PAHs such as, naphthalene, anthracene and phenanthrene usually cause acute toxicity while, HMW PAHs tend to cause chronic toxicity, and slower penetration through cell walls (Neff 1979). Moreover, PAHs are known to bioaccumulate in edible aquatic organisms including fish, and thus, represent a health risk to top predators, including humans (Huang et al. 2006; Díez et al. 2009).

To estimate the potential eco-toxicological risk of sediments to benthic organisms, the determined PAH concentrations were compared with sediment quality guidelines (SQGs) (MacDonald et al. 1996), commonly used in sediment quality assessment. Several methods and approaches have been used to develop these guidelines and each of them has its advantages and its limits, which certainly influence their application in the evaluation of sediment toxicity. Sediment quality guidelines provide a scientifically defensible basis to evaluate the potential effects of sediment-associated contaminants on aquatic organisms. Selection of the most appropriate SQGs for specific applications can be a difficult task for sediment assessors. Therefore, to evaluate the environmental contamination posed by PAHs in surface sediments of Chitrapuzha River, the Canadian interim sediment quality guidelines (CCME 2008) which sets probable effect levels (PEL) and threshold effect level (TEL), as tools for interpreting the significance of PAHs in sediments has been followed. Moreover, sediment toxicity guideline of the US national oceanic and atmospheric administration (NOAA), provides two values: effects range low (ERL, adverse effects of the 10% of tests in a biological effects data base) and effects range median (ERM, adverse effects of the 50% of the tests) (MacDonald et al. 1996; Miki et al. 2014) to estimate potential biological effects. PAH concentrations below the TEL values do not cause adverse effects to aquatic organisms, while concentrations above the PEL are often associated with severe adverse biological effects (CCME 2008). Similarly, ERL values indicated possible adverse biological effects on aquatic organisms, while ERM values suggested a great possibility of posing detrimental biological effects on aquatic organisms (MacDonald et al. 1996). Long et al. (1995) noted that the correlation between impacts and chemical concentrations was fairly good for individual PAHs and total PAHs, and suggested, ERL and ERM concentrations for sediment-sorbed contaminants. Table 6.7 presented the ERL, ERM, PEL and TEL values of PAHs proposed by the sediment quality guidelines and the average concentrations reported in the study area. Increased PAH concentrations were found in sediment samples in Chitrapuzha River, and most of the PAH compounds have concentrations exceeded the probable effect-based guideline values (Table 6.7). Increased PAH levels in surface sediments could be of concern and may have potential to cause adverse biological effects. In general, sediments from the down stream portions in the study area (S3, S4 and S5) were more impacted by PAHs than upstream station S1, based on the differences on average concentrations of total PAHs.

According to Baumard et al. (1998), PAH contents can be described as low, moderate, high, and very high, when ∑PAH concentrations are 0-100, 100-1000, 1000-5000 and >5000 ng/g, respectively. The comparison of the ∑PAH levels in the present study are considered high to very high polluted with PAHs, suggest potential toxic implications for environmental quality and human health. Moreover, the assessment of sediment toxicity was performed, based on the total concentration of potentially carcinogenic PAHs (ΣcPAHs; BaA, Chr, BbF, BkF, BaP, DahA and IcdP) (Qiao et al. 2006). The ΣcPAHs concentrations varied from 1817 to 10687 ng/g dw, with an average value of 1250 ng/g dw (Table 6.7), and accounted for 30-37% in the sediments of Chitrapuzha River. Based on the above criteria, it can be concluded that current concentrations of PAHs in surface sediments of Chitrapuzha River would be likely to cause adverse effects. Further, Long et al. (1995) proposed a mean ERL quotient to evaluate the potential ecological risk possessed by PAHs. For the 20 target compounds for which ERL values exist, each PAH concentration at the site was divided by the ERL for that contaminant and these quotients were then averaged to obtain mean ERL. Mean ERL quotients account for the presence of mixtures of toxic chemicals exceeds their ERL guidelines. This method assumes additivity of toxic effects, and a mean ERL quotient ≥1.0 implies that least one analyte exceeds the ERL and therefore adverse effects are predicted to occur occasionally (Long 2006). In the present study, mean PAH ERL quotient varied from 7 to 37 (Table 6.7), indicating the potential to cause adverse effects in sensitive species. Although, toxicity of bulk sediments may not result only from the PAH compounds measured in the present work, higher mean ERL values suggested a higher risk of toxicity (Long 2006).

Table 6. 7 ERL, ERM, TEL and PEL values and the average concentrations of PAHs in the study area

					Present study	
PAHs (ng/g)	**ERL**	**ERM**	**TEL**	**PEL**	**Min**	**Max**
Nap	160	2100	34.6	391	109	2122
Acy	44	640	20.2	201	402	1899
Ace	16	500	6.71	88.9	804	2212
Fl	19	540	21.2	144	113	2710
An	85	1100	49.9	245	102	509
Ph	240	1500	86.7	544	598	7012
Flu	600	5100	113	1494	806	2710
Py	665	2600	153	1398	131	3072
BaA	261	1600	74.8	693	170	1941
Chr	384	2800	108	846	152	2157
BaP	430	1600	88.8	763	799	4162
BbF	NA	NA	NA	NA	696	2206
InP	240	950	NA	NA	0	221
BgP	430	1600	NA	NA	0	2720
∑PAHs					4882	35653
Σc PAHs					1817	10687
ERL quotient					6.7	36.8
ΣB [a] P_{eq}					851	1257

Yet, occupational exposure limit for total gPAHs has not been established because of the complexity of PAHs in their chemical composition. Several PAH species, including benzo(a) pyrene (as most carcinogenic compound) have been classified into probable (2A) or possible (2B) human carcinogens by the International Agency for Research on Cancer (IARC 1987) (Table 6.2). BaP is a five ring ($C_{20}H_{12}$) compound, which is mutagenic for human cells in culture (Osborne and Crosby 1987) and carcinogenic in whole animal assays (Cerna et al. 2000). According to the literature, the toxic equivalent factor for BaP is one (1), which is highest among all the PAHs. One approach in estimating the carcinogenic potency associated with the exposure of a given PAH compound can be obtained by calculating an index called B [a] p equivalent concentration (Wang et al. 2009), developed by the U.S. Environmental Protection Agency (EPA). The toxicity equivalency factor (TEFi) for each carcinogenic PAH (cPAH) is an estimate of the relative toxicity of a chemical compared to a reference chemical. Benzo(a)pyrene was chosen as the reference chemical, because the chemical toxicity of the compound is well characterized. The total toxic B [a] Peq for other PAHs was calculated using the following equation:

$$\text{Total B [a] } P_{eq} = \Sigma\ i\ C_i * TEF_i$$

Where, C_i is the concentration of individual PAH (BaA, BaP, BbF, BkF, InP, BgP and Chr) and TEFi is the corresponding toxic equivalency factor. US EPA calculated TEFi values for BaA, BaP, BbF, BkF, InP, BgP and Chr are given in Table 6.2. The total B [a] p_{eq} values at different sampling sites varied from 851 to 1257 ng/g (Table 6.7), and the industrial zones and estuarine region in the study area exhibited higher BaP_{eq} values than the reference site. Estimating total B [a] P_{eq} is a suitable approach of assessing the effects of anthropogenic sources in the sedimentary environment. However, this method might underestimate the risk due to the fact that not all PAHs are considered (Wang et al. 2009). In conclusion, comparison with effects-based sediment quality guidelines showed that concentrations of PAHs in surface sediments of Chitrapuzha River may result in possible toxic effects to aquatic organisms. Possible anthropogenic sources of pollution in this area include a diesel power plant, industrial (e.g., a petroleum refinery, a fertilizer manufacturing unit and a petrochemical unit) and wastewater discharges, boating-related activities, agricultural activities, and surface runoff. The proximity to various anthropogenic sources of pollutants warrants a regular monitoring program in the study area for inorganic and chemical organic compounds in sediments, water, and biota in order to have an effective coastal management program to protect the ecological integrity of this valuable ecosystem and the health of humans associated with it. Adverse effects on the organism and/or ecosystem level in surface sediments of Chitrapuzha River can be used as an early warning needle of the potential human health impact. Continued research is necessary to establish the most appropriate indicators to use in describing the sediment condition and the appropriate weighting factors for combining them for an overall pollution assessment.

6.5 Conclusions

Concentration of 14 priority polycyclic aromatic hydrocarbons in the surface sediments of Chitrapuzha River were analysed during pre-monsoon, monsoon and post-monsoon seasons. The total PAHs level, ΣPAHs ranged from 5188 to 33735 ng/g (pre-monsoon), 4906 to 31688 ng/g (monsoon) and 5045 to 32962 ng/g (post-monsoon). Among the 5 sites studied, the station S4 in the industrial zone was found to be the hotspot attaining highest concentration, followed by the estuarine region, S5. In general, the ΣPAHs levels showed a rising trend from the upstream station (S1) to the downstream (S4) region of the study area, and then a slight decline at S5, and the PAH level follows the order S1 < S2 < S3 < S5 < S4. This high average value could be attributed to the combined effect of industrial inputs, coupled with the discharges from apartments on the mainland, oil tanker berth, Cochin Port and Cochin Shipyard, close to this area. Whereas, low levels of PAHs with pyrogenic signature at S1 indicated, the upper reaches of Chitrapuzha River were beyond the influence of industrial (petroleum) pollution, likely due to the larger distance from the industrial units, and thus anthropogenic hydrocarbon sources. The seasonal trend of ΣPAHs in the study area was not consistent from pre-monsoon to post-monsoon seasons at different sites. Seasonal and spatial variations in PAH levels, clearly explain the strong impact of urbanization and industrialization on the overall pollution status of Chitrapuzha River. The average concentrations of ΣPAHs at all the sites revealed highest concentrations during pre-monsoon period while, lowest values during monsoon season. This unique seasonal variation could be attributed to the changes in the discharge of the river as except in the monsoon season, the river is stagnant.

Contrary to typical individual PAHs composition in sediment of other polluted areas, higher proportion of LMW PAHs (2-4 ring) predominated in the surface sediments of Chitrapuzha River, which reflects the significant amount of petrogenic sources and low-temperature pyrogenic processes. Therefore, the predominance of LMW PAHs indicated either a local source of PAHs or a relatively recent introduction of these pollutants into the surface sediments, since, small-size PAHs are more labile, and are expected to degrade faster than HMW PAHs. Moreover, molecular indices based source apportionment revealed that, the PAH distribution profile was dominated by petrogenic derived sources. Statistical tools, PCA and HCA analysis clearly established both petrogenic and pyrolytic inputs as the major source of PAHs in the surface sediments. The results obtained in the present study are on the higher side, as compared to those reported from other parts of the world. Most of the sediment samples contained PAHs concentrations exceeding the international standard values, suggesting possible risk for toxic implications on ecosystem and human exposure in the study area. The carcinogenic potency of PAH compounds was calculated, and found to be significant at the present level of sediments in Chitrapuzha River. The proximity to various anthropogenic sources of pollutants warrants a continual monitoring program in the study area for inorganic and chemical organic compounds in sediments, water, and biota in order to have an effective coastal management program to protect the ecological integrity of this valuable ecosystem and the health of humans associated with it. Further, comprehensive investigations on the fate and distributions of potential toxic contaminants such as pesticides, chlorophenols, dioxins, furans and dioxin-like PCBs are important to provide better baseline information of anthropogenic environmental pollutants in the surface sediments of Chitrapuzha River.

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SUMMARY

Hydrocarbon contamination in the aquatic system is one of the largest threats to environmental quality and human health. The concentration of pollutants in different environmental compartments is related to each other through the action of inter-compartment mass transfer processes. Such mass transfer processes decide the fate of both organic and inorganic pollutants. Therefore, a thorough assessment of the relative concentrations of pollutants in the different environmentally significant compartments of an aquatic system is an essential pre-requisite for any systematic hazard evaluation programme. The present investigation involves such a comprehensive approach, in which the water and surface sediments of Chitrapuzha River have been collected and analysed. The salient features of the study are summarised in the following paragraphs.

Based on specific geographical features, water flow regimes and anthropogenic activities, five sampling locations were selected (stations S1 to S5). Station S1 represents the far upstream portion of the river, and is beyond the influence of industrial pollution and regarded as a reference station. Stations S2, S3 and S4 constitute an industrial zone, which are the recipients of effluent treated water discharge outlets of major industrial establishments. Station S5 represents the downstream portion of the study area, and it receives a considerable quantity of discharges from apartments on the mainland, as well as from the oil tanker berth, Cochin Port and Cochin Shipyard. Surface water and sediment samples were collected during June 2012 (monsoon), April 2013 (pre-monsoon) and December 2013 (post-monsoon), and analysed for hydro biogeochemical parameters as per standard procedures. The sources and distribution of sedimentary organic matter in Chitrapuzha River were examined using bulk (total organic carbon, total nitrogen, total sulphur, elemental ratios and stable isotopic compositions) and molecular-level (fatty acids, n-alkanes and polycyclic aromatic hydrocarbons) analyses.

Anthropogenic activities have a remarkable influence on the hydrographic parameters of the study area. A clear horizontal salinity gradient was observed during the period of the survey with declining values from the estuarine mouth to riverine zone, and the salinity values ranged from not detectable level to 12 psu. Salinity was mainly controlled by fresh water discharges through the river and tidal currents. Dissolved oxygen concentration was higher during monsoon season and the values decreased as the season advanced to pre-monsoon and post-monsoon periods. Relatively higher values were observed in the upper reaches of Chitrapuzha River, as compared to the lower reaches, and it may be due to the higher solubility of oxygen in less saline water. Lowest values were recorded at S2 during pre-monsoon periods which may be explained as the combined effect of a discharge of industrial effluents around S2, low freshwater inflow during the pre-monsoon period, and higher utilisation of oxygen by organic matter. A general pattern in the nitrate ion distribution with normal concentration at S1, and followed by a sudden increase at stations S2 and S3 and then gradual decrease at all stations, independent of the seasonal variations. This pattern of nitrate concentration indicates discharge of industrial effluents rich in nitrogenous compounds around stations S2 and S3 and the decrease in concentration from station S3 to station S5 may be due to the dilution factor along the downstream. The distribution pattern of nitrite content is similar to that of nitrate with very high values at S2 and observed a horizontal gradation from station S2 to S5. This supports the earlier conclusions that the discharge of effluent rich water with higher concentration of nitrogenous materials around stations S2 and S3. The distribution pattern of ammonia followed a similar trend to those of nitrate and nitrite with normal concentration at S1 and then a sudden increase at S2. These observations again support the earlier conclusion of frequent discharge of industrial effluents rich in nitrogenous compounds, around station S2. A significant variation in total soluble inorganic nutrient is observed among the stations, where S2 recorded maximum and S1 exhibited the minimum. The distribution pattern of phosphate concentration also gives a similar trend to the nitrogenous nutrient distributions.

Texture analysis indicated higher contents of sand and clay in the upstream and downstream stations, while the predominance of silt was noted in the industrial zone. Settling of the finer fractions of the sediment resulted in increased clay content at all the stations during non-monsoon seasons. Slightly higher contents of the silt fraction at stations in the vicinity of the industrial zone may be associated with the spillage of phosphogypsum, a waste product from the adjacent fertilizer manufacturing unit. The labile fraction within the total organic matter pool was quantified in terms of biochemical components, and benthic trophic status was identified using various biochemical descriptors. The results of organic carbon content and biochemical components in the surface sediments of Chitrapuzha River displayed comparable concentrations to those reported in highly industrialised and urbanised eutrophic aquatic systems. Biopolymeric carbon chains were dominated by proteins, followed by carbohydrates and lipids in the majority of sampling sites. The concentration of total organic carbon values was comparatively higher than that in the sediments of the northern parts of the Cochin backwater system. The input of organic matter derived from rivers, coupled with the discharges of organic wastes from different industrial, agricultural and aquaculture sectors leads to a large scale accumulation of organic matter in the surface sediments of Chitrapuzha River. The distribution of total organic carbon and total nitrogen was controlled by the grain size of the surface sediments. Concentrations of total nitrogen were relatively lower in the surface sediments, and intermediate values of TOC/TN ratios signalled a combined input of both autochthonous and terrestrial organic matter.

$\delta^{13}C$ values estimated in the surface sediments indicated the input of terrestrial higher plant debris to sedimentary organic matter, while variations in $\delta^{15}N$ values suggested the effect of decomposition processes. The distribution of the terrestrial organic matter exhibited significant spatial differences with a higher contribution in the lower reaches of the study area. The observed values of protein to carbohydrate ratios >1 in the majority of the stations indicated freshly deposited detritus, while the sediments in the upstream station exhibited the predominance of aged organic matter. Similarly, comparatively lower lipid to carbohydrate ratios observed in the industrial zone and estuarine region reflect the lower nutritive quality of organic content during the sampling periods. Higher levels of chloropigments in the surface sediments indicated the possibility of autochthonous production in the study area. The threshold levels of protein, biopolymeric carbon and algal contribution to biopolymeric carbon suggests the eutrophic conditions prevailing in the study area.

The relevance of biochemical descriptors and bulk indices such as elemental composition and isotopic ratios are useful techniques for the evaluation of total quality, benthic trophic status and the relative contribution of marine and terrestrial derived organic matter in surface sediments. However, due to the complex nature, multiple sources and different reactivity of organic compounds, bulk indices such as, elemental stoichiometry and stable isotopic ratios are not completely successful in edifying the sources of sedimentary organic matter. Therefore, the molecular biomarker approach can be effectively used as a tool to characterise the sources of the organic matter in aquatic sediments.

Useful information on the sources of sedimentary organic matter was achieved by the evaluation of fatty acid compositions. Fatty acids ranging from C_{12} to C_{28} were identified, representing a spectrum of organic matter inputs into the surface sediments of Chitrapuzha River. Detected fatty acids include saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, branched fatty acids, long chain fatty acids and bacterial fatty acids. Saturated fatty acids were broadly distributed throughout the study area and confirmed a greater contribution to the overall fatty acids pool. Analysis of variance revealed a significant spatial and seasonal variation of fatty acids with maximum concentrations in the industrial areas (S2 and S3) during post-monsoon season, while the minimum value recorded at the estuarine station S5 during monsoon season. Increased inputs of organic matter associated with industrial run-off events resulted in the higher average fatty acids content. Among the individual fatty acids, palmitic acid ($C_{16:0}$) was the most abundant, which contributed 23 to 53% of the total fatty acid pool. Long chain fatty acids identified in the surface sediments with a strong even order predominance of carbon atoms, contributed 5 to 18%, while, monounsaturated fatty acids constituted 5 to 15% of the total fatty acid pool. Polyunsaturated fatty acids constituted only a meager portion of total fatty acids, and the abundance of odd chain and branched chain fatty acids and lower concentrations of polyunsaturated fatty acids in the study area established higher bacterial reworking of organic matter in the surface sediments of the study area.

Municipal sewages and the effluents from the adjacent industries in the study area influenced the distribution profile of bacterial fatty acids. From the fatty acid biomarker analysis, it is deduced that major inputs of organic matter in the surface sediments derived from phytoplankton, bacteria and terrestrial organic matter. The variations in the sedimentary organic matter sources and the diagenetic pathways due to the environmental settings could also be well documented through this biomarker approach. However, it is difficult to discriminate between the natural and anthropogenic inputs of organic matter through fatty acid biomarker alone. Hence, other lipid biomarkers such as n-alkanes, sterols and terpenoids along with the fatty acids could be more useful to distinguish the various organic matter sources.

The predominance of short chain and long chain odd hydrocarbons obtained in the surface sediments indicates autochthonous and allochthonous inputs of organic matter. A significant spatial and seasonal variation of n-alkane was observed with maximum concentrations near to the industrial areas during pre-monsoon season, while the minimum value recorded at S5 during monsoon season. This higher average value could be attributed to its proximity to the industrial units, which discharge approximately 80 million litres of effluents per day, as per the reference of a published work. Furthermore, the sediment texture has a significant role in the physico-chemical process as well as the species diversity of the depositional environment. The higher terrigenous to aquatic values and elevated average chain length at the upstream and the downstream stations of the study area indicated the preferential preservation of terrestrially derived organic matter over planktonic organic matter.

Biochemical degradation processes could be responsible for the unusual presence of even carbon predominance in the low molecular weight n-alkane homologues. The biochemical degradation pathway operating in surface sediments causes considerable alterations to the planktonic organic matter and the hydrodynamic depositional force plays a dominant role not only on the selective delivery of fluvial terrigenous organic constitutes, but also for the preferential preservation of terrigenous organic matter versus the marine derived organic matter in aquatic sediments. Meanwhile, higher concentrations of even number carbon chains at the industrial zone compared to other two stations (S1 and S5) can be mainly attributed to the direct biogenic contribution from algae, bacteria, fungi and yeast species, as well as the direct influence from petroleum-derived inputs. The calculated hydrocarbon isomeric ratios like carbon preference index, short chain to long chain and pristane to phytane indicates, both biogenic and petrogenic sources of organic matter in the surface sediments. The existence of petroleum contamination in the study area was confirmed by the presence of unresolved complex mixrure appeared in the chromatogram of sediment samples. Carbon preference index value based on short-chain hydrocarbon confirming the significant inputs of organic matter from microorganisms as well as petroleum sources. This was further confirmed by the presence of hopanes in the surface sediments. The principal component analysis indicated terrestrial, bacterial as well as petroleum inputs as the major source of hydrocarbons in the surface sediments of the study area. Thus, the n-alkane study was successful in discriminating biogenic and anthropogenic contributions of various organic matter present in the surface sediments.

Concentrations of 14 priority polycyclic aromatic hydrocarbons in the surface sediments of Chitrapuzha River were analysed, and the total concentration ranged from 5188 to 33735 ng/g in pre-monsoon, 4906 to 31688 ng/g in monsoon, and 5045 to 32962 ng/g in post-monsoon seasons. Among the 5 sampling sites studied, the station S4 located in the industrial zone was found to be the hotspot attaining highest concentration, followed by the estuarine region, S5. In general, the ΣPAHs levels showed a rising trend from the upstream (S1) to downstream (S4) stations, and then a slight decline at S5. This high average value could be attributed to the combined effect of industrial inputs, coupled with the discharges from apartments on the mainland, oil tanker berth, Cochin Port and Cochin Shipyard close to this area. Whereas, low levels of PAHs with pyrogenic signature at S1 indicated, the upper reaches of Chitrapuzha River were beyond the influence of industrial (petroleum) pollution, likely due to the larger distance from the industrial units, and thus anthropogenic hydrocarbon sources. Seasonal and spatial variations in PAH levels in the surface sediments clearly explain the strong impact of urbanization and industrialization on the overall pollution status of Chitrapuzha River.

Contrary to typical individual PAHs composition in sediment of other polluted areas, a higher proportion of low molecular weight PAHs (2-4 rings) predominated in the sediments of Chitrapuzha River, which reflects the significant amount of petrogenic sources and low-temperature pyrogenic processes. Therefore, the predominance of low molecular weight PAHs indicated either a local source of PAHs or a relatively recent introduction of these pollutants into the surface sediments, since, small size PAHs are more labile, and are expected to degrade faster than high molecular weight PAHs. Moreover, molecular indices based source apportionment revealed that the PAH distribution profile was dominated by petrogenic derived sources. Statistical tools like principal component analysis and hierarchical cluster analysis clearly established both petrogenic and pyrolytic inputs of PAHs in the surface sediments. The results obtained in the study indicated the fact that the concentration of PAHs is on the higher side, as compared to those reported from other parts of the world. Most of the sediment samples contained PAHs level exceeding the international standard values (USEPA), suggesting possible risk for toxic implications on the ecosystem and human exposure in the study area. The carcinogenic potency of PAH compounds was calculated, and found to be significant at the present levels in the surface sediments of Chitrapuzha River. The proximity to various anthropogenic sources of pollutants warrants a continual monitoring program in the study area for inorganic and organic chemical compounds in sediments, water, and biota in order to have an effective coastal management program to protect the ecological integrity of this valuable ecosystem and the health of organisms including human beings. Further, comprehensive investigations on the fate and distributions of potentially toxic contaminants such as pesticides, chlorophenols, dioxins, furans and dioxin-like PCBs are important to provide better baseline information of anthropogenic environmental pollutants in the surface sediments of Chitrapuzha River.

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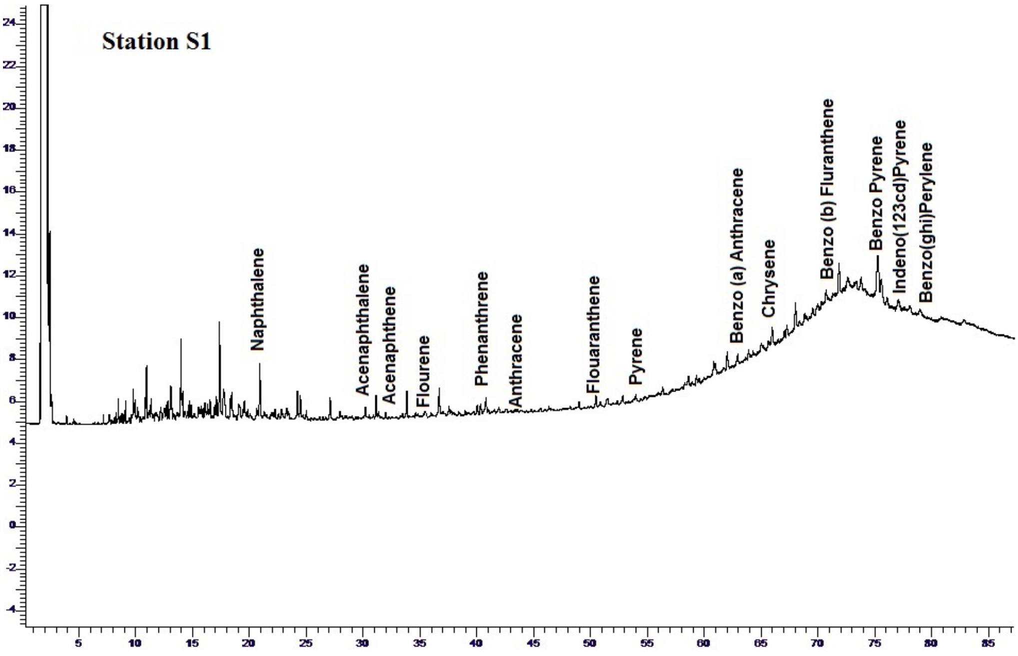
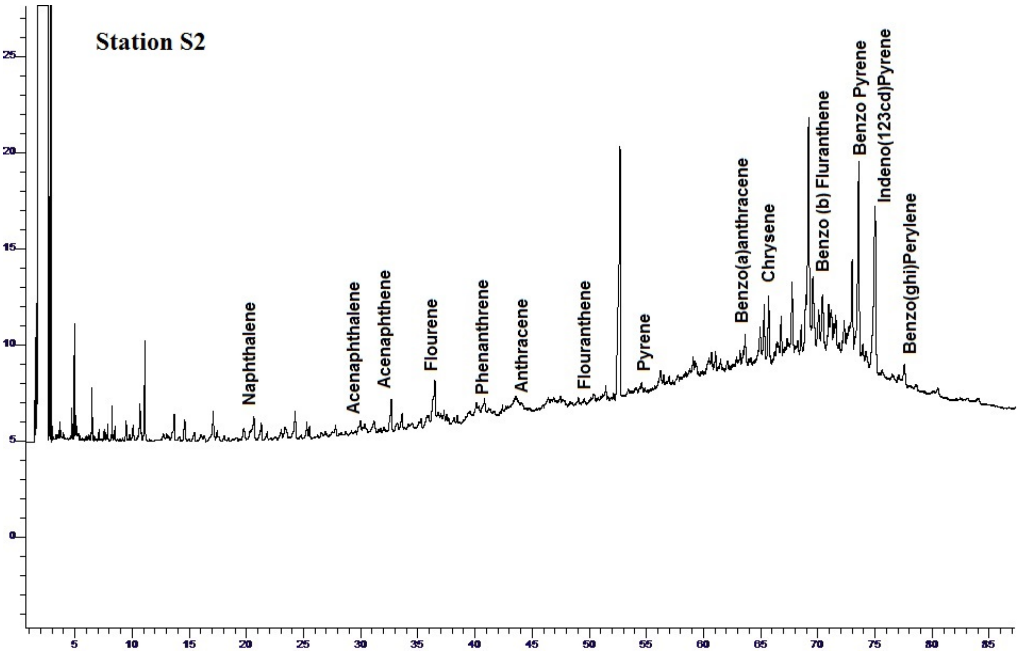
APPENDIXES

Appendix 1.1 Gas chromatograms of polycyclic aromatic hydrocarbons extracted from the surface sediments at different stations of the study area




Station S2
Naphthalene
Acenaphthalene
Acenaphthene
Flourene
Phenanthrene
Anthracene
Flouranthene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo (b) Fluranthene
Benzo Pyrene
Indeno(123cd)Pyrene
Benzo(ghi)Perylene
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80
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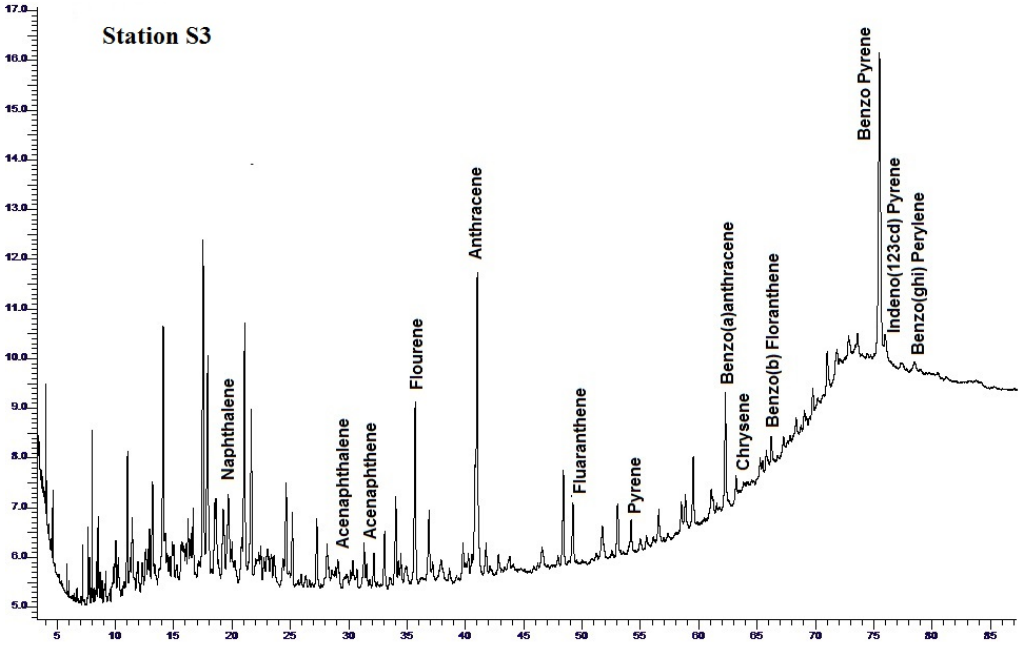
Station S3
Naphthalene
Acenaphthalene
Acenaphthene
Flourene
Anthracene
Fluaranthene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(b) Floranthene
Benzo Pyrene
Indeno(123cd) Pyrene
Benzo(ghi) Perylene
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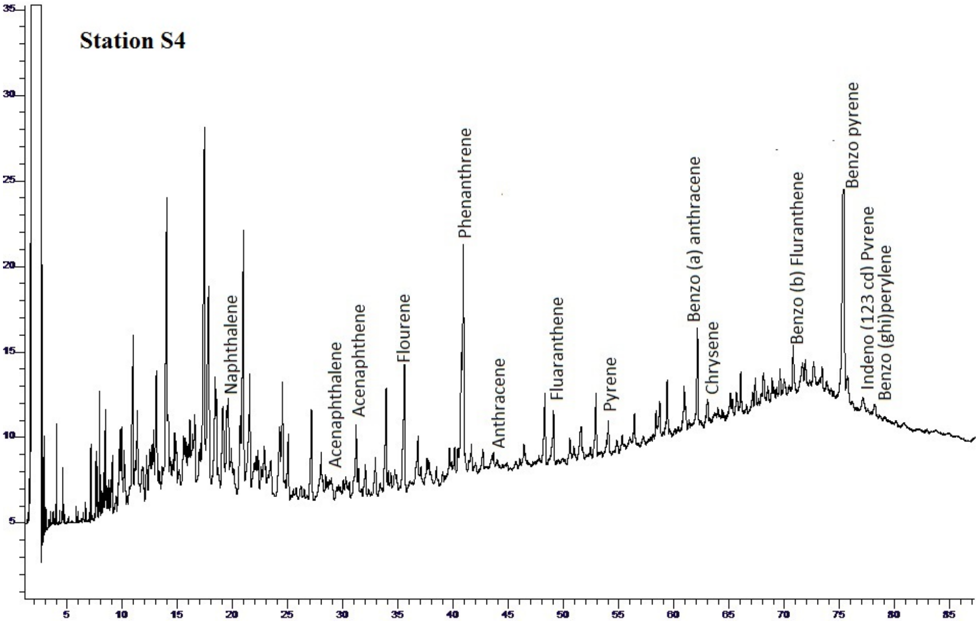
Station S4
Naphthalene
Acenaphthalene
Acenaphthene
Flourene
Phenanthrene
Anthracene
Fluaranthene
Pyrene
Benzo (a) anthracene
Chrysene
Benzo (b) Fluranthene
Benzo pyrene
Indeno (123 cd) Pyrene
Benzo (ghi)perylene
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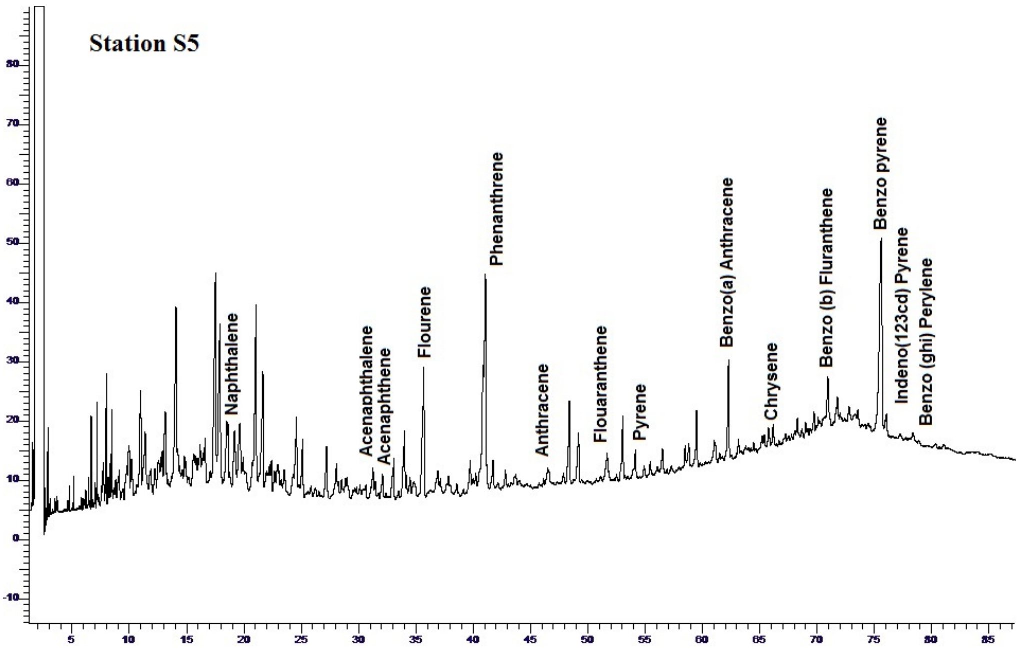
Station S5
Naphthalene
Acenaphthalene
Acenaphthene
Flourene
Phenanthrene
Anthracene
Flouaranthene
Pyrene
Benzo(a) Anthracene
Chrysene
Benzo (b) Fluranthene
Benzo pyrene
Indeno(123cd) Pyrene
Benzo (ghi) Perylene
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LIST OF PUBLICATIONS

1. **K. S. Sanil Kumar** • S. M. Nair **(2015)**. Predominance and Sources of Alkane and Fatty Acid Biomarkers in the Surface Sediments of Chitrapuzha River (South India). **Bulletin of Environmental Contamination and Toxicology**, 94:496 – 502.

2. **K. S. Sanil Kumar** • S. M. Nair • P. M. Salas • Prashob Peter • C. S. Ratheesh Kumar **(2015**). Aliphatic and Polycyclic Aromatic Hydrocarbon contamination in surface sediment of *Chitrapuzha* River, South West India. ***Chemistry and Ecology,*** **DOI: 10.1080/02757540.2015.1125890, (In press).**

3 **K. S. Sanil Kumar** • S. M. Nair **(2015)**. Distribution and sources of sedimentary organic matter in *Chitrapuzha* (Cochin estuary), A Tropical Tidal River, south west coast of India. **Estuarine, Coastal and Shelf Science**. **(Under Review)**.

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Predominance and Sources of Alkane and Fatty Acid Biomarkers in the Surface Sediments of Chitrapuzha River (South India)

K. S. Sanil Kumar · S. M. Nair

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Abstract Surface sediment samples were collected from Chitrapuzha (Cochin) estuarine system to identify the natural and anthropogenic origin of organic matter. The distribution and sources of organic matter were assessed with the help of fatty acid and alkane biomarkers. Fatty acids ranging from C_{12} to C_{28} were identified and $C_{16:0}$ was the most abundant fatty acid, which contributed between 23.5 % and 52.4 % to total fatty acids. The low levels of polyunsaturated fatty acids indicate the effective bacterial recycling of algal fatty acids during the whole settling and depositing process. Aliphatic hydrocarbons ranging from C12 to C33 were identified and the total concentration ranged from 7876 to 43,357 ng g^{-1}. The presence of unresolved complex mixtures and lower pristane to phytane ratios indicates the petroleum contamination in the study area.

Keywords Sediment · Industrial effluents · Biomarkers · Source characterization

The knowledge of the source, distribution, reactivity and transformations of organic matter in estuarine sediments is important to understand the role of estuarine coastal systems in biogeochemical cycles (Zhang et al. 2009). However, understanding these processes is a difficult task due to the complex and multiple sources of organic matter, as well as the difference in reactivity and the spatial variability in different processes occurring in estuarine sediments (Bianchi and Canuel 2011). Therefore, a variety of approaches including elemental analysis (C/N ratios), stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) and biomarkers have been widely used (Mohd et al. 2013).When more detailed information on sources of organic matter is required, molecular source indicators i.e., biomarkers can be applied. The distribution of this biomarkers in sediments ultimately provides useful information about the source, diagenetic alteration, preservation and historical changes in organic matter as well as changes in trophic status (Alfaro et al. 2006).

Fatty acids (FACs) and *n*-alkanes are widely used to trace the sources of organic matter in sediments due to their structural diversity, specificity and relative stability (Gao and Chen 2008). Particularly, FACs are useful biomarkers since they are used to estimate the relative contribution of terrestrial, algal, planktonic and bacterial FACs to the total organic matter pool in sediments (Zimmermann and Canuel 2001). For example, FACs with carbon atoms $\geq$22 are indicative of higher plant inputs (Colombo et al. 1996) while odd carbon numbered and branched chain FACs are used as bacterial biomarkers (Parkes 1987). Unsaturated FACs are mainly associated with algae (Colombo et al. 1996) and act as a diatom marker in marine sediments. The biomarker approach of hydrocarbon is appropriate since it differs, for instances in terms of their residence time, transport mechanisms, according to their physicochemical properties and sources (Pancost and Boot 2004). Generally, *n*-alkanes in nonsiliceous species of phytoplankton and macrophytes are C15, C17 and C19 (Youngblood and Blumer 1973), whereas terrestrial plants contain mainly C29, C31 and C33 with high carbon preference index (CPI) (Xing et al. 2011), a ratio of summation of odd carbon number homologues over a range to the summation of even carbon number over the same range (Elias et al. 2000). *n*-alkanes in the range C14–C22 originate from diatoms (Elias et al. 2000), while bacteria displays predominance of

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n-alkanes C18 and C20 (Han and Calvin1969).Whereas the presence of phytane, a C20 isoprenoid and the sediments containing high concentration of unresolved complex mixture (UCM) indicate petroleum residue (Ye et al. 2007).

This study aims to (1) delineate the sources of sedimentary organic matter in the lower reaches of Chitrapuzha (2) investigate the influence of effluents from industrial units on the distribution profile of *n*-alkanes and (3) characterise the different FACs and *n*-alkanes in the sedimentary system so as to discriminate the biogenic and anthropogenic contributions to organic matter.

Materials and Methods

The study area (Fig. 1), the lower reaches of Chitrapuzha tidal river system (9°52′–10°00N; 76°15′–76°E), is a part of Cochin estuary South India. A host of industries including a major fertilizer plant (FACT), an organic chemical factory (HOCL) and a petroleum refinery (BPCL) are crowed along the small stretch of land by the side of Chitrapuzha River. Sampling was carried out at five stations depending upon the nature and intensity of anthropogenic activities, resulting in several environmental issues raised by national (The Hindu 2011) and local newspapers. Station 1 (S1) serves as a reference site and was located in the upstream portion of the river beyond the influence of industrial pollution. Stations 2 (S2), 3 (S3) and 4 (S4) constitute industrial zone which are the recipients of effluents from major industries. Station 5 is near Thevara ferry point, a tip of main land from which a motor boat ferry service operates to adjacent islands and it receives discharges from apartments on the main land as well as from oil tanker berth, Cochin Port and Cochin Shipyard. The sediment samples were collected using a van Veen grab (0.042 m^2) during June 2012 (monsoon) and March 2013 (non-monsoon).The samples were stored in a deep freezer (−20°C) till the analysis. All the analyses were carried out in triplicates and the average is reported.

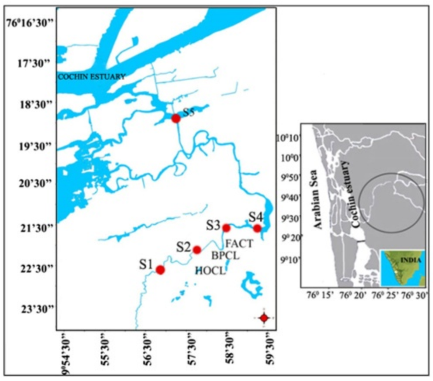

Fig. 1 Location map of the study area

The wet sediment samples were subjected to textural characteristics by pipette analysis (Folk 1980), after removing the inorganic carbonates using 10 % HCl and organic matter using 15 % H_2O_2. A known weight of wet sediment was dispersed overnight in sodium hexametaphate. The sand was separated by wet sieving using a 63 μm sieve. The filtrate containing the silt and clay fractions was subjected to pipette analysis. Total organic carbon (TOC) was estimated using TOC analyser (VARIO TOC SELECT-Elementar) after removing inorganic carbon using 2 M HCl. Biomarkers were extracted by the method described by Harvey (1994). The freeze dried homogenized sediment samples (15–20 g) were extracted with dichloromethane-methanol (2:1) mixture in a Soxhlet apparatus for 72 h. The extracts were concentrated by rotary evaporation, and the residue was subjected to mild alkaline hydrolysis. The neutral fraction was extracted using *n*-hexane and submitted to a silica gel glass column for fractionation. The fractions containing the aliphatic hydrocarbons were eluted with 50 mL n-hexane and then evaporated to 1 mL and injected on a gas chromatograph equipped with flame ionisation detector (GC-FID) with a DB-5 column (30 m × 0.25 mm i.d, 0.25 μm film thickness). The aqueous layer containing the fatty acid was acidified to pH 2, and then treated with 10 mL of 12 % BF_3/MeOH at 70°C for 30 min to form the fatty acid methyl esters (FAMEs). The analysis of FAMEs was performed using gas chromatography–mass spectrometry (GC–MS) using a Perkin Elmer Clarus GC 620, equipped with a fused silica capillary column (30 m × 0.32 mm i.d, 0.25 μm film thickness). Identification of individual compounds was achieved by comparison of retention times with those of standard compounds (C7–C40, Sigma Aldrich and Supelco 37 Component FAME Mix). Analyses of triplicate sediment sub samples indicated hydrocarbon reproducibility to be typically ±7 % and FACs reproducibility to be ±9 % of the mean concentration. Re-extracts yielded less than 2 % of the original extracts both for *n*-alkanes and fatty acids. Data were acquired and processed with M.S Turbomass Version 5.4.2 and Total Chrom work station. The quantification limits were 0.06 μg g^{-1} for FACs and 0.02 ng g^{-1} for *n*-alkanes. Analysis of variance (ANOVA) and Pearson correlation (SPSS Version-13) were employed to evaluate the spatial distribution of alkanes as well as the process governing their distribution.

Results and Discussion

General characteristics of the surface sediments are given in Table 1. The higher values of TOC are reported at the industrial zone (S2, S3 and S4), compared to the other two stations. This is probably attributed to the anthropogenic activities, growth of major industrial units and shipping port operations in this area. Moreover, granulometrically, the fine grained (silty-clay) compositions of the surface sediments in this area enhances the organic matter accumulation, leading to a higher TOC content (Ramaswamy et al. 2008). As organic carbon showed comparatively higher values during non-monsoon, the pre-monsoon season was selected for the FACs and *n*-alkanes analysis.

The FACs distributions suggested different sources of organic matter including algal, bacterial, phytoplankton and zooplankton. The FACs are broadly classified into linear saturated (SFA), branched saturated (Br-FA), monounsaturated (MUFA) and polyunsaturated (PUFA) FACs (Table 2). The total fatty acid (TFA) concentrations ranged from 162 to 390 µg g^{-1} with a maximum value at S3 and a minimum value at S5 (Table 2). The lower values at estuarine station might be due to the out welling of organic matter as a result of high tidal flushing (Ratnayake et al. 2005). SFAs were the major fractions (63 %–82 %) in the study area with predominance of C_{16} and C_{18} pointing zooplankton as the major source (Muri et al. 2004), while significant amounts of $C_{16:1}$ and $C_{18:1}$ (MUFAs) indicate bacterial inputs (Volkman 1986). The intense bacterial activity at the industrial zone and estuarine stations were further confirmed by the presence of iso and anteiso C_{15} Br-FAs (Killops and Killops 1993), which might be due to the influence of leachate from the defunct solid waste treatment plant close to this area. The lower value of unsaturated FACs (MUFA + PUFA) at S4 establishes high bacterial reworking (Joseph et al. 2012) while the higher value of $\Sigma C_{16}/\Sigma C_{18}$ at S1 supports significant contribution of diatom organic matter (Parrish et al. 2000). MUFAs ($C_{16:1}$, $C_{18:1}\omega_7$, $C_{18:1}\omega_9$ and $C_{19:1}$) constituted 5.0 %–15.8 % of TFAs and the distributions reveal algal (Reitan et al. 1994), bacteria and brown algae (Johns et al. 1979) sources. PUFAs constituted only a meagre portion of TFAs (0.28 %–1.8 %) which may be due to its labile nature, and it indicates recent detritus inputs (Carrie et al. 1998).

The distribution profile, total hydrocarbon concentrations (Σn-alkanes) and selected *n*-alkanes ratios are given in Table 2. ANOVA registered significant spatial variations ($p < 0.01$) of *n*-alkanes with maximum concentrations near to the industrial areas (S2, S3 and S4). This high average value could be attributed to its proximity to the industrial units, which discharge 80 million liters of effluents per day (Rejomon et al. 2013). The lower concentrations of Σn-alkanes in the other two sites with the lowest at S5 are likely due to the larger distance from the industrial areas and thus the anthropogenic hydrocarbon sources. Furthermore, the dredging process in this area to facilitate water transportation may further reduce the hydrocarbon level. This is because sediment granulometry showed predominantly silt and clay prior to dredging, which changed to sand with onset of dredging (Nayar et al. 2004). Once the lighter and finer sediments were resuspended in the water column, the coarser and heavier sandy sediments settled quickly on the bottom substratum, further explaining the relatively higher levels of sand and lower concentrations of hydrocarbon (Zimmerman et al. 2003). The *n*-alkane distribution profiles in the study area can be regarded as a combination of two distinct sources i.e., biogenic and petrogenic origin. The predominant C27, C29 and C31 indicate terrestrial higher plant debris (Rielley et al. 1991), while C15, C17 and C19 are pointing to aquatic sources (Jaffé et al. 2001). Meanwhile, the higher concentrations of even number carbon chains at S2 and S3 established both biogenic and anthropogenic (i.e., petrogenic) sources of hydrocarbons (Harji et al. 2008). Pearson correlation analysis revealed that most of the *n*-alkanes exhibited positive correlation with silt and negative correlation with sand signifying the fact that the distributions of *n*-alkanes were mainly controlled by fine grained fraction (Guo et al. 2009; Zhang et al. 2009). A good relationship existed between C13, C14, C18, C20, C21, C22, C23, C24, C25 and C32 implying that these compounds were emitted from similar sources. Pearson's r values were

Table 1 Variations of general sedimentary parameters (average ± SD, n = 9)

Stations	TOC %		Sand %		Clay %		Silt %	
	a	b	a	b	a	b	a	b
S1	2.94 ± 0.06	3.16 ± 0.05	46.35 ± 3.20	45.10 ± 3.64	27.25 ± 2.85	27.6 ± 2.7	26.4 ± 2.1	27.30 ± 2.36
S2	7.22 ± 0.09	7.7 ± 0.09	20.65 ± 2.21	22.10 ± 2.2	23.25 ± 2.45	23.80 ± 2.5	56.10 ± 3.8	54.10 ± 4.6
S3	7.48 ± 0.08	7.6 ± 0.08	21 ± 2.10	20.45 ± 2.45	22.56 ± 2.18	24.28 ± 2.1	56.44 ± 3.55	55.27 ± 4.75
S4	8.91 ± 0.11	8.97 ± 0.10	17.23 ± 1.97	14.57 ± 1.3	23.65 ± 2.14	24.28 ± 2.8	59.12 ± 3.9	58.15 ± 4.23
S5	3.19 ± 0.04	3.26 ± 0.04	44.68 ± 4.8	42.02 ± 3.12	28.10 ± 2.85	29.30 ± 2.9	27.22 ± 2.6	28.68 ± 2.75

a monsoon, *b* non-monsoon

Table 2 Distribution of biomarkers (fatty acids μg g^{-1} and alkanes ng g^{-1}) and their corresponding indices

Fatty acids	S1	S2	S3	S4	S5	Alkanes	S1	S2	S3	S4	S5
$C_{12:0}$	1.09	8.24	9.58	9.08	5.62	C12	642	2244	1944	1362	262
$iC_{14:0}$	ND	ND	ND	ND	ND	C13	1035	1998	1863	1124	361
$C_{14:0}$	2.95	27..45	26..88	12.25	15.48	C14	1573	2574	2412	1868	273
5,9,13-C_{14}	0.41	13..64	24.69	4.16	5.07	C15	74	1523	841	554	62
$iC_{15:0}$	0.89	21.36	22.56	14.12	13.14	C16	1100	2900	1188	1612	191
$aC_{15:0}$	1.36	12.69	16.21	8.01	6.22	C17	102	2412	1818	1628	86
$C_{15:0}$	0.66	16.85	11.23	4.36	5.18	pristane	750	814	774	425	10.1
$iC_{16:0}$	0.61	9.04	12.62	2.36	3.83	C18	989	1944	1988	886	32
$C_{16:1}$	16.94	17.23	14.35	5.32	11.45	C19	36	1988	1966	1530	49
$C_{16:0}$	103.33	105.36	104.26	44.23	54.85	phytane	422	822	802	344	27.8
$iC_{17:0}$	0.15	2.36	8.55	2.03	3.62	C20	1441	1818	1812	882	293
$aC_{17:0}$	ND	ND	ND	ND	ND	C21	1412	2012	1586	1444	171
$C_{17:0}$	8.99	9.33	7.28	2.28	3.55	C22	1269	2160	2284	1124	294
$C_{18:0}$	2.34	28.23	26.45	19.33	12.96	C23	1034	1512	1812	1033	225
$C_{18:1}\omega_7$	1.14	11.98	17.39	1.69	6.2	C24	1067	1885	1889	812	414
$C_{18:1}\omega_9$	1.29	13.99	22.34	3.18	7.05	C25	1014	1577	1202	882	453
$C_{18:2}\omega_6$	ND	6.29	0.23	1.91	ND	C26	864	1237	1265	291	525
$C_{19:1}$	ND	ND	ND	ND	0.88	C27	714	1889	1267	998	644
$C_{20:0}$	8.96	18.26	11.39	2.85	1.39	C28	568	1361	1412	592	624
$C_{20:4}$	0.22	ND	ND	0.75	1.62	C29	762	2882	2653	1882	709
$C_{20:5}$	0.34	ND	0.17	0.7	0.72	C30	632	1452	1706	616	421
$C_{22:0}$	14.94	16.11	15.69	12.63	1.31	C31	814	3220	3065	1818	869
$C_{23:0}$	ND	ND	ND	1.59	ND	C32	484	1444	1564	947	336
$C_{24:0}$	12.85	25.14	20.69	5.43	1.51	C33	1214	1325	1614	988	582
$iC_{26:0}$	9.99	18.44	16.54	11.36	ND	ΣnAlkanes A	18840	43,357	39,151	24,873	7876
$C_{26:0}$	1.04	12.36	18.11	7.23	0.46	Σodd	8211	22338	19,687	13,881	4211
$C_{28:0}$	6.63	8.99	9.74	10.22	ND	Σeven	10,629	21,019	19,464	10,992	3665
TFA	197.12	365.66	390.07	188.01	162.11	Pr/ph	1.78	0.99	0.97	1.31	0.36
SFA	163.78	248.87	234.42	131.48	102.31	nC17/pr	0.14	2.96	2.35	3.60	8.51
BrFA	13.00	63.89	76.48	37.88	26.81	nC18/ph	2.34	2.36	2.48	2.58	1.15
MUFA	19.37	43.20	54.08	10.19	25.58	SC/LC	1.06	1.21	1.01	1.29	0.36
PUFA	0.56	6.29	0.40	3.36	2.34	$CPI_{\mathbf{13-33}}$	0.80	1.07	1.02	1.26	1.15
LCFA	20.52	46.49	48.54	22.88	1.97	TAR	8.74	1.35	1.45	1.27	11.28
ΣOdd	9.65	26.18	18.51	8.23	9.61	ACL	29.22	29.15	29.54	29.31	29.30
ΣOdd + BrFA	22.65	90.07	94.99	46.11	36.42						
$C_{16:1}/C_{16:0}$	0.16	0.16	0.13	0.12	0.21						
$\Sigma C_{16}/\Sigma C_{18}$	25.34	2.18	1.98	1.99	2.68						
$C_{16:1}/C_{16:0}$	0.16	0.16	0.13	0.12	0.21						

SC/LC = Σ(C12–C22)/Σ(C23–C33); TAR = C27 + C29 + C31/C15 + C17 + C19

ACL = 25*C25 + 27*C27 + 29*C29 +31*C31 + 33*C33/C25 + C27 + C29 + C31 + C33;$CPI_{13-33} = \Sigma_{ODD}C13\text{–}33/\Sigma_{EVEN}C12\text{–}32$

ND not detectable

used to further hypothesize the probable source of *n*-alkanes, on the assumption that two or more components may correlate either due to a common origin or environmental behavior. The relationship between levels of C13, C14, C18 and C22 shown to be highly significant with r values ranging from 0.94 to 0.99. A relatively good correlation existed among most of the low molecular weight *n*-alkanes with high molecular weight *n*-alkanes indicated that *n*-alkanes in the study area had presumably undergone similar environmental processes or transport mechanism and could be related to both biogenic and petrogenic orgin (Ekpo et al. 2005).

To distinguish probable sources of hydrocarbons, several indices such as CPI, average chain length (ACL), short chain/long chain ratio (SC/LC), terrigenous/aquatic ratio (TAR), UCM, C17/pristane and C18/phytane were also used in addition to absolute concentrations. The CPI values (Harji et al. 2008) of sediment ranged from 0.80 to 1.26 (Table 2) and the values close to 1 at S2 and S3 indicated significant contributions from oil-derived hydrocarbons from the adjacent petroleum manufacturing unit, while biogenic sources dominated at S1 (Wang and Fingas 2003). Moreover, the presence of UCM (Fig. 2), a structurally complex mixture of isomers of hydrocarbons, which is seen as a 'hump' in the chromatogram (Hong et al. 1995) of sediments from the industrial zones further confirms the contamination from petroleum sources (Commendatore et al. 2000). In contrast, UCM was absent at S1 and S5, suggesting biogenic origin of hydrocarbons. Also the short chain/long chain *n*-alkanes ratios ranged from 0.36 to 1.29 and the estimated value equal to 1 at S3 indicated the influence of petroleum effluents from the adjacent industries (Harji et al. 2008). The *n*-alkane index TAR (Bourbonniere and Meyers 1996) is the ratio between the concentrations of (C27 + C29 + C31) and (C15 + C17 + C19), is used to evaluate the importance of terrigenous verses aquatic inputs. The TAR values ranged from 1.27 to 11.3 (Table 2) and the higher values at S1 and S5 indicated the preferential preservation of terrestrially derived organic matter due to the local dominant fluvial contribution (Rielley et al. 1991). The lower TAR values at the industrial stations are possibly due to higher bacterial activity or a petroleum contribution of short chain (<C20) *n*-alkanes, as suggested by the CPI values. Therefore, contamination of petroleum effluents rich in low molecular weight hydrocarbons (e.g., diesel oil) interferes with the result obtained, and this contamination must be the cause of low TAR values at S2, S3 and S4. The ACL is an additional tool for studying the relative amounts of vascular plants and mature fossil hydrocarbons. The ACL values indicated the preferential preservation of terrestrially derived organic matter over planktonic organic matter (Volkman et al. 1987) in surface sediments of Chitrapuzha. The isoprenoid alkanes, pristane (Pr) and phytane (Ph) were present in the sediment samples with highest concentration at S2. Moreover, the Pr/Ph value closes to 1 at S2 and S3 (Table 2) imply an anthropogenic input of petroleum residues (Gao and Chen 2008). The relatively low values of C17/Pr at S1 and C18/Ph at S5 (Table 2) are likely due to an early stage of biodegradation, and also by the preferential depletion of *n*-alkanes prior to isoprenoids (Jacquot et al. 1996) (Fig. 3).

In summary, the FACs biomarkers were mainly derived from phytoplankton, bacteria and terrestrial organic matter. In contrast, aliphatic hydrocarbons were resulting from mixed origin i.e., biogenic (vascular plants) and anthropogenic (petrogenic) sources. Thus, the study illustrates the usefulness of a multiple biomarker approach in

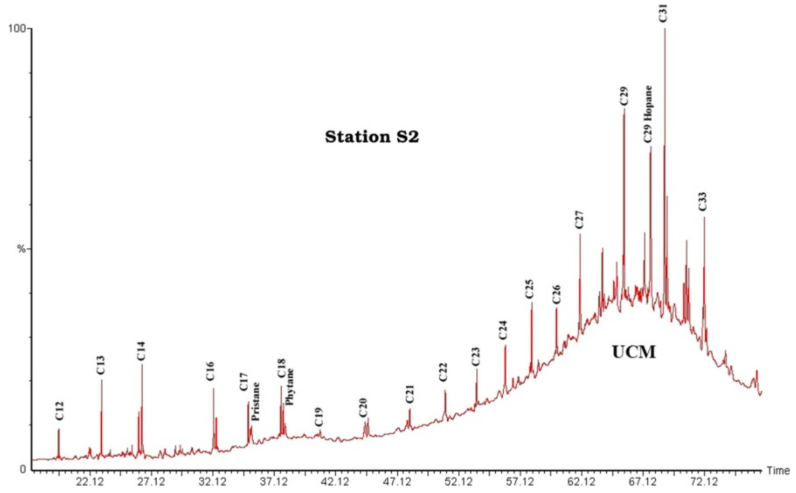

Fig. 2 Alkane Chromatogram showing UCM at stations S2

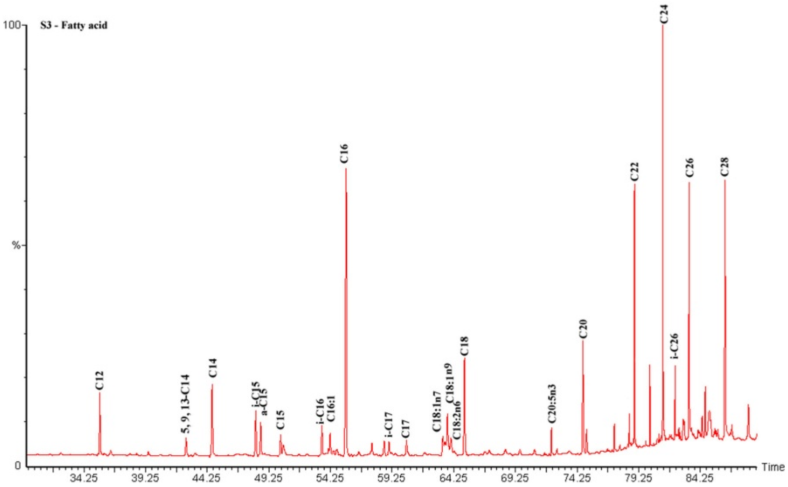

Fig. 3 Chromatogram showing fatty acids at stations S3

discriminating biogenic and anthropogenic sources of organic matter.

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Aliphatic and polycyclic aromatic hydrocarbon contamination in surface sediment of the *Chitrapuzha* River, South West India

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ABSTRACT
Distribution (seasonal and spatial) of aliphatic and polycyclic aromatic hydrocarbons (PAHs) in surface sediments of the *Chitrapuzha* River, Cochin, India, was investigated using gas chromatography. Significantly high concentrations prevailed during the pre-monsoon season with the industrial zones of the river appearing to be hot spots with particularly elevated levels of the hydrocarbons. AHCs ranged between 7754 and 41,173 ng/g with an average of 25,256 ng/g, while total PAHs varied from 5046 to 33,087 ng/g. *n-Alkane indices* and *PAH diagnostic ratios* point to petroleum contamination in the sediments. The significance of PAHs in the sediments was explored using universally accepted interpretation tools. Observed levels of PAHs in sediments of Chitrapuzha are likely to cause adverse effects on biota.

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Aliphatic hydrocarbons; polycyclic aromatic hydrocarbons; surface sediments; ecotoxicological risk

1. Introduction

Aliphatic hydrocarbons (AHCs) and polycyclic aromatic hydrocarbons (PAHs) are introduced into the aquatic environment through natural and anthropogenic sources.[1,2] Due to their hydrophobic and strongly particulate nature they tend to adsorb easily onto the sediment phase, making them a repository of these compounds [3,4] and directly influence bottom-dwelling organisms. An understanding of the influence of these chemicals on sediment quality is essential for evaluating overall aquatic health.

AHCs (*n*-alkanes) are widely used to locate sources of organic matter in aquatic sediments, since many are source specific, and easily accumulated from biogenic and petrogenic sources.[5] Biogenic hydrocarbons are known to be bio-synthesized by a wide range of both marine and terrestrial plants. The difference in characteristic chain length of marine and terrestrial plants renders the distribution of *n*-alkanes an effective tool for assessing sources of organic matter in terrestrial and marine sediments. For example, planktonic organisms generally produce a simple mixture of odd chain *n*-alkanes preferably C15, C17 and C19,[6] while the abundance of C25, C27, C29 and C31 has been used as an indicator of terrestrially derived organic matter.[7] The presence of unresolved complex mixture (UCM) and carbon preference index (CPI) value 1 or close to 1 indicates petroleum contamination.[8]

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PAHs, a group of lipophilic organic pollutants that contain two or more fused aromatic rings, are introduced into the environment through biogenic, petrogenic, and pyrolytic processes.[9] The stable molecular structure of PAHs facilitates their preservation in marine settings and hence PAH analysis is regarded as a valuable paleo-environmental tool for re-creating anthropogenic developments in diverse environments such as lakes, rivers, estuaries, etc. [10] The EU and U.S. Environmental Protection Agency (USEPA) have listed 16 kinds of PAHs as priority pollutants [11] due to their toxicity, persistence, carcinogenicity and oestrogenic effects.

PAHs are classified into low molecular weight (LMW) PAHs (2–3 rings) and high molecular weight (HMW) PAHs (4–7 rings). LMW PAHs include naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), anthracene (An), phenanthrene (Ph) and fluorine (Fl), and HMW PAHs contain fluoranthene (Flu), pyrene (Py), benzo(a)anthrecene (BaA), chrysene (Chr), benzo (a) pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3cd) pyrene (InP), Benzo (ghi) perylene (BgP) and Dibenzo(ah)anthracene (DbA). LMW PAHs are petrogenic, whereas HMW PAHs are mainly derived from pyrogenic processes and the predominance of LMW over HMW PAHs is suggestive of their petrogenic origin.[12] Moreover, PAH isomeric ratios have been widely used to identify the possible sources of PAHs. Canadian interim sediment quality guidelines (ISQGs), probable effects levels (PEL), the effects range low (ERL) and the effects range medium (ERM) values of sediment are also used to estimate the adverse biological effects.

The study area, the *Chitrapuzha* tidal river system, receives considerable amount of domestic sewage, effluents from industrial units, fishery industries and the Cochin seaport, which handles large quantities of diverse industrial chemicals and petroleum products (*The Hindu*, 2011; *The New Indian Express*, 2015). Water and sediment quality of the *Chitrapuzha* River is thus a matter of grave concern because of impacts of chemical pollutants on the ecosystem. Scant reports are available on the distribution of heavy metals and fatty acid biomarkers;[13,14] however, none are available on hydrocarbon contamination in surface sediments, with special reference to source characterisation of AHCs and PAHs. The present study aims to explore the spatial and seasonal distribution of AHCs and PAHs in surface sediments of the Chitrapuzha River, to ascertain the possible sources of AHCs and PAHs, and to evaluate the contamination status of the sedimentary system using qualitative and quantitative indices based on AHCs and PAHs.

2. Materials and methods

2.1. *Study area*

The area of study (Figure 1), the Chitrapuzha tidal river system (9° 40′ and 10° 12′ N and 76° 10′ and 76° 30′ E), forms part of the Cochin estuarine system as well as the National Inland waterway No. 111 (9°52′ and 100°00′N and 76°15′ and 76°25′E). The river originates as a small stream from the high ranges in the eastern boundary of Kerala state and passes through a valley, and finally joins the southern arm of Cochin estuary. Numerous industrial units, including a diesel power plant (BDPP), a fertiliser manufacturing unit (FACT), an organic chemical factory (HOCL) and a petroleum refinery (BPCL), are located along the banks of the Chitrapuzha River. Shipping activities and the discharge and loading of crude oil and its products, and resultant localised oil spills introduce petroleum

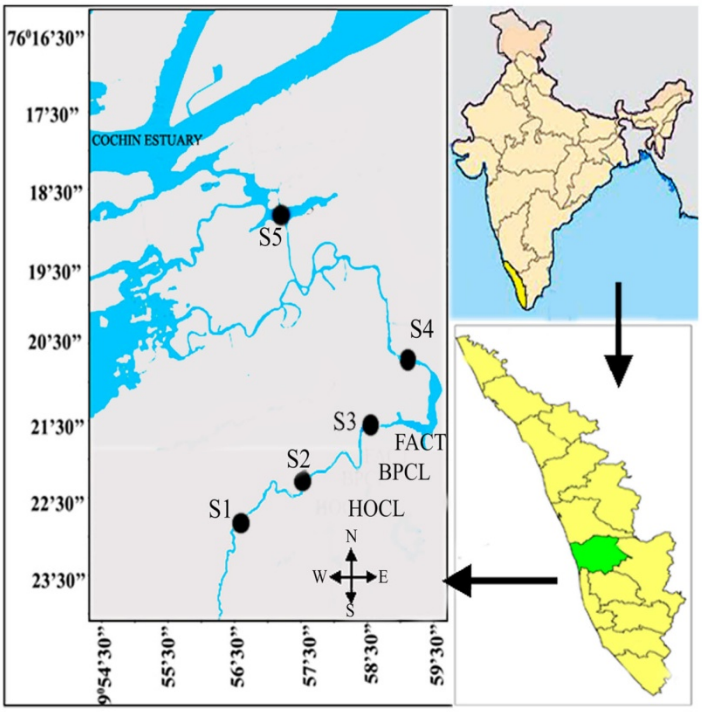

Figure 1. Location map of the study area.

hydrocarbons into the harbour estuary. Hydrocarbon-rich gaseous emissions from industries, road traffic and water transportation eventually contaminate the inshore water and sediment through dry and wet deposition. This area is under the profound influence of southwest monsoon accordingly, three seasons prevail, namely monsoon (MN; June–September), post-monsoon (POM; October–January) and pre-monsoon (PRM; February–May).

2.2. *Sampling*

Based on the aforementioned anthropogenic activities and water flow regimes, surface sediment samples were collected from five locations along the Chitrapuzha River (Figure 1). Three sediment samples were collected from each station, using a van Veen grab (0.042 m^2) during June 2012 (monsoon), April 2013 (pre-monsoon) and December 2013 (post-monsoon). Station one (S1) is beyond the influence of industrial pollution and has an average depth of 3 m. Stations 2 (S2), 3 (S3) and 4 (S4) constitute industrial zones, which are the recipients of industrial effluents. Station 5 (S5) is near the Thevara ferry point, and it receives discharges from apartments on the mainland as well as from the oil tanker berth, Cochin Port and Cochin Shipyard. The samples were packed in ice boxes for transportation to the laboratory where it was stored in a deep freezer (−20°C) till the analysis. All the analyses were carried out in triplicate and the average is reported.

2.3. *Grain size analysis*

Sediment grain sizes were determined by pipette analysis using wet sediment samples.

2.4. *Extraction of AHCs and PAHs*

AHCs and PAHs were extracted by the method described by Harvey [15]. The freeze-dried homogenised sediment samples (15–20 g) were extracted with the dichloromethane–methanol (2:1) mixture in a soxhlet apparatus for 72 h and activated copper was added to remove sulphur. The extracts were concentrated by rotary evaporation, and the residue was subjected to mild alkaline hydrolysis using 0.5 M KOH in methanol at 70°C for 6 h. The neutral fraction was extracted using *n*-hexane and submitted to a silica gel glass column for fractionation. The fractions containing AHCs were eluted with 50 mL *n*-hexane and the second fraction containing PAHs was eluted with 5 mL of hexane and 70 mL of methylene chloride–hexane (30:70). The elutants were concentrated to 0.5 mL under a stream of pure nitrogen and injected on a gas chromatograph equipped with flame ionisation detector (GC-FID) with a 30 m × 0.25 mm i.d. DB-5 column, 0.25 µm film thickness. For AHCs, the injection port was kept at 250°C and the detector temperature was maintained at 325°C. The oven temperature was held at 50°C for 5 min and then increased to 300°C at a rate of 3°C/ min and held for 5 min. Nitrogen was used as a carrier gas with a flow rate of 2 mL/min. Identification of individual compounds was achieved by comparison of retention times with those of standard compounds. Quantification was made based on the calibration with authentic standards (C7-C40, Sigma Aldrich). For PAHs, the GC oven had an initial temperature of 70°C (4 min hold) and was ramped at 8°C/ min to 320°C (8 min hold). The carrier gas was helium at a constant flow rate of 1.0 mL/min. Concentration of individual compounds was obtained by the external calibration method based on a four-point calibration curve for individual compounds (PAH mixt, Supelco, Sigma-Aldrich Corporation). The relative standard deviation in measurements ranged from 5% to 10% ($n = 3$), and the averaged recoveries of the studied PAH components were from 80% to 99%.

2.5 *Analytical quality controls*

All glassware used for the analysis was cleaned by a ultra-sonic bath followed by heating at high temperature in the oven. All reagents used were of analytical grade and standard solutions were prepared with Milli-Q water. All data were subjected to strict quality control procedures, including the analysis of procedural blanks and spiked samples with each set of samples analysed. None of the target compounds were detected in the procedural blanks for sediment samples. Spiked samples (10 ng of *n*-alkane and the PAHs mix standard) were determined with good precision and high recoveries. Calibration standards of *n*-alkanes and PAHs were purchased from Sigma-Aldrich (USA). Concentration of individual compounds was obtained by the external calibration method based on a four-point calibration curve for individual compounds. Analyses of triplicate sediment sub samples indicated *n*-alkane reproducibility to be typically ± 6% and PAHs reproducibility to be ± 8% of the mean concentration. Data were acquired and processed with M.S. Turbomass Version 5.4.2 and Total Chrom work station. All statistical analysis was done using

'Statistical Package for Social Sciences' (SPSS), version-13, to identify the sources of organic matter as well as the processes governing their distribution. For data exploration and description, data set was treated with principal component analysis (PCA) and hierarchical cluster analysis (HCA). In this study, squared euclidean distance measurement and Ward's method were used.

3. Results and discussion

3.1. *Textural characterstics*

Texture analysis (Figure 2) indicated that the sand fraction dominated in the upstream (S1) and downstream (S5) stations while silt particles predominated in the industrial area. The high clay content was reported at S5, while S1 recorded high percentage of sand. Percentage of clay was generally higher in all stations during the non-monsoon period, which is due to the settling of finer fractions of the sediment samples.[16] Contrary to this, stations S2, S3, and S4 in the industrial zones displayed higher values of silt during monsoon. This might be due to the spillage of phosphogypsum, a waste product from the adjacent premises of the fertiliser manufacturing industry.

3.2. *Aliphatic hydrocarbons*

A representative total ion chromatogram of *n*-alkane fraction extracted from the surface sediments of the study region is depicted in Figure 3, and it indicated the presence of *n*-alkanes ranging from C12 to C33. Average concentrations of individual *n*-alkanes are given in Table 1 with total concentrations (Σ*n*-alkanes) ranging from 7754 ± 188 to 41173 ± 196 ng/g. Maximum concentrations of *n*-alkanes (∑ alkanes) were reported in the sediments from the industrial zones (S2, S3 and S4) during the PRM season, while

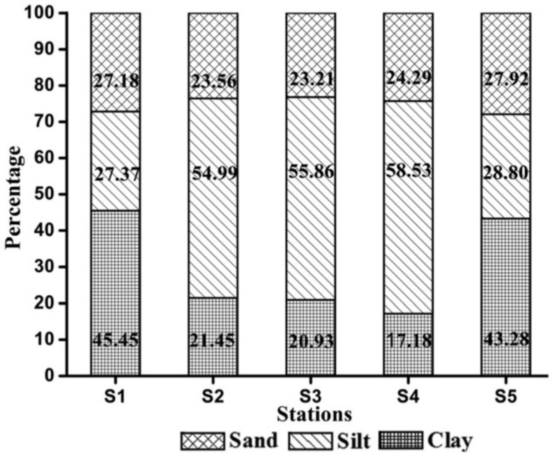

Figure 2. Contribution of sand, silt and clay in surface sediments of Chitrapuzha River.

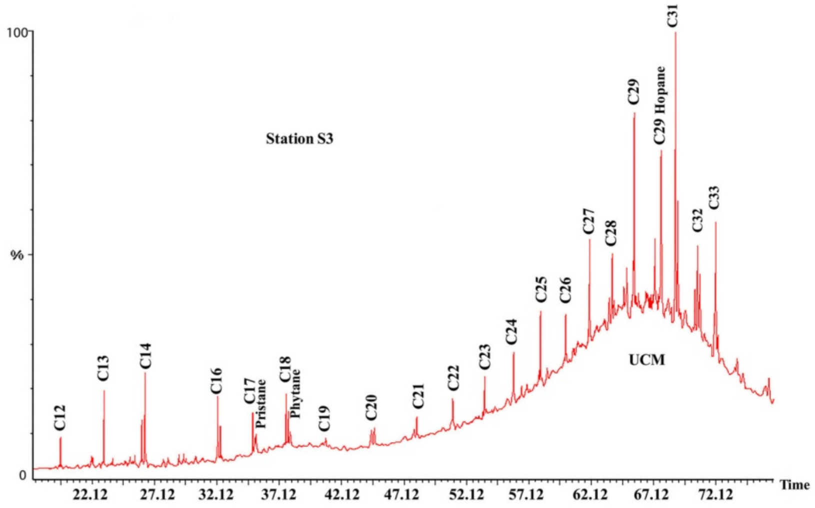

Figure 3. A representative total ion chromatogram of n-alkane fraction extracted from the surface sediments of Chitrapuzha.

Table 1. Average concentration of n-alkanes(± SD) recorded in the surficial sediments.

Stations					
n-alkanes(ng/g)	S1	S2	S3	S4	S5
C12	588 ± 62	2120 ± 113	1845 ± 128	1268 ± 111	256 ± 7
C13	980 ± 63	1873 ± 113	1697 ± 243	1029 ± 113	355 ± 6
C14	1519 ± 62	2450 ± 109	2313 ± 128	1772 ± 115	267 ± 17
C15	64 ± 11	1400 ± 112	742 ± 127	458 ± 112	91 ± 55
C16	1048 ± 60	2777 ± 111	1091 ± 127	1515 ± 113	183 ± 7
C17	58 ± 40	2436 ± 254	1744 ± 128	1531 ± 115	80 ± 6
C18	933 ± 61	1821 ± 112	1832 ± 128	794 ± 115	32 ± 6
C19	43 ± 6	1863 ± 114	1867 ± 128	1432 ± 115	48 ± 8
C20	1388 ± 58	1726 ± 115	1715 ± 127	786 ± 114	294 ± 4
C21	1358 ± 60	1888 ± 113	1489 ± 127	1348 ± 114	167 ± 12
C22	1213 ± 63	2404 ± 264	2148 ± 128	1029 ± 114	298 ± 15
C23	980 ± 62	1388 ± 113	1710 ± 128	938 ± 112	213 ± 18
C24	1011 ± 62	1761 ± 112	1788 ± 130	715 ± 113	407 ± 28
C25	959 ± 61	1453 ± 113	1103 ± 126	786 ± 114	434 ± 14
C26	803 ± 71	1145 ± 116	1164 ± 129	255 ± 31	512 ± 8
C27	659 ± 60	1764 ± 114	1166 ± 129	902 ± 115	645 ± 15
C28	378 ± 108	1204 ± 159	1313 ± 126	496 ± 114	611 ± 16
C29	708 ± 62	2759 ± 112	2554 ± 126	1786 ± 14	703 ± 5
C30	578 ± 63	1329 ± 112	1607 ± 127	521 ± 113	411 ± 13
C31	761 ± 60	3091 ± 121	2962 ± 29	1722 ± 113	857 ± 27
C32	471 ± 22	1320 ± 113	1466 ± 125	850 ± 115	317 ± 23
C33	1200 ± 24	1203 ± 112	1512 ± 128	892 ± 116	573 ± 10
Pristane	698 ± 66	677 ± 68	675 ± 65	358 ± 36	16 ± 6
Phytane	368 ± 32	691 ± 55	705 ± 63	251 ± 22	29 ± 11
∑ ODD	7771 ± 128	21,116 ± 204	18,546 ± 288	12,825 ± 262	4165 ± 164
ΣEVEN	9930 ± 212	20,057 ± 188	18,282 ± 244	10,001 ± 174	3589 ± 213

the minimum value was recorded at S5 during MN (Figure 4). This high average value could be attributed to the anthropogenic activities, growth of major industrial units and shipping port operations in this area. Moreover, several studies have indicated that fine-

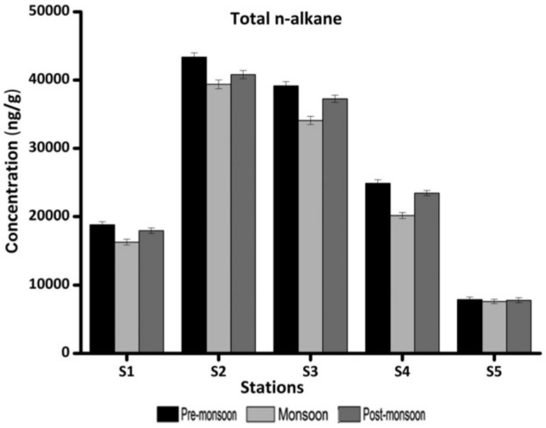

Figure 4. Seasonal and spatial variations of n-alkanes in the study area.

grained sediments (Figure 2) are more susceptible to accumulation and preservation of organic matter.[17] While, at S5, the sandy sedimentary texture as well as dredging operations to facilitate motor boat ferry service to adjacent islands may reduce the hydrocarbon contamination.[18] *n*-Alkane distribution in the study area showed an even over odd predominance at S1, and it is quite different from compositional patterns of *n*-alkanes observed in the marine and freshwater sediments. This indicates the deposition conditions and biological sources of *n*-alkanes, including plankton, a microbial reworking of algal detritus and recent biogenesis of lipid materials in the surface sediments.[19] Meanwhile, the higher concentrations of even number carbon chains at the industrial zones compared with other two stations (S1 and S5) might be attributed to the direct biogenic contribution from algae, bacteria, fungi and yeast species [20] as well as the direct influence from petroleum-derived inputs.[7,19] There is a strong possibility of direct petroleum inputs from the adjacent industries causing the *n*-alkane even predominance in and around the study region, especially at S2, S3 and S4, while the chance for such oil spillage is limited towards the upstream station S1. Thus, even predominance of LMW *n*-alkanes in sediments can be due to the inputs of both natural and anthropogenic organic matter which have differed in their relative proportions from S1 to S5. The strong odd ($\sum$Odd *n*-alkanes) to even carbon predominance of *n*-alkanes in the study area except at S1 revealed the prominent terrigeneous contribution from higher vascular plant wax.[19] Odd number carbon chains tend to dominate in biological materials, whereas petrogenic (fossil carbon) materials usually have a high prevalence of even numbered carbons ($\sum$ even *n*-alkanes). A strong odd and even number carbon chains in the industrial area demonstrated both biogenic and petrogenic inputs of organic matter.

To discriminate biogenic and anthropogenic sources of hydrocarbons in surface sediments, *n*-alkane indices such as CPI, UCM, average chain length (ACL), short chain/long chain ratio (SC/LC), and terrigenous/aquatic ratio (TAR) were also used (Table 2). For example, CPI differentiates *n*-alkane sources, and thus provides supportive evidence for the incorporation of different hydrocarbon sources. CPI is defined as a ratio of the

Table 2. Selected n-alkane indices and PAH isomeric ratios.

Stations					
Diagnostic ratios	S1	S2	S3	S4	S5
n-alkanes					
pr/ph	1.91 ± 0.26	0.98 ± 0.32	0.96 ± 0.16	1.51 ± 0.44	0.58 ± 0.37
C17/pr	0.08 ± 0.03	3.67 ± 0.27	2.61 ± 0.30	4.55 ± 0.46	5.52 ± 0.51
C18/ph	2.56 ± 0.38	2.66 ± 0.55	2.63 ± 0.31	3.60 ± 0.43	1.11 ± 0.21
TAR	12.56 ± 0.84	1.34 ± 0.28	1.52 ± 0.77	1.29 ± 0.27	10.37 ± 1.05
CPI^a	0.79 ± 0.25	1.03 ± 0.09	1.02 ± 0.05	1.28 ± 0.06	1.16 ± 0.13
SC/LC	1.08 ± 0.15	1.24 ± 0.62	1.01 ± 0.06	1.32 ± 0.18	0.36 ± 0.09
ACL	29.27 ± 2.1	29.16 ± 1.9	29.56 ± 2.4	29.34 ± 0.66	29.30 ± 0.53
PAHs					
Flu/Py	5.8 ± 0.65	0.89 ± 0.23	0.85 ± 0.31	0.87 ± 0.18	0.81 ± 0.44
Flu/Flu + h	0.57 ± 0.04	0.40 ± 0.04	0.27 ± 0.03	0.29 ± 0.14	0.28 ± 0.06
Flu/Flu + y	0.85 ± 0.23	0.48 ± 0.08	0.46 ± 0.07	0.45 ± 0.33	0.45 ± 0.29
Ph/An	5.14 ± 0.54	7.53 ± 0.62	14.35 ± 1.15	14.13 ± 0.88	14.07 ± 1.65
Chr/BaA	0.90 ± 0.04	1.06 ± 0.31	1.24 ± 0.04	1.15 ± 0.64	1.16 ± 0.29
InP/BgP + nP	ND	ND	ND	0.05 ± 0.04	0.1 ± 0.2

Notes: SC/LC = $\sum$(C12–C22)/$\sum$(C23–C33); TAR = C27 + C29 + C31/C15 + C17 + C19;
ACL = 25*C25 + 27*C27 + 29*C29 + 31*C31 + 33*C33/C25 + C27 + C29 + C31 + C33;
CPI^a = $\sum_{\mathbf{ODD}}$C13–33/$\sum_{\mathbf{EVEN}}$ C12–32; ND – not detectable.

summation of the odd carbon number homologues over a range, to the summation of even carbon number homologues over the same range.[21] In this study, CPI values were calculated according to Harji et al. [7] and the CPI^a (full range n-alkanes) values were in the range 0.79 ± 0.25–1.28 ± 0.06, and the values close to 1 (Table 2) indicated oil contamination except at S1.[22] However, CPI values alone cannot be employed to identify oil-derived *n*-alkanes in aquatic sediments, especially in cases where river bank vegetation supplies abundant amount of organic matter to the sediment. Therefore, the reality of oil contamination was further confirmed by the presence of UCM (Figure 3), a mixture of hydrocarbons present in significant quantities in petroleum, which are resistant to weathering and biodegradation processes.[13] UCM indicates the anthropogenic inputs of petroleum hydrocarbons and its magnitude are related to the degree of anthropogenic contribution.[14] It is noticeable that these oil-contaminated sediments are found in distinct areas only (S2 S3 and S4), while UCM was absent in the sediments from the upper and downstream portions of the study area.

The total input of *n*-alkanes C27 + C29 + C31 ranged from 12.0% to 28.4%, accounting for an average of 19.0%, and exhibited increased inputs in the estuarine region (S5) which reflected a dominant fluvial contribution of organic matter, while the total contribution of planktonic *n*-alkanes (C17 + C19 + C21) accounted for 3.8–18.9% with maximum at S4 and minimum at S5. Based upon these groups of *n*-alkanes, an index TAR [23] has been proposed to estimate the relative contribution of terrestrial- and marine-derived organic matter. TAR values ranged from 1.29 ± 0.27 to 12.56 ± 0.84 (Table 2) and the higher TAR values at S1 and S5 indicate the preferential preservation of terrestrial organic matter due to the local dominant fluvial contribution.[24] ACL values in the study area have no significant spatial seasonal variations and reported to be around 29 ± 1.5. However, the higher TAR values and elevated ACL at S1 and S5 further indicated the preferential preservation of terrestrial and planktonic organic matter in surface sediments.[25] SC/LC ratios of *n*-alkanes calculated were ranged from 0.36 ± 0.09 to 1.32 ± 0.18 and the ratio 1 at S3 indicated a major contribution of *n*-alkanes from petroleum sources while SC/LC value <1.0 at S5 might be due to the increased terrestrial and bacterial input.[7] On the other hand, ratios >1.0 at S2 and S4 indicated a mixed origin of algal, planktonic and petroleum hydrocarbon sources.[7,19]

Isoprenoid alkanes, pristane (Pr; 2,6,10,14-tetramethylpentadecane) and phytane (Ph; 2,6,10,14-tetramethyl hexadecane) were detected in the study region and the values ranged from 16 ± 6 to 698 ± 66 and 29 ± 11 to 705 ± 63 ng/g, respectively (Table 1). The geological formation of pristane and phytane strongly depends on the deposition conditions existing in the sedimentary environment. For example, under anoxic conditions the cleavage of a phytyl side chain of chlorophyll (a) yields phytol, which undergoes reduction to give phytane as the final product.[26] While the oxic environment promotes the conversion of phytol to pristane by the oxidation of pytol to phytenic acid followed by decarboxylation and reduction. These hydrocarbons are usually present in petroleum,[27] and have been used to assess the microbial degradation of petroleum residues in soils and sediments.[28] The highest concentrations of pristane and phytane were recorded in the surface sediments of the study region except at S5 (Table 1), and Pr/Ph value close to or lower than 1 at S2, S3 and S5 (Table 2) suggests petroleum contamination,[5] likely due to the influence of effluents from the adjacent petroleum manufacturing unit. While at S1 and S4, Pr/Ph ratios were >1, indicating the predominance of biogenic sources of hydrocarbons.[29]

3.2.1. *Principal component analysis (PCA)*

PCA was employed on data sets including *n*-alkanes, TOC and sediment grain size components. PCA indicated three principal components (PC1, PC2 and PC3) accounting for 81.53%, 10.81% and 4.0% (Table 3) of the total variance, respectively. PCA results focus

Table 3. PCA analysis of n-alkanes with textural characteristics and TOC.

Component	PC1	PC2	PC3
Sand	−0.96	−0.09	−0.17
Clay	−0.80	−0.27	−0.51
Silt	0.94	0.18	0.23
Toc	0.95	0.09	0.25
C12	0.70	0.54	0.44
C13	0.49	0.64	0.58
C14	0.53	0.44	0.71
C15	0.67	0.52	0.36
C16	0.55	0.20	0.67
C17	0.85	0.38	0.33
C18	0.41	0.68	0.59
C19	0.85	0.43	0.27
C20	.015	0.65	0.73
C21	0.42	0.31	0.85
C22	0.38	0.70	0.58
C23	0.41	0.62	0.60
C24	0.32	0.78	0.52
C25	0.35	0.54	0.72
C26	−0.04	0.92	0.36
C27	0.61	0.54	0.39
C28	0.42	0.88	0.15
C29	0.76	0.56	0.30
C30	0.41	0.85	0.29
C31	0.69	0.66	0.25
C32	0.68	0.64	0.32
C33	0.20	0.66	0.63
% variance	81.53%	10.81%	4.00%

on the first two principal components, which explained 92% of total cumulative variance. PC1 shows a high positive loading on the variables of *n*-alkanes C12, C15, C16, C17, C19, C29, C31 and C32, TOC and fine-grained sediments (silt) indicating the terrigenous signature and geochemical accumulation of organic matter with respect to the grain size.[16] PC1 was characterised by high loadings of odd number *n*-alkanes including C15, C17, C19, C27, C29 and C31. In general, odd number carbon chains tend to dominate in biological materials, whereas petrogenic (fossil carbon) materials have a high prevalence of even numbered carbons.[17] Therefore, PC1 is considered as a biogenic component. PC2 is distinguished by highly significant loading for *n*-alkanes in the range C18 to C33 except C19 and C21, indicating the biogenic contribution of organic matter and petrogenic-derived organic matter as the main component. The predominance of odd numbered *n*-alkanes with C27 and C31 is a characteristic of terrestrial higher plant debris [24] while C15 and C17 indicate aquatic sources.[26,30] Meanwhile, significant loading of *n*-alkanes with even carbon predominance (C12 to C22) in the sediments was derived mainly from the biogenic contribution of algae, bacteria and fungi [29] and also from petroleum-derived inputs.[7,31] Consequently, PC2 was defined as a biogenic and petrogenic component. Thus, the PCA analysis clearly established the natural and anthropogenic contributions of AHCs in the surface sediments of Chitrapuzha.

3.3. *Distribution and possible sources of PAHS*

Total concentrations of PAHs (ΣPAHs) ranged from 4906 ± 142 to 31688 ± 192 ng/g in monsoon, 5188 ± 122 to 33735 ± 166 ng/g in PRM and 5045 ± 112 to 32962 ± 115 ng/g in POM (Figure 5). Maximum concentration of ΣPAHs was observed at S4, followed by the estuarine region S5 during the PRM season. This result might be related to the combined effect of industrial inputs, coupled with the discharges from apartments on the main land, oil tanker berth, Cochin Port and Cochin Shipyard, close to this area. In general, PAHs' concentrations increase from the upstream (S1) to downstream (S4) portion of the study

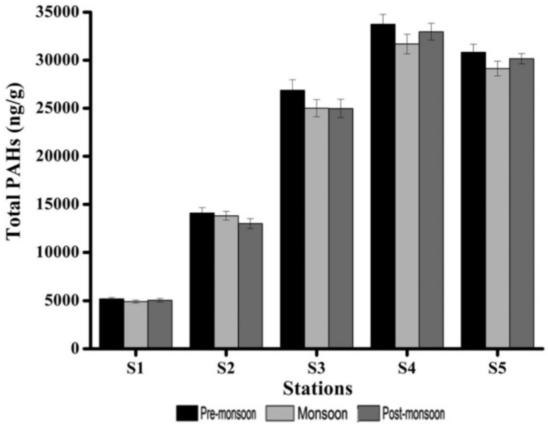

Figure 5. Seasonal and spatial variations of PAHs in the study area.

area, and then a slight decline at S5. This is attributed to the biodegradation and desorption of LMW PAHs during transport and deposition from upper to the lower reaches,[32] and also by the out welling of organic matter as a result of high tidal flushing. The average concentration and PAH profiles (Table 4) at the industrial and estuarine region indicated both pyrogenic and petrogenic sources of organic matter. Whereas low levels of PAHs with pyrogenic signature at S1 indicated that the upper reaches of Chitrapuzha were beyond the influence of industrial (petroleum) pollution. Moreover, the sedimentary texture characterised by the predominance of sand fraction has a significant role in the PAHs diversity of the depositional environment.[16]

Anthropogenic PAHs are formed mainly by two mechanisms: pyrogenic and petrogenic processes. At low to moderate temperature or from the combustion of coal,[33] low-molecular weight PAH compounds are abundant. While at higher flame temperature, such as combustion of petroleum fuels, the high molecular weight PAH compounds are dominant.[34] Thus, it is possible to classify the pollutant sources as either petrogenic or pyrolytic using the isomeric ratios between some individual PAHs (Table 2).[35] For example, Ph/An ratio <10 and Flu/Py ratio >1 are characteristics of pyrolytic origin,[36] while the values >10 and <1 are representative of petroleum-derived PAHs.[37] The calculated Ph/An ratio and Flu/Py ratios ranged from 5.14 ± 0.54 to 14.35 ± 1.15 and 0.81 ± 0.44 to 5.8 ± 0.65, respectively, and exhibited prominent contributions from the petrogenic origin of PAHs while pyrogenic inputs were predominant at S1. Similarly, Flu/(Flu + y) ratio less than <0.4 is consistent with petroleum-derived input; values between 0.4 and 0.5, PAHs mainly from combustion of petroleum and the ratio higher than 0.5 imply combustion of grass, wood or coal.[38,39] The observed values of Flu/(Flu + y) (0.45 ± 0.29–0.85 ± 0.23) established the prevalence of PAHs from petroleum sources in the surface sediments. Furthermore, discrimination of PAH sources can be achieved by using Chr/BaA ratio (>1.0 petrogenic and <1.0 pyrolytic).[36] The ratio Chr/BaA ranging from 0.9 ± 0.04 to 1.2 ± 0.04 confirmed the suspected petrogenic inputs of PAHs in the study area except at S1. The ratio InP/BgP + nP <0.2 points out to the petroleum origin of PAHs, while values ranging from 0.2 to 0.5 specify liquid fossil fuel combustion and the values >0.5 suggest grass, wood and coal combustion.[40] Data recorded at S4 and S5 (0.05 ± 0.04 and 0.1 ± 0.2) further clarify the petroleum contamination. Therefore, we can conclude that the PAHs detected in the study area are predominantly from pyrolytic sources at S1, while petrogenic inputs are the major sources at all other stations. Table 5 presents the ΣPAHs' concentrations in sediments at different locations around the world.

3.3.1. *Sources evaluations using PCA and HCA*

The PCA method, which is widely used to distinguish natural and anthropogenic inputs, was applied to explore the origin and geochemical factors influencing PAH distribution in sediments. PCA (Table 6) indicated three principal components (PC1, PC2 and PC3) accounting for 62.68%, 20.69% and 6.70% of the total variance, respectively. CA loading scores higher than 0.50 were considered and PC1 was characterised by high loadings of PAHs including Nap, Ace, Fl, Ph, An, Flu, Py, BaA, Chr, BaP and BgP. Moreover, BbF and InP also scored a significant eigenvalue. In general, the heavier PAHs are the result of combustion or pyrolitic processes and are usually absent in petrogenic sources.[52] Flu and Py are characteristic markers for pyrolysis or incomplete combustion while diesel engine

Table 4. Average concentrations of individual PAHs (± SD)and sediment quality quality guideline values.

PAHs (ng/g)	S1	S2	S3	S4	S5	TEL	PEL	ERL	ERM
Nap	121 ± 11.5	485 ± 8.02	1498 ± 103.03	2024 ± 85.4	2040 ± 72.79	34.6	391	160	2100
Acy	413 ± 10.06	1637 ± 380.9	1197 ± 69.67	1302 ± 16.25	1106 ± 30.6	20.2	201	44	640
Ace	816 ± 12.0	1612 ± 12.0	2084 ± 111.06	2154 ± 44.3	1847 ± 149.1	6.71	88.9	16	500
Fl	125 ± 11.5	599 ± 6.1	2126 ± 77.6	2667 ± 37.4	2508 ± 15.1	21.2	144	19	540
An	121 ± 17.2	265 ± 13.5	454 ± 49.2	446 ± 32.7	437 ± 60.3	49.9	245	853	1100
Ph	614 ± 15.09	1992 ± 70.08	6497 ± 446.4	6266 ± 38.5	6252 ± 60.9	86.7	544	240	1500
Flu	818 ± 12.0	1308 ± 13.1	2340 ± 310.5	2600 ± 134.5	2438 ± 50.3	113	1494	600	5100
Py	142 ± 11.5	1416 ± 8.7	2749 ± 48.8	3032 ± 34.69	3000 ± 48.8	153	1398	665	2600
BaA	182 ± 12.0	373 ± 10.2	1244 ± 51.8	1850 ± 79.1	1723 ± 73.1	74.8	693	261	1600
Chr	165 ± 13.0	397 ± 16.6	1538 ± 56.3	2117 ± 34.4	1997 ± 35.2	108	846	384	2800
BaP	812 ± 13.5	2350 ± 291.5	2389 ± 55.5	4050 ± 96.8	3463 ± 391.9	88.8	763	430	1600
BkF	ND	ND	ND	ND	ND	–	–	–	–
BbF	710 ± 14.0	1203 ± 215.5	1500 ± 141.3	2172 ± 29.6	958 ± 87.7	–	–	–	–
InP	ND	ND	ND	ND	ND	–	–	–	–
BgP	8 ± 13.8	ND	ND	2365 ± 406.9	2194 ± 256.6	–	–	–	–
DbA	ND	ND	ND	ND	ND	–	–	–	–
$\sum$LMW PAHs	2208 ± 77.2	6590 ± 136.3	13856 ± 264.5	14858 ± 249.6	14190 ± 288.7				
$\sum$HMW PAHs	2838 ± 16.6	7047 ± 144.5	11760 ± 192.0	18229 ± 226.8	15846 ± 193.4				

Notes: Nap, Naphthalene; Ace, Acenaphthene; Acy, Acenaphthylene; Fl, Fluorene; Ph, Phenanthrene; An, Anthracene; Flu, Fluoranthene; Py, Pyrene; BaA, Benz(a)anthracene; Chr, Chrysene; BbF, Benzo(b)fluoranthene; BaP, Benzo(a)pyrene; InP, Indeno(1,2,3 cd)pyrene; BgP, Benzo(ghi)Perylene.

TEL, threshold effect level; PEL, probable effect level; ERL, effects range low; ERM, effect range median; LMW, low molecular weight; HMW, high molecular weight; ND, not detectable.

Table 5. PAHs concentrations (ng/g) in sediments at different locations around the world.

Location	Range of average PAHs (ng/g)	Number of PAHs identified	References
Chitrapuzha, South India	5046–33087	16	Present study
Kim Nguu River, Vietnam	218–751	16	[41]
Songhua River, China	68–654	16	[42]
Zhanjiang Bay	42–934	16	[43]
Yangtze estuary, China	77–2937	16	[44]
Liaodong Bay, China	145–292	16	[45]
South Carolina, USA	64–26,288	16	[46]
Yellow River Estuary, China	11–252	16	[47]
James Ross Island, Antarctica	1–205	16	[48]
Mersey Estuary, UK	626–3766	16	[49]
Norwegian Harbour, Norway	2000–76,000	16	[50]
Olbia Harbour, Italy	160–770	16	[51]

Table 6. Varimax-rotated component matrix of PAHs obtained from PCA.

Compound	PC1	PC2	PC3
Nap	0.991	0.079	−0.053
Acy	0.319	0.608	0.618
Ace	0.823	0.520	0.121
Fl	0.972	0.164	−0.099
Ph	0.928	0.233	−0.108
An	0.914	0.287	0.056
Flu	0.951	0.238	−0.063
Py	0.941	0.279	0.033
BaA	0.988	0.069	−0.084
Chr	0.982	0.096	−0.107
BbF	0.539	0.717	−0.127
BaP	0.899	0.233	0.231
InP	0.520	−0.395	0.298
BgP	0.830	−0.204	−0.005
Toc	0.250	0.932	0.109
Sand	−0.256	0.126	0.854
Clay	0.091	−0.965	−0.082
Silt	0.171	0.960	0.154
% variance	62.68	20.69	6.70

emission predominantly contains Ph, Flu and py.[53] BbF is linked with fossil fuels combustion,[54] while, BaP and InP are associated with traffic emission, whereas BaA appears to be the main tracer with respect to gasoline.[53] The presence of a meaningful BgP loading lead to interpreting this component as a tracer of auto emissions.[18] Consequently, PC1 was defined as a petrogenic and pyrolitic fuel combustion component. PC2 presented its maximum positive loadings for PAHs such as Ace and Acy in sediments, which are abundant in petrogenic sources mainly caused by petroleum spills such as crude oil and fuel oil,[10] and interpreted as the petrogenic source of PAHs.

3.3.2. *Cluster analysis*

HCA is a multivariate technique whose primary purpose is to identify the homogeneous groups of individual PAHs occurring in the sedimentary system. Hierarchical clustering is the most common approach in which clusters are sequentially formed. It provides intuitive similarity relationships between any one sample and the entire data set, and is typically illustrated by a dendrogram/tree diagram.[55] The most similar objects are grouped first, and these initial groups are merged according to their similarities. The result of HCA is indicated in the hierarchical dendrograms (Figure 6) which distinguished the 14 individual PAHs into two statistically significant clusters, cluster-1 and cluster-2. The first group included the high-molecular-weight PAHs with four to six rings (Chr, BaA, BgP, BbF and InP) and some three-ring PAHs such as Nap, Acy and An. The first major group included three subgroups. The first subgroup contained Nap, BaA, BgP and Chr, all of them including four rings, with the exception of Nap. These PAHs are usually detected in pyrogenic sources, for example, combustion of coal, wood, vehicle fuel and waste tyre.[56] The second (Acy and BbF) and third subgroups (Ant and Inp) included the LMW PAHs and HMW PAHs revealing the influence of anthropogenic and natural activities on sediment contamination.[12] The second major group contained Ace, Flu, Fl, Pyr and BaP. The LMW PAHs (Ace and Fl) with three rings mainly from oil spill, volatilisation or combustion of petroleum, while HMW PAHs such as Flu, Pyr and BaP were typically released during combustion of coal, natural gas and vehicle exhaust of gasoline and diesel.[57] From the above statistical data, PAHs in the study area could be attributed to the mixed sources of petroleum combustion and biomass combustion. Moreover, the oil spill, volatilisation or combustion of petroleum was the major sources of PAHs in the industrial areas.[39]

3.4. *Toxicological significance of PAHs in sediments*

To evaluate the environmental contamination posed by PAHs in surface sediments, the Canadian interim sediment quality guidelines,[58] which sets probable effect levels (PELs) and threshold effect level (TEL) as tools for interpreting the significance of PAHs in sediments has been followed (Table 4). Moreover, the sediment toxicity guideline of the US National Oceanic and Atmospheric Administration provides two values: (ERL, adverse effects of the 10% of tests in a biological effects data base) and effect range median (ERM, adverse effects of the 50% of the tests) [59] to estimate potential biological

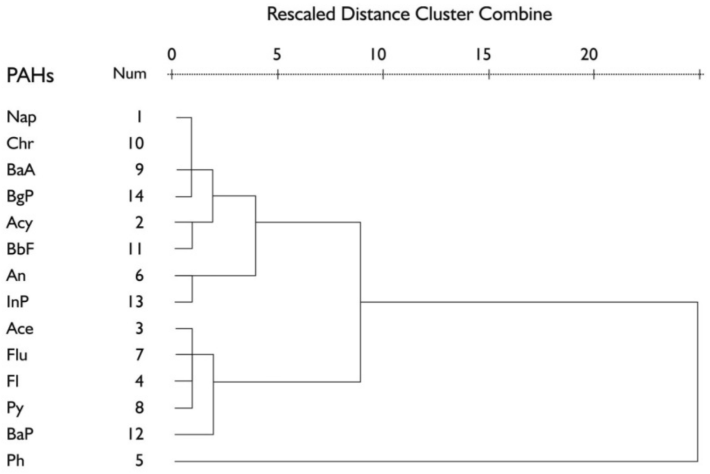

Figure 6. HCA result.

effects. PAH concentrations below the TEL values do not cause adverse effects to aquatic organisms, while concentrations above the PEL are often associated with severe adverse biological effects.[58] Similarly, ERL values indicated possible adverse biological effects on aquatic organisms, while ERM values suggested a great possibility of posing detrimental biological effects on aquatic organisms.[59] Table 5 presents the ERL, ERM, PEL and TEL values of PAHs proposed by the sediment quality guidelines and the average concentrations reported in the study area. Based on the above criteria, it can be concluded that current concentrations of PAHs in sediments of Chitrapuzha would be likely to cause adverse effects.

Additionally, carcinogenic potency associated with a given PAH can be calculated using an index called B [a] p equivalent concentration,[44] developed by the USEPA. The toxicity equivalency factor (TEFi) is an estimate of the relative toxicity of a chemical compared to a reference chemical. Benzo (a) pyrene was chosen as the reference chemical because the chemical toxicity of the compound is well characterised. The total toxic B [a] P_{eq} for other PAHs was calculated using the following equation:

$$\text{Total B[a]}P_{eq} = \sum \text{i}C_i{}^{*}\text{TEF}_i,$$

where C_i is the concentration of individual PAH (BaA, BaP, BbF, BkF, InP, BgP and Chr) and TEFi is the corresponding toxic equivalency factor. US EPA calculated TEFi values for BaA, BaP, BbF, BkF, InP, BgP and Chr are 0.1, 1.0, 0.1, 0.1, 0.1, 0.01 and 0.01, respectively.[60] The total B [a] p_{eq} values at different sampling sites varied from 904 to 4502 ng/g, and the industrial zones and estuarine region exhibited higher BaP_{eq} than the reference site. Estimating total B [a] P_{eq} is a suitable approach of assessing the effects of anthropogenic sources in the sedimentary environment. However, this method might underestimate the risk due to the fact that not all PAHs are considered.[53] Adverse effects on the organism and/or ecosystem level in surface sediments of the Chitrapuzha River can be used as an early warning needle of the potential human health impact. Continued research is necessary to establish the most appropriate indicators to use in describing the sediment condition and the appropriate weighting factors for combining them for an overall pollution assessment.

4. Conclusions

The concentration levels of AHCs and 14 priority PAHs in the surface sediments of River Chitrapuzha were analysed during pre-monsoon, monsoon and post-monsoon seasons. The predominance of short chain, and long chain, odd carbon number *n*-alkanes indicates autochthonous and allochthonous inputs of organic matter in the surface sediments. The higher TAR values and elevated ACL at S1 and S5 showed the preferential preservation of terrestrial-derived organic matter as well as higher plant wax esters. Moreover, the significant loading of *n*-alkanes with even carbon predominance (C12–C22) directed the biogenic contribution of algae, bacteria and fungi. The calculated *n*-alkane isomeric ratios and UCM indicated petroleum as the major source in the industrial zone stations. Among the five sites studied, station S4 in the industrial zones was found to be the hot spot attaining highest PAHs concentration, followed by the estuarine region, S5. Predominance of two–four ring PAHs indicated relatively recent local sources of PAHs, and the molecular indices based source apportionment revealed that the PAH distribution profile was dominated by petrogenic-derived sources. The calculated carcinogenic potency of PAH compounds was significant at the present level of sediments in Chitrapuzha. The proximity to various anthropogenic sources of pollutants warrants a continual monitoring programme for inorganic and chemical organic compounds in sediments, water and biota in order to have an effective coastal management programme to protect the ecological integrity of this valuable ecosystem and the health of humans associated with it.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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