DISTRIBUTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE COCHIN ESTUARY AND BACKWATERS

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By

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Dedication

To my sons Louis Ndegwa, Shadrack Nzaka and Joseph Vangu for enduring my long absence from home.

Certificate

This is to certify that this thesis is an authentic record of the research carried out by Mr. Munga, D. under my supervision and guidance in the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of **Philosophiae Doctor** *of the Cochin University of Science and Technology.*

Cochin – 16
July 2002.

Prof. Dr. Jacob Chacko
Supervising Teacher.

DECLARATION

I hereby declare that this thesis entitled "DISTRIBUTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE COCHIN ESTUARY AND BACKWATERS" is an authentic record of research work carried out by me under the supervision and guidance of Dr. Jacob Chacko, Professor, Department of Chemical Oceanography, School of Marine Sciences, in partial fulfilment of the requirements for the Ph.D. degree of Cochin University of Science and Technology and that no part of it has previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title or recognition.

Cochin-16
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MUNGA, D.

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Preface

The Cochin backwaters is a multiple-use system of great socio-economic importance to the community. Activities that impact directly on the aquatic ecosystem include water transport and harbour operations, fishing, aquaculture, sand mining, coir making and upriver irrigation. Increasing urbanisation and industrialisation along the shores of the water system have inevitably exacerbated the problem of waste management and pollution control.

Polycyclic aromatic hydrocarbons (PAHs) are produced naturally by the diagenetic transformation of organic matter to fossil fuels. However, the PAH load in the environment is enhanced by the extraction and utilisation of fossil fuels. Indeed, urban and domestic activities, such as the manufacturing industry, land-transport and domestic fires, are significant sources of the contaminants. Thus, the increasing production and load of PAHs in the environment is a direct consequence of the socio-economic activities in the neighbourhood. It follows that initiatives at controlling environmental contamination actually touch on the socio-economic culture of the community.

It cannot be gainsaid that environmental assessment provides the scientific basis for viable environmental management initiatives. This thesis, therefore, is the culmination of an attempt to contribute to the growing database on the environmental quality of the Cochin estuarine system. The thesis is presented in 7 chapters as follows;

Chapter 1: Introduction. Pertinent sources of PAHs in the study area are described and factors influencing their distribution and pathway in the aquatic environment are briefly highlighted.

Chapter 2: Materials and methods. Detailed descriptions of the field and laboratory analytical techniques applied in the course of the study are presented.

Chapter 3: Water quality and physico-chemical parameters. Results of hydrographical parameters recorded are presented and discussed with reference to the general water quality.

Chapter 4: Variability of PAH concentrations with hydrographic factors. The variation of concentration levels of the contaminants at a point station under the dynamic estuarine processes is illustrated to highlight the complexity of factors influencing the distribution and speciation of PAHs in the water column.

Chapter 5: Distribution of PAHs in estuarine backwaters. Results of the concentration levels of PAHs in the study area are presented and the spatial and temporal distribution, and important sources discussed.

Chapter 6: Characteristics of PAH assemblages. The PAH distribution patterns are analysed with the view of further elucidating the sources of the contaminants in the aquatic system.

Chapter 7: Conclusion. Overall concluding remarks and recommendations are presented.

A summary of the thesis provides an overview of the work accomplished and salient findings. The thesis ends with a compilation of literature cited.

CONTENTS

Chapter 1

INTRODUCTION

The ubiquity of trace levels of polycyclic aromatic hydrocarbon (PAHs) contaminants in the environment became more evident with the development of sensitive resolution techniques for the analysis of environmental samples. The concern and interest in PAHs arose primarily from the realisation that some of them are carcinogenic to Mammals (Grover, 1973; Grimmer, 1983; Mastrangelo *et al.*, 1996; Mehlan *et al.*, 1997). Early observations on occupational bronchial carcinoma of gas workers and coke plant workers were attributed to the inhalation of PAHs generated in the furnaces. Subsequent studies on carcinogenic properties of particularly benzo[a]pyrene on mammals and population groups experiencing occupational exposure to PAHs raised further concern on the public health. Indeed, contemporary direct sources of PAHs to man include cigarette smoke, automobile exhaust and industrial emissions.

PAHs consist of two or more fused aromatic rings, forming plannar structures with naphthalene ($C_{10}H_8$) being the member with the lowest molecular weight. Graphite, an allotropic form of elemental carbon, is considered the ultimate fused ring aromatic compound. The compounds of primary environmental concern include a large number of PAHs from naphthalene (MW 128.16) to coronene (MW 300.36) differing in the number and position of aromatic rings, and in the number, chemistry and position of substituents on the parent structure. Variable physical and chemical characteristics, such as, an almost logarithmically decreasing vapour pressure and aqueous solubility with increasing molecular weight, influence their behaviour and distribution in the environment and effects on biological systems. PAHs may be categorized into two molecular weight classes based on physical, chemical and biological properties; lower molecular weight 2-3 ring aromatics composed of naphthalenes, fluorenes, phenanthrenes and anthracenes, and higher molecular weight 4-7 ring structures that include chrysene to coronene. In comparison, the former group of PAHs exerts significant acute toxicity to aquatic organisms. On the other hand most proven PAH carcinogens occur in the latter group.

1.1 Sources of PAHs in the Aquatic Environment

The main primary source of PAHs in the environment is the diagenetic transformation of organic material into fossil fuels. The process occurs at low temperatures, 100-150 °C, to form crude oil and coal, both of which are composed of complex mixtures of PAHs. However, it is the various anthropogenic activities that increase the load of PAHs in the environment, and they include industrial activities, such as pyrolysis of kerosene to form benzene, toluene and other organic solvents, and oil refinery operations (Neff, 1979; UNEP/IOC/IAEA, 1992; GESAMP, 1993). Combustion of fossil fuels in industrial and domestic boilers, for electricity power generation, in internal combustion engines, and forest fires produce emissions rich in PAHs. The PAHs may reach the aquatic environment through industrial waste effluents, domestic wastewater discharged into aquatic systems, and spillages of petroleum and its products into water bodies. Airborne PAHs may eventually be introduced into water bodies by wet deposition, dry particle deposition and gas exchange at the air-water interface. Atmospheric PAHs may also be deposited on land and eventually reach the aquatic environment through surface runoff. A minor natural source of PAHs to the aquatic environment is recent biosynthesis. The diverse sources of PAHs can be differentiated by the composition of the hydrocarbon assemblages.

1.1.1 Chemical composition of fossil fuels

PAHs in petroleum

Crude oil or petroleum is a complex mixture of many thousands of organic compounds, of which hydrocarbons predominate, usually representing 50-98% of the total composition of most crudes. 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 reservoirs. On average, crude oil is composed of about 30% alkanes, 50% cycloalkanes, 15% aromatics and 5% nitrogen, sulfur and oxygen heterocyclic compounds (UNEP/IOC/IAEA, 1992) and include varying concentrations of organometals of vanadium, nickel, iron, sodium, calcium, copper and uranium. Refined petroleum products are predetermined boiling range fractions of the starting crude oil and may contain significant quantities of PAHs. Characteristically, crude oils contain significant concentrations of alkyl-substituted naphthalenes (Mono- and di-methyl naphthalenes) and phenanthrenes (mono-and di-methyl phenanthrenes).

PAHs in coal

Coal consists of a complex macromolecular material of variable composition. The pyrolysis of coal produces a large number of complex PAHs and heteroaromatic compounds that have been found difficult to relate directly to the original structure. However, it has been determined that the primary products of coal liquefaction are PAHs, and nitrogen, oxygen and sulfur heterocyclics (Later *et al.*, 1986). The liquefaction PAHs include 3-, 4- and 5-ring structures, such as phenanthrenes, fluorenes and benzo[a]pyrene.

1.1.2 Formation of PAHs by pyrolysis of organic matter

It is recognized that a major proportion of the PAHs in the environment is derived from the incomplete combustion or pyrolysis of organic material. It has been proposed, that during pyrolysis, complex organic molecules are cracked to lower molecular weight free radicals, a process followed by pyrosynthesis in which the free radicals containing one, two or more carbon atoms rapidly combine to produce PAHs (Badger *et al.*, 1960).

There are several factors or conditions that affect the yield and molecular weight distribution of PAHs formed by pyrolysis. These include the chemical composition of the fuel, pyrolysis temperature and duration of the exposure to elevated temperatures. Of the pyrolysis conditions, temperature has a significant influence on PAH generation. In general, high pyrolysis temperatures favour the formation of unsubstituted over alkyl-substituted PAHs (Blumer, 1976; Youngblood & Blumer, 1975). For instance, in coal coking where temperatures reach 2000°C, the pyrolytic products consist of a simple mixture of unsubstituted parent PAHs. In open flames with temperatures between 400-800 °C, relatively higher proportions of alkyl substituted PAHs are formed. As pyrolysis temperatures decrease, the ratio of alkyl-substituted to unsubstituted PAHs increases. Ultimately, crude oils which are usually formed at temperatures below 150 °C, have a predominance of alkyl-substituted over the parent PAHs. Consequently, it is to a certain extent possible to infer the source of PAH assemblages by the distribution of alkyl substituted homologues, particularly differentiating between freshly spilled petroleum or crude oil and incomplete combustion sources. However, weathering processes, which include preferential microbial- as well as photo-oxidation of alkyl substituted PAHs tend to modify the composition of oil to resemble that of combustion generated hydrocarbon products.

1.1.3 Biosynthesis of PAHs

Information on the direct synthesis of PAHs by aquatic organisms is at most inconclusive (Soclo *et al.*, 2000). Relatively more convincing is the indirect biosynthesis of PAHs by the reduction of polycyclic quinone pigments (Aizenshtat, 1973; Wakeham *et al.*, 1980). Polycyclic quinones synthesized by bacteria, fungi, higher plants and some animals include naphthaquinones, common in flowering plants, e.g. Vitamin K2, anthraquinones and perylenequinones. Under reducing conditions that may occur in anaerobic basin sediments and waterlogged soils such aromatic quinones are readily reduced to the corresponding hydroquinones and subsequently to the parent PAH. For example, high concentrations of perylene, unto 260 ppb, were reportedly found in sediments off Southern California at water depths of 600-2060 m and were attributed to indirect biosynthesis processes (Orr & Grady, 1967; Aizenshtat, 1973). It was hypothesized that perylene is formed in anaerobic sediments through reduction of perylenequinones of biological origin. Sedimentary PAH assemblages arising from biosynthesis are typically quite simple in composition.

1.2 Distribution of PAHs in Natural Waters

Once PAHs enter an aquatic system a variety of physical and chemical processes, including biological interaction influence their distribution in the water column. These processes include solubilization, partitioning between water and particulate matter, hydraulic transport, bioaccumulation, photo- and biodegradation, sedimentation and recycling, volatilisation and gas exchange at the air-water interface.

1.2.1 PAH transfer at the air-water interface

The air-water interface is essentially the surface microlayer and plays a vital role in the transfer of substances between the atmosphere and water column, PAHs being no exception. In the atmosphere the low molecular weight (2 – 3 rings) PAHs exist partially in the gaseous phase, whereas the relatively less volatile high molecular weight components prefer the particulate phase. Gaseous PAHs may be introduced into the sea-surface through precipitation or by gaseous exchange at the air-water interface. Particulate PAHs may be deposited on the sea-surface (and on land) in the wet form through precipitation. Particulates may also be deposited as dry fallout by gravitation supported by Brownian movement and trapping by whitecap bubbles on the sea-surface.

The thickness of the surface microlayer is operationally determined by the sampling procedure and varies between 0.03 to 1000 μm (Cincinelli *et al.*, 2001; Falkowska, 1999a; Carlson *et al.*, 1988; van Vleet & Williams, 1980; Larsson *et al.*, 1974; Harvey, 1966; Garret, 1965). The chemistry and composition of the microlayer varies considerably over the range depicting internal stratification (Falkowska, 1999a).

The chemical and biological composition of the microlayer is influenced by environmental factors, which are induced by atmospheric conditions, such as solar radiation, temperature, rain and winds (Carlson, 1983; Falkowska, 1999a). Surface winds create waves, which disturb and break up the microlayer. Waves produce larger surface area for evaporation and other sea-air transfer processes. Strong wind action and large waves lead to disturbance of the microlayer and spray formation, an important transfer process.

It is well recognized that in the microlayer various chemical and microbiological components of seawater undergo accumulation relative to the bulk of the water column, such as dissolved organic matter and particulate organic matter (Falkowska, 1999b). The microlayer is thus essentially organic in nature and many organic pollutants tend to concentrate therein, especially hydrophobic contaminants such as PAHs that adhere to particles and colloids (Liu & Dickhut, 1997). It is understood that rising air bubbles can sorb sub-surface organic material in the water column and burst at the microlayer thereby releasing both surface and sub-surface compounds to the atmosphere (Davey *et al.*, 1990).

1.2.2 Solubility and transport

PAHs have low aqueous solubilities reflecting their non-polar and hydrophobic nature. Solubility generally decreases as the number of aromatic rings or molecular weight increases. Thus, the most soluble PAH, naphthalene has a solubility of about 30 ppm, while 5-ring PAHs have solubilities in the 0.5 – 5.0 ppb range in distilled water. Alkyl substituted PAHs tend to have relatively lower solubilities than the unsubstituted parent PAHs. Some of the exceptions are; benz[a]anthracene is less soluble than methyl or ethyl benz[a]anthracene, and chrysene is less soluble than methyl and dimethyl chrysene (Davis *et al.*, 1942 cited by Neff, 1979). Linear PAHs are generally less soluble than corresponding angular or pericondensed isomers. Thus, anthracene is less soluble than phenanthrene and naphthacene is less soluble than chrysene, benz[a]anthracene or triphenylene. Generally PAHs are slightly less soluble in seawater than freshwater, a consequence of salting out. Solubilities of PAHs tend to increase with temperature. In natural waters, surfactants such as humic and fulvic acids and other surface-active substances may act as PAH solubilizers. However, contrary to expectations Liu and Dickhut (1997) found the solubility of relatively more hydrophilic PAHs increasing with DOC in the surface microlayer. The observation was explained as the dominance of kinetic factors, such as desorption and gas exchange rates, in influencing PAH enrichment in the surface microlayer. Dissolved PAHs are subject to transport by the hydraulic movement of water, with the residence time of the contaminant determined by the residence time of the water body.

1.2.3 Water particle partitioning

Due primarily to the hydrophobic nature and low solubilities, PAHs tend to sorb onto particulate matter. At equilibrium, this partitioning is described by the water-particle partition or distribution coefficient K_p or K_d defined as,

$$K_p \text{ or } K_d = \frac{\text{PAH concentrations in particulate phase (ng kg}^{-1}\text{ dry weight)}}{\text{PAH concentrations in dissolved phase (ng l}^{-1})}$$

While K_p is the theoretical coefficient, K_d usually refers to the observed partitioning coefficient. In practice, however, particulates are most often collected by filtration through glass fibre filters with nominal pore size cutoffs of 0.7 to 1.0 μm, which allow submicron particles and colloidal forms into the dissolved phase. Thus, the observed K_d is usually less than the actual K_p.

Both particle geochemistry and compound properties influence partition coefficients. Particle properties include organic carbon fraction, size/shape and type. Particle sources are either biotic or abiotic and may be allochthonous (due to tributary inputs, runoff, erosion and atmospheric deposition) or autochthonous (primary production, faecal pellet production and precipitation). The types and sizes of particles in natural water are dynamic due to biogenic processing, aggregation and mineralization. The organic carbon fraction is the most important particle property affecting partitioning of non-ionic hydrophobic compounds. PAHs tend to associate strongly with the organic carbon portion of the particulate. Thus, differences in partitioning behaviour among different types of particulates is normally eliminated by normalizing K_d to the organic carbon fraction resulting in the organic carbon partition coefficient K_{oc}, which should be relatively independent of particle type for a given particle size (Karickoff *et al.*, 1979; Mackay 1982). K_{oc} has been found to be positively correlated with the specific surface area of sorbent material and hence negatively correlated with particle size.

In natural waters, partitioning can be influenced by dissolved organic macromolecules, such as humic and fulvic acids. They may solubilize and stabilise PAH contaminants and affect their bioavailability and availability for partitioning (Leversee *et al.*, 1983; Wijayaratne & Means, 1984; McCarthy & Jimenez, 1985). These observations contributed to the observed low field K_d values (compared to the actual K_p) and led to the consideration of a 3-phase system, *i.e.* freely dissolved, particulate and colloidal phases (Gschwend & Wu, 1985; Baker & Eisenreich, 1989; Saliot *et al*, 1990). The fate of, particularly, the highly hydrophobic PAHs (log $K_{ow} > 4$) is closely linked to the fate and residence time of associated particulate matter.

The partitioning process affects transport and fate of hydrophobic contaminants. The process facilitates rapid removal of particulate associated contaminants by sedimentation. Particulate associated contaminants may be less available for such processes as volatilisation and degradation, but more available for food chain uptake and sediment transport.

1.2.4 Sedimentation of PAHs

The sedimentation of suspended particulate matter (SPM) and incorporation of PAHs in bottom sediments are important processes in the removal of the hydrophobic contaminants from the water column. Recycling processes at the water sediment interface include the detrital or microbial food web and resuspension (Swackhamer & Eisenreich, 1991). Incorporation of deposited SPM associated PAHs is made possible where bottom currents allow minimum resuspension and/or further transport process. The burial of the organic contaminants and distribution in the sediments is influenced by bioturbation, caused by the activities of benthic organisms and mixing of surficial sediments (e.g. Robbins, 1986; Schaffner *et al.* 1997). In sediments PAHs partition into the dissolved phase of pore-water, associate with dissolved organic matter (DOM), and may undergo degradation and burial. The distribution of PAHs, and other hydrophobic organic compounds in general, between sediment and pore-water compartments is influenced by sediment characteristics (type, particle size, organic carbon fraction etc.) and dissolved organic carbon (DOC) content. It is reckoned that in sediments most hydrophobic organic compounds in pore-water is bound to DOM colloids of humic and fulvic substances.

1.3 Degradation of PAHs in the Aquatic Environment

PAHs in the aquatic environment are subject to degradation through abiotic and biotic degradative processes. The most important among the processes are photooxidation, chemical oxidation and biodegradation and/or biotransformation.

1.3.1 Photooxidation of PAHs

In the water column the photo–induced oxidation of PAHs occurs by reaction with singlet oxygen, hydroxyl free radicals, ozone and other oxidising agents. The most common photooxidation reaction is the formation of endoperoxides, which on further photolysis or pyrolysis result in ring cleavage and dealkylation to produce a variety of oxidation products Neff (1979). It is understood that photooxidation involves an energy transfer from the triplet state of PAH yielding singlet oxygen ($^1O_2$), which in turn reacts with excited PAH to yield the peroxide or dione. Singlet oxygen from other sources can also similarly react with the PAH. Another photo-induced mechanism is an electronic transfer oxidation involving a PAH-oxygen electron transfer pair. Sigman *et al.* (1998) described a mechanism for electron transfer oxidation through excitation of pyrene-oxygen contact charge transfer pairs, with the photoproducts composed of mainly 1,6-pyrenequinone and 1,8-pyrenequinone.

Photooxidation of PAHs in natural waters may be initiated by the action of photosensitizers, which catalyse the production of electronically excited singlet oxygen from ground state O_2. In the photooxidation of aromatic compounds Ehrhardt & Petrick (1984) used anthraquinone as a photosensitizer because it was found to occur in seawater, whereas Klein & Pilpel (1974) used 1-naphthol.

Studies by Paalme *et al.* (1990) showed that photooxidation was more effective if PAHs are adsorbed on a solid mineral carrier, such as Al_2O_3 or SiO_2, which is of special significance to atmospheric distribution of the contaminants on particulate matter in the form of smoke or soot. Benz[a]anthracene in seawater was found to degrade three times faster under tropical conditions of sun radiation and temperatures than in temperate zones. Another study on the factors affecting the photooxidation of benzo[a]pyrene adsorbed on calcite particles in water revealed that the rate of degradation increased with illumination, oxygen concentration and temperature (Suess, 1972). On the other hand the rate of degradation was not influenced by the concentration of benzo[a]pyrene (range 0.08 – 1.3 $\mu g\ g^{-1}$), pH (range 7-10) or increasing ionic strength of the aqueous mixture. The conclusion was that in natural waters benzo[a]pyrene photooxidation will show seasonal variations due to changes in solar radiation, temperature and dissolved oxygen. The degradation rate will decrease with decreasing depth because of diminishing light owing to absorption by water and suspended matter, and decreasing temperature and dissolved oxygen. Photooxidation of benzo[a]pyrene is expected to be negligible in bottom sediments devoid of solar radiation and oxygen.

Photooxidation is known to selectively degrade alkylated aromatic compounds, in direct contrast to biodegradation. Thus, it is quite possible that a photooxidised and biodegraded crude oil might show a PAH "fingerprint" similar to fresh oil. In this case the use of conservative biomarkers, such as hopanes, and also some molecular indices can be considered (Garret *et al.*, 1998).

In surface waters, dissolved organic matter such as fulvic and humic acids can be photolysed to produce organic free radicals and oxidants such as H_2O_2. In natural water heterogeneous reactions probably play an important role in the photooxidation of PAHs. For instance, H_2O_2 was shown to be produced by green and blue-green algae (Zepp *et al.*, 1987 cited by Swackhamer & Eisenreich, 1991), whereas Readman *et al.* (1982) attributed the removal of naphthalene and possibly phenanthrene and anthracene from the estuarine environment to heterotrophic activity.

1.3.2 Biodegradation of PAHs

In the course of evolution microorganisms have been exposed to numerous xenobiotics, mainly introduced into the biosphere through anthropogenic activities, thereby adapting to their intrusive presence by developing appropriate enzymes, which aid in metabolising such chemicals. Significant effects associated with biodegradation include mineralisation, detoxification cometabolism, activation and defusing (Ashok & Saxena, 1995). Of particular significance is cometabolism or cooxidation in which the metabolised xenobiotic does not serve as a source of nutrient and/or energy. This process demonstrates that for biodegradation to occur it is not essential for the xenobiotic to serve as a source of carbon and energy.

Metabolism of PAHs by aquatic bacteria and fungi

There are several species of bacteria that are capable of partial or complete metabolism of PAHs. Ashok & Saxena (1995) presented a review of species of bacteria capable of metabolising PAHs. The reaction is initiated by the introduction of two hydroxyl groups in the ortho positions as in catechol or in the para positions as in gentisic acid, through a dihydrodiol intermediate. This is brought about by the incorporation of both atoms of molecular oxygen into the aromatic nucleus. For example, naphthalene is oxidised by *Pseudomonas putida* to cis-1,2-dihydroxy 1,2– dihydronaphthalene. Oxidation of the dihyrodiol to 1,2-dihydroxynaphthalene followed by ortho-cleavage yields, by way of intermediates, salicyclic acid, which is degraded through catechol to CO_2 and H_2O (Neff, 1979). Microorganisms are unable to completely oxidize the higher PAHs and thus utilize them as sole carbon sources. However, several species of bacteria can catalyse the oxidation of the PAHs, including the carcinogens benzo[a]pyrene and benz[a]anthracene, to produce various phenolic and acidic metabolites. In this case also, cis-hydroxylation is the principal mechanism for the commencement of the degradation process (Neff, 1979).

Fungi (yeasts and molds), unlike bacteria but like mammals possess a cytochrome P-450 (a heme protein) enzyme system that initiates the degradation process. In this case, a trans-dihydrodiol is produced through an arene oxide intermediate, in contrast to the bacterial pathway in which a cis-dihydrodiol is produced through a dioxetane intermediate.

The successful biodegradation of PAHs depends on a number of intrinsic and extrinsic factors. These factors include physico-chemical properties of the PAH, which include molecular structure, size, the presence of substituents, nutrient status, lipophilicity, aqueous solubility, volatility and concentration, aqueous solubility, volatility and concentration. Influential environmental factors include pH, temperature, oxygen availability, salinity, light intensity, bioavailability, inorganic nutrients and the microbial ecology. PAHs resistance to biodegradation increases with molecular weight or the number of fused aromatic rings. The rates of biodegradation generally decrease with temperature, probably due to decreased rates of enzymatic activity. Most heterotrophic bacteria favour a pH near neutral for growth and biodegradation. PAHs sorbed on particulates or sediments with high organic matter tend to be less bioavailable for effective biodegradation. Increasing salinity tends to reduce the solubility of PAHs, which in turn reduces the bioavailability. The microbial oxidation of PAHs typically requires the presence of oxygen. The activity of PAH degrading microbes can be enhanced by prior exposure of microbial communities to the contaminant. For instance, many strains of bacteria capable of utilizing PAHs have been isolated from oil polluted habitats (e.g. Neff, 1979; Kimura *et al.*, 1990; Hedlund *et al.*, 1999).

The population of bacteria and yeast species and strains capable of degrading PAHs in marine and freshwater, except in heavily polluted areas, are generally low. In addition, since most species cannot utilize PAHs as sole carbon sources, population increases in polluted areas may be hampered by deficiency in alternative organic carbon growth substrates. The concentrations of PAH degrading microorganisms and organic carbon in marine and freshwater sediments are usually much higher. Thus, significant degradation of PAHs may occur in oxygen rich sediments. Otherwise little or no degradation is expected in anoxic sediments or water.

However, recent studies focusing on possible remediation of PAH contaminated river sediments revealed that native bacteria can degrade or metabolise PAHs to a certain extent by utilizing background nutrients as electron donors and acceptors from within (Johnson & Ghosh, 1998). PAH degradation was enhanced by the addition of sulphate as an electron acceptor. MacRae & Hall (1998) on the other hand managed to stimulate the biodegradation of PAHs in anoxic marine sediments by using nitrate as an alternative electron acceptor other than oxygen. While these observations are of significance in efforts at bioremediation of PAH contaminated sediments both *in situ* and *ex situ*, they provide indications of possible, albeit slow, biodegradation of the contaminants in anaerobic aquatic environments subject to certain conditions.

Biotransformation of PAHs by aquatic animals

It is well established that the hepatic cytochrome P-450-dependent (CYP) mixed function oxidase (MFO) system in mammals and many other vertebrates and invertebrates is responsible for initiating the metabolism of xenobiotics, among other exogenous and endogenous compounds. The primary function of the enzyme system is to transform the xenobiotics into more polar, water soluble products that are amenable to excretion. Enzyme activities associated with CYP system, include aryl hydrocarbon hydroxylase (AHH), ethoxyresorufin o-deethylase (EROD) and other antioxidants.

The CYP system is located in the mammalian liver, as well as in several other organs of non-mammalian organisms. The system catalyses the incorporation of one atom of oxygen into water and one atom into the PAH. Oxidative metabolism of PAHs in this system proceeds via highly electrophilic intermediate arene oxides, some of which bind covalently to cellular macromolecules, such as DNA, RNA and protein, and thereby inducing carcinogenesis and mutagenesis. For instance, the carcinogenicity of benzo[a]pyrene is attributed to the isomeric 9,10-epoxy-7,8–dihydro–7,8–dihydroxy benzo[a]pyrenes (Neff, 1979).

Active epoxides may be converted to less toxic products by various enzymatic and non-enzymatic reactions. Major enzymatic biotransformation pathways include microsomal epoxide hydrase which converts epoxides to diols, glutathione s-transferases which catalyse the formation of glutathione (GSH) conjugates, and sulfokinases which convert phenols to sulfates (Neff, 1979).

The presence and activity of the CYP enzyme system has also been established in many aquatic animals, with the level of activity varying from species to species. For example, high AHH activity was reported in the marine worm *Nereis virens*, but very low activity in bivalves and only after long periods of induction by exposure to PAHs, such as the oyster *Crassostrea virginica*, the clam *Myra arenaria* and mussels *Mytilus edulis* (Vandermeulen & Penrose, 1978; Neff, 1979).

Crustaceans have shown low variable hepatopancreatic AHH activity. There are wide inter and intra-species variations in AHH activity among fish. While most CYP activity is localized in the liver, AHH activity has been detected in other fish organs, such as the heart, kidney and gills (e.g. Neff, 1979; Bogovski *et al.*, 1999; Yuan *et al.*, 1999).

It is apparent that there are many variable endogenous and exogenous factors that can influence levels of AHH activity and cytochrome P-450 levels in the tissues of aquatic animals. Endogenous factors may include age, sex, nutritional status, period of molt cycle (for arthropods) etc. Exogenous factors may include temperature, season, and prior exposure to inducers or inhibitors of the microsomal PAH metabolising system.

1.3.3 Heterogeneous degradation of PAHs in the aquatic environment

In the aquatic environment, the various PAH oxidation processes do not proceed independently of one another, but work in tandem. The residence time of PAHs in marine waters is considered to be short. Lower molecular weight PAHs (naphthalene, anthracene, and phenanthrene) are removed primarily by evaporation and microbial activity. Higher molecular weight PAHs are generally comparatively more hydrophobic and tend to associate with suspended particulate matter and are, consequently, removed mainly by sedimentation and photooxidation. Anthracene is also rapidly oxidized. Degradation by animals in the water column is of minor importance.

Bioturbation by benthic fauna, such as marine worms, have been shown to play important roles in sediment-water interactions, sediment resuspension and solute and solid transport processes. Activity of meiofauna and macrofauna causes mixing of sediments and exposure of subsurface sediments to the water-sediment interface where high microbial activity can enhance degradation of PAHs (Neff, 1979; Robbins, 1986; Schaffner *et al.*, 1997). Some macrofauna e.g. polychaete worms, and meiofauna can ingest sediment and are able to degrade PAHs sorbed on them.

1.4 Biological Effects of PAHs in the Aquatic Environment

Freshwater and marine organisms are known to take up PAHs and accumulate them in their tissues to higher concentrations than in the ambient media. Aquatic organisms exposed to PAH contamination may experience acute or sublethal effects. Such adverse effects may have far reaching ecological consequences on aquatic ecosystems.

1.4.1 Uptake and release of PAHs by aquatic biota

There are many studies, laboratory based and backed by field observations that have been performed on accumulation and release of PAHs in solutions by a wide variety of aquatic species, up the different trophic levels. Marine and freshwater algae have shown varying degrees of ability to accumulate and metabolise PAHs (e.g. Neff, 1979; Kriso & Irha 1998). In the studies by Kriso and Irha, marine brown algae *Fucus vesiculosus* accumulated 89 – 99 % of benzo[a]pyrene in solution. The transformation of PAHs in algae was attributed to mixed function oxidase enzyme systems (o-diphenol oxidase, CYP and peroxidase).

Marine worms have been shown to accumulate PAHs in their tissues and some species possess the ability to degrade the genotoxins. It has been observed that electrophilic metabolites tend to remain in tissues during depuration, suggesting that they are covalently bound to tissue macromolecules (e.g. Rossi & Anderson, 1977; Chandler *et al.*, 1997). Molluscs, such as oysters and mussels, have little or no ability to metabolise PAHs. Their pattern of accumulation and release is passive, governed by water-lipid partitioning processes (Stegeman & Teal 1973; Vendermeulen & Penrose, 1978). Crustaceans and prawns on the other hand posses CYP systems and thus the pattern of accumulation and release reflects their more active mode of life and greater PAH metabolising ability. Generally crustaceans and fish tend to release unmetabolised PAHs more readily, but a slow release of electrophilic metabolites. High molecular weight PAHs are released more slowly than low molecular weight hydrocarbons (Neff *et al.*, 1976; Jackson & Bidleman, 1990).

The apparent availability of the hydrocarbons is an important factor affecting uptake and accumulation by aquatic organisms. In natural water PAH associated with dissolved organic matter and with suspended particulate matter is less available for uptake and bioaccumulation (Boehm & Quinn, 1976). PAHs associated with sediments, specifically with the organic carbon fraction have also been found to be less available for uptake and accumulation.

Worms, crustaceans and fish can, in varying degrees of efficiency, accumulate and assimilate hydrocarbons taken through food. (e.g. Leppanen & Kukkonen, 1998). The pattern of release observed is qualitatively similar to depuration of PAHs accumulated from water.

A study on the uptake of PAHs by aquatic plants from sediments revealed that plants can accumulate the contaminants in their tissues to concentrations higher than ambient (McGlynn & Livingston, 1997). At high sediment PAH concentrations, however, aquatic plants cease to bioaccumulate PAHs.

1.4.2 Toxicity effects of PAHs

The toxicity of PAHs to living organisms is manifested in either of two ways. They may bind irreversibly to lipophilic sites in the cell, disrupting cellular processes, or metabolites may covalently bind to cellular structures causing chronic adverse effects. Low molecular weight PAHs (naphthalene, phenanthrene and anthracene) usually cause acute toxicity, a manifestation of their relatively higher aqueous solubility and bioavailability. The higher molecular weight 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, such as osmotic and ion exchanges, and thus disrupts neurotransmission, muscle contraction and osmoregulation. In addition, PAHs interfere with the normal activity of membrane-associated enzymes.

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. This phenomenon, reviewed by Arfsten *et al.* (1996), was aptly demonstrated by the photoinduced toxicity of PAHs (e.g. Bowling *et al.*, 1983; Veith *et al.*, 1983; Ankely *et* al., 1997; Pelletier *et al.*, 1997; Boese *et al.*, 1999). For example, Ankley *et al.* (1997) observed no lethality in 96 hr. exposure of the marine worm *Lumbriculus vareigatus* to aqueous solutions of anthracene and pyrene (37 & 80 $\mu g\ l^{-1}$, respectively) in the absence of ultra-violet light. However, complete mortality occurred on exposing the worms to lower concentrations of the PAHs (10 & 5 $\mu g\ l^{-1}$, respectively) and ultra-violet light. The electrophilic metabolites are primarily attributed with causing chronic toxic effects in the form of mutagenesis, carcinogenesis and teratogenesis, when they bind to cellular macromolecules.

Acute toxicity

The acute toxicity of PAHs to aquatic animals varies between species and different contaminants (Neff, 1979). In general, within a species, the acute toxicity increases with molecular weight of the PAHs. However, the high molecular weight PAHs show low acute toxicity (particularly chrysene, benzo[a]pyrene, and dibenzanthracene), which is attributed to their extremely low aqueous solubility and bioavailability. Acute toxicity increases with alkyl substitution on the parent PAH structure. Comparing the relative susceptibility of aquatic animals to PAH toxicity crustaceans are the most sensitive, followed by polychaete worms, and fish the most resistant. In most cases the concentrations of PAHs that cause acute toxicity to aquatic animals are several orders of magnitude above concentrations found even in the most heavily polluted marine or fresh waters. On the other hand, polluted sediments most often contain PAH concentrations that are much higher than those which cause acute toxicity. However, sediment-associated PAHs tend to be relatively less bioavailable and apparently less acutely toxic.

Sublethal effects

The chronic exposure to sublethal levels of PAHs may produce long-term adverse effects on aquatic organisms that may pose a potential hazard to the survival of the organisms and the well being of the ecosystem. An important aspect of chronic exposure to PAHs that has received considerable attention because of implications on human health is the induction of cancer in sensitive species. Detailed reviews on the sublethal effects of PAHs on aquatic organisms include those by Neff (1979) and Hellou (1996).

The documented effects of the exposure of marine bacteria species to sublethal levels of PAHs include growth inhibition (Neff, 1979). Low concentrations of PAHs (range 0.003 – 2.0 μg ml^{-1}) have been observed to stimulate growth of marine algae. However, exposure to higher concentrations (e.g. benz[a]anthracene at 10-300 μg ml^{-1}) resulted in inhibition of growth. Exposure of marine algae to low concentrations of PAHs has also been found to inhibit photosynthesis (Neff, 1979). Low concentration levels of the contaminants (phenanthrene at 25-500 ppb) have been shown to interfere with normal growth and calcification of corals due to inhibition of photosynthesis by the symbiotic zooxanthallae (Neff, 1979). Contrary to the experience with terrestrial plants, PAHs in sediments were found to inhibit growth of aquatic macrophytes - a rosette monocot and a vittate monocot, both species rooted (McGlynn & Livingston, 1997).

Several studies have shown that the adverse effects of exposure of aquatic animals to sublethal levels of PAHs include interference with reproduction and recruitment of juveniles, cause stress conditions, physiological effects impacting on such processes as osmoregulation and histopathological conditions. For example, sublethal PAH levels have been found to cause embryo-toxicity leading to reproduction failure, abnormal growth of new offspring and negative impact on recruitment of juveniles (Neff, 1979). The juvenile stages are more sensitive to sublethal toxicity of PAHs, with resultant effects such as interference with osmoregulation and molting leading to mortalities of crabs, shrimps and other crustacea. Other sublethal effects include respiratory stress and inhibition of feeding of exposed copepods, mussels, etc. (e.g. Lotufo, 1997; Wirth *et al.*, 1998). DiMichele & Taylor (1978) described various histopathological and physiological effects on the mummichog *Fundulus heteroclitus* to the exposure to as low as 2 ppb naphthalene.

It is noteworthy from the above cases that PAH concentrations that caused significant sublethal effects were higher than normally encountered in all but heavily polluted aquatic environments. However realistic PAH concentrations, not uncommonly encountered in polluted aquatic areas, such as harbours and in the vicinity of oilrigs, have been found to cause significant responses in some sensitive organisms. For example, PAH- contaminated sediments (PAH concentration 25-50 $\mu g\ g^{-1}$) adversely affected the immune response of exposed fish (Flounder *Psedopleuronectes americanus*) (Payne & Fancey, 1989).

PAH–induced cancer in aquatic organisms

The ability of some PAHs to cause cancer in mammals and/or aquatic animals has long been recognized. The high potential for PAHs to cause cancer in human beings has been demonstrated by correlations between occupational or other exposure (e.g. cigarette smoking) and the incidence of cancer (Grimmer, 1983; Mastrangelo *et al.*, 1996; Mehlan *et al.*, 1997). The relative carcinogenicity of PAHs to mammals varies widely. PAHs with 4, 5 and 6 aromatic rings are relatively more carcinogenic than either smaller or larger ring systems. Highly angular PAHs are more carcinogenic than linear or highly condensed structures. The position of alkyl substitution on the ring structure influences the carcinogenicity of a PAH. The carcinogenicity is also related to the structure and reactivity of major metabolites of the PAH. For example, of the monomethyl benz[a]anthracenes, 7–methyl benz[a]anthracene is the most carcinogenic, 6-, 8- and 12– isomers slightly active, and the remaining are inactive (Neff, 1979).

The sensitivity of different aquatic species to PAH–induced carcinogenesis varies depending on the levels of mixed function oxidase (CYP) enzyme activity, the stereochemistry of enzymatic reactions and rate of detoxification of active metabolites. It has been demonstrated that, among other aquatic animals, the CYP system of fish is able to produce carcinogenic or mutagenic metabolites (e.g. Stegeman, 1977).

Most laboratory studies on carcinogenesis of PAHs on aquatic animals applied concentrations that were relatively higher than environmentally realistic levels in water, food and sediments. However, there is a growing body of information on bioassay and field studies in which, particularly PAH polluted sediments have been shown to cause carcinogenic and mutagenic effects on natural populations of aquatic animals (e.g. Vogelbein *et al.*1990; Minissi *et al.*, 1998).

In a test on mutagenecity of polluted sediments on *Vice faba* root tips, Minissi *et al.*, (1998) reported a high level of activity attributed primarily to PAHs. The total amount of PAHs encountered was 4.5 to 625.2 ng g^{-1} of dry sediment. A high prevalence of liver cancer in a population of mummichog *Fundulus heteroclitus* from a creosote–polluted site was reported by Vogelbein *et al.* (1990). Sediments from the site contained extremely high concentrations of PAHs, up to 2200 mg kg^{-1} of dry sediment that were attributed with hepatocarcinogenesis of the fish. It is realised, however, that there are many different environmental contaminants that can induce cancer in sensitive species, and polluted aquatic environments most often contain varieties of potential carcinogens.

1.5 Contemporary Techniques for Sample Preparation and Analysis of PAHs in the Aquatic Environment

1.5.1 Sample collection

The dynamic nature of tide influenced inshore waters and estuaries and the type of sample required (water, sediment or biota) are important factors in determining the techniques for collection of samples. Forthwith, this discussion will concentrate on techniques applied for the sampling and analysis of water and sediments. The designing of environmental monitoring studies involving biota is beyond the scope of the present study.

Contemporary techniques for trace analysis of hydrocarbon components, such as, capillary GC-FID, GC-MS, HPLC-UVF and HPLC-MS are highly sensitive, and thus strict avoidance of contamination during sample collection cannot be over emphasized.

There are a number of devices that have been used for sampling the surface microlayer, varying in design according to the thickness of the water layer sampled. Falkowska (1999a) evaluated the performance of the Teflon plate, glass plate and screen samplers, with average film thicknesses of 10 µm, 90 µm and 250 µm, respectively, under varying wind conditions. The conclusion was that the screen sampler was more practical in terms of a relatively shorter sampling time and larger sample volume, and applicability in inclement weather conditions.

Small volumes of near surface water samples (2.5 – 5 l) can conveniently be collected using a weighted glass reagent bottle (UNESCO, 1984; Knap *et al.*, 1986; Ehrhardt *et al.*, 1990). There are several sampling bottles that have been routinely used for collecting deeper water samples, such as, the Niskin sampler (Boehm & Fiest, 1982; Gryzybowski *et al.*, 1987). Larger volume glass sphere sampling devices (volume 10 l and 200 l) designed for deep-sea sampling (200 – 2,000 m depth) have been used (GESAMP, 1993).

The Van Veen grab sampler is adequate for collecting surficial sediment samples. For studies aiming at detailed sediment vertical profiles, a piston corer or hand held stainless steel corer is appropriate for sample collection and subsequent subsampling.

1.5.2 Extraction techniques

The extraction technique of choice depends on the sample matrix. For natural water samples, especially inshore waters, which contain high levels of suspended particulate matter (SPM), it is normal practice to filter the samples before further processing. SPM is usually filtered using micro glassfibre filter paper (e.g. Whatman GF/C or GF/F) of nominal pore size 0.7–1.2 µm. The SPM and filtered water are then extracted separately. Indeed, in comparing extraction procedures for hydrocarbons in particle–rich waters Siron & Guisti (1990) demonstrated that direct liquid-liquid extraction resulted in low yields because of partitioning of the solute into particulate lipids.

Liquid–liquid extraction

The high values of K_{ow} for PAHs allow the use of direct liquid–liquid extraction (LLE) of water samples. LLE is usually performed at room temperature and thus, low-boiling solvents can be used. For such highly hydrophobic hydrocarbons as the PAHs, only low volumes of the suitable extraction solvent are required. The utilization of low volumes of the extraction solvent in LLE makes this the initial preconcentration step of the technique. Suitable solvents that are commonly used for LLE of PAHs are dichloromethane and chloroform. The procedure usually involves addition of the extraction solvent to the water sample in a separating funnel (40 to 60 ml solvent per litre of sample) and mechanical agitating for 2-15 minutes, followed by separating the two phases. The procedure is repeated two more times using about half the initial volume of solvent and all the extracts combined.

Solid–phase extraction

The application of solid adsorbents to extract dissolved PAHs in water was developed as a response to the need to process high sample volumes for trace organics in relatively less polluted open ocean waters. Early attempts at solid phase extraction applied Amberlite XAD-2 and Amberlite XAD-8, porous polyurethane and polytetrafluoroethylene (PTFE), which had the disadvantage of frequent contamination from aromatic compounds, including some PAHs, such as naphthalene, alkyl naphthalenes and biphenyls (e.g. de Lappe *et al.*, 1983; Gómez-Belinchón *et al.*, 1988; Ehrhardt & Burns, 1990). However, clean PAH–uncontaminated adsorbents are commercially available and Ehrhardt (1987, cited by GESAMP, 1993) described an effective procedure for cleaning solid sorbents.

Introduced relatively recently are n-octadecyl bonded (C_8-C_{18}) silica sorbents which have proved to be exceptionally efficient, without the problems of contamination experienced with the earlier sorbents. Junk & Richard (1988) reported recoveries of greater than 85% for PAHs and pesticides in spiked water samples. In addition, it was demonstrated by Green & le Pape (1987) that hydrocarbons sorbed on Amberlite XAD–2 and octadecyl–silanized silica gel faced no danger of biodegradation or transformation.

In solid phase extraction (SPE) a short glass or plastic column is filled with sorbent material in a fine mesh (150-400 mesh) and thickness of about 0.5 cm. A water sample is passed through the sorbent trap in which hydrocarbons are sorbed and retained. The trap is dried by a nitrogen flow and eluted rapidly with a suitable organic solvent, such that a retention volume only a few times higher than the dead volume is used. An enrichment process involving the elution solvent system needs to be developed for the analytes (PAHs) and the SPE column. However natural waters, especially inshore waters, contain high quantities of surfactants, such as fulvic and humic acids, which can denature the SPE columns and thereby alter their properties.

Soxhlet extraction

This is the most widely used technique for extracting organic contaminants from solid matrices, such as dusts, sands, carbonaceous solid residues and marine sediments. The technique is particularly suitable when the organic contaminants are strongly adsorbed onto the solid matrix. Normally a high boiling solvent is used to increase the extraction power of the process for especially the high molecular weight PAHs. Ideally the solvent should have a high boiling point, but not so high as to cause decomposition of the analytes, or too low such that the solvent tends to escape from the extractor. Solvents that are widely used for soxhlet extraction of solid matrices for PAH contaminants, include hexane, hexane–dichloromethane mixtures and methanol. Usually wet solid samples are dried using precleaned anhydrous sodium sulphate before extraction. The use of a freeze drier often results in losses of low boiling components.

Wet hydrolysis or saponification can be performed in the soxhlet apparatus to increase extraction efficiencies, especially in cases of high lipid solid samples, including biota. The normal procedure involves initial extraction of the sample with methanol (4-6 hrs.), followed by the addition of saponification reagents (KOH solution estimated at 1.0 M equivalent, 56 mg, per every 200 mg of lipid extract) and further refluxing (unto 2 hrs). The non-saponifiable lipids, which contain the hydrocarbons, are separated from the saponifiable lipids by extracting the

KOH/MeOH extract mixture with hexane. To determine the total extractable lipid weight of the sample, the aqueous–MeOH phase is acidified with an equivalent amount of HCl solution. The acidified solution is extracted with hexane, filtered and dried to give the saponifiable lipid fraction.

In the endeavour to reduce extraction time, and hence reduce sample handling and increase the turn-over of sample processing, coupled with the desire for alternative and/or less harmful extraction solvents, novel techniques have been inducted in trace-organic analysis in environmental samples. These techniques include microwave assisted extraction, accelerated solvent extraction and supercritical fluid (CO_2) extraction (SFE). The common advantage across the techniques is the utilisation of lower amounts of solvents compared to conventional soxhlet extraction. In addition, some of the techniques have been automated for routine analysis, e.g. SFE (e.g. Berg *et al.*, 1999).

Sonic or shaker extraction

Extraction of sediment samples by the use of a sonicator or mechanical shaker is an alternative procedure to the use of soxhlet extractors. In this method, a sediment sample is dried with clean anhydrous sodium sulphate and mixed with dichloromethane / acetone or dichloromethane / methanol. The sample mixture is sonicated for about 3 minutes at predetermined settings for efficient extraction. Alternatively, the sample mixture can be shaken for several hours using a mechanical shaker. The solvent is separated from the mixture by decanting. The extraction procedure is repeated two more times and the extracts combined, filtered, dried and concentrated.

1.5.3 Extract Cleanup

The preferred cleanup method for sample extracts is adsorption or column chromatography, whose objective is to remove all interfering lipid co-extractives and split the hydrocarbon extracts into saturated and aromatic fractions. Partial removal of lipids is effected by the KOH / methanol hydrolysis procedure. However, other organic groups of contaminants that may be of interest, especially chlorinated pesticides are unstable under the harsh hydrolysis conditions. It is necessary to calibrate the efficiency of the separation by running through a series of standards and quantifying their recovery in collected fractions. Column re-calibration has to be carried out whenever a new batch of adsorbent material is used and the elution procedure adjusted accordingly.

Normal phase column chromatography using alumina and silica gel is an efficient and convenient cleanup method. An adaptation of this cleanup method was applied in the present study (see sec. 2.5). Pre-cleaned adsorbents are activated at 200 °C for at least 4 hrs and partially deactivated by the addition of 2-5 % v/w water, and allowed to equilibrate. The amount of adsorbent required is estimated by keeping the ratio of adsorbent to lipid weight greater than 100 to 1. Adsorbents are introduced into glass columns by wet packing with hexane. Packing is completed by placing a short layer of anhydrous sodium sulphate (1 g or 1 cm) on top of the column. The lipid extract is reduced to 1 ml in hexane, pipetted into the column and eluted with hexane (approximately 1 bed volume) to give the first fraction containing saturated hydrocarbons (including benzene and alkyl benzenes). Further elution with 10-50 % dichloromethane in hexane gives a second fraction containing unsaturated and aromatic hydrocarbons. The exact composition and volume of dichloromethane–hexane required to elute all the PAHs of interest has to be determined empirically. Examples of elution solvent systems that have been applied are 1.5 bed volumes of 10% dichloromethane in hexane, and 1 bed volume of 50% dichloromethane in hexane.

Superior separation efficiency was achieved using normal phase HPLC by Petrick *et al.* (1988), a procedure that is suitable for separating various organic environmental contaminants including PAHs. It is however necessary to remove most of the lipid co-extractives from the sample extract using alumina chromatography columns before injecting into the HPLC. A simple isocratic HPLC capable of programming an elution scheme of unto 3 sequential solvents is adequate. An HPLC capable of gradient elution programming assures better reproducibility. As is the case with column chromatography separations, the HPLC procedure must be calibrated using appropriate standards, and elution solvent systems have to be determined empirically. UNEP/IOC/IAEA (1992) described an HPLC procedure, adapted from Petrick *et al.* (1988) for the separation of various organic contaminants. The HPLC operating conditions were as follows:

Column		5 µm Nucleosil, 20 cm length x 0.4 cm i.d.
Injection volume		75 – 100 µl
Mobile phase	:	0.5 ml min^{-1}.

Gradient Elution programme 11 ml hexane, 4 ml. 20% dichloromethane in hexane, 10 ml pure dichloromethane.

Expected fractions	Elution volume (ml)
1. Alkanes and alkenes	0.5-2.0
2. Alkylbenzenes, naphthalenes, HCB, PCBs	2.0 – 4.5
3. PAHs and Toxaphene	4.5 - 11.0
4. Pesticides	11.0 – 15.0
5. acids and polar compounds	15.0 – 25.0

(Source: UNEP / 10C/IAEA, 1992)

PAHs can be targeted by analysing the second and third fractions. The fractions may be reduced in volume accordingly by rotary evaporation and/or using a flow of nitrogen gas.

1.5.4 Qualitative and quantitative analysis

There are a number of analytical techniques that have been used for the analysis of PAHs. UV fluorescence spectrometry (UVF) is a semi-qualitative, semi-quantitative technique that can be used for screening PAHs. Detailed information on individual PAH components in samples can be obtained by using GC-FID and GC-MS techniques. HPLC-UV, HPLC-UVF and HPLC-MS have been successfully applied for the analysis of individual components of PAHs.

1.6 Scope and Objectives of the Study

There are various activities that are potential sources of chemical pollutants to the Cochin estuarine backwaters. Urban wastewater and industrial liquid effluents are invariably discharged into the inshore waters. 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. PAH rich gaseous emissions from industries, road traffic and water transportation, eventually contaminate the inshore waters through dry and wet deposition. The water quality of the Cochin backwaters is a matter of concern because of perceived impacts of the chemical pollutants on the ecosystem. Further liberal economic policies are expected to spur industrial growth and urban development, with ensuing problems of waste management and disposal. Indeed, the decline of the productivity of the important molluscan, demersal and prawn fisheries in the Cochin backwaters was attributed to the declining water quality (Saraladevi 1986). In addition, the uptake and accumulation of organic pollutants by aquatic organisms, directly or through the food chain, is a potential hazard to the public who may be exposed to the contaminants through the consumption of contaminated seafood. Therefore, there is need for environmental monitoring, assessment of the environmental quality and a database that can be useful for regulatory purposes. This requires, among other pertinent information, baseline data on the water quality. So far a systematic study on PAHs in the Cochin backwaters is yet to be reported.

In view of the above concerns, therefore, the broad aim of the present study was to establish water quality data and assess the extent of pollution of the inshore waters from hydrocarbon contaminants, especially the environmental genotoxins, PAHs. In order to realise the stated aim, attempts were made to fulfil the following specific short- and long-term objectives;

Short-term objectives:

- Determination of PAH concentrations in water, suspended particulate matter and surficial sediments.
- Establish spatial and temporal distribution of PAHs in the study area.
- Study factors that influence the distribution and levels of PAHs in the study area, namely salinity, temperature, dissolved oxygen, turbidity, pH, organic carbon content of suspended particulate matter and estuarine mixing processes.

Long-term objectives:

- Establish critical environmental factors influencing distribution, transport and transformation of PAHs in the study area.
- Augment a growing body of data on water quality that can be useful as baseline reference, as well as for regulatory purposes.

It was envisaged that the study would provide information on aspects of environmental impact of socio-economic activities in the study area.

Chapter 2

MATERIALS AND METHODS

2.1 Description of the Study Area

The Cochin estuarine system extends between latitude 9°40' to 10°12'N and longitude 76°10' to 76°30'E. It is connected to the Arabian Sea by a permanent bar mouth at Cochin and two seasonal openings, during peak monsoon season, at Andhakaranazhi and north Paravoor. The expansive backwaters are generally shallow, with the exception of two deep navigation channels maintained at atleast 12 m depth that lead into the harbour. Two major rivers discharge freshwater into the estuarine system, the Periyar flowing into the northern part and the Pumpa flowing into the southern part. Other smaller rivers flowing into the estuary are Achankoil, Manimala, Meenachil and Muvatupuzha.

A large number of industrial establishments are located along the banks of the River Periyar, and include coconut retting yards and fish processing units. The Cochin estuarine system is, thus, subjected to emissions and discharges of effluents from manufacturing industries, harbour operations, urban waste from the Cochin City and land runoff.

There were 8 sampling sites located in the study area as follows (Fig. 2.1);

Station No.	Name	Environment	Socio-economic Activities
1	FACT	riverine	industrial area
2	Eloor	riverine	water transport
3	Tatapuram	estuarine	industrial, water transport and urban
4	Bar mouth	towards open sea	water transport and urban
5	Fishing Harbour	estuarine	harbour for fishing boat fleet
6	Venduruthy Bridge	estuarine	land and water transport
7	Chambakara	estuarine	sub-urban
8	Shipyard	estuarine	dry dock and water transport.

2.2 Sampling and Preservation

Sampling sites were occupied once a month from December 1995 to November 1996, with the exception of September 1996. At the Cochin Shipyard sampling site, (Site No. 8) samples were collected at two hourly intervals for about 13 hours to cover short-term variations due to tides. This sampling regime effectively covered the three main seasons, namely Pre-Monsoon (March–May), Monsoon (June–September) and Post-Monsoon (October–February). All samples were taken in duplicate.

Surface micro-layer water samples were obtained using a Garrett-type screen sampler of 60 x 60 cm with a stainless steel screen of mesh size 1.0 x 1.0 mm (Garrett, 1965). A simple weighted bottle holder designed to carry a 2.7 l Winchester reagent bottle with a float to maintain the equipment at about 1.0 m was used to take samples of the sub-surface water layer. The bottom water layer was sampled using a conventional deep-water sampler. Between 0.5 l and 1.0 l of each water sample was filtered through pre-combusted and pre-weighed 0.7 µm pore size glassfibre filter paper (Whatman GF/F) for the determination of particulate organic carbon (POC) content of the suspended particulate matter (SPM). The rest of the water samples were filtered using pre-combusted glassfiber filter paper for the determination of PAHs associated with SPM. The filter paper with the SPM samples was wrapped in aluminium foil and stored at –10 to –20 °C. All samples were processed in duplicate. Sediment samples were obtained using a grab sampler. Surface sediment samples at between 0 and 10 cm depth were taken, wrapped in aluminium foil, labelled and stored at -10 to -20°C.

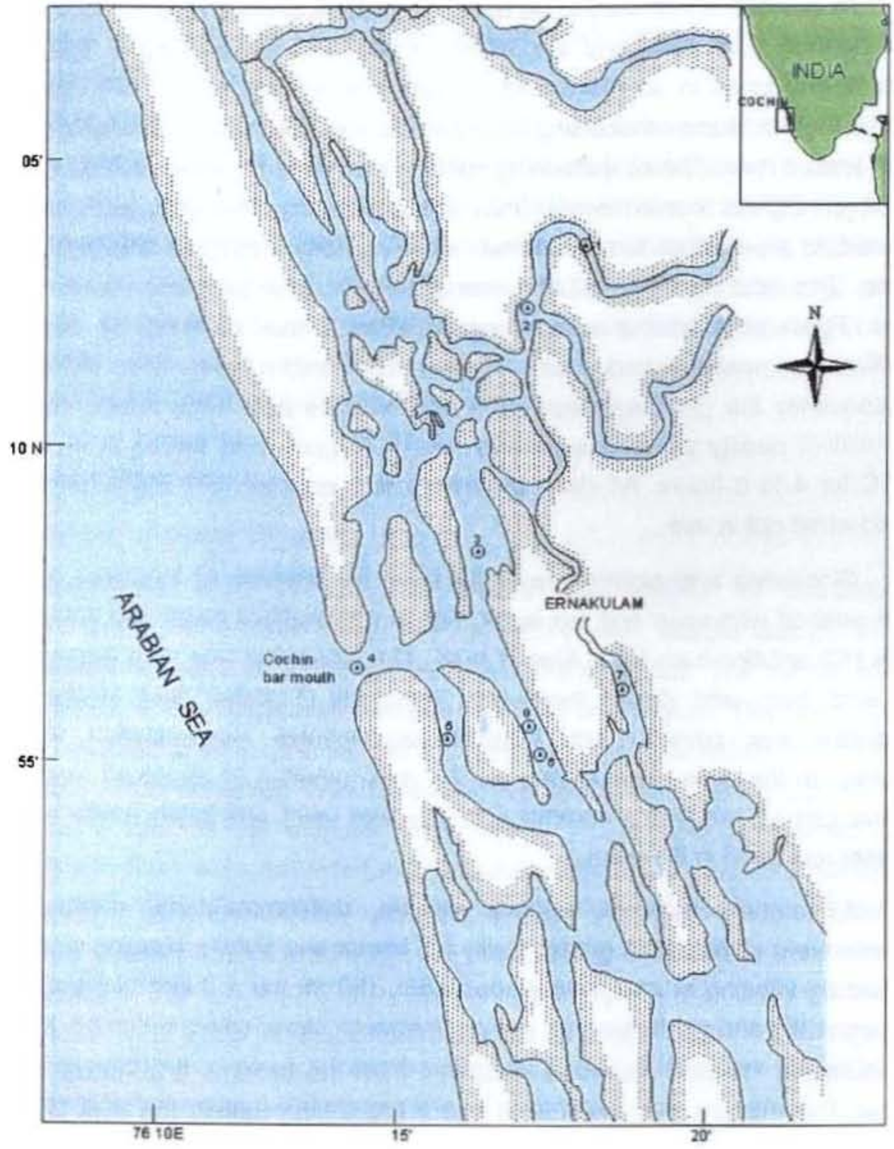

Fig. 2.1. Map of Cochin estuarine system showing location of sampling stations

2.3 Preparation of Apparatus and Chemicals

All glassware that were to be used for analysis of trace organic contaminants was cleaned with soap and tap water, rinsed with distilled water followed by acetone and dried in an oven. After cooling the dry glassware were rinsed with residue-free dichloromethane and immersed in a concentrated $NaNO_3/H_2SO_4$ bath for at least 1 hour. The acid cleaning solution was used to get rid of any remaining traces of organic contaminants from the glassware. The acid bath was kept covered, in an efficient fume chamber, which greatly minimized exposure to acid fumes. The acid cleaning solution was preserved after use and reused several times. Fresh acid solution was prepared when it was observed to have been significantly contaminated with water, absorbed from the atmosphere. Subsequently the glassware was removed from the acid bath, rinsed thoroughly with milli-Q quality water (conductivity 14–18 MΩ.cm) and baked in an oven at 200°C for 4 to 6 hours. All clean glassware was covered with aluminium foil and stored when not in use.

Glassware and plasticware to be used for analysis of inorganic nutrients, were washed with soap and tap water, rinsed with distilled water and immersed in a 5% HCl solution bath for at least 1 hour. The apparatus was then removed from the acid bath and rinsed thoroughly with milli–Q water, and stored. Clean glassware was covered with Parafilm to minimize contamination whenever required. In the case of glassware for the determination of dissolved oxygen and organic carbon, however a chromic acid bath was used, and finally rinsed with milli-Q water and dried in the oven.

All organic solvents, namely hexane, dichloromethane, methanol and acetone were of analytical grade quality (E. Merck and Sisco). Hexane was further purified by soaking in analytical grade H_2SO_4 (50 ml per 1.0 litre of hexane) in a reagent bottle and gently stirring, using a magnetic stirrer with a teflon bit, for about 12 hours. As the acid absorbed impurities from the hexane, it gradually became yellow. The mixture was transferred into a separating funnel, the acid separated and drained back into the reagent for reuse. About 100 ml of concentrated NaCl solution was added to the hexane and the separating funnel shaken to wash off any remaining acid. The mixture was allowed to separate and the aqueous layer drained. The clean hexane was then transferred into a conical flask and pre-cleaned Na_2SO_4 added. The hexane was allowed to dry for several hours. The hexane was then decanted into a 2 l round–bottomed flask and double distilled in an all-glass apparatus. Purified hexane was stored in clean reagent bottles covered with aluminium foil–lined caps. Dichloromethane and methanol were also double distilled in all-glass apparatus and stored in clean reagent bottles. Solvents were freshly purified before use.

All solid reagents, namely anhydrous Na_2SO_4, alumina and silica gel were pre-cleaned by soxhlet extraction with methanol (8 hrs) followed by hexane (8 hours). The pre-extracted reagents were then dried in an oven at 60 °C. Na_2SO_4 was then baked at 450 °C in a furnace, while alumina and silica gel were baked at 200 °C in an oven overnight. Clean reagents were stored in glass bottles with aluminium foil–lined tops. Alumina and silica gel were activated by heating in an oven at 200 °C for 4 hours just before use. Glass wool was similarly pre-extracted, dried and stored in a well covered bottle.

2.4 Extraction Procedure

2.4.1 Processing of water samples

A direct liquid-liquid extraction procedure was applied for extracting hydrocarbons from filtered water samples. About 1.5 l water sample was put in a 2.0 1 separating funnel and 50 ml of dichloromethane added. The funnel was vigorously shaken for 2 minutes with frequent release of pressure. The extraction mixture was allowed to settle and the dichloromethane layer drained into a 250 ml conical flask. The extraction was repeated two more times using 25 ml of dichloromethane and all the extracts were mixed. Total sample volumes of between 2.5 and 5.0 l were extracted in this manner. Combined dichloromethane sample extracts in aluminium foil–covered conical flasks were then kept in the dark and the solvent was allowed to evaporate to near dryness. The samples were then quantitatively transferred to labelled 15 ml glass vials with aluminium-lined screw tops. Small portions of dichloromethane and hexane were used to rinse the sample conical flasks, which were also put into the sample vials. The vials were stored in a refrigerator (< 5 °C) awaiting further processing.

2.4.2 Processing of SPM and sediments

Samples of suspended particulate matter (SPM) and sediments were extracted using soxhlet extractors. Glass thimbles were made by making drainage holes at the bottom of boiling tubes. The holes were plugged with pre-cleaned glass wool and the soxhlet setup cleaned by pre- extracting with dichloromethane/hexane (1:1) mixture; 8-10 solvent cycles. For the extraction of SPM samples, soxhlet extractors with a capacity of 60 ml and 250 ml round–bottomed flasks were used. The SPM filter paper samples were extracted with 150 ml of dichloromethane/hexane (1:1) mixture for 8 hours. In order to monitor recoveries of the procedure the internal standard 9,10 dihydroanthracene (50 µg) was introduced into the soxhlet extractor before commencing the extraction after every 10 successive samples.

After the extraction was completed, the sample extract was allowed to cool and dried with Na_2SO_4. The round-bottomed flask with the sample extract was then attached to a rotary evaporator and the extract concentrated to about 10 ml. The sample was transferred to a labelled clean 15 ml glass vial, and further concentration carried out over a gentle flow of nitrogen gas. Sample vials were stored in a cool, dry and dark place (< 5 °C). Sediment samples (10-25 g) were dried with pre-cleaned Na_2SO_4, about three times the mass of wet sample, and surrogate standard (50 µg of 9,10–dihydroanthracene) added and extraction commenced. Further processing of the samples was carried out in a similar manner to SPM. (If the same sample is to be analysed for saturated hydrocarbons then, elemental sulphur contaminant can be removed using activated copper.)

Blank samples composed of Na_2SO_4 for the sediment samples, and pre-combusted filter paper for SPM samples were processed after every 10 samples.

2.5 Cleanup of Sample Extracts

Normal phase adsorption chromatography using alumina and silica gel was used for clean-up of sample extract to remove lipid co-extractives and separate saturated hydrocarbons from PAHs and other unsaturated hydrocarbons.

Activated sorbent material was partially deactivated by adding 5% (v/w) water and mixing thoroughly using a mechanical shaker (1 hr shaking) and allowed to equilibrate for 24 hrs. 10 ml of alumina and 10 ml of silica gel were mixed in a 100 ml beaker and hexane added to make a slurry. The slurry mixture was then carefully transferred into a chromatography column (45 m length x 1.0 cm i.d.) with teflon stopcock. Small volumes of hexane were used to rinse the beaker and the adsorbent material added into the column. Gently tapping of the column facilitated close packing of the sorbent. Finally about 1.0 g or 1.0 cm of Na_2SO_4 was put on top of the column. Excess hexane was drained without allowing the column to run dry. This column is capable of cleaning about 100 mg of lipid extract.

A lipid sample extract in a vial is made up to 1 ml with hexane and pipetted into the column and allowed to drain. The column is then eluted with 20 ml hexane, to give a first fraction (F1), which contains saturated hydrocarbons. This is followed by dichloromethane/hexane (36 ml) to give the second fraction (F2), which contains unsaturated hydrocarbons. Fractions F1 and F2 were received in separate vials. Sample concentrations were carried out over a flow of nitrogen ($\leq$ 5 ml) and samples stored in a deep freezer (-10 to -20 °C) awaiting further analysis. For the purpose of the present study further analysis was restricted to F2.

2.6 Qualitative and Quantitative Determination of PAHs by GC-FID

A Perkin Elmer AutoSystem XL gas chromatography equipped with a flame ionisation detector (GC-FID) was used for qualitative and quantitative determination of PAHs in sample extracts. The GC-FID was calibrated using a certified reference standard SRM 2260 obtained from the National Institute for Standards and Technology (NIST) of the USA, with the internal standard 9,10-dihydroanthracene obtained from the Marine Environmental Laboratories of the International Atomic Energy Agency (MEL-IAEA), in Vienna.

Samples were spiked with 50 µg of the internal standard (for all samples not previously spiked during the extraction procedure) and concentrated to volumes of less than 0.5 ml, before injecting into the GC. The internal standard solution was also injected into the GC and the actual concentration determined. Close estimates of sample volumes were then made by comparing the concentration of the internal standard in the samples and the concentration in the standard solution.

The operating conditions of the GC-FID were as follows:-

Column PE 5, 30 m length x 0.53 mm i.d. x 1.5 µm film thickness,
Carrier gas N_2 at 40 cm s^{-1} (~ 5 ml min^{-1}),
Injection mode : splitless, injection volume = 2.0 µl,
Injector temperature 250°C,
Detector temperature : 300°C,

Oven temperature program 60°C hold for 2 minutes,
60 to 280°C at 5°C min^{-1},
280°C hold for 40 minutes.

2.7 Method Evaluation

Data quality assurance and method validation were performed by processing and analysing reference standard material composed of PAHs in estuarine sediments, SES 1 obtained from the National Research Council of Canada. An indication of the PAH recoveries in the extraction and cleanup processes of SPM and sediment samples was obtained from the recoveries of the surrogate standard 9,10-dihydroanthracene (see sec. 2.4.2).

Recoveries of the surrogate standard ranged from 73.7 to 93.8 % (mean 82.4 ± 4.6%). The mean concentrations of all the PAHs from 5 determinations in the standard reference material exceeded 80 % of the certified values (overall range of 12 PAHs 74.7 – 94.4 %, mean 84.1 ± 4.6 %), with the exception of the results for naphthalene which were lower and more variable (range 46.1-68.8 %, mean 57.8 ± 8.2%). The results of the analysis of the standard reference material were found acceptable and correction of generated data for recoveries was considered not necessary.

2.8 Environmental Parameters

A selection of hydrographic parameters were routinely monitored in the course of the study, namely, nitrates (NO_3^--N), phosphates (PO_4^{3-}-P), particulate organic carbon (POC), total organic carbon (TOC in sediments), dissolved oxygen (DO), salinity, pH, temperature and turbidity. Standard colorimetric (Hitachi model 160-20 UV-visible spectrophotometer) methods were applied for the determination of inorganic nitrates (Grasshoff, 1983a) and phosphates (Koroleff, 1983). Salinity was determined using a refractometer and the standard silver nitrate titration method (Grasshoff, 1983b). POC was determined by a chromic acid digestion colorimetric method (Parsons *et al.*, 1984), and DO by the Winkler titration method (Grasshoff, 1983c). A pH meter and sensitive thermometer were used to measure pH and temperature, respectively. Water turbidity was measured by a nephelo-turbidity meter (Systronics, India) with formazin reference standard.

Estimations of the sand, silt and clay content of sediment samples were made by wet sieving through a 63 μm sieve and the pipette analysis procedure (Krumbein & Pettijohn, 1938; Carvar, 1971; Lewis, 1984).

2.9 Data Analysis

Data analysis was performed with the aim of establishing temporal and spatial distribution of PAHs and environmental parameters. The temporal variations were considered over the pre-monsoon, monsoon and post-monsoon seasons. Spatial distribution considered horizontally and vertical profiles across the sampling stations. A computer-based spreadsheet, MS Excel, was used for data management and the preparation of graphical presentations. The multivariate statistical procedures, Principal Component and Cluster analysis, were used to analyse distribution patterns in the data, to establish relationships between PAHs and environmental variables, and elucidate sources of PAHs in the study area. A computer-based multivariate statistical package, STATISTICA Kernel release 5.5A '99 edition (StatSoft, Inc., 2002) was used to accomplish the tasks.

Chapter 3

WATER QUALITY AND PHYSICO-CHEMICAL PARAMETERS

3.1 Introduction

The Cochin estuarine system extends between latitude 9°40' to 10°12'N and longitude 76°10' to 76°30'E. It is connected to the Arabian Sea by a permanent bar mouth at Cochin and two seasonal openings, during peak monsoon season, at Andhakaranazhi and north Paravoor. The expansive backwaters are generally shallow, with the exception of two deep navigation channels maintained at atleast 12 m depth that lead into the harbour. Two major rivers discharge freshwater into the estuarine system, the Periyar flowing into the northern part and the Pumpa flowing into the southern part. Other smaller rivers flowing into the estuary are Achankoil, Manimala, Meenachil and Muvatupuzha.

The Cochin estuary experiences a diurnal tidal regime with the mean tidal range at 0.63 metres (Wyatt & Qasim, 1972). The tidal cycle thus influences short term or daily variations of physico-chemical parameters and water quality in the estuary. Seasonal variations of the hydrographic parameters are influenced by the monsoon driven rains (June-September), which increase the volume of freshwater flowing into the estuary. The physico-chemical parameters recorded describe the overall condition and water quality of the Cochin estuarine system. Spatial and temporal distribution of inorganic nitrate (plus nitrite) and phosphate, including DO levels, were used to get an indication of anthropogenic inputs of wastes (Lakshmanan *et al.*, 1987) and their impact on the water quality in the system. The multivariate principal component analysis technique was further applied to gain insight into variations in the water quality. The computer statistical package STATISTICA Kernel release 5.5A '99 edition (StatSoft Inc., 2002) was used for the analysis.

3.2 Results and Discussion

3.2.1 Salinity

Daily variations of water salinity in the Cochin estuarine system are influenced by the diurnal tidal cycle (see Chapter 4). Seasonal salinity variations on the other hand are influenced by the increased freshwater discharge during the monsoon seasonal rains. Salinities were highest during the pre-monsoon season, which is characterised by dry weather and low river flow (Table 3.1, Fig. 3.1). Little or no saline water intrusion was indicated at the riverine Stations 1 and 2, where salinities never exceeded 1‰. The estuary was virtually completely inundated with freshwater as a result of the high volumes of river and runoff discharged during the monsoon season. Thus, average salinities fell to less than 8 ‰ at Station 4, and less than 4 ‰ at the other stations. Salinities increased during the relatively dry post-monsoon season to levels approaching those prevailing during the pre-monsoon season.

A further examination of the salinity variations down the water column, however revealed a salinity gradient attributable to high salinity water at the bottom and low salinity river water at the surface, especially at the deeper Stations 4 and 5, during the monsoon season (Fig. 3.2b). At Station 4, bottom and surface water salinities averaged 17.7 and 2.7 ‰, respectively. The corresponding salinities at Station 5 were 8.3 and 1.4 ‰, which were indicative of the influx of high volumes of freshwater, which effectively inundated the estuary, with the effect extending beyond the Bar Mouth into the sea. Vertical salinity gradients were less pronounced at the deep estuarine stations during the dry seasons, indicating effective mixing (Fig. 3.2a,c). Stratification of the backwaters as depicted by a distinct high salinity bottom layer as a result of the influx of seawater during the monsoon season has been previously observed and reported by Ramamirtham & Jayaraman (1963).

Table 3.1. Seasonal variations of water salinity (measurements in ‰)

Season	Sample*	Station Nos.						
		1	2	3	4	5	6	7
Pre-monsoon	SML	0.4	0.6	15.8	23.3	23.9	19.6	8.0
	SSL	0.4	0.6	21.1	24.1	24.1	20.8	9.3
	BL				26.2	24.6	16.0	
	Mean	0.4	0.6	18.5	24.5	24.2	18.8	8.7
Monsoon	SML	0.1	0.1	2.3	2.5	1.3	0.6	0.2
	SSL	0.0	0.1	0.9	2.7	1.4	0.5	0.1
	BL				17.7	8.3	1.0	
	Mean	0.1	0.1	1.6	7.7	3.6	0.7	0.2
Post-monsoon	SML	0.0	0.1	11.6	21.2	17.0	16.3	5.0
	SSL	0.0	0.6	13.1	21.7	19.7	18.9	7.5
	BL				26.6	23.3	20.3	
	Mean	0.0	0.3	12.3	23.2	20.0	18.5	6.2
Overall mean		0.4	0.8	8.9	14.8	13.2	11.0	5.5

* SML = surface microlayer, SSL = sub-surface layer, BL = bottom layer.

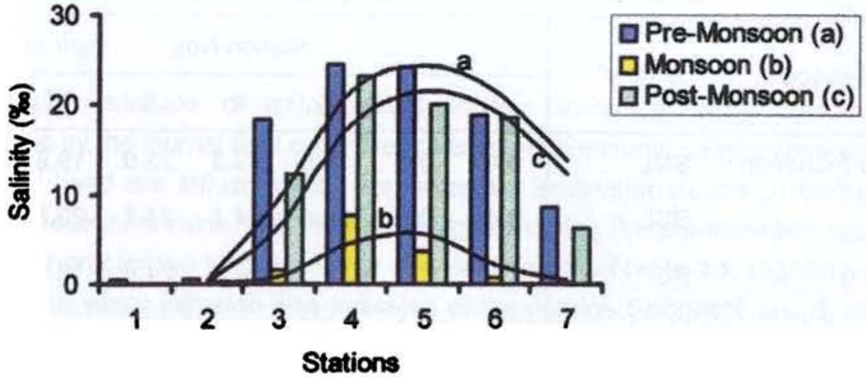

Fig. 3.1. Seasonal variations of water salinity

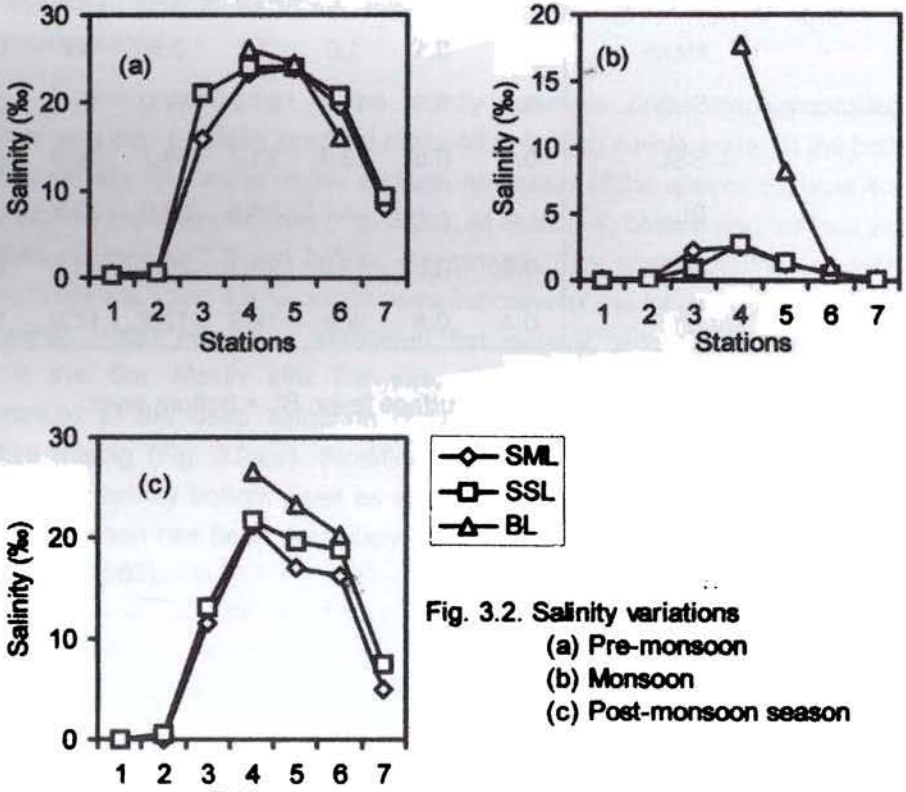

Fig. 3.2. Salinity variations
(a) Pre-monsoon
(b) Monsoon
(c) Post-monsoon season

3.2.2 pH

Measurements of pH averaged from 6.4 at Station 2 to 7.8 at Stations 4 and 5 (Table 3.2). Expectedly the riverine stations experienced more acidic pH values, which became progressively alkaline towards the estuary and the open sea (Fig. 3.3). In the monsoon season, when the entire estuary was inundated with freshwater, mean pH across the stations varied narrowly (range 7.0-7.4) (Fig. 3.4). In comparison, the tidal influence during the relatively dry pre- and post-monsoon seasons was reflected by a wider variation in the pH (6.4-7.8).

Table 3.2. Seasonal variations of water pH (measurements in pH units)

Season	Sample	Station Nos.						
		1	2	3	4	5	6	7
Pre-monsoon	SML	7.1	6.6	7.6	7.7	7.7	7.7	7.2
	SSL	6.4	6.2	7.3	7.7	7.8	7.6	7.2
	BL				7.7	7.9	7.7	
	Mean	6.7	6.4	7.5	7.7	7.8	7.6	7.2
Monsoon	SML	7.1	7.1	7.2	6.9	7.0	7.1	7.2
	SSL	7.0	6.9	7.2	7.4	7.3	7.1	7.4
	BL				7.9	7.6	7.3	
	Mean	7.1	7.0	7.2	7.4	7.3	7.1	7.3
Post-monsoon	SML	7.2	6.7	7.3	8.0	7.5	7.3	7.3
	SSL	6.8	6.5	7.6	7.8	7.7	7.7	7.0
	BL				7.7	7.9	7.9	
	Mean	7.0	6.6	7.4	7.8	7.7	7.7	7.1
Overall mean		6.9	6.8	7.4	7.7	7.7	7.5	7.2

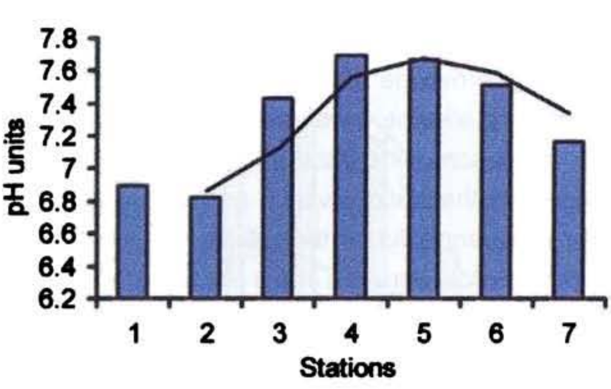

Fig. 3.3. Mean water pH

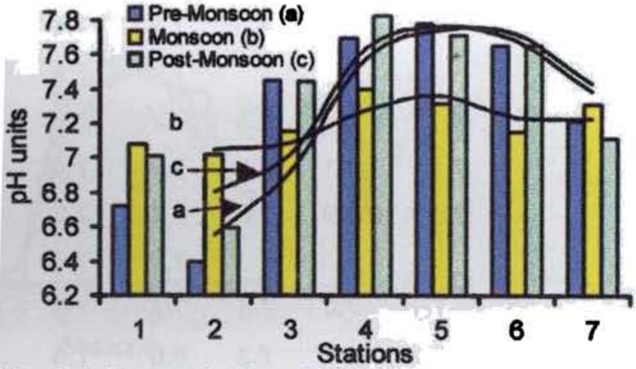

Fig. 3.4. Seasonal pH variations

3.2.3 Temperature

Average water temperatures ranged from a low of 27.7°C during the monsoon to a high of 31.3°C in the pre-monsoon season (Table 3.3). The lowest temperatures occurred during the wet season (27.7 to 29.6°C) and the highest in the pre-monsoon period (29.9 – 31.3°C) (Fig. 3.5). It is noted that temperatures recorded at Station 7 were consistently about the highest. This was because the station was shallow and almost always visited in the early afternoon when water temperatures had peaked.

Table 3.3. Seasonal variations of water temperature (measurements in °C)

Season	Sample	Station Nos.						
		1	2	3	4	5	6	7
Pre-monsoon	SML	29.0	29.8	29.5	29.2	29.5	28.3	30.4
	SSL	31.9	32.4	30.7	30.5	30.9	30.6	32.2
	BL				30.1	30.0	30.8	
	Mean	30.4	31.1	30.1	29.9	30.1	29.9	31.3
Monsoon	SML	28.3	27.9	27.8	27.8	27.9	28.3	29.0
	SSL	28.0	28.0	28.2	29.6	29.2	28.4	30.3
	BL				25.8	27.3	28.0	
	Mean	30.4	31.1	30.1	29.9	30.1	29.9	31.3
Post-monsoon	SML	29.1	29.6	28.5	28.1	29.5	27.6	30.0
	SSL	29.7	29.5	29.3	29.2	29.9	29.5	30.9
	BL				28.5	29.2	28.6	
	Mean	29.4	29.6	28.9	28.6	29.5	28.6	30.5
Overall mean		29.4	29.6	29.2	29.0	29.3	29.1	30.5

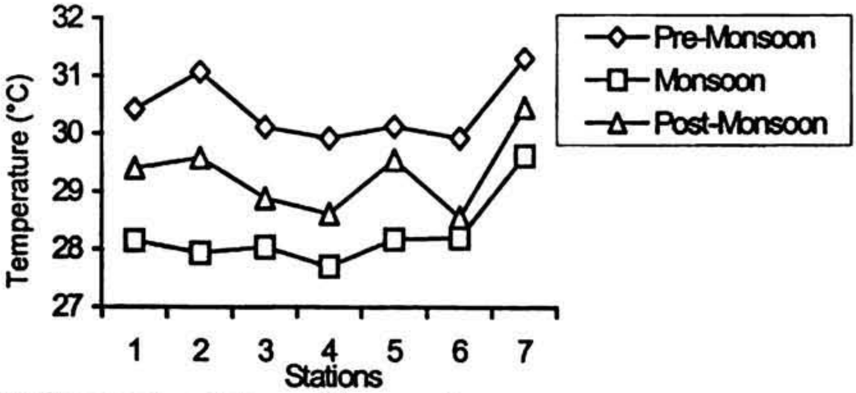

Fig. 3.5. Seasonal variations of temperature

3.2.4 Turbidity

The seasonal average turbidity ranged from 6.0 to 133.8 NTU (Table 3.4). The seasonal trend shows relatively higher levels of turbidity during the monsoon season, an indication of the increased volumes of suspended solids carried downriver through stormwater and runoff (Fig. 3.6). Station 5 experienced about the highest levels of turbidity throughout the study period, followed by Stations 6 and 3. It is noted that the three stations are located in the vicinity of the urban, industrial and harbour areas and are thus subjected to discharges of contaminated wastewater and surface runoff. Station 5 especially, is subjected to wastewater discharge from the busy fish market located adjacent to the harbour. The highest turbidities were encountered in the bottom water layer (Fig 3.7a,b,c), which was attributed to sediment re-suspension and estuarine mixing processes.

Table 3.4. Seasonal variations of water turbidity (measurements in NTU)

Season	Sample	Station Nos.						
		1	2	3	4	5	6	7
Pre-monsoon	SML	133.8	7.5	10.7	15.5	11.7	17.6	9.5
	SSL	18.0	11.3	13.3	8.6	17.2	33.0	11.8
	BL				9.0	59.3	21.1	
	Mean	75.9	9.4	12.0	11.0	29.4	23.9	10.6
Monsoon	SML	8.7	6.3	21.5	9.4	21.7	10.0	10.4
	SSL	11.6	8.4	27.7	11.0	26.0	17.0	12.7
	BL				20.0	77.0	20.5	
	Mean	10.2	7.4	24.6	13.5	41.6	15.8	11.6
Post-monsoon	SML	11.5	10.4	11.3	15.6	9.6	8.2	10.6
	SSL	8.9	9.9	11.0	11.7	12.7	8.1	6.0
	BL				19.7	42.8	20.0	
	Mean	10.2	10.2	11.2	15.6	21.7	12.1	8.3
Overall mean		23.8	9.3	17.2	12.9	24.6	18.0	11.3

An exceptionally high turbidity (264 NTU) was however recorded for the surface micro-layer at Station 1 and was attributed to the discharge of contaminated wastewater from the industries located in the vicinity (Fig. 3.7a). The results, however, indicate that suspended solids did not affect the sub-surface and bottom water layers, probably because of efficient flushing by the river.

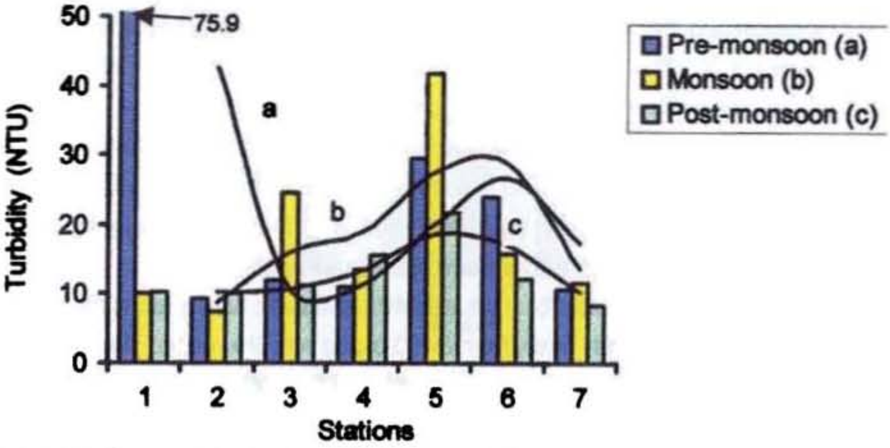

Fig. 3.6. Seasonal variations of water turbidity

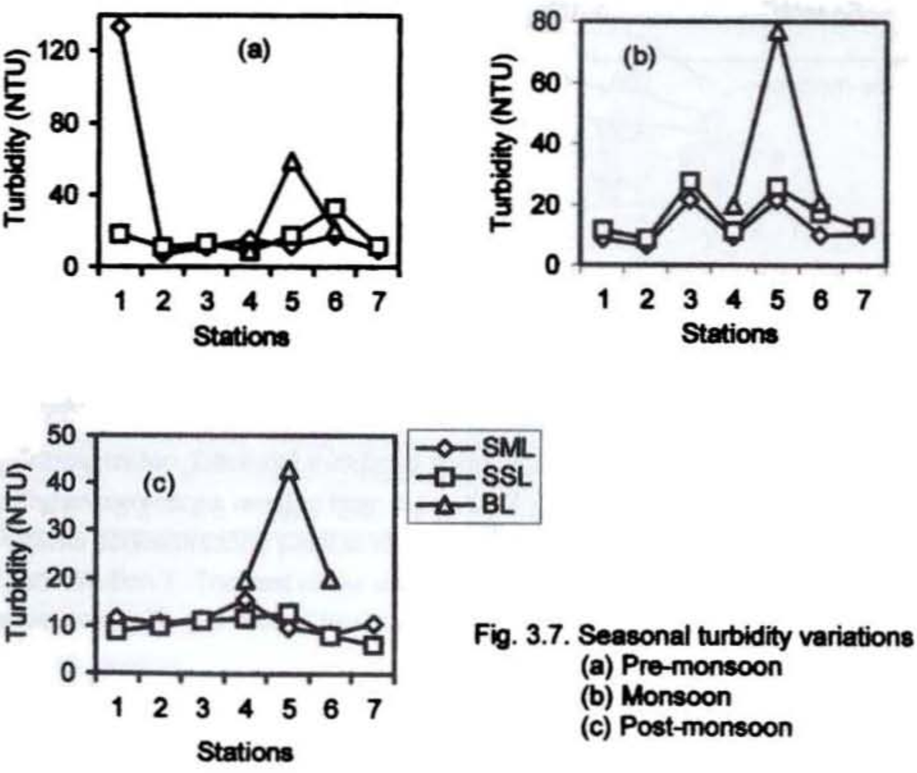

Fig. 3.7. Seasonal turbidity variations
(a) Pre-monsoon
(b) Monsoon
(c) Post-monsoon

3.2.5 Dissolved oxygen

Concentrations of dissolved oxygen (DO) on average varied from 4.3 to 8.7 mgO l^{-1} (Table 3.5). The highest DO concentrations occurred at the Station 1, followed closely by Station 2 (Fig. 3.8). All the estuarine stations gave relatively low DO values, a manifestation of the lowered solubility with increasing water salinities. It is noteworthy that the lowest DO concentrations were measured at Station 7 despite shallow depths and low salinities. The low DO values signify the poor quality of the water as a result of pollution from land-based sources. The station is subject to pollution from a nearby market and domestic sources, which typically discharge organic wastes that exert high BOD loads into the water. On average the highest DO levels were obtained during the wet monsoon season, attributed to turbulence and lowered salinities (Fig. 3.9).

Table 3.5. Seasonal variations of dissolved oxygen(concentrations in mgO l^{-1})

Season	Sample	Station Nos						
		1	2	3	4	5	6	7
Pre-monsoon	SSL	5.6	6.2	5.2	5.0	4.9	4.7	4.3
	BL	5.6	5.2	4.8	4.9	4.9	5.1	4.4
	Mean	5.6	5.7	5.0	4.9	4.9	4.9	4.3
Monsoon	SSL	8.2	7.6	6.3	7.1	7.5	6.5	4.8
	BL	8.7	7.8	6.4	5.5	7.0	6.2	5.4
	Mean	8.4	7.7	6.4	6.3	7.2	6.4	5.1
Post-monsoon	SSL	6.6	6.8	4.9	5.4	5.7	5.2	4.3
	BL	6.8	6.8	5.1	6.0	5.6	5.7	4.7
	Mean	6.7	6.8	5.0	5.7	5.6	5.5	4.5
Overall Mean		6.6	6.5	5.3	5.4	5.6	5.4	4.5

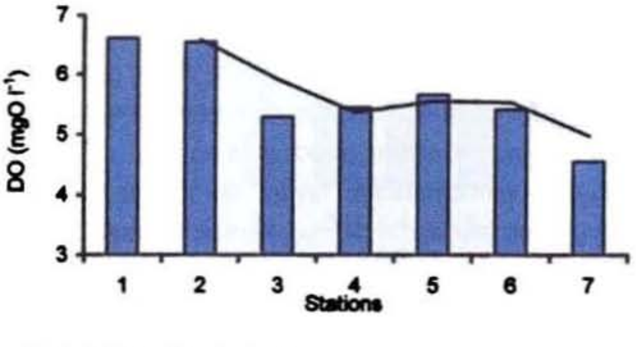

Fig. 3.8. Mean dissolved oxygen

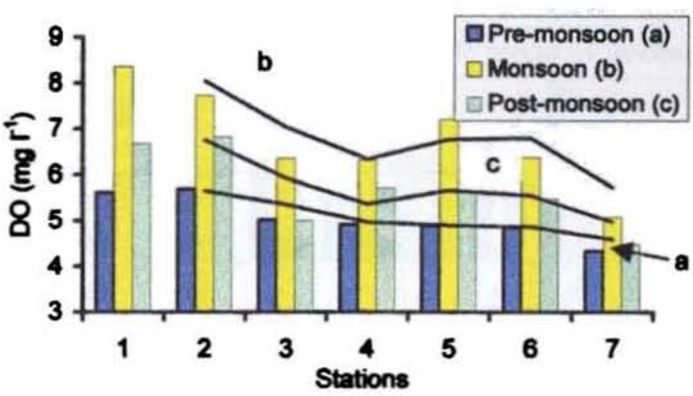

Fig. 3.9. Seasonal variations of DO

3.2.6 Nitrate/nitrite – nitrogen

Concentration levels of inorganic nitrates and nitrites ($NO_3^- + NO_2^- - N$) were high with concentrations ranging from 4.1 to 82.7 µmol $NO_3^- / NO_2^- - N\ l^{-1}$ (Table 3.6). Nitrate/nitrite concentrations peaked at Station 3 ,followed by the riverine Stations 1 and 2, and Station 7. The rest of the estuarine stations showed progressively lower concentrations, with the lowest levels occurring at Station 4 (Fig. 3.10). The high levels of the nutrients signify high inputs through the river and enhanced by land-based sources from the urban and industrial areas. The relatively lower levels at Stations 6, 5 and 4 indicate the effects of removal of the nutrients due to rising salinities (Lakshmanan *et al.*, 1987).

The highest concentrations were obtained during the pre-monsoon season (Fig. 3.11), an observation that is similar to that reported by Balakrishnan & Shynamma (1976). The high concentrations at Stations 1 and 2, indicated high inputs through the river. In addition high inputs from land-based urban and industrial sources were reflected by the high concentration levels at Stations 3 and 6. It is evident that estuarine mixing processes do not adequately remove the nutrients and thus high concentration levels are maintained despite the high prevailing salinities (mean salinity > 18 ‰). Similar trends of the nutrient levels were obtained for the pre- and post-monsoon seasons, with the latter giving lower concentrations. The concentrations during the monsoon season were relatively lower and with a narrow range of variation (Table 3.6, Fig. 3.11). This signifies the diluting effect of the high volumes of freshwater resulting in a more even distribution of the nutrients.

Table 3.6. Seasonal variations of nitrate/nitrite-nitrogen(concentrations in μmol l^{-1})

Season	Sample	Station Nos.						
		1	2	3	4	5	6	7
Pre-monsoon	SML	64.9	70.5	48.6	15.2	63.5	44.5	15.2
	SSL	37.7	48.3	82.7	18.2	16.5	71.8	49.1
	BL				55.1	25.2	46.7	
	Mean	51.3	59.4	65.7	29.5	35.1	54.3	32.1
Monsoon	SML	22.9	36.4	22.0	51.3	34.0	5.9	21.3
	SSL	24.5	23.5	33.9	10.7	19.5	23.3	13.3
	BL				6.0	16.5	11.5	
	Mean	23.7	30.0	27.9	22.6	23.3	13.5	17.3
Post-monsoon	SML	23.9	28.0	57.4	27.7	15.2	16.2	39.3
	SSL	27.3	18.6	39.7	19.9	19.2	17.9	53.5
	BL				15.0	16.0	4.1	
	Mean	25.6	23.3	48.6	20.8	16.8	12.7	46.4
Overall mean		33.5	37.6	47.4	24.3	25.1	26.9	31.9

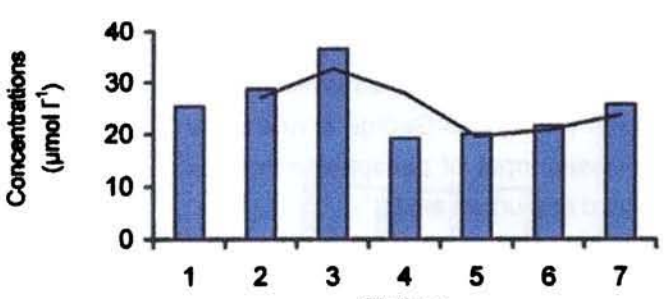

Fig. 3.10. Mean nitrate/nitrite concentrations in water

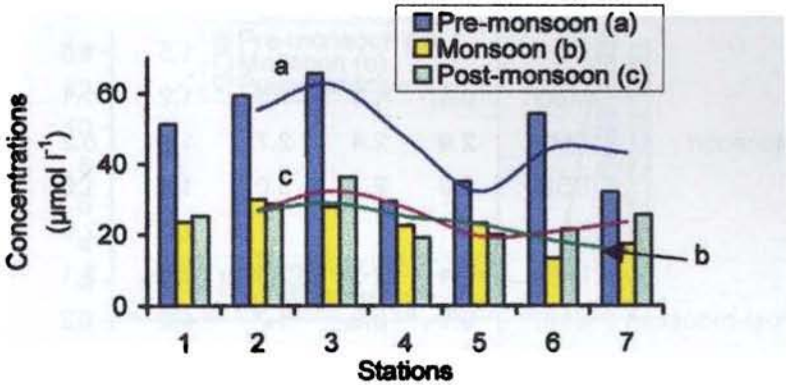

Fig. 3.11. Seasonal variations of Nitrate/Nitrite in water

3.2.7 Inorganic phosphate

Concentrations of inorganic phosphate in the water column ranged from 0.4 to 43.6 μmol PO^{3-}_4-P l^{-1} (Table 3.7). Station 2 had the lowest mean concentration (1.6 μmol l^{-1}) and Station 7 the highest concentration (21.7 μmol l^{-1}) (Fig. 3.12). Stations 4 and 6 had the lowest mean concentration at 1.4 μmol PO^{3-}_4-P l^{-1}, followed closely by Station 2. The relatively higher concentration at Station 1 was apparently due to an episodic occurrence of industrial discharge. The consistently high concentrations at Station 7 confirm the poor water quality due to the discharge of domestic and/or urban wastewater.

Seasonal trends show that within the estuary, with the exception of Station 7, higher values were obtained during the monsoon season than in the pre- and post-monsoon seasons (Fig. 3.13), an observation that is in general agreement with Wyatt & Qasim (1972) and George & Krishnakartha (1963). This observation reflected the increased input of phosphates into the system through runoff from both agricultural land and urban areas.

Table 3.7. Seasonal variations of phosphate-phosphorus(concentrations in μmol l^{-1})

Season	Sample	Station Nos						
		1	2	3	4	5	6	7
Pre-monsoon	SML	0.4	1.0	1.3	1.1	0.9	0.6	43.6
	SSL	0.4	0.9	1.7	1.1	0.9	2.3	34.4
	BL				1.5	1.5	1.1	
	Mean	0.4	1.0	1.5	1.2	1.1	1.3	39.0
Monsoon	SML	2.9	2.4	2.7	1.5	5.2	1.2	10.8
	SSL	2.0	2.7	3.0	1.9	1.9	0.8	12.4
	BL				2.0	2.0	2.0	
	Mean	2.4	2.5	2.9	1.8	3.1	1.3	11.6
Post-monsoon	SML	9.1	0.9	1.7	1.2	1.2	1.8	13.5
	SSL	8.3	1.3	1.2	0.8	0.8	1.4	15.2
	BL				1.1	1.3	1.2	
	Mean	8.7	1.1	1.4	1.0	1.1	1.5	14.4
Overall mean		3.8	1.5	1.9	1.4	1.7	1.4	21.7

However, the peak concentrations obtained at Station 7 during the pre-monsoon season dropped in the succeeding season, indicating the effect of the influx of high volumes of relatively low-phosphate freshwater during the wet season.

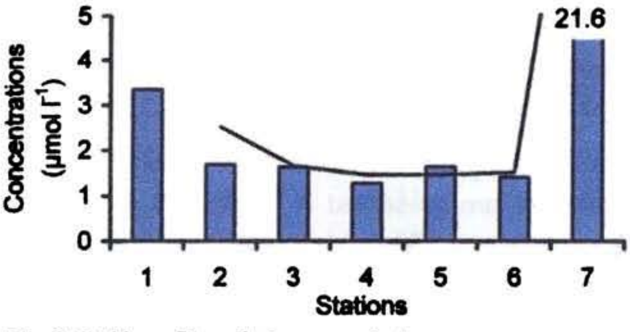

Fig. 3.12. Mean Phosphate concentrations in water

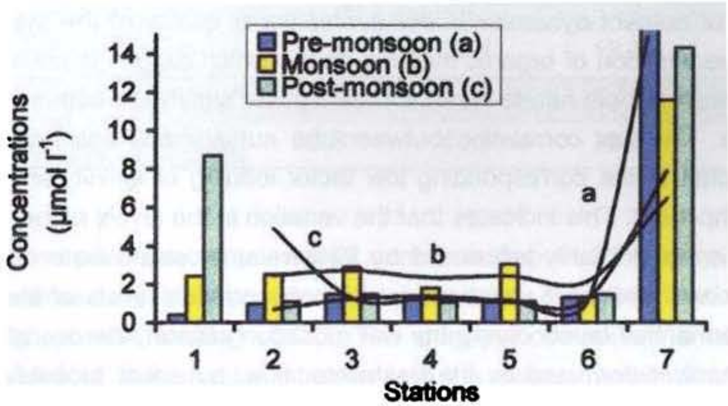

Fig. 3.13. Seasonal variations of Phosphate in water

3.2.8 Principal component analysis

The objective of the technique is to establish linear relationships between different variables and reduce them into fewer important components that can adequately explain most of the variance in the data set. In practice, the technique consists of the determination of the covariance or correlation matrix of the normalized variables, followed by the calculation of the eigenvector matrix which represents the magnitudes and directions of variables in space, and the eigenvalues which represent their corresponding variances (Manly, 1994).

Seasonal variations of the physical and chemical parameters in the sub-surface and bottom layers were considered in the analysis. Data on the surface micro-layer was left out because DO concentrations were not determined. All data were normalised to minimise discrepancies due to differences in units and magnitude of the parameters. The normalised data was then subjected to principal component analysis using STATISTICA (StatSoft Inc., 2002).

The correlation matrix and results of the principal component analysis on the environmental variables are presented in Tables 3.8 and 3.9. It is evident that about 90% of the variance in the environmental conditions is attributed to the first four principal components. The first principal component F1 accounts for about 34% of the variance in the environmental variables, and is characterised by high positive factor loadings of temperature and nitrate/nitrite, moderate loading of phosphate, and a high negative loading of DO. This factor clearly explains the contribution of nutrient dynamics in the overall water quality of the system, which entails remineralisation of organic material during which oxygen is consumed. The positive loadings of both nitrate/nitrite and phosphate signify the common source of the nutrients. The low correlation between the nutrient and salinity variables is further reflected in the corresponding low factor loading of salinity and pH in the principal component. This indicates that the variation in the levels of the nutrients in the system is not primarily influenced by freshwater or saline water flows. Thus, despite observed seasonal variations in the concentration levels of the nutrients, particularly enhanced levels during the wet monsoon season, the overall variations are not primarily determined by the freshwater flow, but most probably by inputs from land-based sources, which include municipal wastewater and industrial effluent. This observation has important ramifications on the water quality and pollution of the estuarine system.

Table 3.8. Correlation matrix of environmental parameters (N = 30, highlighted correlations are significant at $p < 0.5$)

	Salinity	pH	Temperature	DO	Turbidity	Nitrate	Phosphate
Salinity	1						
pH	**0.79**	1					
Temperature	0.06	**-0.32**	1				
DO	**-0.59**	**-0.38**	**-0.51**	1			
Turbidity	0.17	**0.31**	-0.27	0.07	1		
Nitrate	0.09	-0.25	**0.57**	**-0.40**	-0.12	1	
Phosphate	-0.20	-0.15	**0.36**	**-0.35**	-0.17	0.23	1

Table 3.9. Principal components and factor loadings of environmental parameters (absolute values of highlighted loadings are > 0.5)

	Factors			
	F1	F2	F3	F4
Salinity	0.16	**-0.93**	0.08	0.20
PH	-0.20	**-0.92**	-0.21	0.02
Temperature	**0.86**	0.11	0.18	0.02
DO	**-0.72**	**0.58**	0.14	0.01
Turbidity	-0.37	-0.38	0.34	**-0.76**
Nitrate	**0.75**	0.05	**0.50**	-0.08
Phosphate	**0.58**	0.19	**-0.62**	-0.46
Eigenvalue	2.37	2.25	0.86	0.84
% of total variance	33.8	32.1	12.3	12.0
Cumulative eigenvalue	2.37	4.61	5.47	6.31
Cumulative %	33.8	65.9	78.2	90.1

The second principal component F2 contributes about 32% of the variance and is characterised by high negative factor loadings of salinity and pH, and moderately high positive loading of DO. This principal component is associated with the dynamics of saline water intrusion into the estuary and mixing process which are the principal environmental factors influencing the water quality in the system. While pH changes directly proportionally to salinity, the inverse is the case between DO and salinity.

The third principal component F3 accounts for about 12% of the variance and is characterised by moderately high negative loading of phosphate and positive loading of nitrate/nitrite. This indicates an inverse relationship between nitrate/nitrite and phosphate variations in the system, which may signify the net removal of phosphate.

The fourth principal component accounts for a low 12% of the variance in the environmental variables, dominated by turbidity with a high negative factor loading. It is noted that turbidity displayed low correlation with salinity and all other environmental parameters considered, which is reflected by correspondingly low factor loadings. This principal component is associated with processes that influence turbidity in the estuary, which include sedimentation and/or sediment resuspension.

3.2.9 Nutrient levels in the Cochin estuarine system

A comparison of the concentration levels of nitrates and nitrites in water reported in earlier studies and the present study in the Cochin backwaters generally indicated a rising trend (Table 3.10). The results from the present study fall within the range reported by Nair (1990) and Saraladevi (1991). The rising levels of the nutrients over the years are attributed to increasing inputs from land-based sources, which include intensified agricultural activities, and the discharge of poorly treated or completely untreated urban and industrial wastewater.

Table 3.10. Reported concentrations of nitrate and nitrite in Cochin backwaters

System	Concentration range (µg-at N l^{-1})		References
	NO_3^--N	NO_2^--N	
Cochin backwaters	0.0 – 3.0	0.0 – 2.0	Shynamma & Balakrishnan, 1973
Cochin backwaters	0.0 – 22.7	0.0– 45.0	Lakshmanan *et al.*, 1987
Cochin estuary	0.6 – 25.41	0.63 – 6.68	Anirudhan, 1988
Cochin backwaters	0.82 – 71.6	0.3 - 8.90	Nair, 1990
Periyar river estuary	0.0 – 140.4	0.0 – 20.0	Saraladevi, 1991

The concentration levels of inorganic phosphates in the Cochin backwaters also showed a rising trend over the years (Table 3.11). The results obtained from the present study are comparable to those reported by Anirudhan (1988), Nair (1990) and Saraladevi (1991). Again this is indicative of increased inputs from land-based sources.

Table 3.11. Reported concentrations of inorganic phosphate in the Cochin estuarine system

System	Concentration range (μg-at P l^{-1})	References
Cochin backwaters	0.0 – 3.0	Shynamma & Balakrishnan, 1973
Cochin backwaters	0.0 – 150	Lakshmanan *et al.*, 1987
Cochin estuary	0.27 – 11.61	Anirudhan, 1988
Cochin backwaters	0.4 – 9.30	Nair, 1990
Periyar river estuary	1.0 - 290	Saraladevi, 1991

3.3 Concluding Remarks

Salinity variation in the Cochin estuarine system is influenced by the tidal dynamics. Seasonal salinity variations are determined by the SW monsoon rains (June – September) which inundate the entire system with freshwater resulting in low salinities (mean range < 1 to > 24 ‰). Relatively higher salinities prevail in the estuary during the relatively drier pre- and post-monsoon seasons.

The spatial and seasonal variations of pH also reflect the salinity trends (mean range 6.4 – 7.8). Thus, relatively low pH values occur in the riverine Stations 1 and 2, which gradually increase towards the estuary and the sea.

Water temperatures showed seasonal variations, with the highest temperatures experienced during the pre-monsoon, and the lowest occurring during the monsoon season (mean range 27.7 – 31.3 °C).

The water turbidity (mean range 7.4 – 75.9 NTU) was enhanced during the monsoon season, reflecting the increased discharge of suspended solids through stormwater and runoff. The high turbidities recorded at Station 5, followed by Station 6 and Station 3 were attributed to wastewater and runoff discharge from urban, industrial and harbour areas. The highest turbidity values that were encountered in the bottom water were attributed to sediment re-suspension and estuarine mixing processes.

Increasing salinity was primarily attributed with lowered oxygen solubility in the estuarine stations (mean range 4.3 – 8.4 mgO l^{-1}). The lowest DO concentrations encountered at Station 7 indicated the poor quality of the water as a result of pollution from the nearby market and domestic sources.

High concentrations of inorganic nitrate and nitrite (mean range 2.5 – 110.3 µmol NO_3^-/NO_2^- -N l^{-1}) were attributed to high inputs through the river, and urban and industrial areas. Relatively lower concentrations in the estuarine stations (Stations 4, 5 and 6) were attributed to removal in rising salinities. The highest concentrations obtained during the pre-monsoon season demonstrated the high inputs through the river, and urban and industrial areas. Overwhelming high volumes of freshwater flows during the monsoon, lowered and more evenly distributed the nutrients.

The high concentrations of inorganic phosphate encountered were attributed to river input and the discharge of domestic and/or urban wastewater into the estuary. Higher concentrations were obtained during the monsoon season, with the exception of the most contaminated station (with respect to phosphate levels), Station 7, signifying increased inputs due to runoff.

Multivariate principal component analysis indicated that variations in the environmental factors and water quality in the estuarine system were primarily influenced by nutrient dynamics, associated with remineralisation processes, which also indicated inputs from land-based sources. It is instructive that nutrient dynamics was not primarily associated with salinity indicating that nutrient levels in the estuary were mostly influenced by input from urban and industrial areas. Of slightly lower significance was saline water intrusion and by implication mixing processes. The contribution of water turbidity to variations in the environmental conditions was relatively less significant and was associated with sedimentation and resuspension processes.

Chapter 4

VARIABILITY OF PAH CONCENTRATIONS WITH HYDROGRAPHIC FACTORS

4.1 Introduction

The concentration of PAHs in the water column is subject to the dynamics of estuarine processes brought about by the tidal cycle and resultant mixing processes. Particulate associated PAHs in the estuary are subject to the levels of suspended sediments, which are derived from inflowing river and seawater and/or by resuspension of settled sediment as a result of tidal stirring. Thus, the concentrations at a sampling point depend on not only contaminant fluxes but also on physical and chemical factors and biological interactions. This raises a question over the practice of collecting representative water samples from the estuarine system by point sampling at predetermined time intervals, such as monthly or seasonally. Data obtained from the Shipyard Station (Station 8) over a period of approximately 10 hours gave a good indication of the variation of concentrations of PAHs with the tidal cycle and hence associated physical and chemical parameters.

4.2 Results and Discussion

4.2.1 Variation of PAH concentrations with tides

The variations of physico-chemical factors with tides, including concentrations of PAHs at the Shipyard Station are summarized in Table 4.1.

In the water column the lowest salinities occurred in the surface micro-layer with the highest salinities in the bottom layer, demonstrating the process of the denser saline seawater at the bottom mixing with low salinity river water on the surface (Fig. 4.1). The salinity differences were more pronounced during flooding tide and approached uniform mixing after high tide. The mixing trend with the tidal cycle is corroborated by pH variations in the water column (Fig. 4.2).

The water turbidity was generally lowest in the surface micro-layer, but with fairly uniform turbidity in the water column at about low tide (Fig. 4.3). The turbidity in the bottom water layer, however increased considerably during flooding tide, an indication of enhanced flocculation during mixing and resuspension of sedimentary material (Olsen *et al.*, 1982).

Dissolved oxygen levels were high at about low tide, with relatively higher concentrations in the surface layer, which is attributed to the dominance of high-oxygen low-salinity river water (Fig. 4.4). DO levels however decreased with flooding tide which was attributed to the lower solubility of oxygen in rising salinities.

Variations in water temperatures showed rising temperatures due to normal heating with intensification of sun radiation, peaking in the early afternoon. The temperatures then decreased with flooding tide as a result of the introduction of cooler seawater into the estuary and decrease in the intensity of sunlight (Fig. 4.5). Such diel variations whereby water temperatures increased towards evening were observed and reported by Balakrishnan & Shynamma (1976). The surface micro-layer experienced relatively lower temperatures compared to the sub-surface and bottom waters (the cooling effect of evaporation as a result of wind action and radiation). The temperature differences between the surface micro-layer, and the sub-surface and bottom water layers were narrow in the morning and increased steadily as the day wore on.

The variation of concentrations of nitrate/nitrite can be attributed to removal with increasing salinities and resuspension of nutrient-rich sedimentary material (Fig. 4.6). Nitrate/nitrite levels in the surface micro-layer and sub-surface layer show a decrease to a minimum, which coincides with minimum salinity at about mid-flood tide, followed by a gradual increase. The decrease signifies the dominance of removal processes at the prevailing high salinities (> 17‰). It is evident that the rising salinities and enhanced turbulence with flooding tide, flocculation and resuspension processes result in increased concentrations of nutrients. These results indicate that the nutrient sources and/or fluxes remained stable in the short-term.

In the bottom layer, nitrate/nitrite levels similarly decrease with salinity to a minimum, at about mid-flood tide, followed by a rapid increase. The rise in nutrient levels is in this case attributed to enhanced resuspension of nutrient rich sedimentary material, which is also reflected by a general rise in concentrations in the water column.

Table 4.1. Variations of physico-chemical parameters and PAH concentrations with the tidal cycle

Time	Temp	pH	Salinity	Turbidity	DO	Nitrate	ΣPAHs species§				TPAH	SPAH
							DPAH		PPAH			
(hh:mm)	(°C)		(‰)	(NTU)	(mg l^{-1})	(µmol l^{-1})	(µg l^{-1})	(%)*	(µg l^{-1})	(%)*	(µg l^{-1})	(µg kg^{-1})
7:55 AM	29.6	7.5	21.3	12.2	6.9	8.4	1.32	70.6	0.55	29.4	1.87	ND#
10:18 AM	29.5	7.4	18.5	17.3	6.7	9.6	1.96	86.3	0.31	13.7	2.27	ND
12:05 PM	30.6	7.5	17.4	7.7	5.8	4.8	2.00	95.2	0.16	4.8	2.10	102.3
2:08 PM	31.0	7.8	23.8	39.3	4.6	5.2	0.94	62.2	0.57	37.8	1.51	370.1
4:00 PM	30.2	8.0	27.8	27.9	4.5	8.3	0.39	47.0	0.44	53.0	0.83	108.3
6:03 PM	29.4	8.1	30.1	22.7	5.0	9.8	1.26	95.5	0.48	4.5	1.32	ND
Mean	30.1	7.7	23.2	21.2	5.6	7.7	1.3	76.1	0.4	23.9	1.7	193.5
SD	0.7	0.3	5.1	11.4	1.1	2.2	0.6	19.6	0.2	19.6	0.5	152.9

* % proportion of TPAH

ND - not determined.

§ DPAH = dissolved ΣPAHs, PPAH = particulate ΣPAHs, TPAH = DPAH + PPAH, SPAH = sedimentary ΣPAHs.

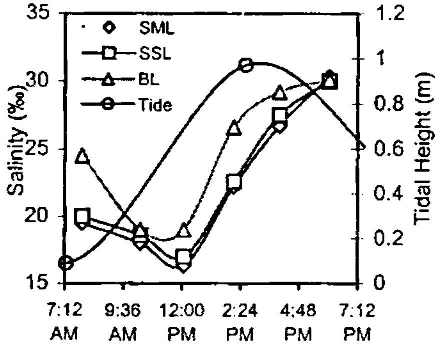

Fig. 4.1. Salinity variations with tidal height

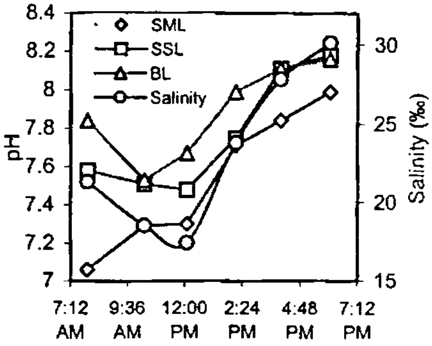

Fig. 4.2. Variation of pH with salinity

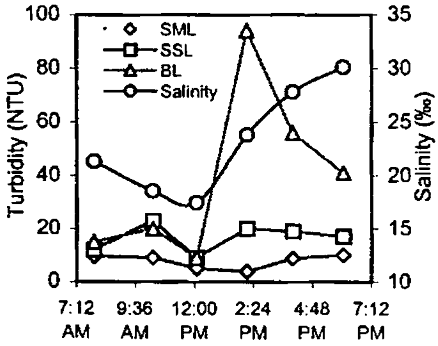

Fig. 4.3. Variation of turbidity with salinity

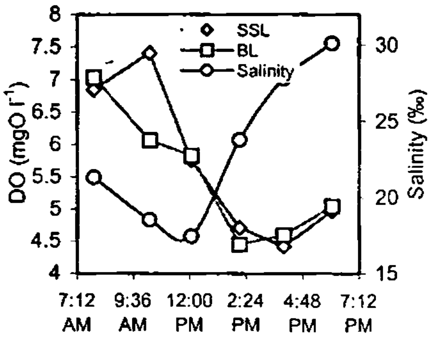

Fig. 4.4. Variation of DO with salinity

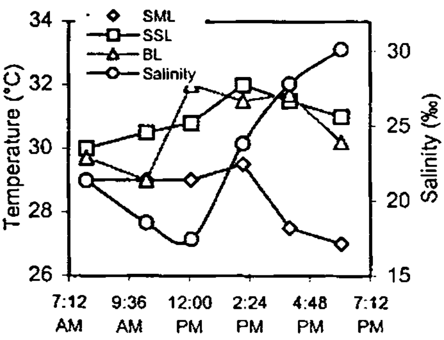

Fig. 4.5. Variations of temperature with salinity

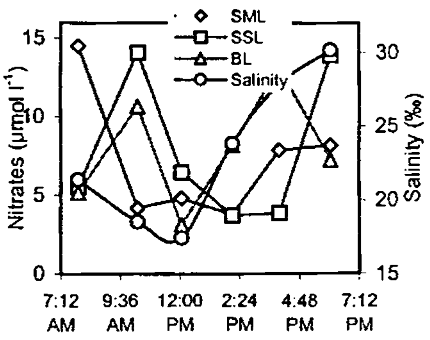

Fig. 4.6. Variation of nitrate/nitrite with salinity

A graphical presentation of variations of PAH concentrations with salinity and tides is given in Figures 4.7 and 4.8. Dissolved PAHs (mean 1.3 ± 0.6 µg l^{-1}) showed an increase with decreasing salinity and conversely a decrease with increasing salinity (Fig. 4.7). Particulate PAH levels (mean 0.4 ± 0.2 µg l^{-1}) showed a decrease with decreasing salinity followed by a modest increase with increasing salinity. Overall PAH concentrations (mean 1.7 ± 0.5 µg l^{-1}) tended to increase with decreasing salinity and vice versa. In the short-term the observations indicated a stable flux of PAHs into the system.

At Station 8 a delayed response of salinities to changing tides was recorded, with maximum salinity occurring well after high tide and low salinity occurring long after the attainment of low tide. A similar observation had been earlier reported at a point 1 - 2 km from Station 8 towards the Bar Mouth (Balakrishnan & Shynamma, 1976). The result of delayed response of salinities to changing tides was an increase in PAH concentration levels with rising tide to a maximum at about mid-tide, then falling to a minimum at about high-tide. Subsequently, the PAH concentrations began to rise with ebbing tide (Fig. 4.8).

It is evident that mixing at the Shipyard Station effectively begins at about mid-flood tide, when the salinity minimum is attained. At that point the water is experiencing low turbidities (see Fig. 4.3) and most of the PAHs introduced into the system are in the dissolved form (60-95%). Thereafter the turbidities rise sharply resulting in decreased concentrations of dissolved PAHs and an increase in the proportion of PAHs associated with particulates (75.0%). With the increased levels of suspended particulate matter as a result of flocculation and sediment resuspension processes during mixing, most of the PAHs get sorbed or associated with the particulates and are removed from the water column, resulting in a PAH concentration minimum at about the high tide. These observations have important implications on the results obtained from point sampling in a dynamic estuarine system, whereby continuously changing hydrographic factors influence the concentration levels of the hydrophobic contaminants.

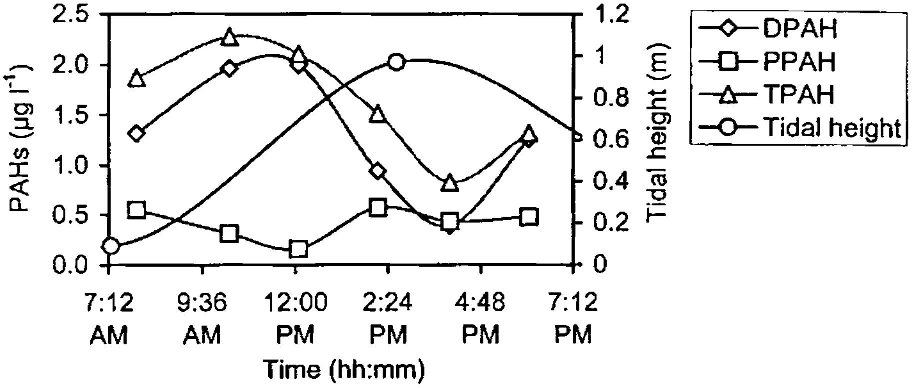

Fig. 4.7. Variation of PAHs with tidal height

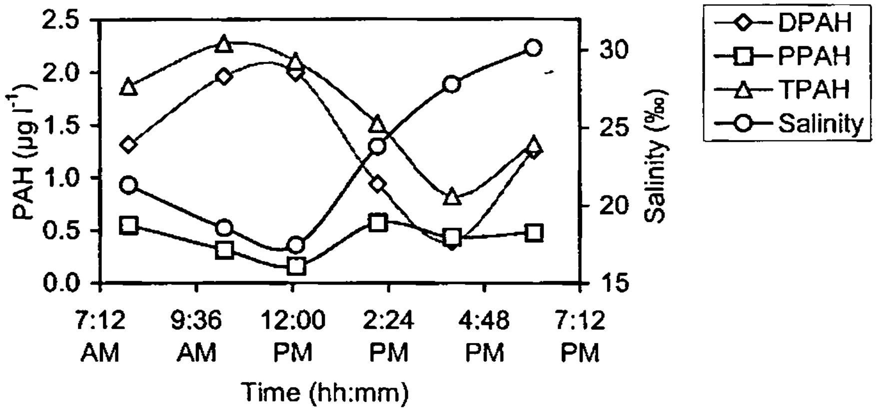

Fig. 4.8. Variation of PAHs with salinity

4.2.2 Correlations

The relationship between the variation of PAH concentrations in the water column and hydrographic parameters is summarized in a correlation matrix (Table 4.2). Taking into consideration the small size of the data set, total PAHs (TPAH) and dissolved phase PAHs (DPAH) are significantly negatively correlated with salinity, pH and turbidity, which reflect the influence of the tidal cycle and associated estuarine processes.

Table 4.2. Correlations between PAH levels and environmental factors (N = 12, highlighted correlation coefficients are significant at $p < 0.5$)

	Temperature	pH	Salinity	Turbidity	DO	Nitrate	DPAH	PPAH
Temperature	1							
pH	0.33	1						
Salinity	0.23	**0.99**	1					
Turbidity	0.25	0.46	0.49	1				
DO	**-0.63**	**-0.70**	**-0.64**	**-0.50**	1			
Nitrate	-0.21	0.13	0.21	0.27	0.08	1		
DPAH*	-0.04	**-0.61**	**-0.61**	**-0.56**	0.31	0.02	1	
PPAH*	-0.16	0.25	0.28	-0.22	0.15	0.06	0.08	1
TPAH*	-0.08	**-0.51**	**-0.50**	**-0.59**	0.34	0.04	**0.96**	0.34

* Total PAHs (TPAH) = Dissolved PAHs (DPAH) + Particulate PAHs (PPAH).

4.3 Concluding Remarks

The concentrations of PAHs in the water column are subject to the dynamics of estuarine processes in the course of the tidal cycle. At Station 8 the changes in the tide were reflected by salinity variations, with flooding tide characterised by denser high salinity bottom water mixing with low salinity surface water flowing downriver. Consequences of the changing tide included rising water pH and falling DO concentrations as a result of decreased oxygen solubility with rising salinity. The tidal current and mixing of saline and freshwater resulted in increased turbidity, especially in the bottom water layer, which indicated enhanced flocculation of suspended particulate matter and re-suspension of sedimentary material.

The concentrations of inorganic nitrate and nitrite in the water column were influenced by removal processes which predominate under low tidal-current conditions that prevail during low and high tide. On the other hand, flooding tide (and most probably ebbing tide), increased tidal currents and turbulence tend to enhance turbidity due to flocculation and sediment re-suspension resulting in increased concentrations of nitrate and nitrite.

The concentrations and speciation of PAHs in the water column were influenced by changing salinities and mixing processes in the estuary. Thus at low salinities and low turbidities, most of the PAHs were in the dissolved form. During flooding tide and higher turbidity, an indication of increased SPM, a greater proportion of the PAHs got associated with particulate matter and was removed from the water column, resulting in overall decreased concentrations of the contaminants in the water column. Therefore, concentrations and speciation of PAHs encountered in the water column were highly influenced by the tidal dynamics and resultant changes in the physical and chemical properties of the water.

Chapter 5

DISTRIBUTION OF PAHS IN ESTUARINE BACKWATERS

5.1 Introduction

The extent of pollution of the Cochin backwaters with hydrocarbon contaminants was first reported by Nair *et al.* (1995), who used a gravimetric technique to estimate the distribution of petroleum hydrocarbons in surficial sediments in the estuary. Conspicuous by the scarcity, comprehensive studies on hydrocarbon contamination of the Cochin estuarine system using contemporary analytical techniques are yet to be reported. The present study, of which results are presented below, is thus one of the few attempts at remediating the dearth of information on the subject.

5.2 Results and Discussion

5.2.1 Distribution of PAHs in the water column

An overview of the results of the concentration levels of PAHs in the Cochin estuarine system are summarized in Table 5.1 and graphical presentations in Figures 5.1 and 5.2. Mean contaminant levels in the water column were obtained by taking the means of the concentrations in the surface micro-layer, sub-surface layer and bottom layer, where applicable. Monthly averages of total PAHs (ΣPAHs) in the water column ranged from a minimum of 0.04 µg l^{-1} to a maximum of 7.21 µg l^{-1}. In general, the PAH levels showed a rising trend over the study period, with the increase becoming more evident after June, which was about the beginning of the rain season (Fig. 5.1). The generally low concentrations recorded from January to May are however also attributed to some losses due to PAH degradation during storage of samples. Relatively higher contamination levels were indicated for

Stations 1, 2, 3 and 7 (Fig. 5.2). The distribution of the contaminants encountered in the Cochin estuarine system over the study period is aptly presented in Fig. 5.3.

Table 5.1. Average concentrations of ΣPAHs in the water column

Month	Station Nos.							Mean	Max	Min
	1	2	3	4	5	6	7			
Dec-95	0.15	0.40	ND*	0.36	0.25	0.91	0.22	0.38	0.91	0.15
Jan-96	0.31	0.36	2.27	0.42	0.44	0.52	0.67	0.71	2.27	0.31
Feb-96	0.42	6.62	0.17	0.49	0.28	1.37	0.47	1.40	6.62	0.17
Mar-96	2.46	0.46	1.16	0.54	0.36	0.23	0.89	0.87	2.46	0.23
Apr-96	0.18	0.51	0.21	0.48	0.80	0.04	0.56	0.40	0.80	0.04
May-96	3.94	1.86	0.26	0.74	0.54	0.72	1.73	1.40	3.94	0.26
Jun-96	1.04	0.47	0.71	2.00	0.87	1.07	1.53	1.10	2.00	0.47
Jul-96	1.64	2.37	5.45	ND*	1.62	1.81	2.28	2.53	5.45	1.62
Aug-96	1.98	6.01	3.60	2.57	3.48	2.67	3.12	3.35	6.01	1.98
Oct-96	3.91	5.37	5.74	2.55	2.25	2.69	5.22	3.96	5.74	2.25
Nov-96	7.21	3.70	6.08	2.51	4.27	3.16	6.49	4.77	7.21	2.51

* Not determined.

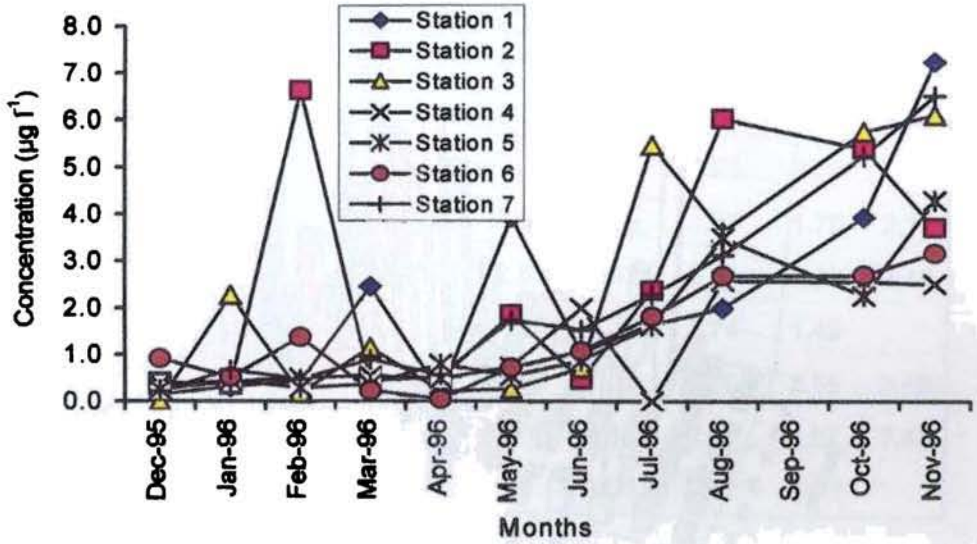

Fig. 5.1. Monthly distribution of ΣPAHs

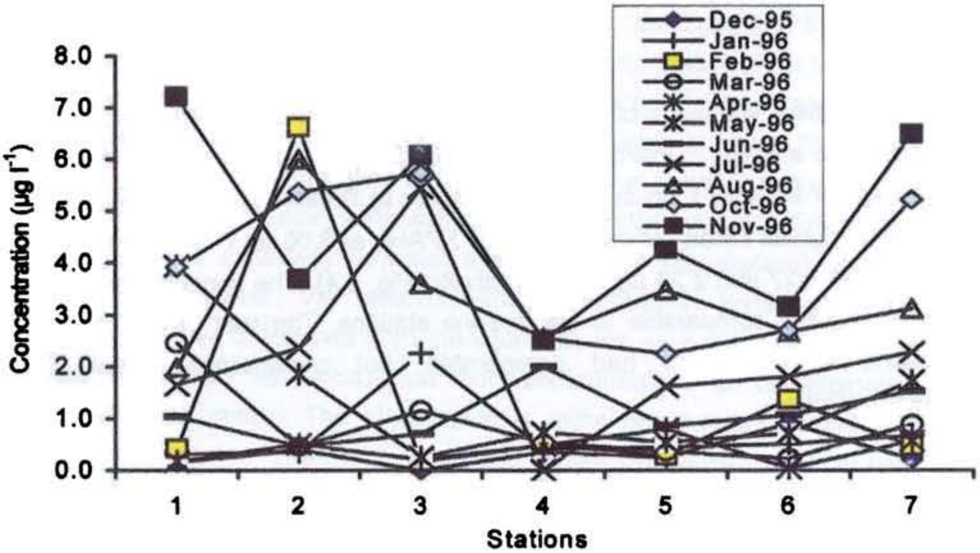

Fig. 5.2. Geographical distribution of ΣPAHs

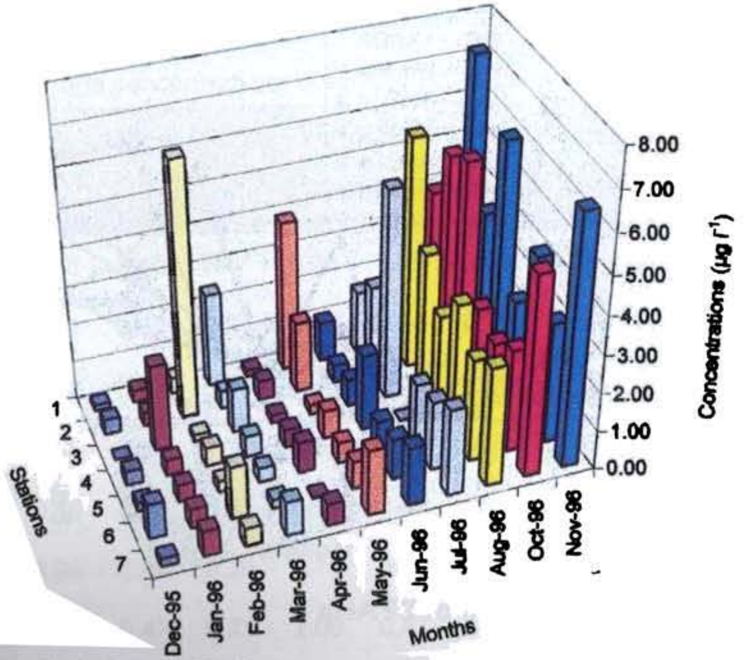

Fig. 5.3. Distribution of ΣPAHs

Seasonal variations of PAHs in the study area are presented in Tables 5.2 and 5.3. On average, ΣPAHs ranged from 0.25 µg l^{-1} in bottom water at Station 5 to a high of 5.69 µg l^{-1} in surface micro-layer at Station 3 (Table 5.2). Station 3 gave the highest mean concentrations of ΣPAHs at 3.00 µg l^{-1} followed by Station 1 and 2, at 2.27 and 2.24 µg l^{-1}, respectively (Fig. 5.4). The concentration levels at Station 7 were comparable to the riverine stations. The rest of the stations i.e. Stations 4, 5 and 6 had comparable, but considerably lower ΣPAHs concentrations.

Table 5.2. Seasonal variations of ΣPAHs in water (concentrations in μg l^{-1})

Season	Sample	Station Nos.						
		1	2	3	4	5	6	7
Pre-monsoon	SML	1.96	1.09	0.51	0.67	0.59	0.32	0.90
	SSL	1.09	3.76	0.50	0.74	0.65	0.59	1.07
	BL				0.28	0.25	0.60	
Monsoon	SML	1.20	3.93	3.91	2.25	1.81	1.76	2.12
	SSL	1.76	2.71	2.60	3.32	2.41	2.30	2.49
	BL				1.27	1.74	1.49	
Post-monsoon	SML	3.35	3.67	5.69	2.83	2.09	2.35	3.69
	SSL	4.22	1.24	3.70	1.13	2.00	1.87	3.47
	BL				1.03	1.32	0.60	

It is realized that the station with the highest level of contamination, Station 3, experiences combined effects of effluent from the manufacturing industry, emissions and discharges due to water transport, and discharge of urban wastewater. The two riverine stations, Stations 1 and 2, are impacted by mainly effluent, gaseous and liquid, from industrial establishments. Station 7 on the other hand is exposed to waste from domestic activities, water transport and an oil refinery (petroleum) in the neighbourhood. The two estuarine stations, Stations 5 and 6, are also subjected to emissions and discharges due to water transport and urban activities. In addition, heavy vehicular traffic, a potential source of PAHs, use the Venduruthy Bridge causeway between the City centre (Ernakulam) and the Willingdon Island. However, the relatively low concentration levels of PAHs encountered is indicative of removal from the water column as a result of estuarine mixing processes, such as flocculation and sedimentation of contaminated suspended particulate matter. The observation is more pronounced for the Bar mouth Station which opens towards the open sea, where the diluting effect of less contaminated seawater also plays a part in maintaining the low concentrations encountered.

Table 5.3. Mean ΣPAHs concentrations (µg l^{-1}) in water

Season	Station Nos.						
	1	2	3	4	5	6	7
Pre-Monsoon	0.84	1.99	0.95	0.48	0.47	0.54	0.65
Monsoon	2.21	1.56	2.14	1.37	1.01	1.20	1.84
Post-Monsoon	3.76	3.16	5.91	1.80	2.26	2.25	3.97
Mean	2.27	2.24	3.00	1.22	1.25	1.33	2.15

As is evident in Fig. 5.5 relatively higher concentrations of PAHs were obtained during the monsoon and post monsoon seasons at most stations.

The PAH concentrations in the surface micro-layer were generally higher or comparable to that in the sub-surface layer (Fig. 5.6a, b, c). PAH concentrations in the bottom water at the three deep Stations 4, 5 and 6, were significantly lower ($p < 0.5$) during the three seasons. The vertical gradient of PAH concentrations in the water column indicates removal of the contaminants by not only the physico-chemical estuarine processes, as observed at Station 8 (see Chapter 4), but also through interaction with biotic systems resulting in bioaccumulation and biodegradation, and abiotic processes such as photodegradation.

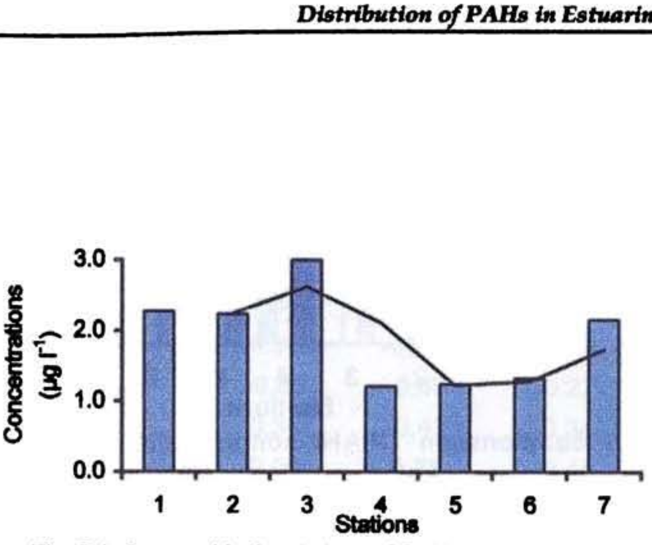

Fig. 5.4. Geographical variations of PAHs

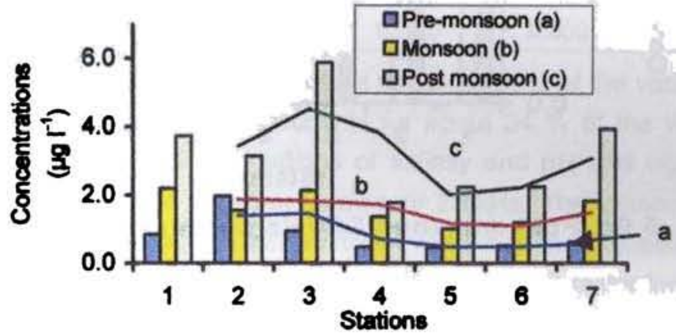

Fig. 5.5. Seasonal variations of PAH concentrations

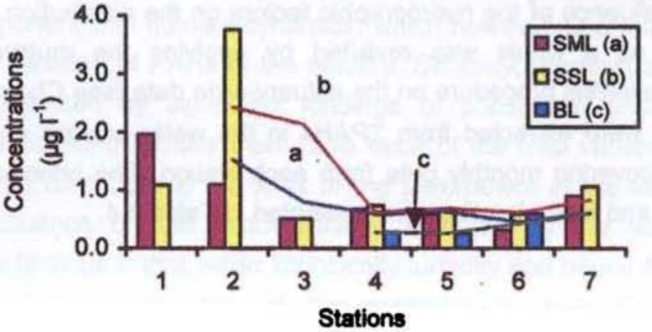

Fig. 5.6a. Pre-monsoon ΣPAHs concentrations

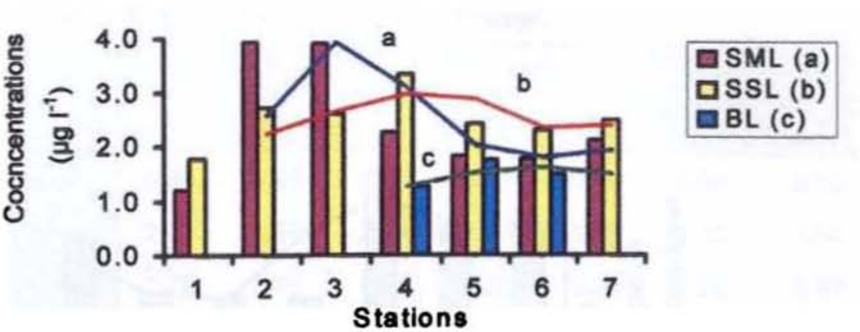

Fig. 5.6b. Monsoon ΣPAHs concentrations

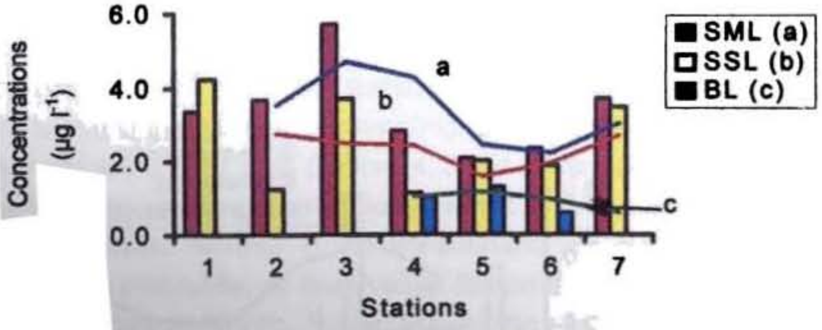

Fig. 5.6c. Post-monsoon ΣPAHs concentrations

5.2.2 PAH distribution with physico-chemical factors

The influence of the hydrographic factors on the distribution of PAHs in the study area as a whole was revisited by applying the multivariate principal component analysis procedure on the estuary-wide data (see Chapter 4). Principal components were extracted from ΣPAHs in the water column and hydrographic parameters covering monthly data from each station. The principal components, eigenvalues and factor loadings are presented in Table 5.4.

Table 5.4: Principal components and factor loadings for ΣPAHs and hydrographic parameters (absolute values of highlighted loadings are > 0.50)

	Factors			
	F 1	F 2	F 3	F 4
ΣPAHs	**-0.78**	0.03	0.23	-0.01
Salinity	**0.94**	0.02	0.02	-0.21
PH	**0.84**	-0.33	0.31	-0.01
Temperature	0.04	**0.87**	-0.19	-0.04
DO	**-0.65**	**-0.63**	-0.22	-0.02
Turbidity	0.34	-0.43	-0.36	**0.73**
Nitrate	0.09	**0.75**	-0.48	0.04
Phosphate	-0.12	**0.60**	**0.55**	**0.51**
Eigenvalue	2.75	2.37	0.89	0.84
% of total variance	34.38	29.57	11.14	10.48
Cumulative eigenvalue	2.75	5.12	6.01	6.85
Cumulative %	34.38	63.95	75.09	85.57

The first four principal components account for about 85% of the variance in the data. The first principal component accounts for about 34 % of the variance and is characterised by high positive loadings of salinity and pH and significant negative loading of DO and high negative loading of ΣPAHs. The component is associated with the mixing between saline and fresh water and the processes that influence the concentrations of PAHs in the estuary. The negative factor loading of ΣPAHs signifies inverse relationship with salinity, and likewise pH. The second principal component accounts for about 30 % of the variance in the data. High nitrate, phosphate and temperature loadings and a negative loading of DO associate the component with nutrient dynamics, which however was not primarily attributed with the variation of PAHs in the estuary. Similarly, the third and fourth components characterised by significant loadings of phosphate and turbidity, respectively, and accounting for less than 12 % each of the total variance signify those processes that contribute to the level of the parameters in the estuary but have no direct influence on the concentrations of PAHs in the water. The implication of these findings is that, while intrinsically turbidity and hence SPM have been shown to influence speciation of the contaminants (see Chapter 4), processes that are primarily associated with removal of the contaminants from the water column, such as sedimentation which results from estuarine mixing, have prominence in the PAH pathway in the estuary.

5.2.3 Enrichment and water-particle partitioning of PAHs

An indication of the degree of enrichment of PAHs in the surface microlayer in comparison with concentrations in the sub-surface water layer is presented in Table 5.5. The Enrichment Factor (EF) is defined as the ratio of the concentration of the contaminant in the surface microlayer and the corresponding concentration in the sub-surface layer.

Table 5.5. Enrichment factors (EFs) of ΣPAHs in the water surface microlayer

Phase	Station Nos.							
	1	2	3	4	5	6	7	8§
Dissolved*	6.9	2.9	1.2	2.0	1.5	2.7	2.5	2.20
SPM associated*	2.3	3.2	6.0	10.9	45.8	0.0	2.9	5.2
SPM normalised*	1.2	1.5	2.7	6.7	42.1	0.0	1.2	11.36

* Mean EFs for period: Dec 95 - Nov 96

Dec 95 data

§ Jul 98 data

On average there was modest enrichment of ΣPAHs in the surface microlayer compared to the sub-surface layer. Mean Enrichment Factors (EFs) in the dissolved phase ranged from 1.2 at Station 3 to 6.9 at Station 1 (Table 5.5). Enrichment factors for individual PAHs however showed wider variations. Thus seasonal averages of EFs for individual PAHs reached 2 orders of magnitude, with some outliers attaining 3 orders of magnitude (EFs range < 1.0 to 621). The highest EF value was obtained for benz[a]anthracene at Station 5 (EF = 621), followed by benzo[k]fluoranthene (EF = 284) at Station 7, and 1-methyl phenanthrene (EF = 117) at Station 3. The average EFs for individual PAHs in the dissolved phase decreased in the order benz[a]anthracene > benzo[k]fluoranthene > pyrene > acenaphthylene > naphthalene > chrysene > phenanthrene > 2-methyl phenanthrene > 2,3,5-trimethyl naphthalene > fluoranthene > 1-methyl phenanthrene (range of EFs 2.2 – 29.9). The generally higher EFs of the 4- and 5-ring PAHs, relative to the lower molecular weight 2- and 3-ring assemblages reflect their higher stability and resistance to removal from the microlayer through such surface processes as volatilisation and photooxidation.

The EFs for ΣPAHs associated with SPM in the surface microlayer are comparatively higher than values obtained for the dissolved phase. The EFs range from 0 to 45.8 (mean EF = 10.9 ± 15.9) (Table 5.4). The corresponding EFs for SPM or mass normalized ΣPAHs are comparable to the former EFs (range 0 – 42, mean 9.5 ± 14.8) and are not significantly different ($p < 0.05$). The mean EFs of individual PAHs associated with SPM however range from 0 to a maximum of 536 for pyrene followed by fluoranthene with EF = 297. The EFs of the SPM associated and SPM normalised PAHs decrease in the order pyrene > fluoranthene > phenanthrene > acenaphthene > benzo[b]fluoranthene > chrysene > 2,6-dimethyl naphthalene > 1-methyl phenanthrene > 1-methyl naphthalene > benz[a]anthracene > 2,3,5-trimethyl naphthalene > biphenyl (EFs range: 3.4 – 103). With the exception of pyrene and fluoranthene, the generally lower EFs for the 4- and 5-ring, relative to the lower ring PAHs is contrary to the hydrophobicity and higher affinity of the former PAHs to particulate matter. This finding is attributed to the higher concentration of SPM in the microlayer, which tend to "dilute" the PAHs and also provide a route for the removal of the SPM associated contaminants by sedimentation. The "diluting" effect of the SPM is illustrated by the generally lower EFs of the SPM normalised PAHs relative to the SPM associated PAHs (Table 5.5).

It has been shown elsewhere, however, that meteorological factors, especially wind velocity greatly influence accumulation of particulate and dissolved phase PAHs at the surface microlayer (Lui & Dickhut, 1997; Falkowska, 1999a). SPM, POC and DOC concentrations in the microlayer have been found to decrease exponentially with wind speed and hence cause a decrease in EFs. Wind activity causes mixing of SPM resulting in the transport of associated PAHs into the water column, and enhance desorption and subsequent dissolution of the organic contaminants. It is, therefore, appreciated that the EFs for PAHs in the surface microlayer were also subjected to such environmental elements as wind activity, which was however not factored in the present study. The effect of the wind factor may partly explain the relatively low EFs encountered. For example, Liu & Dickhut (1997) reported EFs for particulate ΣPAHs and individual PAHs as high as 4,000 and 5,300, respectively.

Overall, however, the data showed a good relationship between PAHs associated with SPM and the fraction organic carbon of particulate matter. This is illustrated in a scatter diagram of an extract of the data of the concentration of ΣPAHs in dry mass of SPM as a function of the POC % in SPM (Fig. 5.7). A significant correlation ($r = 0.72$, $p < 0.05$) between particulate PAHs and POC was obtained.

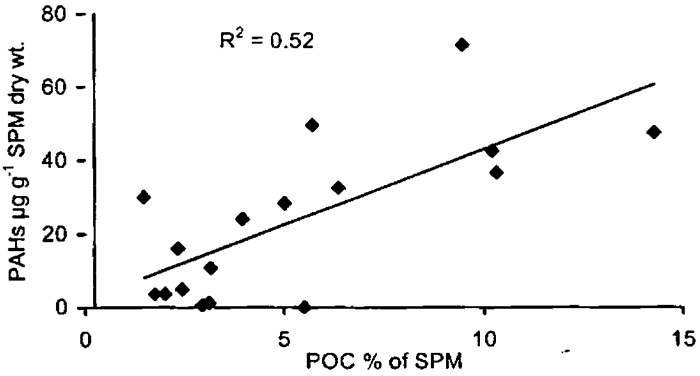

Fig. 5.7. Particulate PAHs as a function of POC

The mean water-particle distribution coefficients K_d and organic-carbon normalized distribution coefficients K_{oc} for PAHs in the Cochin estuarine backwaters is presented in Table 5.6. K_d values ranged from 7.2×10^3 to 6.8×10^5, whereas K_{oc} ranged from 2.1×10^5 to 1.1×10^7. The average K_d values in the surface micro-layer, sub-surface layer and bottom water layer were in the same range of magnitude and comparable. Similarly the mean K_{oc} values in the different water layers were comparable, but an order of magnitude higher than K_d values. The high values of the distribution coefficients indicate that partitioning of PAHs between the dissolved phase and particulate matter (SPM), which facilitates removal from the water column by sedimentation, is an important process in the environmental pathway of the contaminants in the Cochin estuarine system.

Table 5.6. Water-Particulate K_d and K_{oc} values for ΣPAHs in water

Distribution Coefficients		Station Nos.					Mean
		2	4	5	6	8	
SML	K_d	3.8E+5	-	1.5E+5	1.9E+4	6.1E+4	1.5E+5
	K_{oc}	2.7E+6	-	2.7E+6	3.3E+5	9.7E+5	1.7E+6
SSL	K_d	6.8E+5	3.6E+5	-	1.9E+4	7.2E+3	2.7E+5
	K_{oc}	1.1E+7	1.1E+7	-	1.1E+6	2.3E+5	5.8E+6
BL	K_d	-	6.7E+5	1.3E+4	1.0E+5	8.5E+3	2.0E+5
	K_{oc}	-	-	4.5E+5	5.2E+6	2.1E+5	2.0E+6
Mean	K_d	5.3E+5	5.2E+5	8.2E+4	4.6E+4	2.6E+4	
	K_{oc}	6.9E+6	1.1E+7	1.6E+6	2.2E+6	4.7E+5	

5.2.4 Distribution of PAHs in sediments

The results of sporadic measurements of the PAH concentrations associated with surficial sediments are presented in Table 5.7. Levels of ΣPAHs ranged from 24.2 μg kg^{-1} to 274.6 μg kg^{-1} dry mass (average 100.0 μg kg^{-1}) were recorded. The lowest concentrations were obtained from Station 1, followed by Stations 2 and 7. These are significant observations bearing in mind that the concentrations of PAHs encountered in the water column were quite high, only surpassed by levels found at Station 3 (Fig. 5.4). The concentrations of the contaminants in sediments are closely associated with the sediment size distribution in the study area. Thus, bottom sediments at Stations 1 and 2 were predominantly sandy, and at Station 7 the substrate was composed of about 70% sand (Table 5.8, Fig. 5.8). About the highest sedimentary PAH concentrations were found at Stations 3 and 4, both of which have bottom substrate composed of high levels of silt and clay, notwithstanding Station 3 having the most contaminated water column. The relative concentration of PAHs in sediments was 3 orders of magnitude higher than levels in the water column, and showed the significance of the sediments as a sink.

Table 5.7. ΣPAHs in sediments (concentrations in μg kg^{-1})

Month	Station Nos.					
	1	2	3	4	6	7
Nov-95			201.2			
Dec-95			136.5			
Feb-96	49.8			85.1		42.7
Mar-96		42.1				
Apr-96	29.3				132.5	
Jul-96					275.6	
Nov-96	24.2	181.9				

Table 5.8. Sediment grain size distribution (mean composition in %)

Grain size	Station Nos.						
	1	2	3	4	5	6	7
Sand	98.1	97.0	10.0	29.5	48.4	55.3	68.3
Silt	1.0	1.5	40.2	38.7	37.9	32.5	17.5
Clay	0.9	1.5	49.8	31.8	13.7	12.2	14.2

The concentrations of PAHs in the water can be described as low pollution level compared with that of similar areas elsewhere (Kalf *et al.*, 1997; Liu *et al.*, 2000; Cincinelli *et al.*, 2001). The concentrations of PAHs encountered indicate slight to fairly contaminated sediments, and potential acute biological effects are expected to be low (Kalf *et al.*, 1997; Kim *et al.*, 1999). However, potential sub-lethal toxicity effects of low concentration levels of PAHs have been reported; for example, the induction of cytochrome P450 1A, which can lead to the formation of carcinogenic and mutagenic metabolites, including the highly bioactive diol epoxides, and DNA adducts in marine organisms (e.g. Law *et al.*, 1997; Aas *et al.*, 2000). The situation may be exacerbated by photo-induced toxicity of the mixture of PAHs, impacting on aquatic organisms (e.g. Pelletier *et al.*, 1997; Boese *et al.*, 1999; Choi & Oris, 2000).

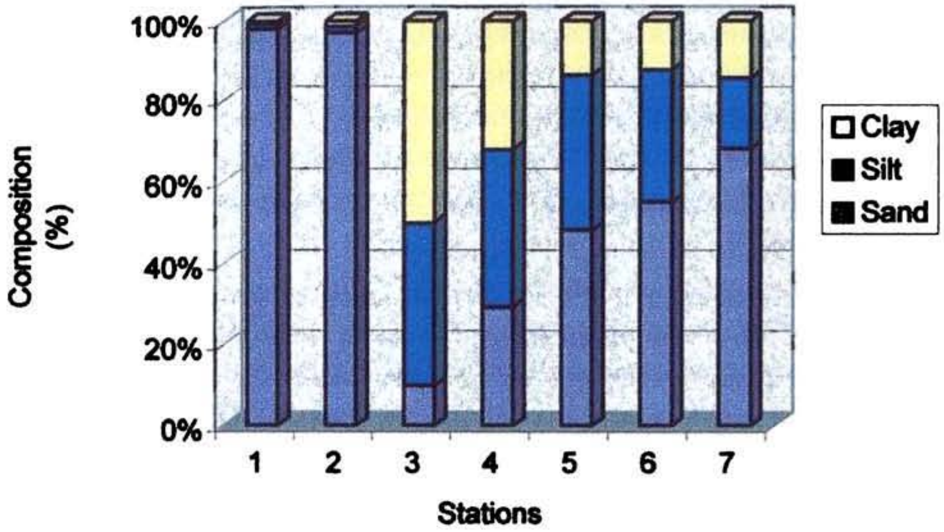

Fig 5.8. Sediment grain size distribution

5.3 Concluding Remarks

Polycyclic aromatic hydrocarbons in the Cochin estuarine backwaters are widely distributed with concentrations varying widely temporally and spatially. Monthly averages of ΣPAHs in the water column varied from a minimum of 0.04 µg l^{-1} to a maximum of 7.21 µg l^{-1}. Seasonal variations of ΣPAHs ranged from 0.25 to 5.69 µg l^{-1}. Relatively higher concentrations were obtained during the monsoon and post monsoon seasons at most sampling stations.

The station experiencing the highest level of contamination overall was Station 3 (Tatapuram), which was attributed to combined impacts of industrial manufacturing, water transport and the discharge of municipal wastewater. The next highest contaminated sites were the riverine Stations 1 and 2, which was attributed to PAH rich gaseous emissions and liquid waste discharges from industrial establishments. The level of contamination at Station 7 was comparable to Station 1 and 2, but was attributed inputs due to domestic activities (e.g. disposal of domestic waste and emissions from domestic fires), some emissions and discharges due to water and land transport, and effluent from a petroleum refinery located in the neighbourhood. Stations 5 and 6 and including Station 4 (bar mouth) had relatively lower levels of PAH contamination, even though they are also subjected to emissions and wastewater discharges due to transportation (both water and land) and urban activities. The lower PAH concentrations encountered at Stations 4, 5 and 6 were attributed to removal as a result of estuarine mixing processes, such as flocculation and sedimentation of contaminated suspended particulate matter. Indeed, principal component analysis indicated that the variation of PAHs in the estuary was primarily influenced by saline water flow and hence mixing processes.

The concentration of PAHs in the water surface microlayer was generally comparable or higher than in the sub-surface layer, while bottom concentrations were significantly lower than in the surface layers. Mean EFs of dissolved ΣPAHs in the microlayer ranged from 1.2 to 6.9. EFs for individual PAHs in the dissolved phase varied widely ranging from < 1.0 to 621. Four and 5-ring PAHs generally had higher EFs than the 2- and 3-ring structures, which reflected their stability and resistance to removal from the microlayer through such processes as volatilisation and photooxidation. The EFs for ΣPAHs associated with SPM in the surface microlayer ranged from 0 to 45.8 (mean 10.9 ± 15.9). The corresponding EFs for SPM or mass normalised ΣPAHs ranged from 0 to 42.0 (mean 9.5 ± 14.9). However, the EFs of individual PAHs associated with SPM varied from 0 to a maximum of 536. With the exception of pyrene and fluoranthene, the 4- and 5-ring PAHs generally had lower EFs. This was attributed to higher concentrations of SPM in the microlayer, which tended to have a dilution effect on the contaminants. A good correlation was found between particulate PAHs and fraction organic carbon of the SPM ($r = 0.72$, significant at $p < 0.05$). Estimates of distribution coefficients for PAHs in the Cochin estuarine system ranged from $7.2\text{x}10^3$ to $6.8\text{x}10^5$ for K_d and $2.1\text{x}10^5$ to $1.1\text{x}10^7$ for K_{oc}. The high values of distribution coefficients were further indication of the important role the partitioning process between the dissolved and particulate phases plays in the environmental pathway in the study area.

The limited amount of data collected on sedimentary PAHs in the study area gave an indication of the extent of contamination of surficial sediments relative to the overlying water. Concentration levels of ΣPAHs ranged from 24.2 to 274.6 µg kg^{-1} sediment dry mass. The highest concentrations were obtained from Station 6, followed closely by Station 3. About the lowest concentrations were obtained from

Stations 1 and 2, followed by Station 7. The concentration levels were associated with sediment grain size distribution. Thus, Stations 1 and 2 had predominantly sandy bottom substrates (over 97 % sand), and the lowest PAH concentrations. On the other hand, Station 3 with sediments composed of about 90 % clay and silt was among the most contaminated. The levels of PAH contamination in the sediment of up to 3 orders of magnitude the concentration in the water column signified the role of the sediments as a sink.

The level of contamination of the Cochin estuarine system is indicated as low water pollution and slight to fairly contaminated sediments. While acute biological effects of the PAH contamination is expected to be low, there is a high potential for sub-lethal effects on marine organisms, such as the induction of cytochrome P450 1A and DNA adduct, which may be exacerbated by photo-induced carcinogenesis.

Chapter 6

CHARACTERISTICS OF PAH ASSEMBLAGES

6.1 Introduction

It is recognized that the composition and distribution of PAHs in the environment is influenced by their production sources. The production processes have been linked to certain characteristic PAH patterns, namely pyrolytic and petrogenic or diagenetic processes (Soclo *et al.*, 2000). Petrogenic PAHs are characterised by homologous families of 2- to 4- ring PAHs, namely naphthalenes, fluorenes, phenanthrenes and chrysenes, where the respective parent PAHs are less abundant than the alkyl substituted homologues. Pyrogenic PAHs, generated by the combustion of fossil fuels and recent organic matter (e.g. internal combustion engines, wood and forest fires etc.) are dominated by the parent compounds of 3-, 4-, 5- and 6-ring PAHs. Fluoranthene and pyrene are usually the most abundant (Page *et al.*, 1999).

However, 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. The original distribution patterns of the sources are further modified by processes that result in PAH degradation in the aquatic environment, such as photooxidation in the water layer (e.g. Neff, 1979; Ehrhardt & Petrick, 1984), and biodegradation in the water layer and sediments (Neff, 1979; Ashok & Saxena, 1995). Whereas, net cumulative processes in sediments impart a certain measure of stability, which may allow "fingerprinting" of PAH sources, dynamic conditions prevail in the water column. In a situation of chronic contamination of an area, however, average distribution patterns 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 low molecular weight and high molecular weight PAHs

(LMW/ HMW), ratio of concentrations of phenanthrene and anthracene (Phen/Ant), the ratio of fluoranthene and pyrene (Fluoran/Pyr), ratio of chrysene and benz[a]anthracene (Chr/BaA) and ratio of total of alkyl substituted naphthalene and phenanthrene, and the total of the respective parent PAHs. It is possible to determine the main source-processes of the PAHs by association of the molecular indices (e.g. Lake *et al.*, 1979; Neff, 1979; Budzinski *et al.*, 1997; Fernandes *et al.*, 1997; Notar *et al.*, 2000; Soclo *et al.*, 2000).

In the present study the distribution and covariability of individual PAHs in the water column, as well as in surficial sediments was examined with the aim of establishing the major source-processes of the contaminants. The multivariate principal components and cluster analysis procedures using STATISTICA were applied.

6.2 Results and Discussion

6.2.1 Identification of PAH sources

PAHs in water

The concentrations of individual PAHs in the water column are presented in Table 6.1. The data for each PAH was normalised in order to reduce the mean to 0 and variance 1 to minimise discrepancies due to diverse magnitudes. Principal components were extracted from the data with the PAHs as the variables. The first 5 principal components accounting for 64.5% of the total variance were considered (Table 6.2).

The first principal component explains about 28 % of the total variance of the concentrations of individual PAHs in the study area. It is the most important principal factor and is characterised by high loadings of acenaphthene, 2,3,5-trimethyl naphthalene, fluorene, fluoranthene, pyrene, perylene and benzo[ghi]perylene, and moderately high loadings of 2-methyl naphthalene, phenanthrene, benzo[k]fluoranthene, and benzo[a]pyrene. The principal factor features loadings of 2- and 3-ring PAHs including alkylated homologues. This group of PAHs is associated with petrogenic sources of the contaminants. Such sources include leakages and spillage from petroleum handling facilities, discharges of contaminated wastewater from the municipality and discharge of contaminated bilge from ships. On the other hand, the high loading of the 4- and 5-ring PAHs which include pyrene point to pyrogenic sources of the contaminant. The pyrogenic sources of PAHs include combustion of fossil fuels and recent organic matter, such as in water and land transportation, industrial manufacturing and wood fires.

Table 6.1. PAHs in the water column

Station No	Sample	Concentrations of PAHs $(\mu g\ l^{-1})$											
		Nap	2-MeNap	1-MeNap	Bip	DiMeNap	AcNapy	AcNaph	TriMeNap	Flu	Phe	Ant	MePhe
1	SML	0.015	0.000	0.002	0.000	0.000	0.002	0.000	0.003	0.001	0.055	0.129	0.028
	SSL	0.000	0.000	0.047	0.000	0.000	0.007	0.000	0.001	0.003	0.015	0.204	0.011
2	SML	0.016	0.002	0.331	0.002	0.010	0.001	0.000	0.008	0.003	0.029	0.261	0.016
	SSL	0.005	0.003	0.016	0.002	0.005	0.009	0.024	0.040	0.069	0.058	0.130	0.008
3	SML	0.009	0.000	0.093	0.000	0.000	0.019	0.014	0.005	0.009	0.095	0.234	0.052
	SSL	0.006	0.001	0.053	0.001	0.002	0.002	0.001	0.003	0.005	0.039	0.071	0.007
4	SML	0.016	0.000	0.025	0.000	0.000	0.000	0.000	0.004	0.001	0.023	0.107	0.028
	SSL	0.013	0.001	0.017	0.002	0.000	0.001	0.001	0.003	0.002	0.009	0.093	0.014
	BL	0.043	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.001	0.010	0.046	0.004
5	SML	0.003	0.001	0.048	0.000	0.000	0.001	0.000	0.025	0.010	0.022	0.151	0.035
	SSL	0.022	0.002	0.089	0.002	0.000	0.000	0.001	0.000	0.003	0.014	0.162	0.064
	BL	0.007	0.000	0.041	0.002	0.001	0.002	0.000	0.001	0.005	0.028	0.000	0.009
6	SML	0.206	0.000	0.001	0.001	0.000	0.000	0.000	0.002	0.000	0.027	0.077	0.018
	SSL	0.032	0.001	0.098	0.001	0.000	0.002	0.000	0.003	0.001	0.009	0.103	0.043
	BL	0.010	0.001	0.003	0.001	0.002	0.001	0.000	0.008	0.005	0.032	0.045	0.001
7	SML	0.005	0.002	0.006	0.002	0.000	0.002	0.038	0.011	0.007	0.043	0.185	0.007
	SSL	0.005	0.001	0.148	0.001	0.000	0.000	0.000	0.008	0.007	0.029	0.171	0.021

Table 6.1. (continued)

Station No.	Sample	Concentrations of PAHs* (µg l^{-1})											
		Floran	Pyr	BaA	Chr	BbF	BkF	BeP	BaP	Per	IndPyr	DBA	Bper
1	SML	0.150	0.279	0.028	0.019	0.268	0.002	0.133	0.016	0.005	0.040	0.000	0.031
	SSL	0.076	0.033	0.043	0.169	0.610	0.010	0.284	0.085	0.023	0.282	0.021	0.000
2	SML	0.169	0.247	0.006	0.031	0.557	0.004	0.247	0.013	0.000	0.181	0.000	0.000
	SSL	0.131	0.403	0.099	0.122	0.397	0.057	0.245	0.030	0.051	0.282	0.000	0.078
3	SML	0.136	0.256	0.081	0.245	0.490	0.002	0.270	0.026	0.001	0.134	0.014	0.000
	SSL	0.044	0.061	0.094	0.392	0.282	0.003	0.144	0.007	0.006	0.145	0.040	0.000
4	SML	0.088	0.135	0.019	0.094	0.279	0.006	0.150	0.003	0.003	0.126	0.000	0.000
	SSL	0.064	0.073	0.145	0.144	0.269	0.023	0.157	0.012	0.000	0.085	0.006	0.000
	BL	0.028	0.040	0.017	0.018	0.148	0.021	0.087	0.003	0.005	0.060	0.000	0.000
5	SML	0.000	0.084	0.103	0.012	0.900	0.002	0.142	0.008	0.002	0.096	0.000	0.000
	SSL	0.031	0.086	0.054	0.007	0.443	0.021	0.166	0.009	0.005	0.216	0.000	0.009
	BL	0.050	0.054	0.024	0.015	0.272	0.003	0.136	0.004	0.000	0.107	0.000	0.000
6	SML	0.062	0.108	0.013	0.014	0.315	0.003	0.132	0.002	0.003	0.131	0.000	0.000
	SSL	0.023	0.038	0.125	0.065	0.345	0.027	0.196	0.031	0.022	0.147	0.035	0.000
	BL	0.017	0.029	0.086	0.020	0.113	0.003	0.073	0.003	0.007	0.075	0.000	0.000
7	SML	0.096	0.106	0.015	0.027	0.367	0.137	0.098	0.060	0.008	0.119	0.017	0.048
	SSL	0.152	0.088	0.117	0.194	0.576	0.011	0.262	0.016	0.013	0.275	0.048	0.013

* Nap = naphthalene, 2-MeNap = 2-methyl naphthalene,
1-MeNap = 1-methyl naphthalene, Bip = biphenyl,
DiMeNap = 2,6-dimethyl naphthalene, AcNapy = acenaphthylene,
AcNaph = acenaphthene, TriMeNap = 2,3,5-trimethyl naphthalene,
Flu = fluorine, Phe = phenanthrene. Ant = anthracene,
MePhe = 1-methyl phenanthrene, Floran = fluoranthene,
Pyr = pyrene, BaA = benz[a]anthracene, Chr = chrysene,
BbF = benzo[b]fluoranthene, BkF = benzo[k]fluoranthene,
BeP = benzo[e]pyrene, BaP = benzo[a]pyrene, Per = perylene, IndPyr = indeno[1,2,3-cd]pyrene, DBA = dibenz[ah]anthracene,
Bper = benzo[ghi]perylene.

The second principal factor accounts for about 16 % of the variance in the PAH data and includes high factor loadings of anthracene, benzo[b]fluoranthene, benzo[e]pyrene, and indeno[1,2,3-cd]pyrene. The source of the 5-ring PAHs is predominantly high temperature combustion processes. Anthracene is also a product of combustion of organic material, such as wood. Thus, the second principal factor is associated mostly with high temperature combustion processes or pyrolysis, such as burning fossil fuel in internal combustion engines and bunker fuel in industrial boilers.

The third principal factor accounts for a low 9.7 % of the total variance of PAHs and is characterised by significant factor loadings of benzo[k]fluoranthene and benzo[a]pyrene, and low loading of acenaphthene. The 5-ring PAHs as well as acenaphthene are pyrolytic products. However, their distribution contributed relatively less to the variations in PAH levels in the study area.

Table 6.2. Factor loadings of individual PAHs (loadings with absolute values > 0.50 are selected)

PAHs	Factors				
	F 1	F 2	F 3	F 4	F 5
Naphthalene					
2-Methyl naphthalene	0.59				
1-Methyl naphthalene					
Biphenyl				-0.55	
2,6-Dimethyl naphthalene					
Acenaphthylene				0.54	
Acenaphthene	0.70		0.57		
2,3,5-Trimethyl naphthalene	0.75				
Fluorene	0.80				
Phenanthrene	0.62				
Anthracene		0.78			
1-Methyl phenanthrene					
Fluoranthene	0.71				
Pyrene	0.83				
Benz[a]anthracene					0.69
Chrysene					0.59
Benzo[b]fluoranthene		0.69			
Benzo[k]fluoranthene	0.59		0.70		
Benzo[e]pyrene		0.85			
Benzo[a]pyrene	0.61		0.73		
Perylene	0.75				
Indeno[1,2,3-cd]pyrene		0.80			
Dibenz[ah]anthracene					
Benzo[ghi]perylene	0.83				
Eigenvalue	6.67	3.78	2.22	1.51	1.29
% of total variance	27.8	15.7	9.7	6.3	5.4
Cumulative eigenvalue	6.67	10.44	12.66	14.18	15.47
Cumulative %	27.8	43.5	52.8	59.1	64.5

The contributions of the fourth and fifth principal factors to the variance in the PAH concentrations are even lower and relatively less significant at 6.3 % and 5.4 %, respectively. The former principal factor is associated with the rather low contributions of biphenyl and acenaphthylene to the variance, whereas the latter principal component accounts for the low contributions of benz[a]anthracene and pyrene, both basically of pyrogenic origin.

The significance of the results of the principal component analysis on the distribution of individual PAHs in the estuarine system is that the main sources of the contaminants are petroleum hydrocarbons and combustion processes. However, the relative contribution of the two main sources of PAHs cannot be delineated with the present data. One probable reason is that on the ground the socio-economic activities attributed with the contaminants are not strictly zoned, particularly as it regards to Stations 3, 4, 5 and 7, which are located within the harbour area and in the vicinity of the municipality.

PAHs in sediments

The average concentrations and distribution of individual PAHs in sediments are presented in Table 6.3. Principal components were extracted from the normalised data of PAH concentrations and *variance maximizing (Varimax) rotation* (StatSoft Inc., 2002) applied to the results for better classification of the original PAH variables (Table 6.4). The first two principal components which accounted for about 76 % of the variance in the PAH concentrations were considered.

The first principal component, after rotation, accounted for about 35 % of the variance and included high factor loadings of 2,6-dimethyl naphthalene, acenaphthylene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene, perylene and benzo[ghi]perylene. Apart from 2,6-dimethyl naphthalene, the odd one out, the factor is dominated by PAHs of pyrolytic origin, which include 3, 4, 5- and 6-ring PAH structures, especially combustion of fossil fuels.

Table 6.3. Distribution of PAHs in Sediments (concentrations in ng g^{-1} sediment dry mass)

PAH	Station Nos.						Mean	SD
	1	2	3	4	6	7		
Naphthalene	2.8	24.9	13.5	3.8	2.4	1.4	8.1	9.3
2-Methylnaphthalene	0.3	2.1	0.9	1.0	0.8	0.8	1.0	0.6
1-Methylnaphthalene	0.2	1.2	0.6	0.6	0.7	0.6	0.6	0.3
Biphenyl	0.1	1.7	0.5	0.7	0.6	0.5	0.7	0.5
2,6-DiMenaphthalene	0.3	1.4	1.4	1.3	2.2	1.8	1.4	0.6
Acenaphthylene	0.3	1.3	0.5	0.4	5.1	1.3	1.5	1.8
Acenaphthene	0.2	6.2	1.2	0.1	2.1	1.3	1.9	2.2
2,3,5-TriMethyl naphthalene	0.1	2.1	0.3	0.4	0.5	0.3	0.6	0.7
Fluorene	0.3	2.2	1.0	0.9	0.7	0.5	0.9	0.7
Phenanthrene	0.1	2.9	1.9	0.5	2.6	0.2	1.4	1.3
Anthracene	3.4	11.0	8.6	9.4	10.5	7.1	8.3	2.8
1-Methylphenanthrene	10.2	1.6	22.4	0.7	31.3	0.4	11.1	13.0
Fluoranthene	0.3	8.7	5.1	2.2	10.2	1.2	4.6	4.1
Pyrene	0.2	9.1	6.0	2.3	8.5	1.5	4.6	3.8
Benz[a]anthracene	0.1	4.1	2.8	1.6	4.9	0.8	2.4	1.9
Chrysene	0.4	7.0	4.4	3.2	6.6	1.6	3.9	2.7
Benzo[b]fluoranthene	4.7	23.9	19.4	18.0	31.5	7.0	17.4	10.1
Benzo[k]fluoranthene	0.8		0.0	0.0		0.7	0.4	0.4
Benzo[e]pyrene	0.5	7.0	4.6	4.0	5.6	1.0	3.8	2.6
Benzo[a]pyrene	0.1	7.3	3.9	1.9	5.9	1.0	3.4	2.8
Perylene	0.7	13.7	7.5	15.9	28.9	5.0	11.9	10.0
Indeno[1,2,3-cd] pyrene	6.0	6.6	5.0	0.8	7.3	1.5	4.5	2.7
Dibenz[ah]anthracene	0.1	13.0	3.0	5.0		0.7	4.4	5.2
Benzo[ghi]perylene	2.9	22.9	12.2	10.1	37.6	4.5	15.0	13.1
ΣPAHs	34.4	181.9	126.6	85.1	204.1	42.7	112.5	70.9

Table 6.4. Principal components and factor loadings for PAHs in sediments - Varimax raw rotation (loadings with absolute value > .60 are selected)

PAH	Factors			
	F 1	F 2	F 3	F 4
Naphthalene		0.88		
2-Methylnaphthalene		0.96		
1-Methylnaphthalene		0.85		
Biphenyl		0.97		
2,6-DiMenaphthalene	0.88			
Acenaphthylene	0.92			
Acenaphthene		0.91		
2,3,5-TriMenaphthalene		0.97		
Fluorene		0.95		
Phenanthrene				
Anthracene	0.72			
1-Methylphenanthrene			0.68	
Fluoranthene	0.74			
Pyrene	0.63			
Benz[a]anthracene	0.76			
Chrysene	0.69			
Benzo[b]fluoranthene	0.79			
Benzo[k]fluoranthene				-0.79
Benzo[e]pyrene		0.61		
Benzo[a]pyrene	0.60	0.63		
Perylene	0.94			
Indeno[1,2,3-cd]pyrene			0.96	
Dibenz[ah]anthracene		0.96		
Benzo[ghi]perylene	0.87			
Eigenvalue	8.34	9.73	3.17	2.30
% of total variance	34.8	40.5	13.2	9.6
Cumulative eigenvalue	8.34	18.07	21.24	23.54
Cumulative %	34.8	75.3	88.5	98.1

The second principal component accounts for about 41 % of the variance in the data and comprises high factor loadings of predominantly 2- and 3-ring PAHs, including alkyl-substituted homologues. The factor is strongly associated with petrogenic sources of the contaminants.

A plot of the factor loadings of the individual PAHs on the 2 principal components illustrates the daunting nature of the attempt to separate the two groups of PAHs and hence exclusively identifying the source processes and actual sources on the ground. The first plot of the unrotated factor loadings for Factor 2 against Factor 1 shows a group composed of more than 80 % of the PAHs defined under the latter factor (Fig. 6.1a). An improved demarcation of the PAHs is realised after rotation of the factor loadings, which results in two major groups, with one group identified with Factor 1 (PAHs of pyrolytic origin) and the other with Factor 2 (PAHs of petrogenic origin) (Fig. 6.1b).

Cluster analysis was applied on the sedimentary PAHs as an alternative test to further elucidate the classification. The procedure involved the calculation of Euclidean distances between individual PAHs from the distribution data using STATISTICA. The results are presented in a tree cluster diagram (Fig. 6.2).

Two major groups or clusters of PAHs are discernible at a linkage distance of 0.94. Cluster 1 comprises naphthalene, 2-methyl naphthalene, biphenyl, fluorine, 2,3,5-trimethyl naphthalene, acenaphthylene, dibenz[ah]anthracene and 1-methyl naphthalene. Cluster 2 comprises phenanthrene, pyrene, benzo[a]pyrene, chrysene, fluoranthene, benz[a]anthracene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, perylene and anthracene. The rest of the groups are single member clusters of PAHs that are much farther apart, namely acenaphthylene, 2,6-dimethyl naphthalene, indeno[1,2,3-cd]pyrene, 1-methyl phenanthrene and benzo[k]fluoranthene.

Cluster 1 is dominated by the low molecular weight 2- and 3-ring PAHs including the methyl-substituted PAHs associated with petrogenic sources of PAHs. Cluster 2 on the other hand is dominated by 4- and 5-ring PAHs associated with pyrogenic sources. The major clusters again indicate the main source-processes of the PAHs and agree quite closely with the results of the principal component analysis.

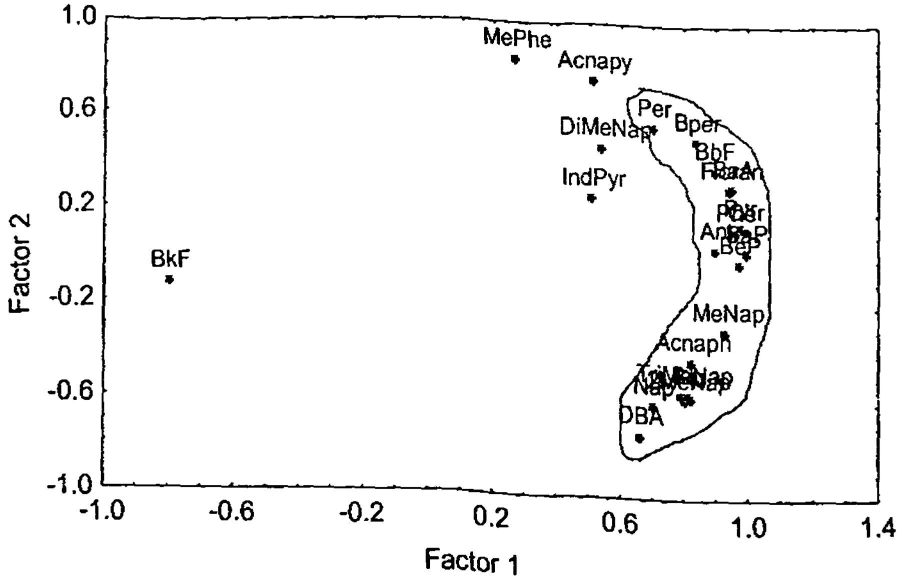

Fig 6.1a. Plot of factor loadings for sedimentary PAHs - unrotated

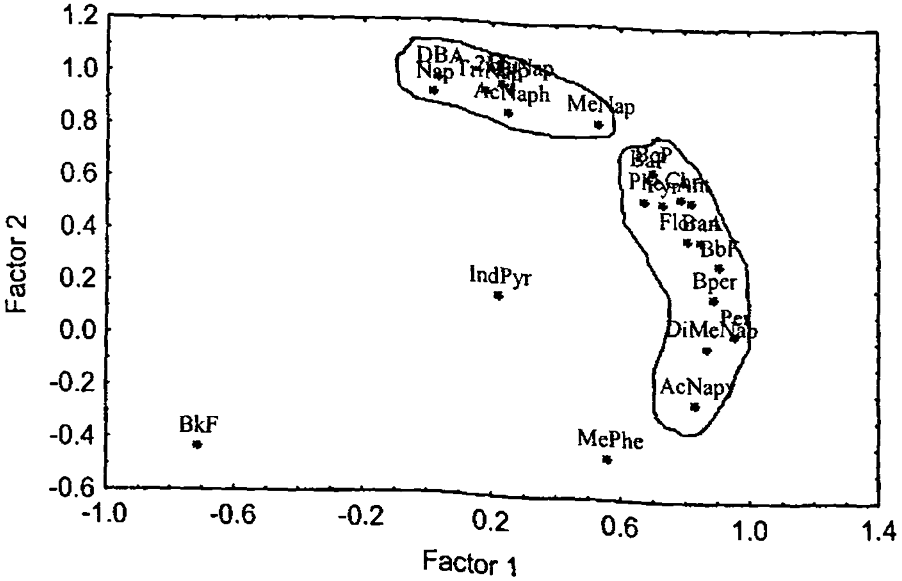

Fig. 6.1b. Plot of factor loadings of sedimentary PAHs - Varimax raw rotated

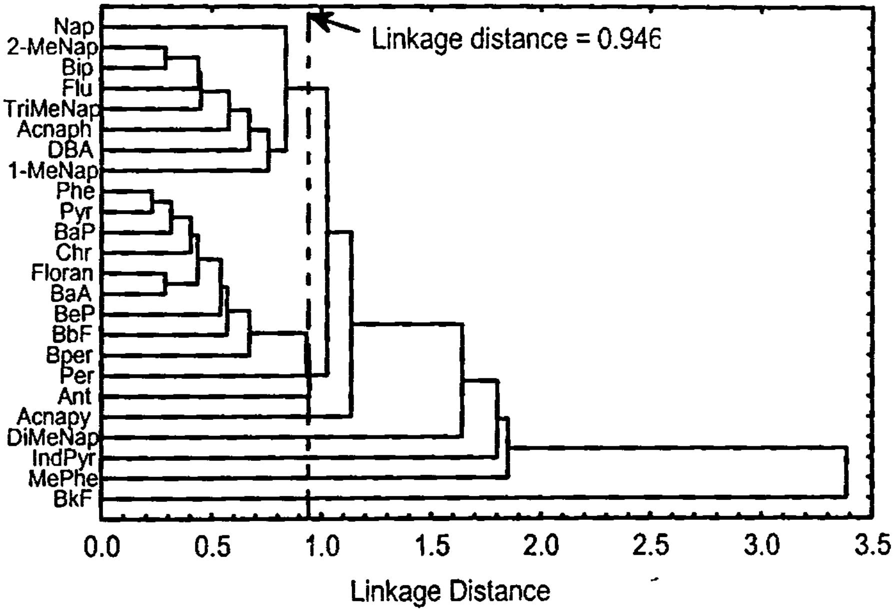

Fig. 6.2. Cluster diagram of sedimentary PAHs

6.2.2 Molecular indices

A further attempt to elucidate the source-processes and sources of PAHs in the study area was accomplished by examining a selection of molecular indices for PAHs in the water column and sediments (Tables 6.5 and 6.6).

The molecular indices that were considered for the water column included the mean ratios of the concentrations of alkyl substituted to the parent PAHs (Al-PAHs/PAHs), 1-methyl phenanthrene to phenanthrene (MePhe/Phe), phenanthrene to anthracene (Phe/Ant), fluoranthene to pyrene (Floran/Pyr) and low molecular weight to high molecular weight PAHs (Table 24). For Al-PAHs/PAHs, with the exception of Stations 4 and 6, the ratios exceed 1.0. The higher values of the ratios (ratio > 1.0) indicate a predominance of petrogenic sources of PAHs in the respective stations. The second index, MePhe/Phe ratio, showed values exceeding 1.0 for Stations 4 and 5, which indicated petrogenic sources of PAHs in the respective stations. All stations had the Phe/Ant ratios below 1.0. Station 7, however, showed a ratio of Floran/Pyr exceeding 1.0. It has been suggested that ratios of Phe/Ant < 10 and Floran/Pyr <1.0 indicate combustion sources of the PAHs (Benlahcen *et al.*, 1997; Notar *et al.*, 2001). All the stations gave LWM/HMW ratios that were less than 1.0, which was an additional indication of a pyrolytic source of the contaminants. Therefore, to sum-up, the molecular indices presented give indications of petrogenic sources of PAHs in the water column, and a strong indication of pyrolytic sources of the contaminants in all the stations. It is appreciated, however, that conditions prevailing in the water column (intense radiation, high temperatures, etc.) favour weathering or a certain degree of degradation of petrogenic PAHs, which tend to modify the distribution of the contaminants to be more like combustion products.

Table 6.5. Molecular indices of PAHs in the water column

Station Nos.	Ratios*				
	Al-PAHs/PAHs	MePhe/Phe	Phe/Ant	Floran/Pyr	LMW/HMW
1	1.08	0.56	0.21	0.73	0.15
2	4.06	0.27	0.22	0.46	0.24
3	1.46	0.45	0.44	0.57	0.20
4	0.86	1.09	0.17	0.73	0.16
5	3.29	1.67	0.21	0.36	0.16
6	0.58	0.92	0.30	0.58	0.24
7	2.47	0.40	0.20	1.28	0.18
Mean	1.97	0.77	0.25	0.67	0.19
SD	1.33	0.49	0.09	0.30	0.04

* Al-PAHs/PAHs = ratio of concentrations of alkyl substituted PAHs to the parent PAHs,

MePhe/Phe = ratio of concentrations of 1-methyl phenanthrene to phenanthrene,

Phe/Ant = ratio of concentrations of phenanthrene to anthracene,

Floran/Pyr = ratio of concentrations of fluoranthene to pyrene,

LMW/HMW = ratio of concentrations of low molecular weight PAHs (2- and 3-ring PAHs) to high molecular weight PAHs (4-, 5- and 6-ring PAHs).

Similar indices were considered for PAHs associated with sediments, but also included were the ratios of concentrations of chrysene to benz[a]anthracene (Chr/BaA) and benzo[e]pyrene to benzo[a]pyrene (BeP/BaP) (Table 6.6). High values of the Al-PAHs/PAHs and MePhe/Phe ratios were obtained with the exception of Station 2 and 4. The indices indicate petrogenic sources of the PAHs at the respective stations.

Table 6.6. Molecular indices of PAHs in sediments

Station Nos.	Ratios*					
	Al-PAHs/PAHs	MePhe/Phe	Phe/Ant	Floran/Pyr	Chr/BaA	LMW/HMW
1	3.88	178.16	0.02	1.41	4.97	0.86
2	0.30	0.56	0.26	0.95	1.72	0.40
3	1.66	11.53	0.23	0.86	1.57	0.57
4	0.93	1.43	0.05	0.95	1.98	0.24
6	7.10	12.24	0.24	1.20	1.37	0.33
7	2.54	2.35	0.03	0.80	2.06	0.48
Mean	2.74	34.38	0.14	1.03	2.28	0.48
SD	2.48	70.63	0.12	0.23	1.34	0.22

* Chr/BaA = ratio of concentrations of chrysene to benz[a]anthracene,

BeP/BaP = ratio of concentrations of benzo[e]pyrene to benzo[a]pyrene.

A rather high MePhe/Phe ratio obtained for Station 1 was considered an outlier. The Phe/Ant ratios for all the stations did not exceed 1.0. On the other hand, Stations 1 and 6 had Floran/Pyr indices exceeding 1.0, which when considered with the Phe/Ant ratio (ratio < 10) indicated pyrolytic sources of the contaminants. All the 6 stations had Chr/BaA ratios above 1.0, which suggested petrogenic sources of the PAHs (Soclo *et al.*, 2000). A LMW/HMW ratio of less than 1.0 for all the stations indicated the dominance of combustion sources of PAHs. This index, however, does not preclude weathered PAHs from petrogenic sources. Therefore, the PAH signal from the sediments indicates both petrogenic and pyrogenic sources of the contaminants in the study area, with an undetermined contribution of weathered petroleum hydrocarbons.

6.3 Concluding Remarks

The PAH assemblages were studied with the aim of ascertaining distribution patterns that conform to the perceived source-processes and possibly identify actual sources in the study area. Principal component analysis was applied on the PAH data from the water column and sediments.

Of the principal components extracted from PAH distribution in the water column, the first two accounting for 28 % and 16 % of the total variance, respectively, were associated with more than 60 % of the PAHs. The first principal component comprised the contribution of 2- and 3-ring PAHs, alkyl substituted homologues, and 4-, 5- and 6-ring PAHs and was associated with both petrogenic and pyrogenic sources of the contaminants. The second principal component encompassed 5-ring PAHs and also anthracene, and was associated with high temperature combustion processes. Petrogenic sources of PAHs include leakages and spillages of petroleum products in the harbour area during bunkering and other shipping activities, the discharge of contaminated wastewater from the urban area and emissions resulting from the combustion of fossil fuels and recent organic matter. However, the relative contribution of the two main source-processes and associated point sources in the study area could not be defined. The limitation experienced was attributed to the presence of a variety of potential sources with no zonation in the study area.

The principal components extracted from the distribution of PAHs in sediments also featured two main factors, which accounted for about 76 % of the variance. The first principal component was dominated by factor loadings of 3-, 4-, 5- and 6-ring parent PAHs, and was associated with pyrogenic sources of PAHs. The second principal component with factor loadings of 2- and 3-ring PAH and their alkyl-substituted homologues was associated with petrogenic sources of PAHs. A fairly good definition of the two groups of PAHs associated with the production processes was accomplished by *variance maximizing (Varimax) rotation* of the factors and applying Cluster Analysis procedure.

An examination of a selection of molecular indices of the PAHs in the water column and sediments provided further insight into the sources of PAHs in the study area. The ratio of the concentrations of alkyl-substituted PAHs to parent PAHs (Al-PAHs/PAHs > 1) in the water column at most of the stations indicated petrogenic sources of the PAHs. The ratios of the concentrations of 1-methyl phenanthrene to phenanthrene (MePhe/Phe < 1) at some of the stations, and low molecular weight to high molecular weight PAHs (LMW/HMW < 1) in most of the stations indicated pyrolytic sources of the contaminants. Most of the stations had ratios of the concentrations of phenanthrene to anthracene (Phe/Ant < 1) and fluoranthene to pyrene (Floran/Pyr < 1) that indicated a predominance of pyrogenic sources of PAHs.

In sediments, ratios of Al-PAHs/PAHs > 1 and MePhe/Phe > 1 in most of the stations indicated petrogenic sources of PAHs. Two stations, 1 and 6, had the ratios Phe/Ant < 10 and Floran/Pyr > 1, which indicated pyrogenic sources of the contaminants. All the six stations had the ratio of the concentrations of chrysene to benz[a]anthracene (Chr/BaA > 1) indicating pyrogenic sources of PAHs. The ratio LMW/HMW < 1 at all the stations indicated the dominance of combustion sources of PAHs, but may include weathered petroleum hydrocarbons.

The molecular indices obtained from the concentrations of the PAHs encountered in the water column and sediments provided an indication that both petrogenic and pyrogenic sources of the contaminants prevail at all the stations.

Chapter 7

CONCLUSION

The study has established the level of contamination of the Cochin estuarine system with the environmental genotoxins polycyclic aromatic hydrocarbons (PAHs). PAHs are widely distributed in the study area with monthly totals of the concentrations of the 24 PAHs investigated (ΣPAHs) ranging from 0.04 to 7.21 µg l^{-1}. The main sources of PAH contaminants are water transport and shipping activities, industrial and urban/domestic activities. Station 3 was the most contaminated and was attributed to emissions and discharges due to water transport, industrial effluents and urban wastewater. The riverine Stations 1 and 2 were the next most contaminated and this was attributed to emissions and discharges from industrial establishments. Station 7 was comparable to the riverine stations, but was exposed to domestic waste and contamination due to water transport and effluent from a petroleum refinery. Significant contamination of Station 5 with PAHs was attributed to the large fleet of fishing boats operating from there. Station 6 and also 5, were subjected to contamination due to water transport and urban activities. Existing in the vicinity of Station 6 is the busy Venduruthy bridge causeway, where heavy vehicular traffic is a potential source of PAHs. Relatively low concentrations of the contaminants encountered at Stations 4, 5 and 6 were attributed to removal from the water column as a result of estuarine mixing processes, such as flocculation and sedimentation of contaminated suspended particulate matter. On average, higher concentrations of PAHs were obtained during the pre- and post-monsoon seasons (range ΣPAHs, 0.50 – 4.70 µg l^{-1}) than in the wet monsoon season (range ΣPAHs, 1.48 – 3.32 µg l^{-1}).

A monitoring exercise carried out at 2-hourly intervals at Station 8 (Shipyard) revealed that concentrations and speciation of PAHs in the water column were influenced by the dynamics of the tidal cycle and resultant changes in the physical and chemical properties of the water. Estuarine mixing processes were attributed with flocculation of PAH associated SPM, sedimentation, resuspension of sedimentary material by the churning-up action of the bottom current and the removal of PAHs from the water column. Application of the multivariate Principal Component Analysis procedure on the distribution of PAHs in the Cochin system indicated that the concentrations are basically influenced by estuarine mixing processes, especially those processes that are primarily associated with removal of the contaminants from the water column, such as flocculation and sedimentation of suspended particulate matter.

Comparison of the relative concentrations of PAHs between the surface microlayer and sub-surface water layer revealed moderate enrichment in both the dissolved and particulate phases. However, seasonal averages of the Enrichment Factors (EFs) of individual PAHs reached up to 3 orders of magnitude higher than the EFs for ΣPAHs (range <1.0 to 621). The average EFs for individual PAHs in the dissolved phase decreased in the order benz[a]anthracene > benzo[k]fluoranthene > 1-methyl naphthalene > pyrene > acenaphthylene > naphthalene > chrysene > phenanthrene > 2-methyl naphthalene > 2,3,5-trimethyl naphthalene > fluoranthene > 1-methyl phenanthrene (range of EFs 2.2 – 29.9). The generally higher EFs of 4- and 5-ring PAHs relative to 2- and 3-ring PAHs were attributed to a higher resistance of the former group to removal through such processes as volatilisation and photooxidation. Comparatively higher EFs for ΣPAHs associated with SPM and for mass normalised ΣPAHs were obtained (range 0 – 45.8). The mean EFs for individual PAHs were even higher (0 – 536) decreasing in the order pyrene > fluoranthene > phenanthrene > acenaphthene > benzo[b]fluoranthene > chrysene > 2,6-dimethyl naphthalene > 1-methyl phenanthrene > 1-methyl naphthalene > benz[a]anthracene > 2,3,5-trimethyl naphthalene > biphenyl (EFs range: 3.4 – 103). The generally lower EFs for the 4- and 5-ring PAHs were attributed to the diluting effect of higher concentrations of SPM in the microlayer.

A good relationship was established between particulate PAHs and the organic carbon fraction of SPM. High values of distribution coefficients were obtained (K_d range $7.2\text{x}10^3 - 6.8\text{x}10^5$, K_{oc} range $2.1\text{x}10^5 - 1.1\text{x}10^7$) which was a further indication of the importance of the partitioning process, between the dissolved and the particulate phases, in the environmental pathway of the contaminants in the study area.

The concentration levels of PAHs in surficial sediments were moderately high, up to 3 orders of magnitude higher than in the water column (range 24.2 – 274.6 µg kg^{-1} dry sediment). The concentrations were associated with sediment grain size distribution. Thus, Stations 3 and 6 with bottom sediments composed of up to 90 % clay and silt were about the most contaminated. On the other hand, Stations 1 and 2, with bottom substrate composed of over 97 % sand had the lowest concentrations of PAHs. The higher concentrations of PAHs relative to the water column signified the sink-function of the bottom sediments.

Application of principal component analysis on PAH assemblages in the water column resulted in 2 principal components which were associated with more than 60 % of the PAHs. The first principal component encompassed a cross-section of low molecular weight PAHs, their alkyl substituted homologues and high molecular weight PAHs, and was associated with both petrogenic and pyrogenic sources of PAHs. The second principal component was dominated by 4-, 5- and 6-ring PAHs and was associated with high temperature combustion of fossil fuels. The relative contribution of the two source-processes and point sources on the ground could not be defined, probably because of the presence of a number of different potential sources within the study area. Distribution patterns in bottom sediments also revealed the main source-processes to be petrogenic and pyrogenic. A fairly good definition of the two groups of PAHs was accomplished by applying *variance maximising rotation* of the principal factors and Cluster Analysis procedure.

Examination of the molecular indices at most of the stations indicated a prevalence of both petrogenic and pyrogenic sources of PAHs in the study area. Thus, in water the ratio of alkyl-substituted and corresponding parent PAHs, Al-PAHs/PAHs (range 0.58 - 4.06), indicated the dominance of petrogenic sources of PAHs. The ratio of 1-methyl phenanthrene and phenanthrene, MePhe/Phe (ratio < 1) for majority of the stations signified combustion sources. Ratios of low molecular weight to high molecular weight PAHs (LMW/HMW), phenanthrene to anthracene (Phe/Ant) and fluoranthene to pyrene (Floran/Pyr) (all ratios < 1) for all the stations indicated a predominance of pyrogenic sources of PAHs. In the case of sediments, the ratios Al-PAHs/PAH (ratio > 1) and MePhe/Phe (ratio > 1) in most of the stations indicated petrogenic sources of the contaminants. Stations 1 and 6 had ratios of Phe/Ant < 10 and Floran/Pyr < 1 indicating pyrogenic sources of PAHs. All the stations had the ratios Chr/BaA > 1 and LMW/HMW < 1 indicating the dominance of pyrogenic sources of PAHs. However, the presence of LMW PAHs was also an indicator of weathered petroleum hydrocarbons.

The PAH concentration gradient down the water column, is a consequence of the removal of the contaminants by physico-chemical processes arising from estuarine mixing (i.e. flocculation, partitioning, sedimentation and resuspension) in conjunction with other biotic and abiotic processes, such as biodegradation and photodegradation. Compared to the situation in similar environmental set-ups elsewhere, i.e. estuarine harbour areas, the Cochin system is indicated as low water pollution and slight to fairly contaminated bottom sediments. While acute biological effects of the PAH contamination is expected to be low, sub-lethal effects on marine organisms, such as the induction of cytochrome P450 1A and DNA adduct, photo-induced carcinogenic activity and adverse effects on endocrine systems of commercially important marine species cannot be ruled out. Such sublethal effects have the potential of long-term negative impacts on the productivity of the system.

The vibrant economy, expected increase in industrial and urban activities, along with the ensuing problems of waste management is bound to exert increasing pressure on the environment. Such a scenario forecasts an increased production of PAHs and contamination of water systems. A viable strategy in alleviating pollution of the environment with PAHs is to address the major sources, and they include shipping activities in the harbour (to reduce leakages of petroleum products during handling, and indiscriminate waste disposal etc.), water and land transport (encourage use of clean, efficient engines), industrial activities (control gaseous emissions and untreated liquid effluent), and the management of urban wastewater (domestic and urban runoff) which is more often allowed to discharge untreated into water systems.

The present study has been by no means exhaustive. It is appreciated that PAHs are part of a larger group of hydrophobic organic pollutants that are potentially carcinogenic and mutagenic and are expected to be present in the Cochin estuarine system. Consequently a cause-effect study is complicated by the myriad array of contaminants of which individual potential and possible synergistic impacts have to be taken into account. A comprehensive assessment of PAHs and related organic pollutants should include flux studies, speciation and interaction with biotic systems. Information on bio- and photodegradation in the local set-up would provide an insight on the persistence of the contaminants and environmental capacity of the Cochin tropical ecosystem. The net accumulation of hydrophobic organic compounds in especially undisturbed bottom sediments makes them a valuable source of information as pollution indicators, and recent pollution history can be acquired by employing sediment-dating techniques.

Summary

The spatial and temporal distribution of the environmental carcinogens Polycyclic Aromatic Hydrocarbons (PAHs) in the Cochin estuarine backwaters and pertinent factors influencing variability of their concentrations were investigated. The levels of PAH contamination were investigated in water and sediments from December 1995 to November 1996; effectively covering the dry pre-monsoon (February - May) the wet South West monsoon (June - September) and the post-monsoon (October - January) seasons. There were 8 sampling stations selected within the study area. Two riverine stations (Stations 1 and 2) were located in the vicinity of an industrial area while the others were located in the estuarine environment exposed to water transport as well as industrial, urban and harbour activities.

Surface micro-layer, sub-surface (at 1 m depth) and bottom water samples were collected at monthly intervals, with the exception of one station which was occupied for one day at intervals of 2 hours for about 13 hours to follow variations due to the tidal cycle. Surface micro-layer samples were collected using a Garret screen sampler (a 60 cm x 60 cm stainless steel screen of 1 mm mesh-size) capable of collecting a thin-layer of 200 - 250 µm thickness. Sub-surface samples were obtained using a 2.5 l weighted glass bottle with a buoy attached to maintain the sampler at about 1 m depth. Bottom water samples were collected using a conventional deep-water sampler (Biotech sampler). Appropriate precautions were taken in the collection of water samples for the determination of physico-chemical parameters. Water samples were filtered (Whatman GF/F filter paper) to separate suspended particulate matter (SPM). Standard colorimetric methods were used for the determination of inorganic nitrates/nitrites and phosphates in pre-filtered water samples. Dissolved oxygen concentrations were determined by the Winkler titration method. Water turbidity was determined using a nephelometer. Salinity measurements were made using a refractometer and the standard silver nitrate titration method. A sensitive thermometer was used to measure water temperatures, whereas field and laboratory pH meters were used for pH

determinations. Surficial sediment samples were also collected sporadically using a grab sampler.

Filtered water samples were extracted for PAHs by direct liquid-liquid extraction with dichloromethane. Dried SPM and sediments were soxhlet extracted with dichloromethane/hexane (1:1). Sample extract cleanup was performed by normal phase alumina/silica gel column chromatography. Qualitative and quantitative determination of PAHs in sample extracts was carried out by capillary gas chromatography with flame ionisation detection. A certified reference standard for PAHs was used for instrument calibration, with 9,10-dihydroanthracene as the internal standard. Samples were analysed for 24, 2-6 rings individual PAHs which included methyl substituted naphthalenes and phenanthrenes, biphenyl, acenaphthylene, acenaphthene, fluorene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]pyrene, dibenz[ah]anthracene and benzo[ghi]perylene.

On a seasonal average, water salinity ranged from a minimum of <1 ‰ at the riverine stations to a maximum of 26.6 ‰ in the estuary. Low salinities were experienced during the wet monsoon season because of high volumes of freshwater inundating the entire estuary. pH varied from 6.4 upriver to 8.0 in the estuary. Water temperatures ranged from a minimum of 27.3 °C during the monsoon to a maximum of 31.9 °C in the pre-monsoon season. Turbidity ranged from 6.0 to 133.8 NTU, with the lowest value being recorded during the post-monsoon, and the highest in the pre-monsoon season at the riverine industrial area station (Station 1). Dissolved oxygen showed a minimum of 4.3 mgO l^{-1} at an estuarine station to a maximum of 8.7 mgO l^{-1} at a riverine station during the monsoon. Elevated concentrations of dissolved inorganic nitrates and nitrites ranged from 4.1 to 82.7 µmol NO_3^-/NO_2-N l^{-1}. Relatively higher concentrations were obtained during the pre-monsoon season (mean range 29.5 – 65.7 µmol NO_3^-/NO_2-N l^{-1}) compared to the monsoon (range 13.5 – 30.0 µmol NO_3^-/NO_2-N l^{-1}) and post-monsoon (range 12.7 – 48.7 µmol NO_3^-/NO_2-N l^{-1}) seasons. Inorganic phosphate concentrations varied from 0.4 to 43.6 µmol PO_4^{3-}-P l^{-1}. Generally, higher levels of phosphate occurred in the pre-monsoon season at all stations, with the exception of Station 7. The consistently high nutrient loads and low dissolved oxygen concentrations at especially Station 7 were attributed to urban waste inputs.

The results provide indications that PAHs are widely distributed in the study area with monthly mean ΣPAHs ranging from 0.04 to 7.21 μg l^{-1}. Relatively higher levels of PAHs were obtained during the monsoon (ΣPAHs 1.48 - 3.32 μg l^{-1}) and post-monsoon (ΣPAHs 1.61 - 4.7 μg l^{-1}) seasons, than in the pre-monsoon season (ΣPAHs 0.50 – 2.42 μg l^{-1}).

The station experiencing the highest level of contamination overall was Station 3 (mean ΣPAHs 2.82 μg l^{-1}), which was attributed to combined impacts of industrial manufacturing, water transport and the discharge of municipal wastewater. The next highest contaminated sites were the riverine Stations 1 and 2 (mean ΣPAHs 2.26 and 2.73 μg l^{-1}, respectively), which was attributed to PAH rich gaseous emissions and liquid waste discharges from industrial establishments. The level of contamination at Station 7 was comparable to Stations 1 and 2, but was attributed to inputs due to domestic activities, emissions and discharges due to water and land transport, and effluent from a petroleum refinery located in the neighbourhood. Stations 5 and 6 and including Station 4 had relatively lower levels of PAH contamination (mean ΣPAHs 1.50, 1.43 and 1.32 μg l^{-1}, respectively), even though they are also subjected to emissions and wastewater discharges due to transportation and urban activities. The lower PAH concentrations encountered at Stations 4, 5 and 6 were attributed to removal as a result of estuarine mixing processes, such as flocculation and sedimentation of contaminated suspended particulate matter.

The concentration of PAHs in the water surface microlayer was generally comparable or higher than in the sub-surface layer, while bottom concentrations were significantly lower ($p < 0.5$) than in the surface layers. Mean enrichment factors EFs of dissolved ΣPAHs in the microlayer ranged from 1.2 to 6.9. EFs for individual PAHs in the dissolved phase varied widely ranging from < 1.0 to 621. Four and 5-ring PAHs generally had higher EFs than the 2- and 3-ring structures, which reflected their stability and resistance to removal from the microlayer through such processes as volatilisation and photooxidation. The EFs for ΣPAHs associated with SPM in the surface microlayer ranged from 0 to 45.8 (mean 10.9 ± 15.9). The corresponding EFs for SPM or mass normalised PAHs ranged from 0 to 42.0 (mean 9.5 ± 14.9). However, the EFs of individual PAHs associated with SPM varied from 0 to a maximum of 536. With the exception of pyrene and fluoranthene, the 4- and 5-ring PAHs generally had lower EFs. This was attributed to higher concentrations of SPM in the microlayer which tended to have a dilution effect on the contaminants. A good correlation was found between particulate PAHs and fraction organic carbon of the SPM ($r = 0.72$, significant at $p < 0.05$). Estimates of distribution coefficients for PAHs in the Cochin estuarine system ranged from 7.2×10^3 to 6.8×10^5 for K_d and 2.1×10^5 to 1.1×10^7 for K_{oc}. The high values of distribution coefficients were further indication of the important role the partitioning process between the dissolved and particulate phases plays in the environmental pathway of the contaminants in the study area.

Multivariate principal component analysis indicated that variations in the environmental factors and water quality in the estuarine system were influenced by nutrient dynamics, associated with remineralisation processes, which also indicated inputs from land-based sources. It is instructive that nutrient dynamics was not primarily associated with salinity indicating that nutrient levels in the estuary were mostly influenced by input from urban and industrial areas. Of slightly lower significance was saline water intrusion and by implication mixing processes. The contribution of water turbidity to variations in the environmental conditions was relatively less significant and was associated with sedimentation and resuspension processes. The principal factors indicated that variation in the concentrations of ΣPAHs in the estuary was primarily influenced by the estuarine mixing processes.

It was demonstrated that the concentrations and speciation of PAHs in the water column were influenced by the changing salinities and mixing processes due to tidal dynamics. At low salinities and low turbidities, most of the PAHs (> 50 %) were in the dissolved form. During flooding tide, with rising salinity and higher turbidity, a greater proportion (> 50 %) of the PAHs was associated with SPM and was removed from the water column, resulting in overall decreased concentration of the contaminants in the water column.

Concentration levels of ΣPAHs in sediments ranged from 24.2 to 274.6 μg kg^{-1} sediment dry mass. The highest concentrations were obtained from Station 6, followed closely by Station 3. About the lowest concentrations were obtained from Stations 1 and 2, followed by Station 7. The concentration levels were associated with sediment size distribution. Thus, Stations 1 and 2 had predominantly sandy bottom substrates (over 97 % sand), and the lowest PAH concentrations. On the other hand, Station 3 with sediments composed of about 90 % clay and silt was among the most contaminated. The levels of PAH contamination in the sediment of up to 3 orders of magnitude of the concentration in the water column signified the role of the sediments as a sink.

Principal component analysis resulted in two principal factors describing the distribution patterns of PAHs in the water column in general. The two principal components accounted for 28 % and 16 % of the total variance, respectively, and were associated with more than 60 % of the PAHs. The first principal component comprised the contribution of 2- and 3-ring PAHs, alkyl substituted homologues, and 4-, 5- and 6-ring PAHs and was associated with both petrogenic and pyrogenic sources of the contaminants. The second principal component encompassed 5-ring PAHs and also anthracene, and was associated with high temperature combustion processes. However, the relative contribution of the two main source-processes and associated point sources in the study area could not be defined. The limitation experienced was attributed to the presence of a variety of potential sources with no zonation in the study area.

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Supplementary information on the sources of PAHs in the study area was obtained from a selection of molecular indices. In the water column, the ratio of the concentrations of alkyl-substituted PAHs to parent PAHs (Al-PAHs/PAHs > 1) at most of the stations indicated petrogenic sources of the PAHs. The ratios of the concentrations of 1-methyl phenanthrene to phenanthrene (MePhe/Phe < 1) at some of the stations, and low molecular weight to high molecular weight PAHs (LMW/HMW < 1) in most of the stations indicated pyrolytic sources of the contaminants. Most of the stations had ratios of the concentrations of phenanthrene to anthracene (Phe/Ant < 1) and fluoranthene to pyrene (Floran/Pyr < 1) that indicated a predominance of pyrogenic sources of PAHs.

In sediments, ratios of Al-PAHs/PAHs > 1 and MePhe/Phe > 1 in most of the stations indicated petrogenic sources of PAHs. Two stations, 1 and 6, had the ratios Phe/Ant < 10 and Floran/Pyr > 1, which indicated pyrogenic sources of the contaminants. All the six stations had the ratio of the concentrations of chrysene to benz[a]anthracene (Chr/BaA > 1) indicating pyrogenic sources of PAHs. The ratio LMW/HMW < 1 at all the stations indicated the dominance of combustion sources of PAHs, but may include weathered petroleum hydrocarbons. The molecular indices obtained from the concentrations of the PAHs encountered in the water column and sediments provided an indication that both petrogenic and pyrogenic sources of the contaminants prevail in all the stations.

The level of contamination of the Cochin estuarine system is indicated as low water pollution and slight to fairly contaminated sediments. While biological effects of the PAH contamination are expected to be low, there is a high potential for sub-lethal effects on marine organisms, such as the induction of cytochrome P450 1A and DNA adduct, which may be exacerbated by photo-induced carcinogenesis.

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