Ph.D Thesis

Ecotoxicological Assessment of Antifouling Biocides in the Sediments of Cochin Estuarine System



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Ecotoxicological Assessment of Antifouling Biocides in the Sediments of Cochin Estuarine System

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Certificate

This is to certify that the thesis entitled "**Ecotoxicological Assessment of Antifouling Biocides in the Sediments of Cochin Estuarine System**" is an authentic record of the research work carried out by Mr. Deepulal P.M, under my supervision and guidance at the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Kochi-682016, in partial fulfilment of the requirements for Ph.D degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any University.

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Declaration

I hereby declare that the thesis entitled **“Ecotoxicological Assessment of Antifouling Biocides in the Sediments of Cochin Estuarine System”** is an authentic record of the research work carried out by me under the guidance and supervision of Dr. Sujatha C.H, Reader and Head, Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, and no part of this has previously formed the basis of the award of an degree, diploma, associateship, fellowship or any other similar title or recognition.

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Preface

Estuaries are dynamic ecosystems which has a connection with the open sea through which the seawater enters according to the rhythm of the tides. They are amongst the most polluted areas throughout the world, with about 60% of the world's population living along estuaries and the coast. As a result, estuaries are suffering from degradation by many factors including sedimentation from soil erosion, from deforestation, overgrazing, over fishing, drainage, eutrophication due to excessive nutrients from sewage and animal wastes, pollutants including heavy metals, biocides, polychlorinated biphenyls, radionuclides and hydrocarbons. Estuaries are usually biologically highly productive zones. Due to heavy pollution load the biological productivity shrinks as it progress. They also acts as a filter for some dissolved constituents in river water, these precipitate in the zone where river water meets seawater. More important is the trapping of suspended mud and sand carried by rivers which leads to delta formations around estuaries.

The pollutants discharged into the estuaries are originate from two main sources-industrial and sewage. The former may be toxic which includes heavy metals, residues from antifouling paint particles and pesticides, while large discharges of sewage will contain pathogenic micro-organisms. The contamination is enough to destroy the amenities of the waterfront, and the toxic substances may completely destroy the marine life and damage to birds, fishes and other marine organisms. Antifouling biocides are a type of chemical used in marine structure to prevent biofouling. These antifouling biocides gradually leach from the ships and other marine structures into water and finally settled in sediments. Once a saturation adsorption is reached they desorbed into overlying water and causes threat to marine organisms. Previous reports explained the imposex and shell thickening in bivalves owing to the effect of biocides. So bivalves are used as indicator organisms to understand the status of pollution. The nervous system is one of the best body part to understand the effect of toxicant. Acetylcholine esterase enzyme which is the main neurotransmitter in nervous was used to understand the effect of pollutants. Present study uses Acetylcholine esterase enzyme as pollution monitoring indicator.

The thesis is divided into seven chapters. Chapter 1 is the **Introduction** and its deals with general introduction of present study and includes the main objectives of the present study. Chapter 2 is **Materials and Methods** and deals with the nature of sampling location and analytical methods employed for the present study. Chapter 3 **is General Sedimentary Character of the Sediments** and it includes the general sedimentary parameters and biochemical composition like carbohydrates, lipids and proteins of sediments. Chapter 4 **is Evaluation of Redox Condition of Sediments using Rare Earth Elements.** This chapter explains the redox environment of sediment using the rare earth elements and also gives source of sediments. Chapter 5 is **Role of Trace Metals as Biocides**. This covers the main trace metals which are used in biocides and their distribution pattern in Cochin estuary. Chapter 6 is **Antifouling Biocides- Organic and Organometallics**. This includes the antifouling biocides used in shipping industry and the leaching status in the Cochin estuary. Chapter 7 is **Ecotoxicological Studies of Antifouling Biocides**. It deals with the inhibitory action of enzyme acetylcholine esterase in "*Villorita cyprinoides* var *cochinensis*" in the presence of biocides.

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List of Abbreviations

Ach	Acetylcholine
AChE	Acetylcholine esterase
BT	Butyltin
CF	Contamination Factor
DBT	Dibutyltin
DPT	Diphenyltin
EF	Enrichment Factor
HREE	Heavy Rare Earth Elements
I_{geo}	Geoaccumulation Index
LREE	Light Rare Earth Elements
MBT	Monobutyltin
MPT	Monophenyltin
MREE	Middle Rare Earth Elements
OT	Organotin
REEs	Rare Earth Elements
TBT	Tributyltin
TOT	Triorganotin
TPT	Triphenyltin
OTC	Organotin compound
CHO	Carbohydrate
LIP	Lipid
PRT	Protein
BDL	Below detection limit

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Chapter 1

Introduction

1.1 General Introduction

An estuary is a dynamic system in which the distribution pattern of dissolved and particulate components are results from the mixing of saline water and fresh water. It acts as a transition zone in which continental material is trapped and the rest of which is transported to the open sea. They are having widely different composition and a variety of chemical and biogenic reactions taking place independently and simultaneously. Estuaries have a long history of contamination, which has resulted in extensive areas of polluted sediments, mainly associated with the most industrialised parts of the catchment.

The history of an estuarine system is largely determined by its sediment supply. Sediments represent one of the ultimate sinks for pollutant discharged into the environment (Luoma and Bryan 1981). The prime source of sediments is obviously the land and a small quantity is transported through the atmosphere, besides coastal erosion and transport of ships and vessels. To estimate the influence of this transport on estuarine development, the total amount of sediments retained in near shore areas must be evaluated. Generally surface sediments are considered as a pollution indicator of water and lake ecosystems

(Casas et al. 2003). It act as both carriers and sinks for contaminants, reflecting the history of pollution (Singh et al. 2005), and also provide a record of catchment inputs into the aquatic ecosystems (Mwamburi 2003). Sediments also serve as a pool of metals that could be released to the overlying water as a result of natural and anthropogenic processes such as bioturbation and dredging respectively, causing adverse health effects.

Although the sea level rise has influenced both estuarine and open shelf environment in a similar fashion. The conditions for sediment deposition in estuaries are much more favourable than on the open shelf. The estuaries often act as an effective barriers against strong waves and currents, so that fine-grained material can settle, whereas shelves are too exposed. This is undoubtedly an important factor, but in addition a number of water and sediment transport mechanisms exist which actively results in the escape of sediments. Some of these mechanisms extend outward over the inner shelf. Since particles are trapped in estuaries, their residence time is generally much longer than that of the estuarine water mass, even if they remain in suspension. During their stay in sediment they are subjected to important changes in size and composition.

Flocculation and changes in biological grain-size changes are the two most important processes that affecting sedimentation. Reactions affecting sediments have more than theoretical interest because they can control the formation of economic mineral deposits, the acoustic and engineering properties of the sediment, exchange pollutants between the sediment and the overlying water and other important aspects of sediment formation. Many diagenetic reactions are thought to begin soon after sediment deposits, but identifying incipient reactions in the solid phase is difficult.

1.2 Estuarine Pollution

Estuaries and coastal areas are extremely important source for nutrients and pollutants in the marine environment. The main emphasis on estuarine research is to study the effects of pollution. Because of rapid population growth and uncontrolled development, many coastal areas and estuaries exhibit severe pollution impacts by introduction of a wide variety of chemical contaminants. Many of the chemical substances are known to concentrate in the water, accumulate in estuarine sediments (McCain et al. 1988) or bioaccumulate in sediment-dwelling organisms (Meador et al. 1995). Pollutants enter estuaries through storm drains, industrial discharges, runoff from lawns, streets, and farmlands, discharges from sewage treatment and atmospheric deposition. Figure 1.1 represents the schematic path way of contaminants.

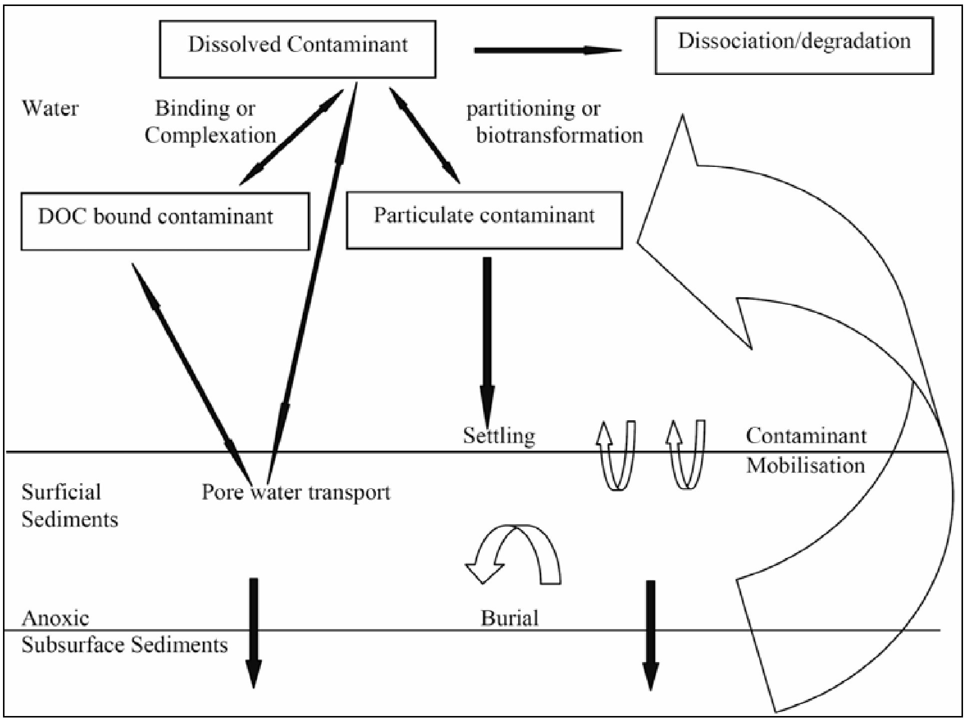

Figure1.1 The Schematic diagram of transport and transformation of Contaminants in sediments

Estuaries can be affected by contamination in the following major ways: oxygen depletion (e.g. hypoxia, anoxia) (Saiz Salinas 1997), toxic substances accumulation or bioaccumulation (e.g. toxic organic compounds, petroleum products, and heavy metals) (Bryan and Langston 1992), spills (e.g. oil spills) (Colombo et al. 2005), pathogens (e.g. from sewage) (Lipp et al. 2001), and thermal pollution (e.g. heated effluent from power plants) (Kaizer et al. 2005). In addition, sediment quality also has received increasing concern because they are known to act both as a repository for contaminants and as a pollutant source with potential impacts on the quality of estuarine ecosystem (Budzinski et al. 1997; Santos et al. 2009). Researchers all over the world have studied the trends of estuarine pollution (Van Geen et al. 1997; Davis et al. 2000; Deepulal et al. 2012).

Most of the pollutants imported to estuaries from land will eventually deposit at the bottom and inturn produce harmful effects on the aquatic organisms, especially benthos, and finally affect the ecosystem health. Sedimentary environment is a complex system and restrained by multiple dynamic factors. It controls to a large degree the exchange of the pollutants on the interface between the sediment and water, thus affecting the pollutant behaviour and its ecological effect. Generally, in the estuarine water, if sediment deposits quickly, the exchange of the pollutant on the interface between water and sediment will be inhibited, whereas in the water where deposition is slow, the exchange will be remarkable.

In the context of estuarine ecosystems, the rate of sedimentation has the following characteristics:

a) Enrichment: The carrying capacity of sediment contaminated with pollutants is much higher than water, and therefore the concentrations of contaminants in sediments are usually several orders of magnitude higher than in water. Toxic pollutants invariably accumulate first in sediments and organisms.

b) Consequence and stability: Pollutant distributions in sediment deposition are consequential and relatively stable both spatially and temporally. The analysis of sediment deposition profiles could reveal both the average levels of contamination and the historical evolution of the environment.

c) Pollution Potential: Sediment can be both a ‘‘sink’’ for pollutants, and also a ‘‘source’’. Under certain conditions, pollutants can be released from sediment and endanger organisms, accumulate in food chains, and cause secondary contamination.

d) De-contamination: Once sediment is contaminated, it is extremely difficult to de-contaminate.

1.2.1 Pollution due to Antifouling Biocides

One of the major sources of pollutant in estuaries and coasts originate from shipping and industrial activities. Ships are coated with special type of paints which prevents marine organisms to attach the hull of the ship and thereby increasing the life span of ships. These paints are known as “antifouling biocides”, which prevents barnacles, algae and other marine organisms that attach the ships hulls. The major antifouling biocides used in the shipping industry are organotin (OT) based formulations, and was introduced in late 1960s. The toxic effect of organotin compounds (OTC) were not known until the abnormal thickening of oyster shell identified in Archechon Bay (Southern France) in late 1970's along with the reports of imposex in dog whelk "*Nucella Lapillus*" reported in Western England. Similar toxic effect of organotin compounds were observed around the world and International Maritime Organization forced ban on organotin based formulation in ships lesser than 25 feet in the year 2003 and put complete ban on January 2008 on all kinds of ships. After the ban on organotin based paints, shipping industry introduced "Irgaorl-1051" a triazine based herbicide as biocide. Later chemicals like chlorothalonil, dichlofluanid, diuron, sea nine-211 etc were introduced as antifouling biocides in shipping industry. The Irgarol-1051 is readily photo degraded in water and settled down on sediments and the rest of the other biocides are less understood for their mechanism and process.

Triorganotin-based formulations are extremely efficient, but the adverse environmental effects at very low concentrations of the biocide have resulted in the phasing out of these paints (Champs 2003). As a consequence of the ban on using organotin compounds in antifouling coatings in small boats, imposed by many countries in 2003, there has been a regain in the use of copper-based antifouling paint formulations. Because of the toxic effects exerted by paints containing triorganotin compounds on non-target organism, Cu, mainly as Cu_2O, is now the principal inorganic biocidal component of most antifouling applications. Yachts and motor boats may leach in excess of ~2Kg of Cu per annum (Boxall et al. 2000). It represents an important, contemporary source of Cu to the coastal environment (Matthiessen et al. 1999; Helland and Bakke 2002; Schiff et al. 2004). In locked or semi-enclosed marinas, harbours and boatyards, water and sediment significantly contaminated by Cu and it has been reported by Comber et al. (2002). Consequently, mostly contemporary antifouling paints employ Cu(I), in the form of cuprous oxide or cuprous thiocyanate as the main biocidal constituent. To improve the efficacy of Cu(I), many formulation also contain Zn oxide and/ or an organic or organometallic booster biocide, such as dichlofluanid, Irgarol 1051 or Zn or Cu pyrithione (Comber et al. 2002). Cr, Pb, Cd were also used in antifouling formulation as co-biocides or as pigment in paints. In intertidal and benthic sediments, antifouling paint particles represent a relatively persistent source of biocides (Tolhurst et al. 2007), and pose a threat to birds and invertebrates that incidentally ingest and digest contaminated residues (Turner et al. 2008b).

1.2.2 Pollution Indicators

Mussels, in particular *Mytilus edulis* species have been established as one of the most widely used monitoring organisms for evaluating the impact of marine pollution. While systematic monitoring was launched with the “Mussel Watch” program in the US in the 1970s (Goldberg 1975), mussels have lately been involved in several European research programmes, e.g. MEDPOL (Gabrieldes 1997), and BIOMAR (Narbonne et al. 1999). The advantages of mussels are several which include their abundance and cosmopolite spreading in most temperate waters around the world, and their lifestyle as stationary filter feeders inhabiting the benthic environment, where pollutants usually end up and accumulate. Mussels readily bioaccumulate both organic (Moore 1985) and metal (Viarengo 1985) pollutants, therefore, often rendering them more accurate insitu bioindicators of pollution than fish (Cajaraville et al. 2000).

Mussels have many attributes that make them ideal bioindicators (Ostapczuk et al. 1997). They are economically important as a source of protein for humans (Yap et al. 2004). They are also long-lived, sedentary and suspension feeders, which allow them to accumulate high levels of metal pollutants from their immediate environment, and yet they are tolerant to natural fluctuations in metal levels. Moreover they are also easy to identify, abundantly available for sampling all year round (Yap et al. 2004). The ability of mussels to accumulate pollutants in their tissues (Chan 1988; Lakshmanan and Nambisan 1989) makes them well suited in providing an indication of the pollution status in the surrounding medium.

The recent development of biomarkers based on biological responses in organisms to pollutants has provided biochemical tools which are essential for implementing the monitoring programmes of contaminants effects. Cholinesterase inhibition has been used for years as a marker of exposure to insecticides, not only in humans but also in wild life, in order to monitor the effects of contaminants on living organisms (Galloway et al. 2002). In the marine environment, the choice of a monitoring target species mainly concerned are bivalve molluscs such as the common mussel or the oyster, essentially because of their capacities to accumulate contaminants. However, molluscs, unlike vertebrate species, have proved relatively insensitive to inhibitors.

Using biomarkers for coastal and marine pollution has many advantages when compared to chemical analysis. Among the biological effects of pollutants, biochemical changes occur more quickly than physiological responses and provide information on the sensitivity of organisms with regard to uptake, biotransformation, and detoxification patterns (Galloway et al. 2002). Biomarkers allow an integrated measurement of bioavailable contaminants causing biochemical responses, providing early indication of potential pollution. Biocides, which are widely used in antifouling paints, enter the marine environment by leaching. The toxic effects of biocides are caused by the irreversible inhibition of enzyme acetylcholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine (ACh) in the cholinergic synapse of the central and peripheral nervous system (Sturm et al. 2007). AChE has been reported as a biomarker to neurotoxic compounds (which include Organo phosphate pesticides), in biomonitoring studies that assess negative effects on aquatic organisms and environmental quality (Escartín and Porte 1997; Radenac et al. 1998; Mora et al. 1999; Galgani and Bocquené 2000; Dailianis et al. 2003). Bivalves living in sandy-mud bottoms are often used for ecotoxicological monitoring because they are filter-feeding organisms and can accumulate contaminants in their tissues to relatively high levels. For instance, bivalve enzyme activity, including AChE, has already been extensively used in monitoring environmental pollution in laboratory and field studies (Le Bris et al. 1995; Dellali et al. 2001; Doran et al. 2001; Alves et al. 2002; Mohamed et al. 2003). In the laboratory study, exposure to neurotoxic compounds caused the decreased AChE activity of blue mussels were already reported (Escartín and Porte 1997; Mora et al.1999; Alves et al. 2002). Variations in the AChE activity of marine mussels have frequently been observed in field studies which link to pollution levels, suggesting the presence of AChE inhibiting substances (Dailianis et al. 2003; Lionetto et al. 2003). The study of Dellali et al. (2001) revealed that the AChE activity of clams (*Ruditapes decussatus)* is generally lower than mussels (*Mytilus gallovincialis*) but showed greater spatio-temporal variability. AChE activity of invertebrates, particularly bivalves, differs from vertebrates. The classifications, characteristics and tissue localisation of cholinesterase (ChE) in vertebrates are generally valid for invertebrates, but there are differences (Kristoff et al. 2006). The ChE in invertebrates are able to cleave suitable substrates among acetylthiocholine (ATC), Butylthiocholine (BTC) or Propionylthiocholine (PrTC). The ChE may exhibit a wide variety of substrate specificities (Kristoff et al. 2006). Earlier studies by the Bocquené et al. (1997) and Galgani (1992), ChE activity of invertebrates like bivalves was measured with acetylthiocholine (ATC) as substrate.

1.3 Antifouling Biocides

First systematic studies of organotin were done by Sir Edward Frankland (1825-1899) who in 1853 synthesised diethyltin- diiodide and later in 1859 tetraetlyltin (Van der kerkm 1975) was synthesised, and today more than 800 organotins are known. Most of these compounds are anthropogenic origin except methyltins, which can also be produced by biomethylation (Guard et al. 1981).

For nearly 100 years, the organotins have not been utilised, mainly because no commercial application realised (Champ and Seligman 1996; Hoch 2001). This changed in the 1940s when the plastics industry, particularly the production of polyvinyl chloride (PVC), began to expand (Blunden et al. 1984). The PVC polymer becomes unstable under the influence of heat and light, resulting in discolouration and embrittlement. It was found that by adding certain organotin derivatives this kind of degradation process can be prevented (Yngve 1940). Even today mono/dialkyltin derivatives are used in PVC processing. The biocidal properties of trisubstituted organotin species were discovered in the late 1950s at the institute of organic chemistry, Utrecht, The Netherlands, (Van der kerk and Luitjen 1954) and used as preservatives in timber industry. Further the uses of organotin compounds includes fungicides, miticides, molluscicides, nematicides, ovicides, rodent repellent, wood preservatives and antifouling paints, primarily containing tributyl-, and triphenyl- toxic additives (Bennett 1996).

Due to the widespread use of these OTC (organotin compounds), significant amounts are distributed in various aquatic ecosystems. Attention has mainly been given to tributyltin in water and sediments because of its high toxic effect to aquatic life even at very low concentrations (Chagot et al. 1990). But other organotin, like triphenyltin (TPT), also showed strong biocidial effects (Horiguchi et al. 1997). Reports have shown that OTCs are obviously released into the aquatic environment from various sources and could represent a risk factor for aquatic and terrestrial ecosystem in the later days.

1.3.1 Types of Antifouling Paints

There are different types of antifouling paints existing in the market; they are free association antifouling paints and self-polishing antifouling paints.

a) Free association antifouling paint, in which the biocides are physically mixed in the paint matrix and are released into the aquatic environment by diffusion. The toxic component leaches exponentially from the paint with time, beginning with an uncontrolled high release rate soon after the application, which decreases slowly with time, but later in a uncontrolled limit. These slow leaching rate is due to the microchannels in the paint surface which could be clogged up (for example by aragonite) and inhibits the further release of the toxic component. The effective period of these paints is about 2 years. Following the legislative regulation, such antifouling paints was banned in many countries (Hoch 2001).

b) Self-polishing antifouling paint, in which the toxic component is chemically bonded with a polymer causing a delay of the organotin release into the water. Such release from the paint surface is caused by chemical reactions with seawater. The copolymer paints were introduced in 1974 and they were the most commonly used and effective type of antifouling paints in the 1980s. Because of the biocidal components are released only at the painted surface, and their releasing rate is low, ultimately enhance their lifetimes to 5-7 years (Hoch 2001).

1.3.2 Distribution and Effect of Organotin Compounds in various Ecosystems

Due to the extensive use for various human activity, large amounts of organotin compounds have been introduced in the ecosystems. Thus, significant concentrations of these pollutants and their metabolites have been detected in varying segments of the aquatic environment: water, suspended matter, sediment and biomass. Due to the presence of negligible amount in the atmosphere, OTC interaction from this source are unlikely to be significant (Blunden and Evans 1990).

A) Organotins in aquatic systems

OTCs are of great interest, particularly in the aquatic ecosystem due to their extreme toxicity, their wide spread use and their relatively high persistence. These toxicants can enter the aquatic system by different routes as high shipping activities, harbours and shipyards. TBT from antifouling paints enter into the water body, gives rise to contamination of both water and sediments of marinas, lakes and coastal areas. But far away from coastal regions high concentration of this contaminant were also detected. Hardly and Cleary (1992) analysed samples of the sea-surface microlayer and near-surface bulk water at 5 stations upto 200 km, offshore in the North Sea. In a zone extended from 100 to 200 km off shore, the OT (organotin) contents in the surface micro layer exceed 20 ng Sn/l, which is 10 times higher than the concentration required to induce imposex in dog whelks (Becker et al. 1997). An important process for such an elimination of OTs from the water column is the adsorption onto suspended matter and sedimentation occurred.

B) Organotins in sediments

Organotin concentrations in the open Arcachan Bay, France, ranges to 1 ng Sn/l to 100 ng Sn/l from 1977 to 1981 and again decreased to about 1ng Sn/l in the late 1980s and 1990s Thus supporting forced in January 1982, for the ban in the use of OT-based antifouling paints (Ruiz et al. 1996). In the aquatic environment, total organotin compounds have low aqueous solubility and low mobility, and they are easily adsorbed onto suspended particulate matter (SPM). This is an important process in the fate and transport of OT in the aquatic system. The deposition of the SPM leads to OT scavenging in sediments, where considerable amounts of the trisubstituted OTs and their degradation products can be detected. The studies of Schebek et al. (1991) at Rhine river sediments were found high concentrations (5- 50 ng/g dry wt) of organotins in harbour location. They found that the use of TBT as antifouling paints, the manufacturing and industrial use of several OT compounds and the effluents from municipal sewage treatment plants are the potential sources of organotins in the river Rhine. The sediments analysed in this study showed a general increase in concentrations of OT species towards the lower Rhine.

Quevauviller et al. (1994) measured the OT concentrations in a sediment core and found a concentration gradient upto 15cm depth. These results indicate that the OTs persisted within the sedimentary column over a large period of time. The half-life of OT in sediments is in the range of several years rather than days or weeks in the water column. De Mora and Delletier (1997) summarised degradation rates of TBT in sediments ranging between 1.8 and 3.8 years, Waldock et al. (1990) reported half-lives upto 5 years. Therefore, the subject is a growing concern and debate on the persistence of OTC existing and the transformation kinetics and their possible release from sediments are a new era of scientific research topics.

Resuspension of particles and remobilisation of the pollutants can be caused by dredging, swirling or desorption. Concerning the extreme high amounts of OT pollutant in sediments, it is important to improve the knowledge about the remobilisation processes. The presence of these OT species on suspended matter or sediments makes them available to filter feeding or sediment-feeding organisms.

C) Organotins in organisms

A wide spread deleterious effect induced by OT pollution is “imposex”, a superimposition of male sex organs on female sea snails. It is generally accepted that imposex is induced in adult female snail even at TBT concentration in water approximately to 1 ng/l. Results of laboratory experiments showed that this effect is also initiated and promoted by TPT at the same concentration (Horiguchi et al. 1997). Imposex gives rise to reproductive failure and as a consequence to population decline. TBT bioaccumulation levels in dog whelks were determined to be 1000 times higher than the concentration in the surrounding water (Gibbs and Bryan 1986). In coastal regions of the North Sea, the dog whelk population is considered to be extinct as a result of high organotin concentrations. A connection between the extinction and the TBT pollution is proved by the increased occurrence of imposex in dog whelks, which were found close to the centres of high boating and shipping activity and correlate significantly with the Sn concentrations which were determined in the tissue of the animals. A change of females into hermaphrodite individuals, unable to reproduce is not only observed in coastal regions but also in populations living far away from the coast. About 100 species of snails around the world are affected. TBT pollution may also cause the death of larvae flowing into the bay, and thus results in failure to re-populate (Horiguchi et al. 1997).

Despite the high concentrations of toxic OTCs formed in aquatic invertebrates, little is known about the accumulation and toxic effects of OTC in higher trophic vertebrate predators, which may be reposed to these pollutants via food ingestion. Significant concentrations were found in various tissues of cetaceans from the Western North Pacific, Bay of Bengal, and Japanese coastal waters (Iwata et al. 1995), in bottlenose dolphins from Italian (Kannan et al. 1996) and US coastal waters (Kannan et al. 1997a), Stellar Sea lions from Japan (Kim et al. 1996), Ganga River dolphins from India (Kannan et al. 1997b), Harbour porpoise from the Black Sea (Madhusree et al. 1997) and sea otters from California coastal waters (Kannan et al. 1998a). In general, coastal species exhibit higher OT accumulations than species from off-shores areas.

Takahashi et al. (1997) determined OT concentrations between 21 and 980 ng/g wet wt in various tissues of deep-sea organisms, collected from Suruga Bay, Japan. The measured amounts are comparable with those found in organisms living in coastal shallow waters from Tokyo Bay that is strongly influenced by human and industrial activities. This indicates that OT contamination not only affects the aquatic life off-shore but also affecting deep-sea organisms.

Aquatic life is affected by OT pollution but also birds, those are in the higher trophic level of the food chain show high levels of xenobiotics and can be considered as bioindicators for monitoring the environmental pollution (Thompson et al. 1990). Comparative studies of the OTC concentrations in wild birds and their natural food stuffs give information about the enrichment of OTs during ingestion. Based on the whole body concentrations of BTs in some cormorants (42-160 ng/g) wet wt) from Lake Biwa, Japan, in comparison with the contamination in the fish ingested by these birds (10-55 ng/g wet wt), the biomagnification factors were assessed to be in the range of 1.1-4.1 (Guruge et al. 1996). Although only limited data of TBT concentrations in birds exist, evidence for wide spread contamination has been obtained. TBT and its decomposition products, MBT, and DBT, are found to accumulate in feathers, muscles, liver and kidney of various water birds (Kannan et al. 1998b). During 1989-1992, sea ducks were collected from various harbours and marines along the west coast of British Columbia. The concentrations of OTCs in the livers of these animals range between 28 and 1100 ng/g wet wt (Kannan et al. 1998b). An interesting result is that mollusc-feeding sea ducks seemed to accumulate high concentration of BTs than predatory birds taking up fish, small mammals or other little birds.

1.3.3 Irgarol 1051

Irgarol 1051 (2-methylthio-4-tertbutylamino-6-cyclopropylamino-s-triazine), is a herbicide which is used as antifouling biocides in shipping industry. It is used in tin free antifouling paints as a co-toxicant in addition to biocides, which reduce the primary growth and colonisation by algae that are resistant against copper and zinc metal oxides (Scarlett et al. 2000). In the past, several studies on the environmental contamination of aquatic systems by Irgarol 1051 were carried out. Little is known about the long-term toxicity and degradation of Irgarol 1051, which possess a low water solubility (7 mg/l) and a relatively high octane/water partition coefficient (log K_{ow}=3.95). According to the manufacturer, the degradation of Irgarol 1051 in sea and fresh water sediments under aerobic condition is slow with half-lives of about 100-22 days, and under anaerobic conditions it is even considerably slower. (Ciba Geigy 1988). In comparison to other triazines Irgarol 1051 exhibit a strong affinity to sediments rather than the pore water.

1.3.4 Chlorothalonil

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is a foliar, nonsystemic, broad spectrum organochlorine fungicide and has been used widely in the agriculture fields for more than 30 years as preservative in paints and adhesives (Caux et al. 1996). Chlorothalonil has moderate acute toxicity (Caux et al. 1996; Aprea et al. 2002). Human exposure to the fungicide is associated with dermatitis, severe irritation of the eyes, skin and gastrointestinal tract, also allergic skin responses have been noted in farm workers. Although Chlorothalonil is not highly toxic to mammals, it has been classified in the B2 group, which is considered as a ‘probable human carcinogen’ by the U.S. Environmental Protection Agency due to the carcinogen hexachlorobenzene that is produced as a by-product during the synthesis of the active ingredient (Cox 1997). Several published studies were reported on the environmental fate and behavior of Chlorothalonil in sediments (Regitano et al. 2001; Sakkas et al. 2002; Kwon and Armbrust 2006)

1.4 Aim and Scope of the Study

The Cochin estuary is one of the largest estuarine systems along the west coast of India. This water body is a very important hot spot for residing many pollutants. Reports on the effect of antifouling biocides along the ports and harbours of India are lacking. Very few such studies were conducted in Zuari-Mondovi estuarine system and Bombay port by Bhosle et al. (2006). The majority of the previous studies were related to organotin based antifouling biocides and no reports have been published previously regarding on the replacement of organotin compounds, i.e. Irgarol-1051, Dichlofluanid and Chlorothalonil and their ecological effects along Indian coasts. The present study aims to investigate the identification, quantification, distribution and toxicological effects of antifouling biocides in the sediments of varying sampling sites in the Cochin estuary. Recently trace metals are used as co-biocides in antifouling formulations, these selected trace metals which are used in antifouling formulations were considered for the present study to understand their concentrations.

The objectives of the present study are:

a) Evaluation of geochemical setting of the sedimentary environment using general sedimentary parameters.

b) Assessment of the biochemical composition of the sediments, for understanding the quantity and quality of organic matter which influence the distribution of antifouling biocides.

c) Assessment of the redox condition of the sediments in the Cochin estuary by employing the distribution pattern of rare earth elements (REEs).

d) Distribution pattern of the main trace metals, used in antifouling biocides, in the sediments of Cochin estuarine system.

e) Quantification of antifouling biocides in the sediments of the study area to understand their concentration levels.

f) Investigation of ecotoxicological effects of antifouling biocides.

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Chapter 2

Materials and Methods

Contents

2.1 Description of Study Area

Cochin estuary, the largest estuarine system in the southwest coast of India, is a part of the Vembanad-Kol wetlands (09°40' 12 - 10°10'46N and 76° 09' 52 -76°23'57 E), included in the Ramsar Site (No.1214). This estuary is topographically divisible into two arms; a southern one extending from barmouth to Thanneermukkam and a northern one extending from Cochin barmouth to Azhikode. This tropical aquatic system is under the profound influence of the monsoon, which contributes to nearly 71% of the annual rainfall (Jayaprakash 2002) and constantly prevailing three seasonal conditions viz. monsoon (June-September), post-monsoon (October-January) and pre-monsoon (February-May). Tides at Cochin are a mixed semi-diurnal type, with the maximum spring tide range about 1m (Srinivas 1999), resulting in incomplete flushing. The existence of the abundant mangrove vegetation of these regions has been shrinking in area due to land reclamation and developmental activities. The Cochin estuary has been shrinking in its area from 315 km^2 in 1912 to 256 km^2 in 1980 (Gopalan et al. 1983).

The estuary is experiencing increased anthropogenic interventions since the last five decades. Indiscriminate reclamation, urban development, discharge of untreated and partially treated sewage, industrial effluents, increased shipping activities and siltation are the major threats to this sensitive ecosystem. As per the Environmental Protection Act in 1986, the Cochin estuary has been classified under the ‘‘Ecologically Sensitive Zone’’. The brief description of the sampling stations are given below,

Station-1 (ST-1): Barmouth

ST-1 is located near the Barmouth, Cochin estuary (Figure 2.1) and it is the gateway of the Arabian Sea. Intense traffic due to boating and shipping activities is taking place in this region with the regular dredging for the uninterrupted movement of ships. Water flow rate is high in this region. Average depth in this region is 14-16 m.

Station-2 (ST-2): Bolgatty Island

The sampling point is situated nearer to the Bolgatty Island, where river Periyar joins the estuary. Newly built International Container Terminal and marina for leisure craft are close to this station. As compared to the first sampling station, the depth of the sampling site is less (around 3-5 m).

Station-3 (ST-3): Sulphur jetty

This station is situated near to the Sulphur berth, which is exclusively used for the sulphur transport. Imports of sulphur via large ships are taking place and sulphur transportation to the destination by large barges taking place and making it an active zone.

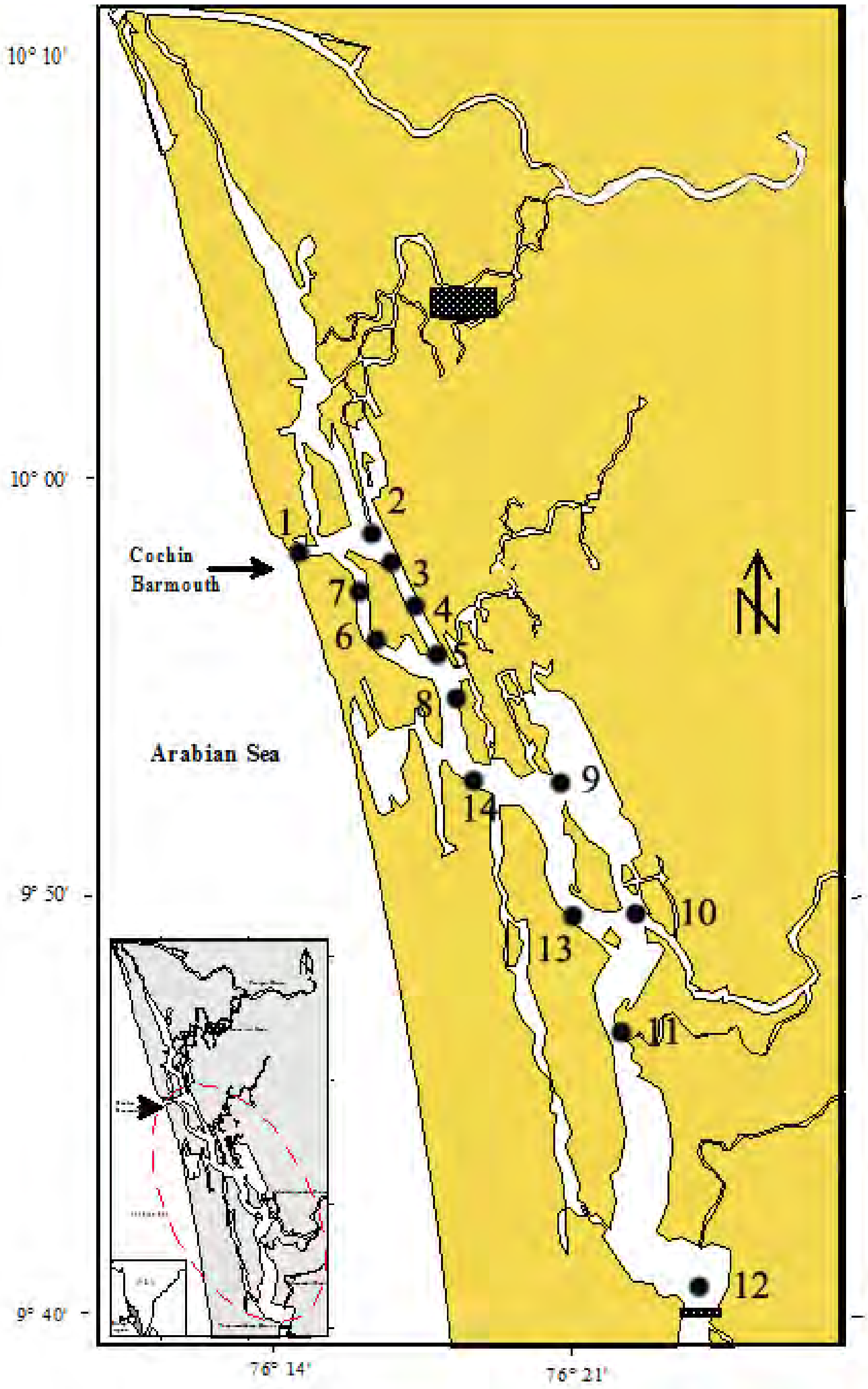

Figure 2.1 Map of Cochin estuary showing sampling locations

Station-4 (ST-4): Shipyard

India’s one of the largest ship building and repairing facility is located near to this sampling station. On the opposite side of shipyard the ship repairing facility of Indian Navy is also located.

Station-5 (ST-5): Thevara Bridge

Station 5, where fresh water from Vembanad Lake split by Willington Island, is taking place at this point.

Station-6 (ST-6): Mattancherry Fishing harbour

This station is known for its largest fleet of fishing boats along the west coast of India and has 8-10 m average depth.

Station-7 (ST-7): Indian Oil Corporation -Oil storage facility

Station 7 is located near the storage facility of petroleum products. Also it contributes the berths for passenger ships and is placed in the northern tip of Willington Island.

Station-8 (ST-8): Kumbalam

This station is near to Kumbalam island and is situated to the southern part of the Cochin estuary where the river Chithrapuzha joins the estuary.

Station-9 (ST-9): Poothotta

Poothotta is the middle part of Vemband Lake and is a coastal village in Ernakulam, Kerala. It is the home town for the Poothotta Coir Vyavasaya (Industrial) Co-operative Society, a government owned firm involved in the manufacture of coir products. The place is located below the sea level. It features an impressive system of dykes and bunds to facilitate paddy cultivation. Adjacent to this stations there is a boat terminal, which connects the districts Ernakulam to Kottayam and Alappuzha .

Station-10 (ST-10): Murinjapuzha Outlet

Ittipuzha is another river which falls in the Cochin estuary. The samples were taken at the discharge point of the river.

Station-11 (ST-11): Ittipuzha Outlet

This river also flows into the Cochin estuary and samples were taken from the discharge point.

Station-12 (ST-12): Thanneermukkam Bund

This station is situated at the southern part of Vembanad Lake. A salinity barrier (bund) is built near to this station to prevent the intrusion of saline water during post-monsoon season, and thereby protecting the paddy field from saline water flow. Samples were taken from near the bund. Thanneermukkom Bund was constructed in 1974 and was functional since 1976. It is the largest mud regulator in India. This barrier essentially divides the lake into two parts - one with brackish water perennially and the other half with fresh water fed by the rivers draining into the lake. This barrier has helped the farmers in Kuttanad, where farming is done below sea level. However it has created ecological problems, primarily, the rampant propagation of the Water Hyacinth in fresh water. The backwaters were abundant with fish as a part of the staple food for the people residing in this region. The salt water barrier has caused deterioration of the catch of fish in their region and the fishermen are opposed to the bund from 2005 onwards.

Station-13 (ST-13): Panavally

Panavally is an island in the middle part of Vembanad Lake. Samples were taken from the Boat jetty situated near the island. As there is no bridge, connecting this island from main land, boats are the means of transportation for people residing in this area.

Station-14 (ST-14): Perumbalam

Perumbalam is another island which is bigger than Panavally which is located in the middle part of Vembanad Lake. The samples were taken from the shores of boat jetty. Mussels collection from the estuary and Lake are the major activity along this place. Machines are deployed in this place to collect mussels from bottom.

2.2 Methodology

Sediment samples were taken from the same 14 stations described above which spread across the Cochin estuary on May 2007 to May 2008. Van Veen grab ($0.042 m^2$) sampler was used to collect the surface sediments from these estuarine stations. Samples were transported to the laboratory in ice box and stored in a deep freezer (-20^0C) until analysis. All the analyses were carried out in triplicates and the average results were reported.

2.2.1 General Sedimentary Parameters

The sediment textural characteristics (sand, silt, and clay) were determined by pipette analysis (Krumbein and Pettijohn 1938) after removing the inorganic carbonates using 10% HCl and organic matter by 30% H_2O_2. This analysis is based on Stoke's law. Sediment was dispersed for overnight with sodium hexametaphosphate. It was then wet sieved through a 63 µm sieve to collect the sand fraction. The mud fraction was divided into silt and clay fractions by the timed gravimetric extraction of dispersed sediments (Folk 1974). Sediment samples were freeze-dried and finely powdered using agate mortar for further analyses. Powder X-Ray diffraction analysis was carried out to find the mineralogy of the sediments (Moore and Reynolds 1997). Total Carbon, Nitrogen and Sulphur were determined using Vario EL III CHNS Analyser. Sediment organic carbon was estimated by the method of El Wakeel and Riley (1956) and later modified by Gaudette and Flight (1974). The amount of Total Organic Matter (TOM) was obtained by multiplying the Organic Carbon (OM) values with 1.724 (Nelson and Sommers 1996).

The chloroplastic pigments (chlorophyll-a and pheaopigments) were analysed spectrometrically according to Lorenzen (1967). Pigments were extracted with 90% acetone (24 hr in the dark at 4^0C). After centrifugation, the supernatant was used to determine the functional chlorophyll-a and acidified with 0.1N HCl to estimate the amount of pheaopigments. Protein (PRT) were determined after extraction with NaOH (0.5M, 4 h) as suggested by Hartee (1972). The absorbance was evaluated at 650nm using UV-VIS Spectrophotometer. Bovine albumin solutions were used as standard. Carbohydrates (CHO) were analysed according to Gerchav and Hacher (1972). This method is based on the same principle as the method of Dubios et al. (1956), and it specifically adopted for carbohydrate determination in sediments. The absorbance was measured at 490nm using Glucose as standard. Lipids (LIP) were extracted by direct elution with chloroform and methanol according to Bright and Dyer (1959). Absorbance was measured at 367nm. Tripalnitine solutions were used as standard. Protein, carbohydrates and lipid concentrations were converted to carbon equivalents by using the following conversion factors; 0.49, 0.40, and 0.75 µg of C µ/g respectively (Pusceddu et al. 2000). The sum of total protein, carbohydrate and lipid carbon equivalents was reported as BioPolymeric Carbon (BPC). The protein to carbohydrate ratio (PRT: CHO) and lipid to carbohydrate (LIP: CHO) ratio were also calculated.

2.2.2 Trace Metals

1g of the dried finely powered sediment sample was repeatedly digested using 1:5 mixture of conc. $HClO_4$ and conc.HNO_3, and then evaporated to dryness (Loring and Rantala, 1992). The dry residue was dissolved in 0.1 HNO_3 and made up to 25ml. Further analysis was conducted by Atomic Absorption Spectrophotometer (Perkin Elmer 3110). The accuracy of the metal analyses was checked using triplicate analysis of a certified reference material (BCSS-1, National Research Council of Canada). The triplicate analysis of BCSS-1 showed a good accuracy and recovery rate.

2.2.3 Rare Earth Elements

The following sample preparation procedure was adopted for the dissolution of samples.

Open acid digestion method by Parijat Roy et al. (2007) was adopted. A test portion (0.5g) of the sample was added to each PTFE Teflon beakers. Each of the samples was moistened with a few drops of ultra- pure water. 10ml of an acid mixture (7:3:1 HF-HNO_3-$HClO_4$) was added to each sample. Samples were swirled until completely get moist. The beakers were then covered with lids and kept overnight for digestion after adding 1ml of 5µg/ml Rhodium (Rh) solution which act as an internal standard and the beakers were heated on a hot plate at 200^{0}C for about 1 hour, the lids were removed and the contents were evaporated to incipient dryness until a crystalline paste was obtained. The remaining residues were then dissolved using 10 ml of 1:1 HNO_3 –milli-Q water and kept on a hot plate for 10 minutes with gentle heat (70^{0}C) to dissolve all suspended particles. Finally, the volume was made up to 250 ml and stored in polyethylene bottles. The samples were then analysed using the Perkin Elmer Sciex ELAN DRCII ICP-MS at National Geophysical Research Laboratory Hyderabad, India. MAG-1 is used as reference materials for REEs analysis. Triplicate analyses were performed and the average value was reported.

2.2.4 Antifouling Biocides

The analytical method for the antifouling biocide extraction was carried out by the below appended means:

For the organotin analysis the methods of Morabito et al. (1995), modified by Sangeeta et al. (2009) were followed. 2-5g wet/dry sediment is taken in a dry boiling tube. To this 15ml of 0.03% tropolone in methanol and 1ml conc.HCl were added. Tripropyltin chloride (TPrTCl) equivalent to 100 ng Sn were added as an internal standard. The samples were then vertexed for 1 hr to obtain organotins (OTs). It is then centrifuged at 2000 rpm for 10 mins and the supernatant (upper layer) were collected in a separating funnel. Repeated the vertexed for another 1hr with the same sample, centrifuged and collected in a previously used separating funnel. To this 20 ml dichloromethane and 100ml 5% NaCl were added and shaken for 15 min. The processes were repeated with another volume of dichloromethane and

NaCl for 15 min. Dichloromethane layer were collected after passing through anhydrous sodium sulphate (Na_2SO_4) to remove moisture. Immediately, 1ml iso-octane was added to dichloromethane layer to avoid evaporative loss. Then the volume was reduced to 0.2ml by passing N_2 or using rotary evaporator. Next 1ml pentyl magnesium bromide (Grignard reagent) were added carefully. Milli-Q water was added repeatedly until effervescence ceases. An extra volume of 1ml water was added, before the addition of Hexane (2ml) to this reaction mixture and repeated (2ml×2) twice. Hexane layer was pipetted out and passed it through the florisil or silica column (4g). 1:1 hexane-toluene mixture was used as the mobile phase. Sample was collected in vials and concentrated by using N_2 gas to 1ml. Finally injected into GC/MS.

For calibration, a standard mixture was prepared by adding 100µl of standard solution containing TBT (107ng Sn), DBT (103ng Sn), MBT (101 ng Sn), MPT (106 ng Sn), DPT (99.4ng Sn), TPT (102 ng Sn) and TPrT (105 ng Sn). Rest of the processes were similar to that has been done for sediment samples.

The GC-MS system (Shimadzu QO 2010) with electron impact ionization mode (70ev) was used for the analysis. The organotins were separated and detected using RESTEK Rtx-5MS capillary column (30m, 0.32mm i.d. 0.25 µm) and selected ion monitoring (SIM) mode. Two micro litres of sample or blank or standard mixture was injected using a programmable column injector. The injector was kept at 60^0C for 1 min and then temperature was raised at 100^0C min^{-1} to 240^0C, and maintained at the same temperature for the run time. Initial column temperature was 80^0C. After 2 min, the oven temperature was programmed to reach 280^0C at

10^0C min^{-1}. The interference temperature of the MS was maintained at 280^0C. Helium was used as the carrier gas (3 ml min^{-1}). The total run-time was 24 min. Data analyse was done by data processing software Shimadzu GC-MS solution Version 2.21.

Quantification of organotins was performed by using TPrT as internal standard. Fresh standards were prepared to obtain calibration curves along with procedural blanks. The minimum detection concentration for butyltins and phenyltins is 0.2 ng Sn/g.

For biocides like Irgarol 1051, chlorothalonil and dichlofluanid, the method of Voulvoulis et al. (1999) were adopted. 50 g of dry sediment sample were taken in 250 ml Teflon bottles and spiked with 1ml working standard solution. 60 ml of acetone and 50ml of dichloromethane (6:5 ratio) were added. The bottles were shaken on a rotary shaker overnight and centrifuged at 2000 rpm for 10 min. The supernatant was filtered through a Buchner funnel and collected in a round bottom flask. Anhydrous sodium sulphate (Na_2SO_4) was added and the solution was reduced by volume using rotary evaporator. Dried with a gentle stream of nitrogen and the extract was re-dissolved in 60 ml acetone and transferred into a separating funnel. 120 ml of 2% sodium sulphate were added and the compounds were subsequently extracted with portion of petroleum ether (3×30ml). The ether fractions were combined and again reduced to incipient dryness. 1ml of acetone was then added to re-dissolve the residue which was transferred to a vial for further analysis by GC-MS (Agilent 6890). Separation was achieved on a fused silica capillary column (DB5). The sample was injected splitless with the injector temperature maintained at 260^0C. The chromatographic temperature conditions were as follows; 70^0C held for 3 mins, increased at 30^0C /min to 120^0C held for 1min, then 15^0C/ min to 180^0C, held for 3mins and 25^0C/ min to 300^0C final temperature, held for 15 min. Helium was used as carrier gas with a flow rate of 0.5ml/ min. The mass spectrometer was in the selected ion monitoring (SIM) mode at 70eV ionization energy and scanned from 50 to 500 Dalton. The individual compounds were identified by comparison with authentic standards and interpreted by mass spectrometric fragmentation patterns.

For biocide Chlorothalonil, method of Voulvoulis et al. (1999) and Kazos et al. (2008) were adopted. 100 g of dried sediment sample were extracted with 60 ml of acetone and 50 ml of dichloromethane (6:5 ratio) for 17 hrs and centrifuged at 2000 rpm for 10 min. The supernatant was filtered through a Buchner funnel and collected in a round bottom flask. Sodium sulphate (Na_2SO_4) was added and the solution was reduced in volume by rotary evaporation. It was then taken down just to dryness with a gentle stream of dry nitrogen and then the extract was re-dissolved in 60ml of acetone and transferred to a separating funnel. 120 ml of 2% sodium sulphate were added and the compounds were subsequently extracted with portion a petroleum ether (3×30ml). The ether fractions were combined and again reduced to incipient dryness. 1ml of acetone was then added to redissolve the residue which was transferred to a vial for further HPLC analysis.

Liquid chromatograph (Perkin Elmer 200 series) with a UV spectrometric detector operated at 242 nm was used to analyse Chlorothalonil. Separation was achieved with a C8 column preceded by a guard column of similar packing at 30^0C. A gradient elution was carried out with acetonitrile (solvent A) and phosphate buffer solution 10nM, pH 5.0 (solvent B). The mobile phase composition was programmed as follows: initially 30% A, then a linear gradient to 70% A in 16 min, kept constant upto 18 min, then returning linearly to 30% A in 20 min and equilibrated for 10 min. The flow rate was at 0.6 ml/ min and the injection volume was 20 µl. The solvents were degassed by sonication. Data acquisition and data analysis were performed with the Total Chrom software package supplied by Perkin Elmer.

All solvents and florisil were purchased from Merck (Darmstadt, Germany). Gas chromatographic and HPLC standards for biocides were purchased from Sigma-Aldrich (USA).

2.2.5 Acetylcholine esterase Enzyme Assay

The method used was a combination of the modified method of Ellman et al. (1961) later by Dellali et al.(2001) and Bonacci et al. (2004).

Total soft tissues were homogenised at 4^0C in a 1:5 ratio (w/v) of 0.1M phosphate buffer optimised at 12000×g for a period of 30min, after which supernatant were obtained and immediately used for assay of AChE activity.

AChE activity was measured using acetylthiocholine iodide (ASChl) as substrate. ASChI is hydrolysed by AChE, producing thiocholine and acetic acid. The thiocholine released from the hydrolysis was made to react with 5,5’-di-thi-bis-2-nitrobenzioc acid (DTNB) yielding the yellow compound 5-thio-z-nitro-benzoic acid (TNB) which absorbs at 412nm. 50µl of supernatant was placed into a reaction mixture consisting of 850 µl phosphate buffer (0.1M, pH 7.5), 1.875 mM DTNB and 50µl of 2 mM ASChI to start the enzymatic reaction. The optical desity at 412 nm was measured using a spectrometer at regular intervals continuously for 30 minites. The reaction time was maintained at 25^0C throughout the period. AChE activity was expressed in nmol/min mg/ protein. The quantity of protein present in the supernatant was determined using the Breadford method (Breadford 1976) with bovine serum albumin as the standard. Absorbance for protein determination was measured at 595 nm.

2.2.6 Statistical Techniques

All data were subjected to statistical analysis wherever necessary. Pearson correlation was employed to find out the inter relationship between different parameters. Principal Component Analysis was done to find out the factors contributing to different biogeochemical processes occurring in estuarine system. Cluster analysis was done to identify the relationship between sampling stations.

A) Enrichment Factor (EF)

EF is a good tool to determine the source of metal between anthropogenic or naturally occurring sources (Morillo et al. 2002; Adamo et al. 2005; Valdes et al. 2005). EF was calculated to determine whether the levels of metals in sediments were of anthropogenic origin (i.e. Contamination) or not. The geochemical normalisation was performed using Fe as the reference element for the following reasons (1) Fe is associated with fine solid surfaces; (2) its geochemistry is similar to that of many trace metals; and (3) its natural sediment concentration tends to be uniform (Daskalakis and O’connor 1995).

The following equitation was used to estimate the EF of metals from each sediment station using Fe as a normaliser to correct the differences in sediment grain size and mineralogy:

$$EF= \quad (M_e/ Fe)_{\text{sample}} / (M_e/Fe)_{\text{average shale value}}$$

Where (M_e/ Fe) sample and (M_e/Fe) average shale value are respectively, the metal concentration (mg/kg dw) in relation to Fe levels (% dw) in sediment samples and average shale values taken from Turekian and Wedepohl (1961)respectively. EF values were interpreted as suggested by Brich (2003) where EF<1 indicates no enrichment; <3 is minor; 3-5 is moderate; 5-10 is moderately severe; 10-25 is severe; 25-50 is very severe; and >50 is extremely severe enrichment.

B) Contamination Factor (CF)

Various calculation methods for quantifying the degree of metal enrichment in sediments have been put forward. One of them is the Contamination Factor (CF) (Perkey et al. 2004; Muthu and Jayaprakash 2007). The CF, calculated as the ratio between the sediment metal content at a given station and the normal concentration level reflects the metal enrichment in the sediment:

$$CF = C_S / C_{\mathbf{b}}$$

CF was classified into four groups in Hokanson (1980) and Perkey et al. (2004). When CF<1, there is no metal enrichment by natural or anthropogenic inputs; $1 > CF < 3$ for a particular metal means that the sediment is moderately contaminated by the element; $3 > CF < 6$ means that there is considerable contamination; and if $CF > 6$, then there is very high contamination for that metal. Taylor’s (1972) crustal average contamination of the trace metals for the background concentrations were used in this study.

C) Geoaccumulation Index (I_{geo})

Geoaccumulation index (I_{geo}) was introduced by Muller (1969) and allows the determination of the sediment analysis with organic and inorganic pollutants comparing the present concentration with background levels. Concentrations of geochemical background are multiplied each time by a factor of 1.5 in order to allow content fluctuations of a given substance in the environment and very small anthropogenic influences. Values of geoaccumulation index can be defined as follows;

$$I_{geo} = \log2 \left[(C_n / (1.5 \times Bn)\right]$$

Where 'C_n' is the measured concentration of the heavy metal (n) in the examined bottom sediment and 'Bn' is the geochemical background value in the average shale (Turekian and Wedepohl 1961) of element 'n'; 1.5 is the background matrix correction factor due to lithogenic effects. The index of geoaccumulation includes even grades from 0 (non-contaminate) to 6 (very strong).

D) Cluster Analysis (CA)

Cluster analysis (CA) is a multivariate technique, whose primary purpose is to classify the object of the system into categories or clusters based on their similarities. The objective is to find an optimal grouping for which the observations or objects within each cluster are similar, but the clusters are dissimilar to each other. Hierarchical clustering is the most common approach in which clusters are formed sequentially. Hierarchical clustering provides intuitive similarity relationships between any one sample and the entire data set, and is typically illustrated by a dendrogram/tree diagram (Singh et al. 2004; Shrestha and Kazama 2007). The most similar objects are grouped first, and these initial groups are merged according to their similarities. Eventually as the similarity decreases all subgroups are fused into a single cluster. The dendrogram provides a visual summary of the clustering processes, presenting a picture of the groups and their proximity, with a dramatic reduction in dimension of the original data. The Euclidean distance usually gives the similarity between two samples, and a distance can be represented by the difference between analytical values from the samples. CA was applied using a single linkage method, the distances or similarities between two clusters A and B is defined as the minimum distance between a point in A and a point in B.

$$D(A,B) = \min \{ d(y_i, y_j), \text{ for } y_i \text{ in A and } y_j \text{ in B} \}$$

Where $d(y_i, y_j)$ is the Euclidean distance in Equation

E) Factor Analysis (FA)

Factor analysis (FA) is designed to transform the original variables into new uncorrelated variables called factors, which are linear combinations of the original variables. The FA is a data reduction technique and suggests how many variances are present in the data. Principal component method (PCA) is used for extraction of different factors. The axis defined by PCA is rotated to reduce the contribution of less significant variables (Richard and Dean 2002; Alvin 2002). This treatment provides a small but approximately the same amount of information as the original set of observations. The FA can be expressed as:

$$F_i = a_1x_{1j} + a_2x_{2j} + \ldots\ldots.. + a_mx_{mj}$$

Where' F_i'is the factor, 'a' is the loading, 'x' is the measured value of variable, 'I' is the factor number, 'j' is the sample number and 'm' the total number of variables.

Factor scores can be expressed as:

$$Z_{ij} = a_1f_{1j} + a_2f_{2j} + \ldots\ldots\ldots\ldots.. + a_mf_{mj} + e_{ij}$$

Where 'z' is the measured variable, 'a' is the factor loading, 'f' is the factor score, 'e' the residual term accounting for errors or other sources of variation.

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Chapter 3

General Chemical Characteristics of the Sediment

3.1 Introduction

The characteristics of organic matter deposited in surface sediments of marine environment are being widely used in the correlation of several oceanographic processes, such as surface productivity, input of land- derived materials into the ocean, dynamics of water masses, redox potential and sedimentation rates (Jacob et al. 2008). The preservation of organic matter is greater owing to the low availability of oxygen in low-energy depositional environments or rapid burial of organic matter by mineral particles in the regions where sedimentation rate is high.

The nature of the organic materials deposited in sediments will depend on the types of contributing agents to the sediments. For example, lignins from higher plants are the main sources of aromatic compounds in contemporing sediments, while plankton and bacteria contribute primary aliphatic materials. Inputs of sedimentary organic matter are classified as autochthonous as they originate at or close to the site of deposition, or allochthonous if transported from another environment. Autochthonous inputs to most aquatic environments include the remains of phytoplankton and of organisms that feed directly or indirectly on phytoplankton, and the remaining of phytoplankton deposited in the upper layers of sediment. Allochthonous organic material mostly derives from higher plants, usually transported by water from adjacent areas of land to the deposition site.

Estuaries are among the most productive systems of the world, and therefore autochthonous biological production is a major source of organic materials to sediments. However, estuarine sediments are also preferential sites for the accumulation of particles of marine and terrestrial origin (Cifuentes 1991). In addition, in polluted estuaries, waste inputs may strongly influence the amount and quality of materials arriving at sediments. Therefore, the abundance and composition of organic materials in estuarine sediments depends on a complex array of factors, which involves the sources and the physico-chemical processes occurring in overlying water layers and in the sediment.

Animal-sediment relationships are fundamental for the studies on the distribution, development and maintenance of benthic communities. These relationships are complex and often they are not clear whether seabed sediment or benthic systems controls and define the characteristics of the other. The grain size of sediments determines the degree to the number of species within a community; therefore, it functions as an environmental influencing factor on biological diversity (Etter and Gressle 1992). This relationship encounters the infannual distribution, which affects not only deposit but also suspension (Snelgrove and Butman 1994). Contrastingly, inhabiting biota influences sediment composition and structure (Reise 2002). The uptake of organic matter strongly depends on the species composition of the benthic community (Josefson et al. 2002).

To determine the origin of the organic matter and the importance of the processes occurring in the transformation of organic materials in sediments, several methodological approaches have been employed. The biochemical composition of sediments (i.e. proteins, lipids, and carbohydrates) seems not only a valid approach to determine the origin of particles and the factors controlling their diagenesis (Colombo et al. 1996); but also a useful tool to properly value the quality of organic materials as available food for benthic consumers (Fabiano and Danovaro 1994; Mayer et al. 1995: Danovaro and Fabiano 1995; Gremare et al. 1997; Rossi and Lardicci 2002; Cividanes et al. 2002). In addition, the biochemical composition of sediments is proposed for assessing the tropic status of coastal marine systems (Dell’Anno et al. 2002; Pusceddu et al. 2003).

Having established the major contributors to sedimentary organic matter, it is vital to consider their chemical composition in order to understand the processes that lead to the fossil forms of carbon preserved in ancient sediment. All organisms are composed of the same basic chemical classes, of which the geochemically important are; carbohydrates, proteins and lipids. Lipids are believed to play a dominant role in petroleum formation and so organisms relatively rich in lipids, such as the planktons are important contributors to oil source rocks.

The term carbohydrate (CHO) derives from the facts that many members of this group of compounds have the general formula $C_n (H_2O)_n$ i.e., contain only carbon, hydrogen and oxygen. They are polyhydroxy-substituted carbonyl compounds. Carbohydrates comprise the most abundant group of organic compounds found in the biosphere, and they are present in the marine environment as structural and storage components of marine organisms and terrestrial remains (Cowie and Hedges 1984; Parsons et.al. 1984; Biersmith and Benner 1998). Carbohydrates represent a significant fraction of particulate and dissolved organic material in the water column and sediments (Burdige et al. 2000; D'souze and Bhosle 2001).

Proteins (PRT) account for most of the nitrogen present in organism. They are polymers of α-amino acids. Where, amino acid and carboxylic acid group are attached to the same carbon atom. In the general structure, the α-carbon atom can be seen to be chiral for all amino acids but not glucine another amino acid. This reflects the stereo specificity of enzymes involved in the formation and utilisation of these compounds. Proteins are made from the same 20 different amino acids. In plants, amino acids are generally synthesised from glutamic acid by transfer of the amino group to other carbon skeletons (transamination). Animals cannot synthesise all the amino acids needed for protein formation and thereby obtain the essential amino acids directly or indirectly from plants. Proteins can be a sizable fraction of the bulk organic material in an organism.

Lipids (LIP) can be defined as all the substance produced by organisms that are effectively insoluble in water but extractable by solvents that dissolve. This broad definition is suitable for our purposes and encompasses a wide variety of compound classes including photosynthetic pigment. However, applications of the term lipid can vary, sometime being restricted to fats, waxes, steroids and phospholipids and sometimes to fats alone. Simple organic compounds like aliphatic carboxylic acids and alcohols can be found among the lipids, but most lipids exists as combination of these simple molecules with one another or with other compound classes such as carbohydrates and proteins. The phospholipids, glycolipids and other lipids are all important constituents of membrane that isolate the contents of cells from the surrounding environment in various organisms. These cell membranes chiefly comprise lipids and proteins. Phospholipids are the main type of membrane lipid. A brief reports on the biochemical composition of earlier works are summarised in table 3.1.

3.2 Results

Geochemical parameters of sediments collected from 14 stations of the study area were analysed in the present study. The main biogeochemical parameters estimated were proteins, total carbohydrates, lipids, chlorophyll-a and pheo pigments. The total carbon, total nitrogen, total phosphrous and total sulphur were also determined (Table 3.2 to 3.5). The mineralogy of sediments collected from the study area was also determined. The ratios C/N, PRT/CHO, LIP/CHO, protein carbon, total carbohydrate carbon, lipid carbon and biopolymeric carbon (BPC) were also calculated.

Table 3.1 Previous studies on biochemical components in various sedimentary environments

Area	Lipids (μg/g)	Proteins (μg/g)	Carbohydrates(μg/g)	References
St. Lawrence Estuary	820-1470	110-400	7580-10700	Colombo et al. 1996
Galician Coast sediments, Spain	50-1483	400-4016	32-675	Cividances et al. 2002
Mundaka Estuary	300-5000	0.00-16700	200-5700	Cotano and Villate 2006
Western Continental shelf, India	-	90-1020	1080-9880	Jacob et al. 2008
Eastern Continental shelf, India	-	170-550	1280-4430	Jacob et al. 2008
Mangroves, Cochin estuary, India	-	200-1240	1550-7860	Geetha et al. 2008
Cochin estuary, India	312-2815	205-1924	250-1229	Ratheesh kumar 2011
South Pacific	7200	5750	5800	Neira et al. 2001
Marsala Lagoon	300-4500	2200-12100	800-70500	Pusceddu et al. 1999
Tyrrhenian Sea	010-20	300-1700	300-1900	Fabiano and Danovaro 1995
Ligurian Sea	90-630	20-300	300-5300	Fabiano and Danovaro 1995
Ionian and Aegean Seas	50-190	70-160	1200-2500	Danovaro et al. 1993

Table 3.2 Geochemical parameters estimated in the sediments of the study area during 2007 (pre-monsoon)

Stations	Sand%	Silt%	Clay%	OC(%)	TN(%)	TC(%)	TS(%)	IC(%)	TP (mg/kg)	TKN (%)
ST-1	85.47	2.13	12.40	0.55	0.21	1.64	0.34	1.09	665.00	0.20
ST-2	5.05	50.83	44.11	2.59	0.25	2.85	1.33	0.27	960.00	0.23
ST-3	5.16	72.13	22.72	2.30	0.25	2.55	1.13	0.25	1477.50	0.27
ST-4	10.30	40.38	49.32	2.16	0.22	2.69	0.51	0.53	815.00	0.09
ST-5	12.68	42.09	45.23	2.21	0.31	1.13	0.53	-1.08	1080.00	0.07
ST-6	6.02	46.25	47.73	2.48	0.27	2.79	1.19	0.31	1151.25	0.11
ST-7	2.26	48.69	49.04	2.51	0.26	2.58	1.18	0.08	823.75	0.26
ST-8	15.04	34.52	50.44	2.32	0.27	2.85	1.13	0.53	1135.00	0.30
ST-9	48.92	32.55	18.53	1.80	0.16	2.07	0.53	0.27	901.25	0.12
ST-10	95.98	1.00	3.02	0.47	0.00	0.64	0.44	0.16	495.00	0.03
ST-11	80.35	10.79	8.86	0.12	0.07	0.61	0.00	0.49	0.07	8.48
ST-12	69.06	13.41	17.53	0.98	0.16	0.98	0.33	0.00	330.00	0.07
ST-13	26.17	30.97	42.86	2.00	0.24	2.38	0.48	0.38	1375.00	0.18
ST-14	65.27	18.66	16.07	0.98	0.12	1.26	0.36	0.28	506.25	0.10

Table 3.3 Geochemical parameters estimated in sediments of the study area during 2008 (pre-monsoon)

Stations	Sand%	Silt%	Clay%	OC(%)	TN(%)	TC(%)	TS(%)	IC(%)	TP(mg/kg)	TKN (%)
ST-1	93.90	2.74	3.35	0.17	0.21	0.85	1.15	0.68	1033.75	0.01
ST-2	18.29	47.17	34.54	1.67	0.24	2.30	1.15	0.64	1073.75	0.19
ST-3	2.92	36.14	60.94	1.99	0.21	2.01	0.75	0.02	1261.25	0.28
ST-4	52.64	35.47	11.89	0.92	0.17	2.74	0.65	1.82	238.75	0.13
ST-5	72.70	10.64	16.66	1.13	0.25	2.39	0.78	1.26	850	0.26
ST-6	6.91	37.74	55.35	1.70	0.21	2.64	1.17	0.94	1073.75	0.23
ST-7	0.34	32.43	67.23	1.89	0.33	2.99	1.29	1.11	922.5	0.26
ST-8	2.25	93.12	4.63	1.61	0.31	2.92	1.17	1.30	186.25	0.19
ST-9	59.18	22.63	18.18	1.64	0.23	2.47	0.64	0.83	242.5	0.21
ST-10	78.77	9.13	12.10	1.02	0.11	1.07	0.00	0.05	227.5	1.15
ST-11	74.04	13.40	12.56	0.72	0.13	1.36	0.00	0.64	545	0.07
ST-12	90.33	3.42	6.25	0.49	0.05	0.62	0.00	0.14	121.25	0.05
ST-13	9.31	85.40	5.29	0.26	0.21	0.36	0.58	0.10	570	0.04
ST-14	67.65	16.34	16.01	0.84	0.09	1.15	0.31	0.31	161.25	0.09

Table 3.4 Biochemical composition of sediments in the study area during 2007 (pre-monsoon)

Stations	Chlorophyl a (μg/g)	Pheophytin (μg/g)	Proteins (mg/g)	Carbohydrates (mg/g)	Lipids (mg/g)	C/N	PRT/ CHO	LPD/ CHO	PRT-C	CHO-C	LPD-C	BPC (mg/g)
ST-1	5.79	12.97	2.29	3.43	0.19	0.00	0.67	0.06	1.12	1.37	0.14	2.64
ST-2	7.35	17.00	8.07	11.87	0.98	11.53	0.68	0.08	3.96	4.75	0.74	9.44
ST-3	17.06	38.52	6.20	8.35	0.79	9.99	0.74	0.09	3.04	3.34	0.59	6.97
ST-4	9.43	23.26	8.17	8.49	0.72	12.29	0.96	0.08	4.00	3.40	0.54	7.94
ST-5	15.04	36.45	3.79	6.29	0.93	0.00	0.60	0.15	1.86	2.51	0.70	5.07
ST-6	18.62	46.65	5.73	6.57	1.00	10.43	0.87	0.15	2.81	2.63	0.75	6.19
ST-7	7.24	17.01	5.91	6.54	0.94	9.94	0.90	0.14	2.90	2.62	0.70	6.22
ST-8	5.74	14.20	6.56	7.93	0.04	10.49	0.83	0.00	3.21	3.17	0.03	6.41
ST-9	5.91	7.59	9.62	2.33	1.39	13.08	4.13	0.60	4.71	0.93	1.04	6.69
ST-10	5.75	11.27	3.12	3.85	0.18	0.00	0.81	0.05	1.53	1.54	0.14	3.21
ST-11	8.48	20.73	2.00	3.66	0.24	0.00	0.55	0.06	0.98	1.46	0.18	2.62
ST-12	5.87	14.75	4.30	7.10	0.14	0.00	0.61	0.02	2.11	2.84	0.10	5.05
ST-13	18.18	43.97	8.16	4.70	1.42	9.84	1.74	0.30	4.00	1.88	1.07	6.94
ST-14	9.83	24.11	4.46	7.25	0.57	10.60	0.61	0.08	2.18	2.90	0.42	5.51

Table 3.5 Biochemical composition of sediments in the study area during 2008 (pre-monsoon)

Stations	Chlorophyl a (μg/g)	Pheophytin (μg/g)	Proteins (mg/g)	Carbohydrates (mg/g)	Lipids (mg/g)	C/N	PRT/ CHO	LPD/ CHO	PRT-C	CHO-C	LPD-C	BPC
ST-1	0.05	2.39	0.51	0.87	0.05	0.00	0.58	0.06	0.25	0.35	0.04	0.63
ST-2	7.15	17.33	7.01	6.05	1.49	9.69	1.16	0.25	3.44	2.42	1.12	6.98
ST-3	9.09	12.15	6.71	7.66	1.87	9.58	0.88	0.24	3.29	3.07	1.40	7.76
ST-4	4.62	13.42	2.28	7.60	0.64	16.53	0.30	0.08	1.12	3.04	0.48	4.64
ST-5	5.00	15.01	4.18	6.97	0.88	9.72	0.60	0.13	2.05	2.79	0.66	5.49
ST-6	10.78	26.15	3.96	8.14	1.21	12.76	0.49	0.15	1.94	3.26	0.90	6.10
ST-7	11.26	26.44	6.31	9.02	1.62	9.16	0.70	0.18	3.09	3.61	1.22	7.92
ST-8	1.52	10.65	6.05	7.91	1.99	9.49	0.77	0.25	2.97	3.16	1.49	7.62
ST-9	10.41	26.91	9.67	6.58	0.51	10.89	1.47	0.08	4.74	2.63	0.39	7.75
ST-10	3.35	8.79	3.72	5.81	0.32	9.57	0.64	0.05	1.83	2.32	0.24	4.39
ST-11	7.31	18.54	1.75	3.14	0.40	10.39	0.56	0.13	0.86	1.26	0.30	2.42
ST-12	9.23	22.90	3.31	3.23	1.23	0.00	1.03	0.38	1.62	1.29	0.92	3.84
ST-13	0.05	2.14	1.34	1.86	0.20	0.00	0.72	0.11	0.66	0.74	0.15	1.56
ST-14	7.81	19.25	3.02	4.94	0.54	0.00	0.61	0.11	1.48	1.97	0.41	3.86

The minerals like Tochilinite, spinel and quartz were present in ST-1 during both sampling years, 2007 and 2008. Mg, Al and Fe were present as major elements in these minerals. In ST-2 Preisingerite was seen as major mineral which contain bismuth as the major element. In the case of ST-3, ST-4, ST-5, ST-6 & ST-7, quartz was found to be the contributing mineral, which is silicon dioxide. ST-8 revealed the presence of Parkinsonite and Quartz as major minerals. These minerals contain Pb, Mo as trace metals. Spangolite which is the source of Cu and Al was present in ST-9. Quartz was also present as a prominent mineral. In ST-10, Cd containing Apatite was found as major mineral. Apatite contains calcium phosphate as the major constituent. Sinoite and Quartz were the major minerals constituent in ST-11. Sinoite is a natural silicon oxynitride which is mainly formed by condensation at high temperature and pressure from a gas of solar composition. ST-12, near the Thaneermukkam Bund contains Uraninite and Fedotovite as major minerals. The Uraninite is very well known ore of Uranium, which is mainly used in atomic energy plants. Fedotovite mainly consists of copper potassium sulphate. ST-13 contains quartz as major mineral. In the case of ST-14 Watanabeite and Quartz were present as major minerals. The Watanabeite is a mixture of Cu, As and Sb.

Same mineralogical pattern was found during 2008 (pre-monsoon) and results the idea that these minerals are profoundly derived from terrestrially. Therefore 2007 XRD data were presented in figures 3.1 to 3.14

Textural character of sediment is very important parameter which determines the adsorption capacity of sediments. High percentage of sand were found at stations ST-1, ST-9, ST-10, ST-11, ST-12 & ST-14 in 2007 and ST-1, ST-5, ST-9, ST-10, ST-11, ST-12 & ST-14 in 2008. Mainly these stations are influenced by river water flush out and high water currents. Mud (silt + clay) fraction is an important parameter. It showed high values in ST-2, ST-3, ST-4, ST-5, ST-6, ST-7, ST-8 & ST-14 in 2007 and ST-2, ST-3, ST-4, ST-6, ST-7, ST-8 & ST-13 in 2008.

The total organic carbon content during 2007 (pre-monsoon) was widely distributed. It ranged from 0.12% to 2.59% during 2007 (pre-monsoon) and 0.26%-1.99% during 2008 pre-monsoon. High organic carbon content was reported in stations ST-2, ST-3, ST-4, ST-5, ST-6, ST-7, ST-8, ST-9 & ST-13 during 2007, where as in 2008, the high values were found at stations ST-2, ST-3, ST-5, ST-6, ST-7, ST-8 & ST-9. In both the years similar pattern was observed in the case of organic carbon.

The Total Carbon values ranged from 0.64% to 2.85% during 2007 and from 0.36% to 2.99% during 2008. High values were observed at stations ST-1, ST-2, ST-3, ST-4, ST-6, ST-7, ST-8, ST-9 & ST-13 in 2007 and ST-2, ST-3, ST-4, ST-5, ST-6, ST-7, ST-8 & ST-9 in 2008. Similar trend was observed in both years. The inorganic carbon content ranged from 0.00% to 1.09% in 2007 and 0.02% to 1.82% in 2008. Higher concentrations were observed at stations ST-1 & ST-5 in 2007 and ST-4, ST-5, ST-7 & ST-8 in 2008. All other stations showed similar pattern during both the years.

Figures 3.1 to 3.14 XRD Spectrum of all sampling stations

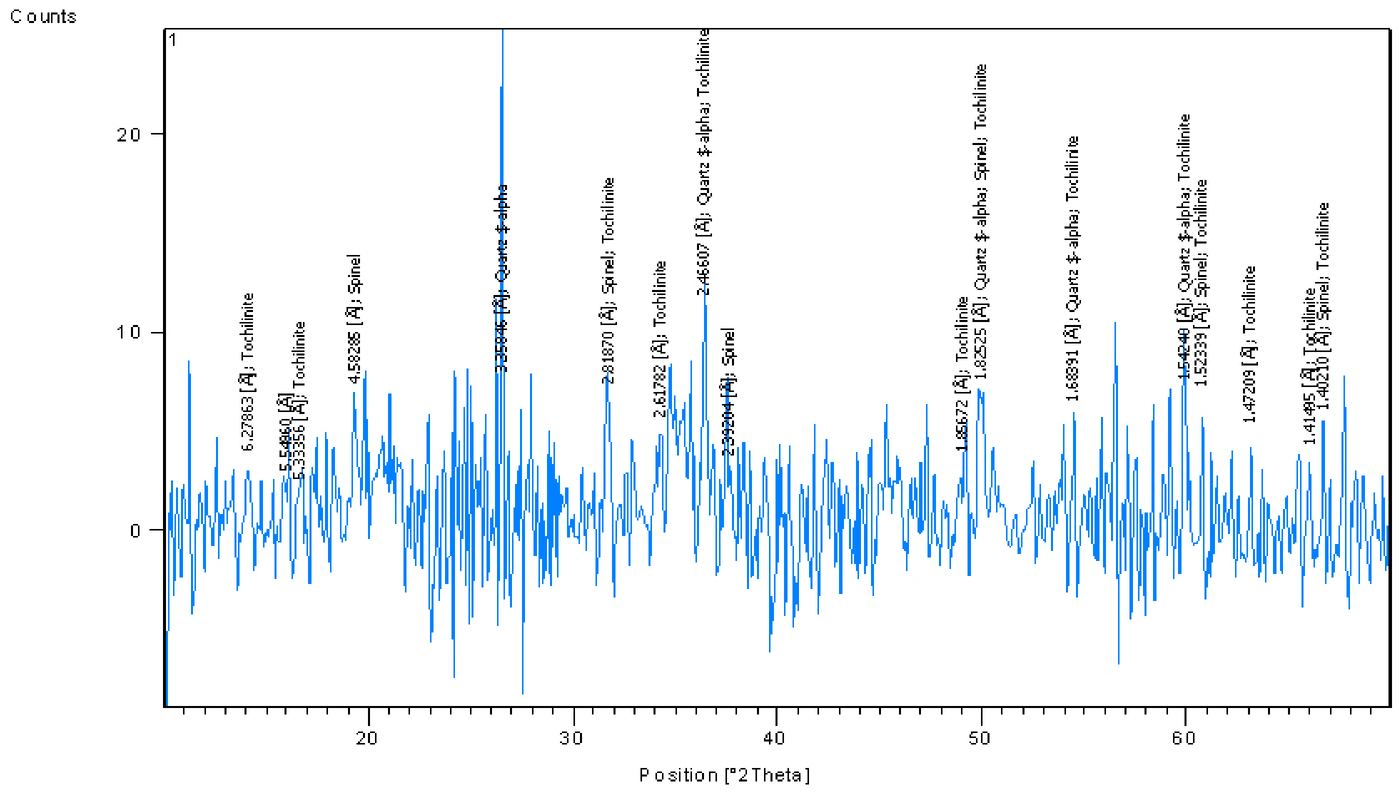


Figure 3.1

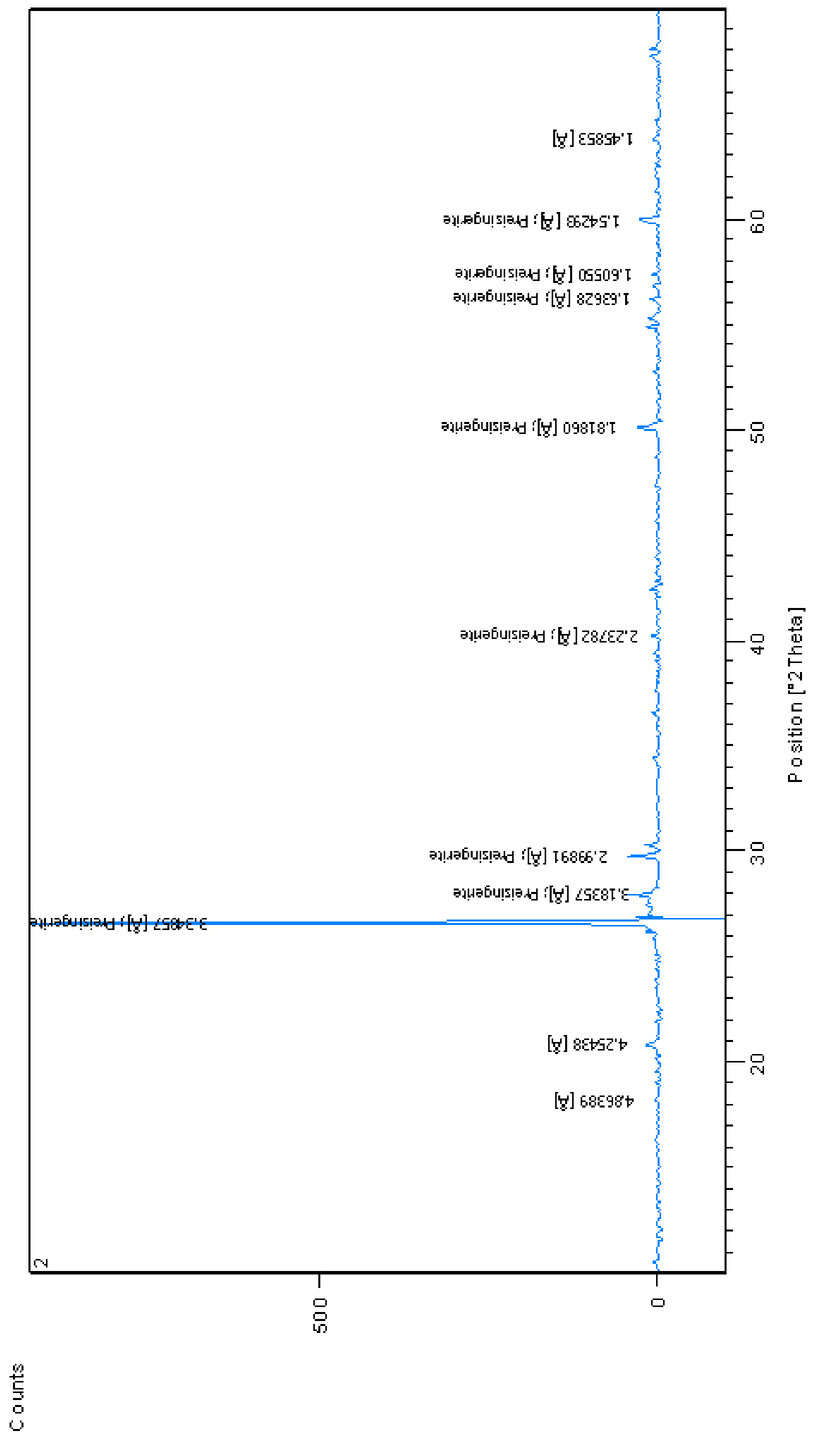

Counts
500
0
2
Position [°2Theta]
20
30
40
50
60
4,86389 [Å]
4,25438 [Å]
3,34857 [Å]; Preisingerite
3,18357 [Å]; Preisingerite
2,99891 [Å]; Preisingerite
2,23782 [Å]; Preisingerite
1,81860 [Å]; Preisingerite
1,63628 [Å]; Preisingerite
1,60550 [Å]; Preisingerite
1,54298 [Å]; Preisingerite
1,45853 [Å]

Figure 3.2

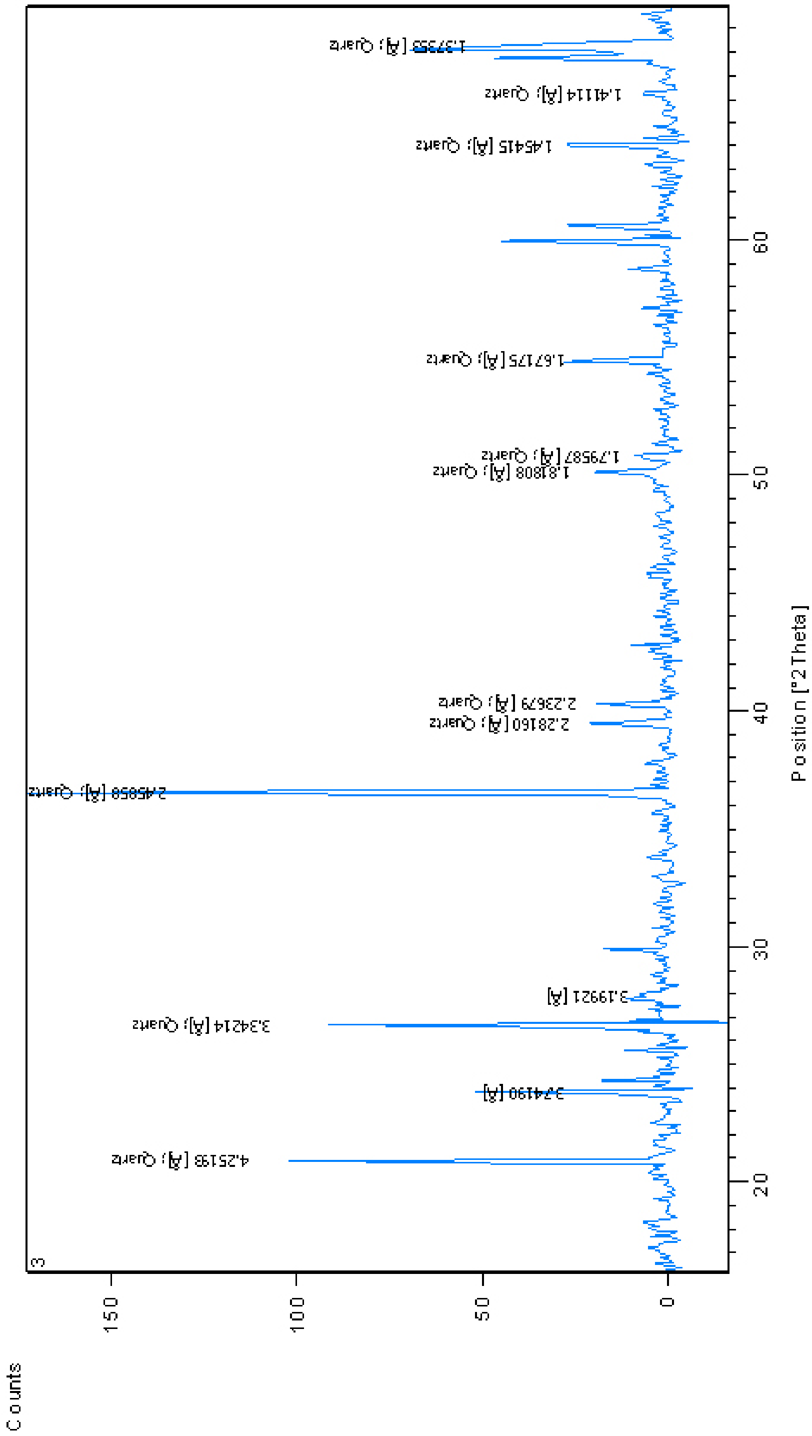

Counts
150
100
50
0
3
4.25198 [Å]; Quartz
3.74190 [Å]
3.34214 [Å]; Quartz
3.19921 [Å]
2.45858 [Å]; Quartz
2.28160 [Å]; Quartz
2.23679 [Å]; Quartz
1.81808 [Å]; Quartz
1.79587 [Å]; Quartz
1.67175 [Å]; Quartz
1.45415 [Å]; Quartz
1.41114 [Å]; Quartz
1.37355 [Å]; Quartz
20
30
40
50
60
Position [°2Theta]

Figure 3.3

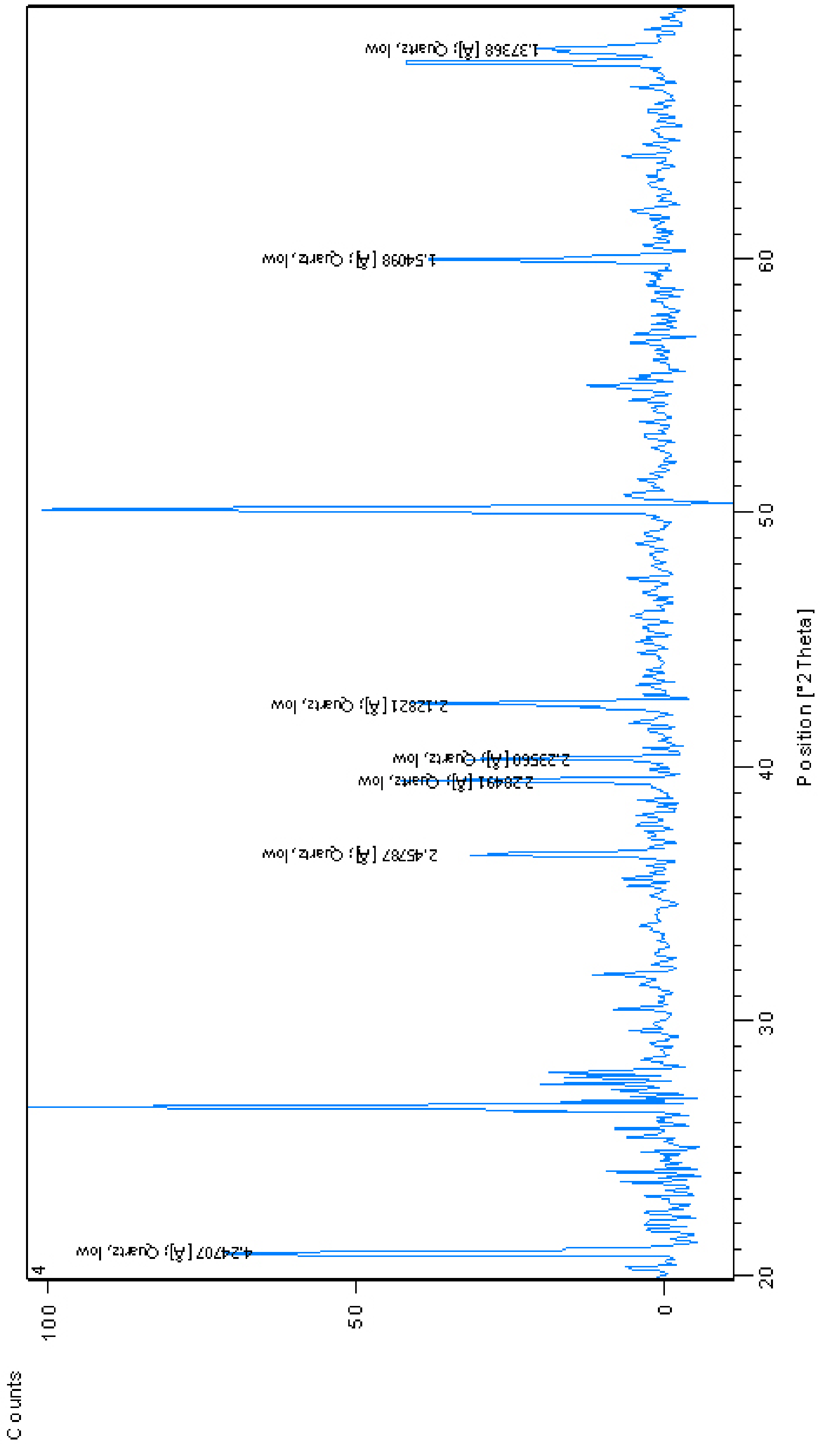

Counts
100
50
0
4
4.24707 [Å]; Quartz, low
2.45787 [Å]; Quartz, low
2.28491 [Å]; Quartz, low
2.23560 [Å]; Quartz, low
2.12821 [Å]; Quartz, low
1.54098 [Å]; Quartz, low
1.37368 [Å]; Quartz, low
20
30
40
50
60
Position [°2Theta]

Figure 3.4

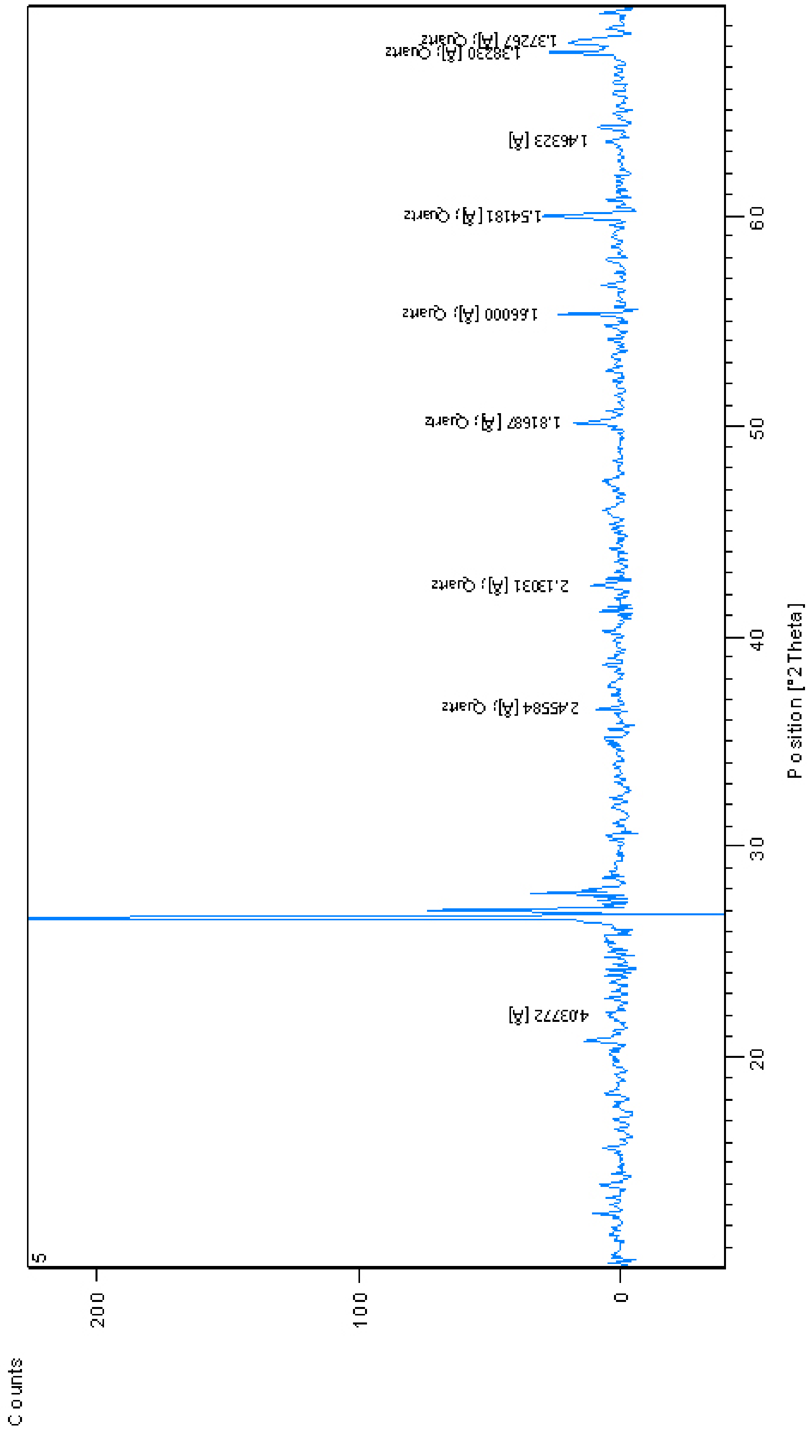
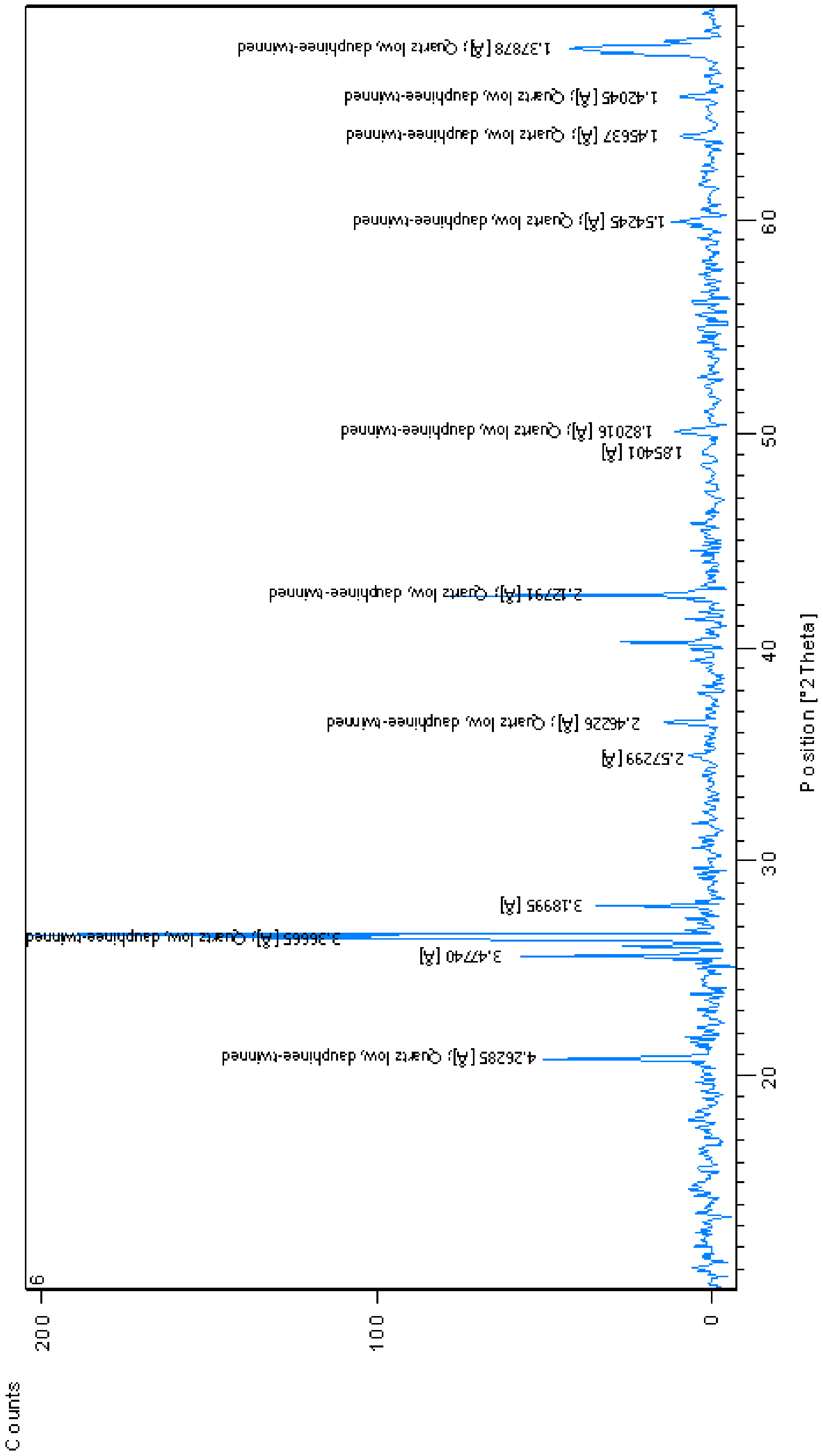

Counts
0
100
200
5
4.03772 [Å]
2.45584 [Å]; Quartz
2.13031 [Å]; Quartz
1.81687 [Å]; Quartz
1.66000 [Å]; Quartz
1.54181 [Å]; Quartz
1.46323 [Å]
1.38230 [Å]; Quartz
1.37267 [Å]; Quartz
20
30
40
50
60
Position [°2Theta]

Figure 3.5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2.57299 [Å]
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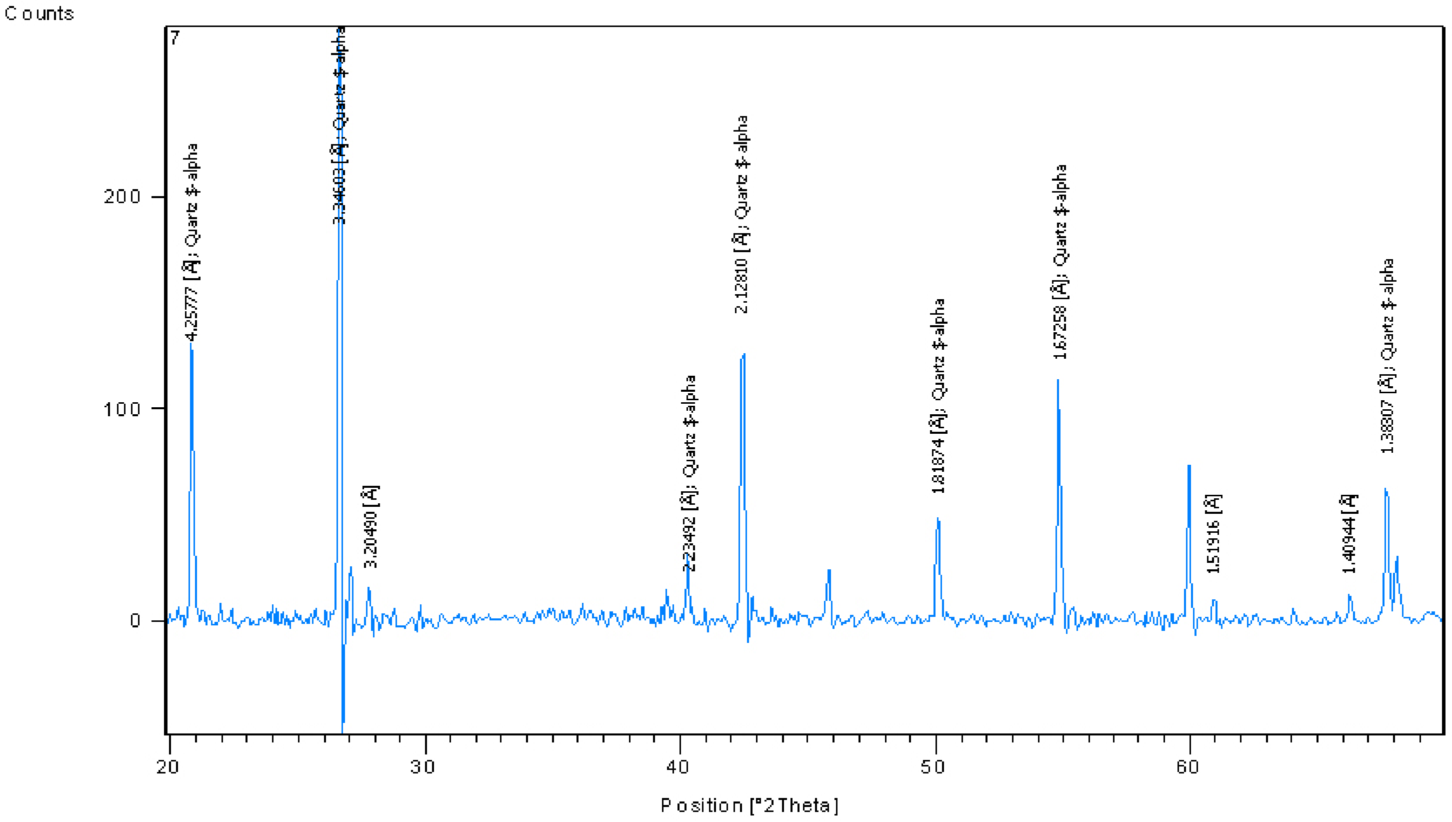
1.37878 [Å]; Quartz low, dauphinee-twinned

Figure 3.6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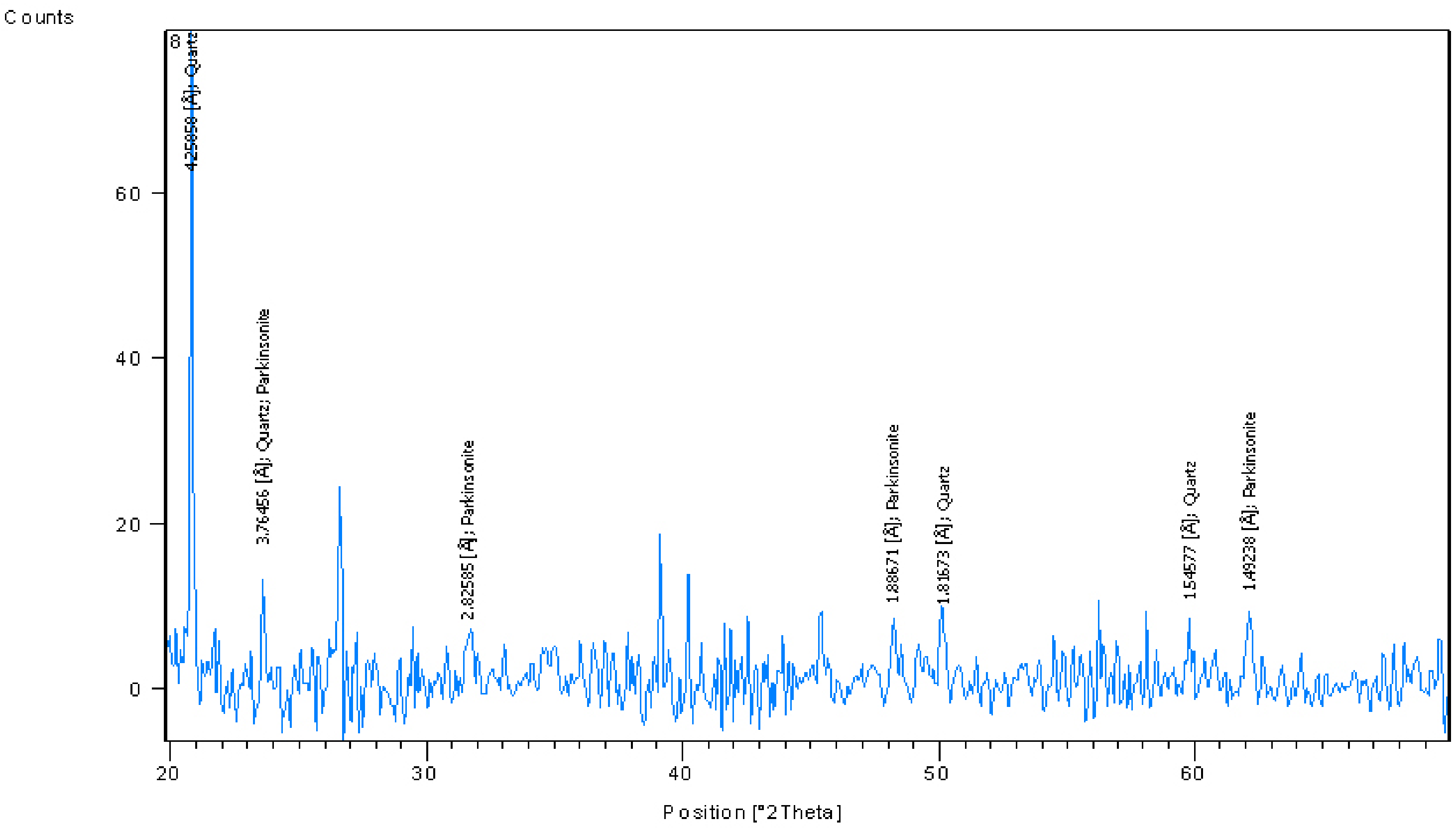
60
Position [°2Theta]

Figure 3.7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1.88671 [Å]; Parkinsonite
1.81673 [Å]; Quartz
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1.49238 [Å]; Parkinsonite
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30
40
50
60
Position [°2Theta]

Figure 3.8

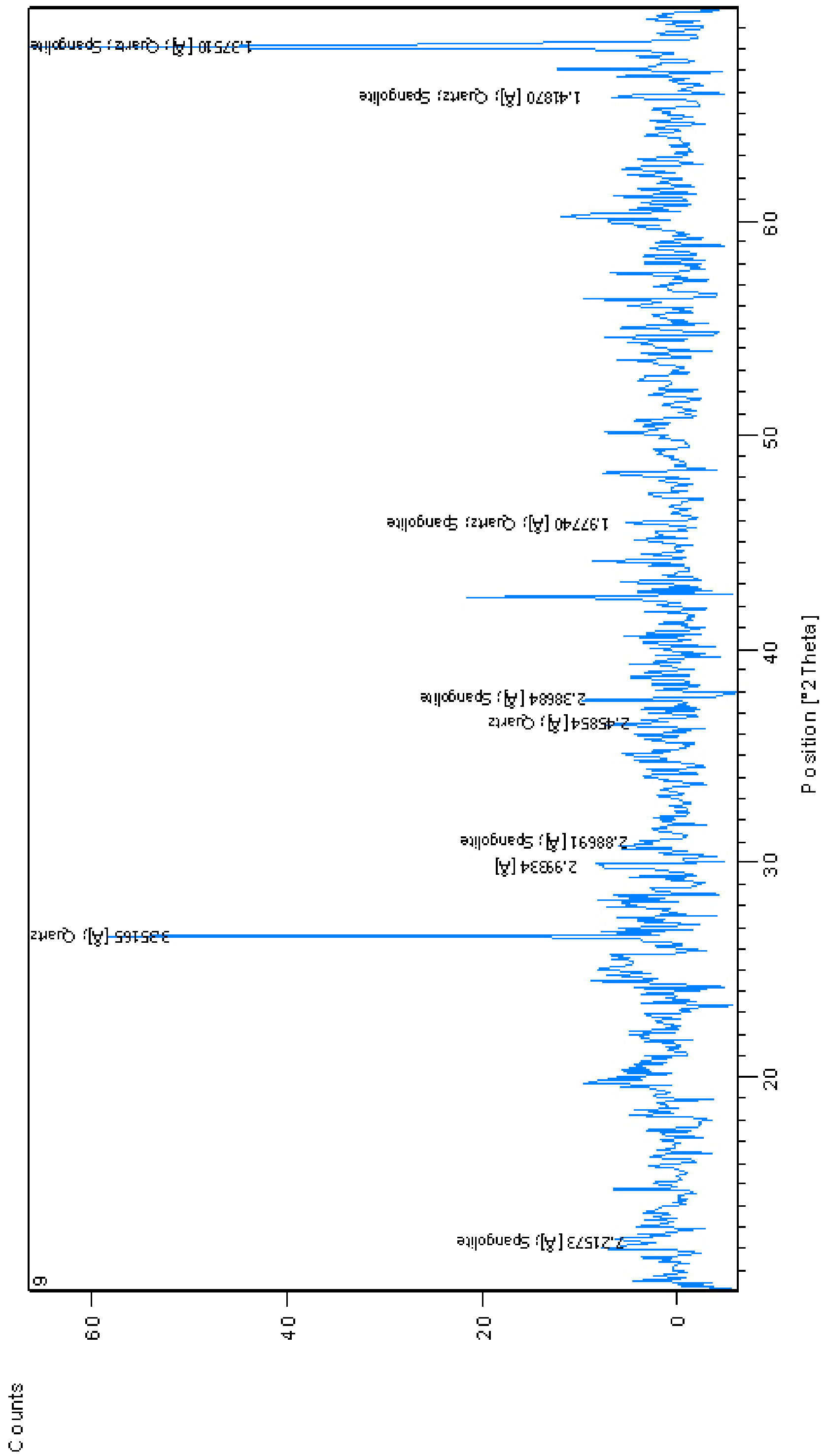
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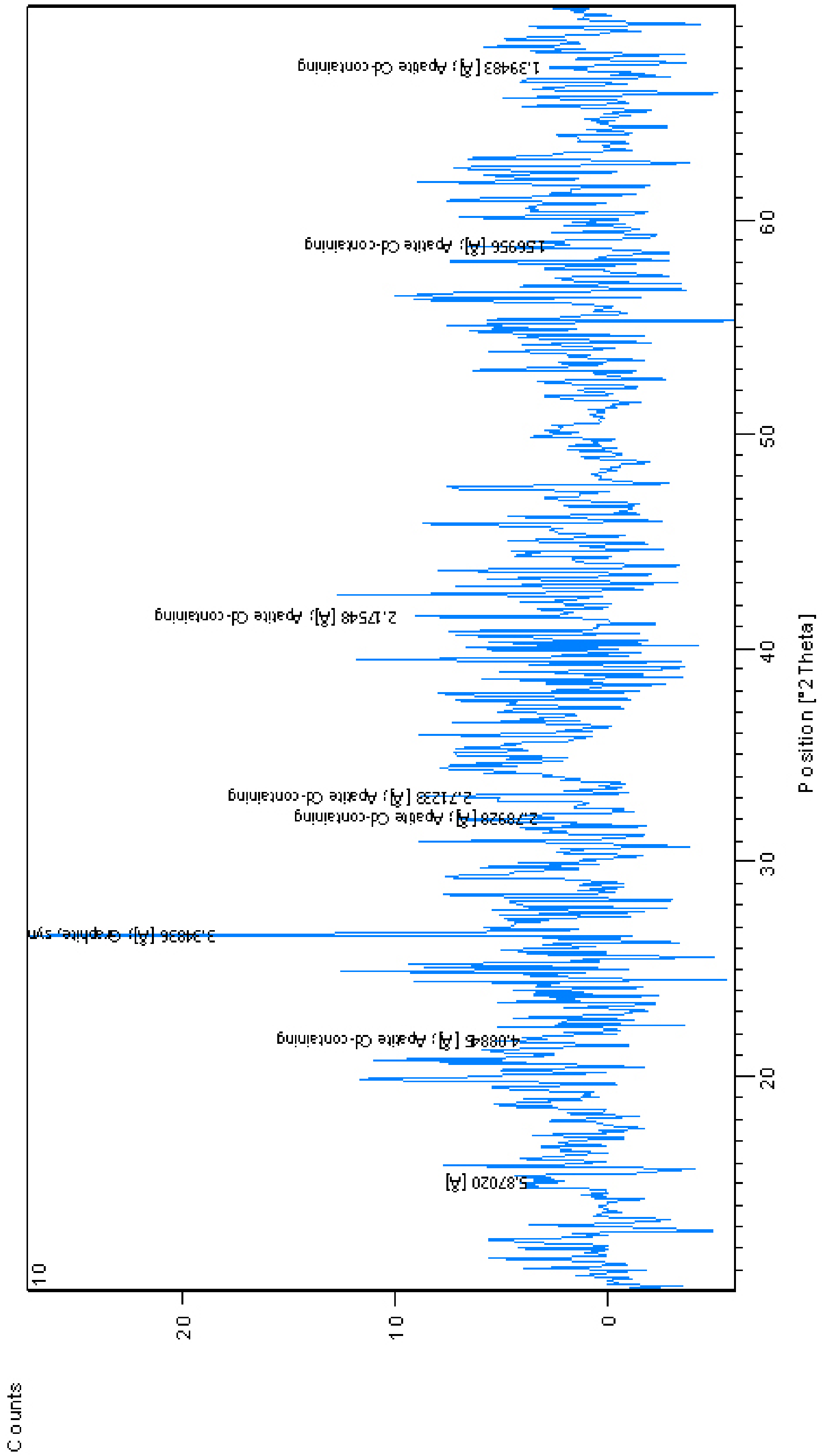
Figure 3.9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30
40
50
60
Position [°2Theta]

Figure 3.10

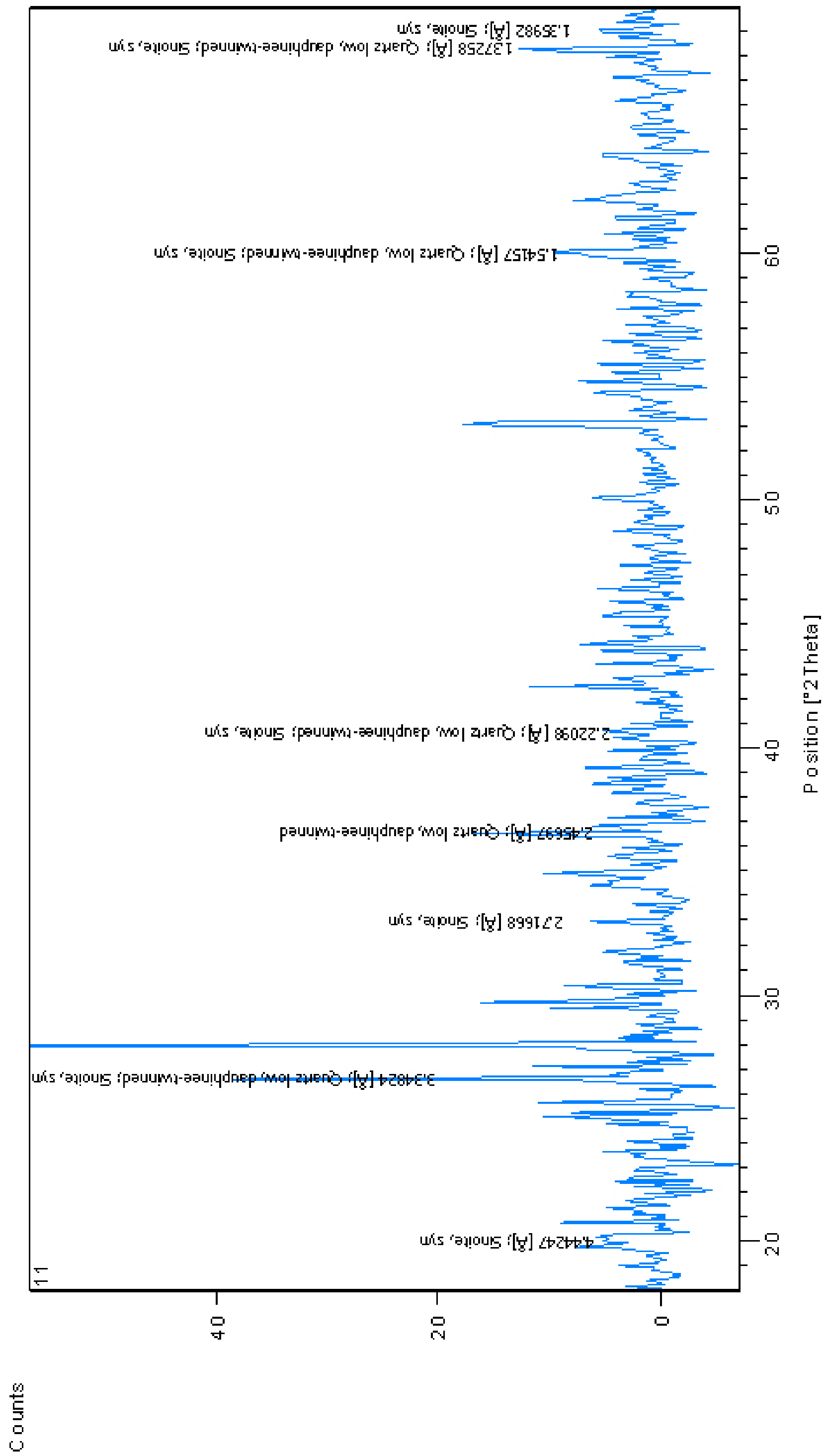
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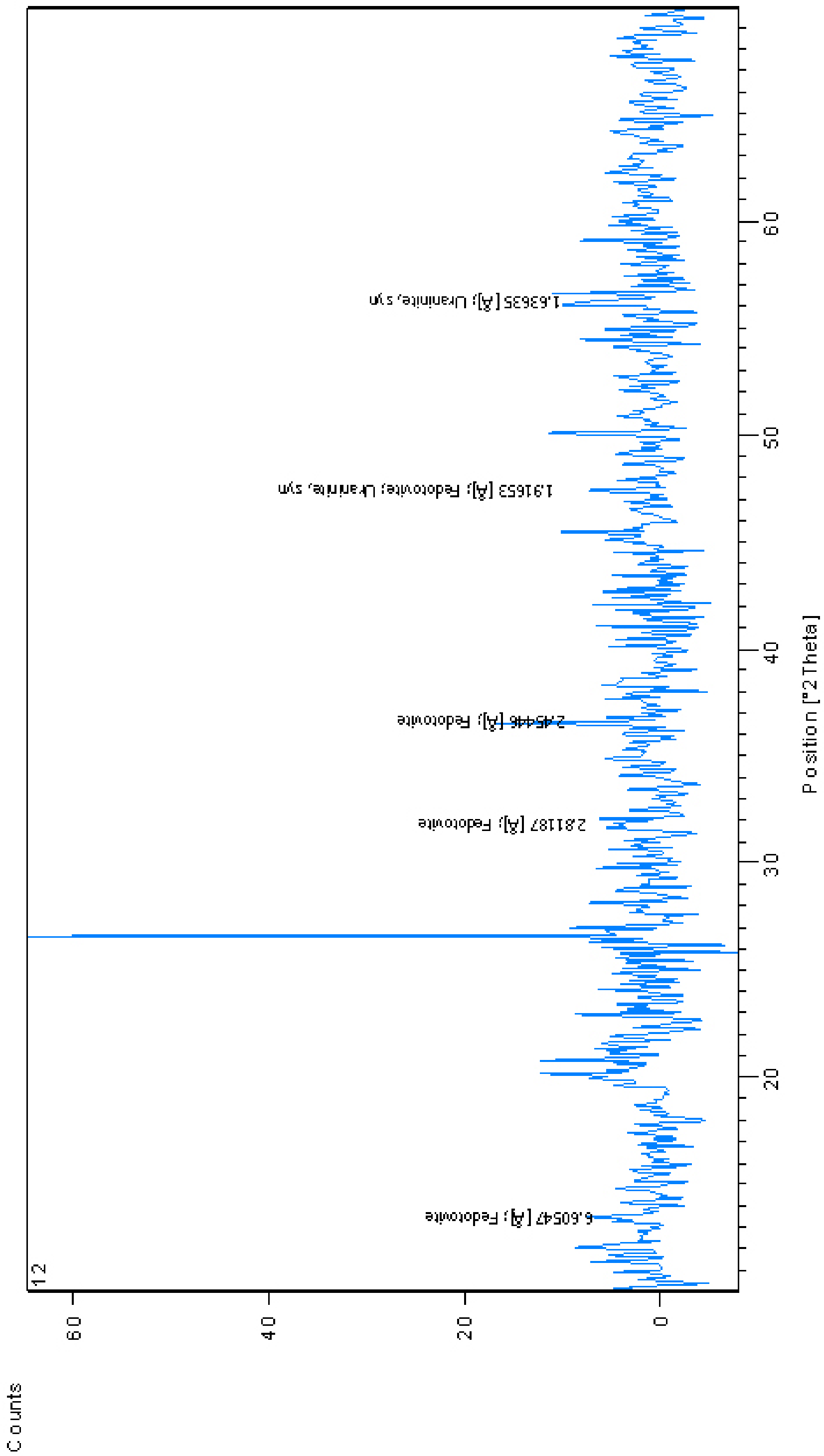
Figure 3.11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1.63635 [Å]; Uraninite, syn
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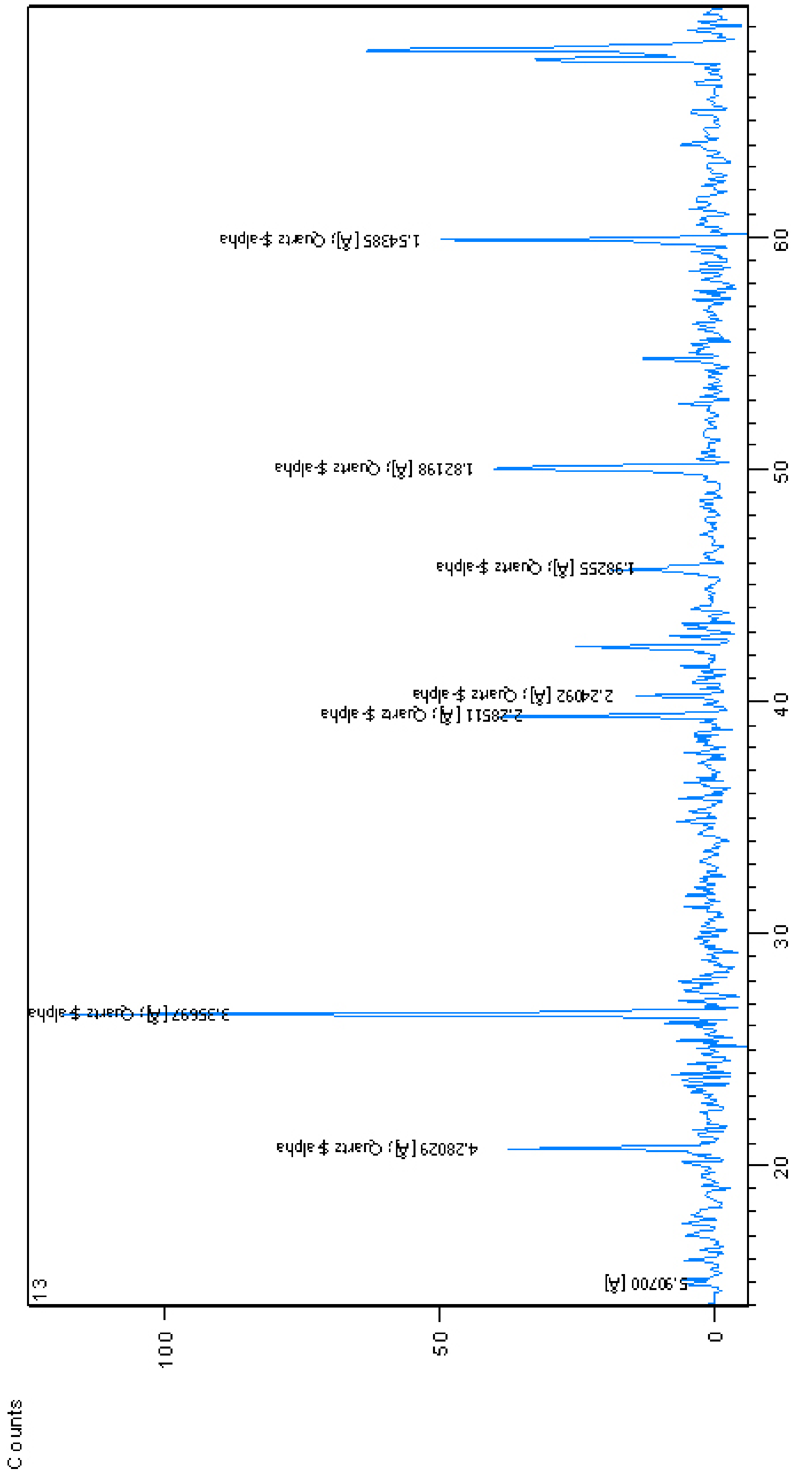
30
40
50
60
Position [°2Theta]

Figure 3.12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1.54385 [Å]; Quartz $-alpha
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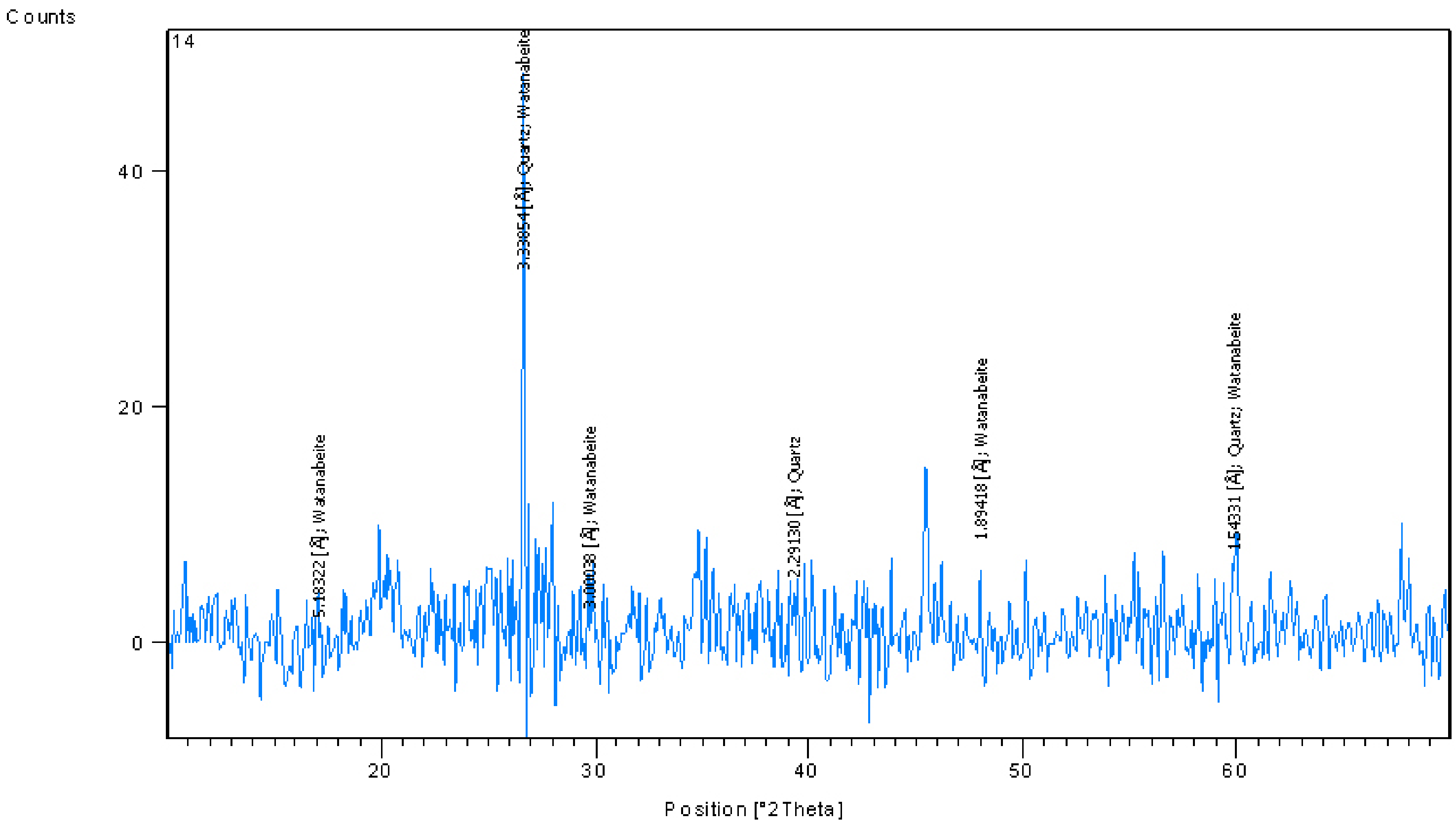
30
40
50
60

Figure 3.13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3.00038 [Å]; Watanabeite
2.29130 [Å]; Quartz
1.89418 [Å]; Watanabeite
1.54331 [Å]; Quartz; Watanabeite

Figure 3.14

The Total nitrogen values ranged from 0.07% to 0.31% during 2007 and from 0.05% to 0.33% during 2008. Very low percentage of total nitrogen were observed at stations ST-10, ST-11, ST-12 & ST-14 in 2007 and also at ST-4, ST-10, ST-11, ST-12 & ST-14 in 2008.

The Total Sulphur percentage varied from 0.033% to 1.33% in 2007 and 0.31% to 1.29% in 2008. Low and almost steady distribution were observed during 2007 and 2008 in the case of total sulphur.

Total Phosphorus, which is an important parameter for determining the basic sedimentary character which varied from 325.07 ppm to 1477.50 ppm in 2007 and 227.5 ppm to 1261.25 ppm in 2008. Higher concentrations were observed at stations ST-2 to ST-10, ST-13 & ST-14 in 2007 and ST-1, ST-2, ST-3, ST-5, ST-6, ST-7 & ST-11 in 2008. Low concentrations were found at stations near to the riverine outputs.

During 2007 (pre-monsoon) the protein concentration in sediments ranged from 2 mg/g to 9.62 mg/g. The low values were recorded in stations ST-1, ST-10 and ST-11 for proteins. The carbohydrate during 2007 pre-monsoon showed concentration ranges from 2.33 mg/g to 11.87mg/g. It showed that lower concentration in stations ST-1, ST-9, ST-10 & ST-11 and having higher concentration in ST-2. Lipids gave low concentration during 2007 pre-monsoon in nearly all stations except ST-6, ST-9 and ST-13. The concentration of lipid varied from 0.04 mg/g to 1.42 mg/g and had lower concentration in stations ST-1, ST-8, ST-10 and ST-12. Chlorophyll-a concentration varied from 5.74 to 18.62. It has lower concentration in stations ST-1, ST-8, ST-9, ST-10 & ST-12 and having higher values in ST-3, ST-5, ST-6 and ST-13. The pheophytin showed similar trend as that of chlorophyll-a during 2007 (pre-monsoon season). It has lower concentration in stations ST-1, ST-8, ST-9, ST-10 & ST-12 and exhibit higher concentration in ST-3, ST-5, ST-6 and ST-13.

In 2008 (pre-monsoon) protein concentration varied from 0.51 mg/g to 9.67 mg/g. It gave very low concentration in stations ST-1, ST-4, ST-11 & ST-13. The total carbohydrate concentration during 2008 (pre-monsoon) varied from 0.87 mg/g to 9.02 mg/g. Total carbohydrate showed lower concentration in stations ST-1, ST-11, ST-12 & ST-13 and having higher values in ST-6, ST-7 & ST-8. The lipid concentration along the study area during 2008 pre-monsoon varied from 0.05 mg/g to 1.99 mg/g. It gave higher concentration at stations ST-2, ST-3, ST-6, ST-7, ST-8 & ST-12 and displayed lower concentration at ST-1, ST-10, ST-11 and ST-13. In the case of chlorophyll-a, during 2008 (pre-monsoon) values ranged from 0.05 to 11.26. It has higher concentration at stations ST-2, ST-3, ST-6, ST-7, ST-9, ST-12 & ST-13 and gave lower values in ST-1, ST-8 & ST-13. Pheopigments in 2008 pre-monsoon varied from 2.14 to 26.91. It recorded higher concentration in ST-2, ST-6, ST-7, ST-9, ST-11, ST-12 & ST-14 and having lower values in ST-1 & ST-13.

3.3 Discussion

3.3.1 Behaviour of Biochemical Composition

The grain size distribution of the environment strongly depends on the hydrodynamic conditions that prevail over the region. Several previous studies explained the grain size distribution in the Cochin estuarine system and the present study agrees with those previous reports. The river mouth remains highly energetic and rich in suspended sediments. The higher but variable sand content may be attributed to the dredging activities which were undertaken in the harbour area and is located near the vicinity of the main navigation channel. In the microtidal and shallow water depth estuaries, wind and wave effects are responsible for the dominant physical processes of particle resuspension and deposition that affect grain size distribution and its variability (Lopez-Laborde and Nagy 1999).

High levels of carbohydrates (CHO) in sediments have been ascribed to the accumulation of aged organic detritus, due to the faster utilisation of protein than carbohydrates by bacteria (Venturini et al., 2012). Higher CHO concentration should be associated to bloom and the following deposition of the detrital material on the bottom. Cochin estuary may be related to high levels of anthropogenic impact, which promote the enhancement of primary production due to high nutrient loads, but also, the dominance of aged detritus due to chemical contamination.

Protein (PRT) is another biochemical compound exclusively present in the Cochin estuary. Fishing harbour and fish processing units are located along the bank of the estuary and the wastes from these industries are dumped into water system where the tissue undergoes decomposition to liberate protein, which are ultimately settled into the sediments (Balasubramaniam et al. 2012). The PRT/CHO ratio in sediments is used as an index for determining the origin of materials present and to distinguish the presence of fresh materials of recent deposition (Danovaro et al. 1993; Cividances et al. 2002). The dominance of PRT over the other biopolymers has been reported in hypertrophic and eutrophic systems with high nitrogen accumulation rates (Dell'anno et al. 2002). PRT/CHO >1 has been ascribed to fresh material of recent formation, where as PRT/CHO <1 has been attributed to more degraded organic matter (Danovaro et al. 1993). It implies that carbohydrates are dominating in the organic pool which is a salient feature of detrital heterotrophic environment (Danavaro 1996; Pusceddu et al. 2003). In organically enriched sediments, PRT can be degraded slowly than CHO does, due to the inhibition of protease enzyme, which causes a tendency of PRT to combine with refractory compounds (Covazzi Harriague et al. 2007; Dell'Anno et al. 2008). PRT:CHO ratios >1 were obtained at the station ST-9 & ST-13 during 2007 and stations ST-9 and ST-12 during 2008 suggesting, intense detritus mineralisation and an increment in their protein content due to bacterial activity. Therefore in productive areas such as estuaries and coastal regions, values of PRT/CHO indexes are high (Galois et al. 2000; Pusceddu et al. 2000). The accumulation of phytodetritus and anthropogenic waste in these stations may contribute to the prevalence of PRT. Finally the study during the two consecutive years (2007 and 2008) reveals the presence of aged organic matter along the sampling stations.

To assess the tropic state, earlier Dell'Anno et al. (2002) suggested a suite of environmental variables to monitor the organic enrichment status of the coastal zone in the Mediterranean Sea, based on variables of biochemical composition of sedimentary organic matter. According to Dell'Anno et al. (2002) hypertrophic systems are characterised by proteins and carbohydrate concentration of 1.5 to 4 mg/g and of 5 to 7 mg/g respectively. Finally, mesooligotrophic areas display protein concentrations of <1 mg/g and carbohydrates concentration of < 5mg/g. The result displayed in this work shows that very high concentration of PRT and CHO which gives an indication of highly eutrophic conditions prevailing in the Cochin estuarine system. It is established that the carbohydrates are diagenetically weaker than proteins under anoxic conditions, whereas the reverse is true under oxic conditions (Harvey et al. 1995). Depilation of oxygen due to oxidation of macrophytic weed matter has been reported earlier in the Cochin estuarine system (Sheeba 2000).

Higher Lipid concentration could be attributed to anthropogenic sources such as petroleum and domestic sewage inputs. High Lipid concentrations in sediments of highly industrialised and urbanised estuaries have been reported because the methods used to extract lipids from sediments may also recover significant quantities of organic contaminants, in particular hydrocarbons reflecting the degree of pollution (Galois et al. 2000). The higher concentrations of lipids recorded in the pre-monsoon may be related to the accumulation of plankton detritus deposited from the water column after the usual spring bloom (Gomez-Erache et al. 2001). Diatoms and faecal pellets of zooplankton are assumed to be important carriers of lipids to marine sediments (Baldi et al. 2010).

The Lipid content and LIP/CHO ratio have been used as biochemical index to describe the energetic (food) quality of the organic content in the sediment (Gremare et al. 1997, 2002; Isla et al. 2006). Lipids in the sediments are probably associated with the labile fraction of sedimentary organics and were considered as the best descriptor for meofauna abundance and biomass over enzymatically hydrolysable amino acids or proteins (Gremare et al. 1997, 2002; Cartes et al. 2002; Isla et al. 2006). In the present study during 2007 (pre-monsoon) results very high CHO content and low Lipid content, and established the very low LIP/CHO ratios. The LIP/CHO values ranges from 0.02 to 0.60 during 2007 (pre-monsoon) and from 0.05 to 0.38 for pre-monsoon 2008. This gives the inference that Cochin estuary behave as a detritus trap for organic matter accumulation with low nutritional values.

The chloroplastic pigments, mainly chlorophyll-a, serve as key variables for studying the productivity whereas phytopigment concentrations are traces for determining the amount of organic matter produced during photosynthesis. So the chlorophyll content in the sediment can be used as a descriptor of the trophic state and productivity of the most estuarine and shallow coastal systems (Cahoon and Cooke 1992; de Jong and de Jonge 1995; Lucas et al. 2000; Manini et al. 2001). In shallow coastal ecosystems, most of the primary production is accounted by microphytobenthos and/ or micro algae rather than phytoplankton (Dell' Anno et al. 2002). Sedimentary chlorophyll-a concentration have not been reported previously for the Cochin estuarine system. Chlorophyll-a concentration obtained was compared with other estuaries like Orda (Kowaleswska et al. 2004) Palmones (Moreno and Neill 2004) and intertidal systems (Shaffer and Onuf 1985; Brotas and Plante-Cuny 1996) and showed very high concentrations. Since all these environmental conditions have been classified them as eutrophic, and thereby the present study could suggest the Cochin estuarine system is eutrophic.

Different biotic and abiotic factors affect the spatial and seasonal variation of chlorophyll pigments in surface sediments (Moreno and Niell 2004). Chlorophyll-a concentrations showed distinct seasonal variations with maximum concentration in the pre-monsoon 2007 compared to the pre-monsoon 2008 and chlorophyll-a also gave positive correlation with clay fraction. This result confirms the results of Moreno and Niell (2004) and reported that chlorophyll-a is positively correlated with the clay content and negatively correlated with hydrodynamic energy. Pigment concentrations are dependent on the light availability and oxygen content in the water column (Kowalewska and Szymczak 2001; Kowalewska et al. 2004). The light availability at the sediment surface is affected by the variability in the hydrodynamic conditions (Moreno and Niell 2004). The lower concentration in 2008 pre-monsoon may be due to the latter days of pre-monsoon and onset of monsoon. In this case an increase in the concentration of suspended particulate matter due to the terrestrial and river run off enhances the water column turbidity leading to a reduction of sufficient light penetration for benthic primary production (Madhu et al. 2007; 2010).

The ratios of chlorophyll-a to phytopigment content are utilised as indicators of fraction of the metabolically active autotrophic component (Dell’ Anno et al. 2002). A very low chlorophyll-a to phytopigment ratio were obtained during two sampling seasons. The ratio ranged from 0.40 to 0.78 during 2007 (pre-monsoon) and had values from 0.02 to 0.75 for 2008 (pre-monsoon). The low values of this ratio could be the results of increase in turbidity and chemical contamination which in turn affects the photosynthetic potential (Bhadauria et al. 1992; Porter et al. 1999). Chlorophyll-a to phaeopigment ratios <1 indicate the prevalence of detritus in primary material in the sediments. According to Dell’Anno et al. (2002) the predominance of phaeopigments may be attributed to high turbidity, chemical contamination or other factors affecting photosynthetic potential of primary producers. The primary production is mainly controlled by sun light and in the Cochin estuary the average light penetration depth is around 40-70cm. besides, it is also controlled by the saline and turbidity front, while nutrients do not play a relevant role due to their availability at saturated levels (Calliari et al. 2005). Even though chemical contamination could be an important factor for controlling primary production in the polluted estuary turbidity may be more relevant in the adjacent coastal area.

The atomic C/N ratio has been widely used to distinguish the origin of organic matter (OM) in estuaries based on the generalisation that fresh marine autogenic organic matter has C/N ratios between 4 and 10, whereas organic matter derived from terrestrial vascular plants has C/N ratios of 20 or greater (Meyers 1994; Twichell et al. 2002). The basic reason for such difference in C/N ratios between vascular plants (>17) and micro algae (5-7) are simply due to the carbohydrates-rich (eg: cellulose/ protein-poor and proteins rich/ carbohydrate poor) nature of each source respectively (Bianchi 2007). Furthermore, the microbial decomposition of organic matter tends to increase the C/N ratio as nitrogen being mineralised faster than carbon (Andrews et al. 1998; Lehmann et al. 2002).

In pre-monsoon, 2007 the C/N showed to 13.08, indicates that the organic matter is derived from terrestrial vascular plants. In 2008 pre-monsoon, similar trend was observed and it gives 0 to 13.08. These showed that the organic matter is utilising very fast referring the oxidation conditions. Therefore the results infer that organic matter is derived from terrestrial origin.

Based on total protein (PRT) concentrations most of the stations were classified as hypertrophic, (PRT >4 mg/g) as proposed by Dell’Anno et al. (2002). This clearly indicated that the study area is probably more close to eutrophic conditions. BPC >5 mg C/g also suggested hypertrophic environment. An algal contribution of BPC < 12% in most of the stations suggested eutrophic condition (Pusceddu et al. 2011), however, even lower values (between 3.19 and 6.34%) suggested hypertrophic condition. PRT enrichment has very high BPC values and low algal contribution to the BPC observed indicated the high eutrophication in of this area, which can be considered as hypertrophic.

3.3.2 Bio-geochemical Interactions and Tropic status

The correlation analysis of sedimentary parameters showed that BPC, TOC, PRT, TCHO had high significant correlation with other parameters except chlorophyll-a and pheo-pigments; therefore in both sampling year shows the presence of allochothonous organic matter inputs to the sediments (Tables 3.6 & 3.7 correlation table during 2007 and 2008 pre-monsoon). The BPC and TOC content did not display any sufficient relationship with phytopigment concentration. The contribution of autotrophic component to the BPC pool was significantly lower in two sampling periods. A low autotrophic contribution to the BPC pool appears to be a common feature in areas receiving waste inputs from industrial area, maritime traffic, or eutrophic conditions which were reported earlier (Rheinheimer 1998; Danovaro et al. 1999; Dell’Anno et al. 2002; Pusceddu et al. 2011). Sporadic introduction of terrestrial organic matter into the Cochin estuarine system has been reported previously (Balachandran et al. 2008).

Table 3.6 Correlation between sedimentary parameters during 2007

	Sand%	Silt%	Clay%	OC(%)	TN%	TC%	TS%	TP	Chlor a	Pheo	PRT	TCHO	LPD	C/N	PRT/ TCHO	LPD/ TCHO	PRT C	TCHO C	LPD C	BPC	Chlo-a/ Phaeo
Sand%	1.00																				
Silt%	**-0.92**	1.00																			
Clay%	**-0.90**	**0.66**	1.00																		
OC(%)	**-0.97**	**0.89**	**0.88**	1.00																	
TN%	**-0.85**	**0.72**	**0.83**	**0.83**	1.00																
TC%	**-0.82**	**0.74**	**0.77**	**0.85**	**0.72**	1.00															
TS%	**-0.78**	**0.77**	**0.65**	**0.83**	**0.63**	**0.81**	1.00														
TP	**-0.76**	**0.75**	**0.62**	**0.81**	**0.76**	**0.74**	**0.67**	1.00													
Chlor a	-0.50	0.53	0.36	0.42	0.46	0.27	0.21	**0.63**	1.00												
Pheo	-0.50	0.50	0.41	0.41	0.48	0.26	0.21	**0.58**	**0.99**	1.00											
PRT	**-0.63**	**0.59**	**0.55**	**0.72**	0.41	**0.75**	0.48	**0.60**	0.15	0.09	1.00										
TCHO	**-0.63**	**0.60**	**0.55**	**0.59**	0.46	0.52	**0.63**	0.31	0.12	0.18	0.32	1.00									
LPD	**-0.56**	**0.58**	0.44	**0.61**	0.43	0.47	0.30	0.59	**0.54**	0.47	**0.70**	0.02	1.00								
C/N	**-0.66**	**0.64**	**0.54**	**0.70**	0.39	**0.82**	**0.59**	**0.56**	0.21	0.17	**0.86**	0.41	**0.59**	1.00							
PRT/TCHO	0.00	0.06	-0.06	0.14	-0.05	0.18	-0.07	0.21	-0.09	-0.21	**0.63**	-0.47	**0.58**	0.42	1.00						
LPD/TCHO	-0.11	0.17	0.03	0.23	0.07	0.18	-0.05	0.30	0.14	0.03	**0.60**	-0.46	**0.77**	0.41	**0.95**	1.00					
PRT C	**-0.63**	**0.59**	**0.55**	**0.72**	0.41	**0.75**	0.48	**0.60**	0.15	0.09	**1.00**	0.32	**0.70**	**0.86**	**0.63**	**0.60**	1.00				
TCHO C	**-0.63**	**0.60**	**0.55**	**0.59**	0.46	0.52	**0.63**	0.31	0.12	0.18	0.32	**1.00**	0.02	0.41	-0.47	-0.46	0.32	1.00			
LPD C	**-0.56**	**0.58**	0.44	**0.61**	0.43	0.47	0.30	**0.59**	**0.54**	0.47	**0.70**	0.02	1.00	**0.59**	**0.58**	**0.77**	**0.70**	0.02	1.00		
BPC	**-0.80**	**0.77**	**0.69**	**0.84**	**0.56**	**0.80**	**0.66**	**0.62**	0.25	0.23	**0.88**	**0.71**	**0.60**	**0.83**	0.23	0.25	**0.88**	**0.71**	0.60	1.00	
Chlo-a/Phaeo	0.21	-0.07	-0.33	-0.06	-0.27	-0.04	-0.10	0.02	-0.30	-0.44	0.39	-0.51	0.36	0.21	**0.90**	**0.81**	0.39	-0.51	0.36	0.03	1.00

bold underlined gives correlation si significant at the 0.01 level

bold gives correlation si significant at the 0.05 level

Table 3.7 Correlation between sedimentary parameters during 2008

	Sand%	Silt%	Clay%	OC(%)	TN%	TC%	TS%	TP	Chlo- a	Pheo	PRT	TCHO	LPD	C/N	PRT/ TCHO	LPD/ TCHO	PRT C	TCHO C	LPD C	BPC	Chloro/ Phaeo
Sand%	1.00																				
Silt%	**-0.79**	1.00																			
Clay%	-0.63	0.03	1.00																		
OC(%)	**-0.66**	0.24	0.77	1.00																	
TN%	**-0.67**	**0.54**	0.41	**0.57**	1.00																
TC%	-0.52	0.26	0.52	**0.81**	**0.68**	1.00															
TS%	**-0.62**	0.43	0.47	0.49	**0.86**	**0.63**	1.00														
TP	-0.41	0.00	**0.67**	0.35	0.47	0.21	**0.61**	1.00													
Chlo- a	-0.17	-0.33	**0.69**	**0.60**	-0.05	0.39	-0.01	0.15	1.00												
Pheo	-0.09	-0.27	0.49	0.53	0.00	0.47	0.02	-0.05	**0.93**	1.00											
PRT	-0.43	0.18	0.48	**0.85**	0.47	**0.62**	0.32	0.09	**0.55**	0.53	1.00										
TCHO	**-0.56**	0.22	**0.64**	**0.89**	0.49	**0.89**	0.42	0.13	0.50	0.50	**0.66**	1.00									
LPD	**-0.66**	0.39	**0.59**	**0.76**	0.46	**0.60**	0.46	0.28	0.41	0.35	**0.59**	**0.68**	1.00								
C/N	-0.31	0.10	0.38	**0.64**	0.34	**0.80**	0.22	0.12	0.31	0.33	0.42	**0.75**	0.30	1.00							
PRT/TCHO	-0.06	0.04	0.05	0.32	0.11	0.02	0.01	-0.06	0.33	0.32	**0.74**	0.00	0.23	-0.12	1.00						
LPD/TCHO	-0.27	0.18	0.20	0.25	-0.03	0.06	0.03	0.04	0.33	0.29	0.28	0.14	**0.75**	-0.15	0.37	1.00					
PRT C	-0.43	0.18	0.48	**0.85**	0.47	**0.62**	0.32	0.09	**0.55**	0.53	**1.00**	**0.66**	**0.59**	0.42	**0.74**	0.28	1.00				
TCHO C	-0.56	0.22	**0.64**	**0.89**	0.49	**0.89**	0.42	0.13	0.50	0.50	**0.66**	1.00	**0.68**	**0.75**	0.00	0.14	**0.66**	1.00			
LPD C	**-0.66**	0.39	**0.59**	**0.76**	0.46	**0.60**	0.46	0.28	0.41	0.35	**0.59**	**0.68**	**1.00**	0.30	0.23	**0.75**	**0.59**	**0.68**	1.00		
BPC	**-0.59**	0.26	**0.63**	**0.96**	**0.54**	**0.81**	0.43	0.16	**0.57**	**0.55**	**0.91**	**0.89**	**0.79**	**0.59**	0.43	0.35	**0.91**	**0.89**	**0.79**	1.00	
Chloro/Phaeo	-0.16	-0.32	**0.67**	**0.63**	-0.17	0.31	-0.17	0.22	**0.78**	**0.56**	0.50	**0.55**	0.47	0.41	0.20	0.33	0.50	**0.55**	0.47	**0.58**	1.00

bold underlined gives correlation si significant at the 0.01 level

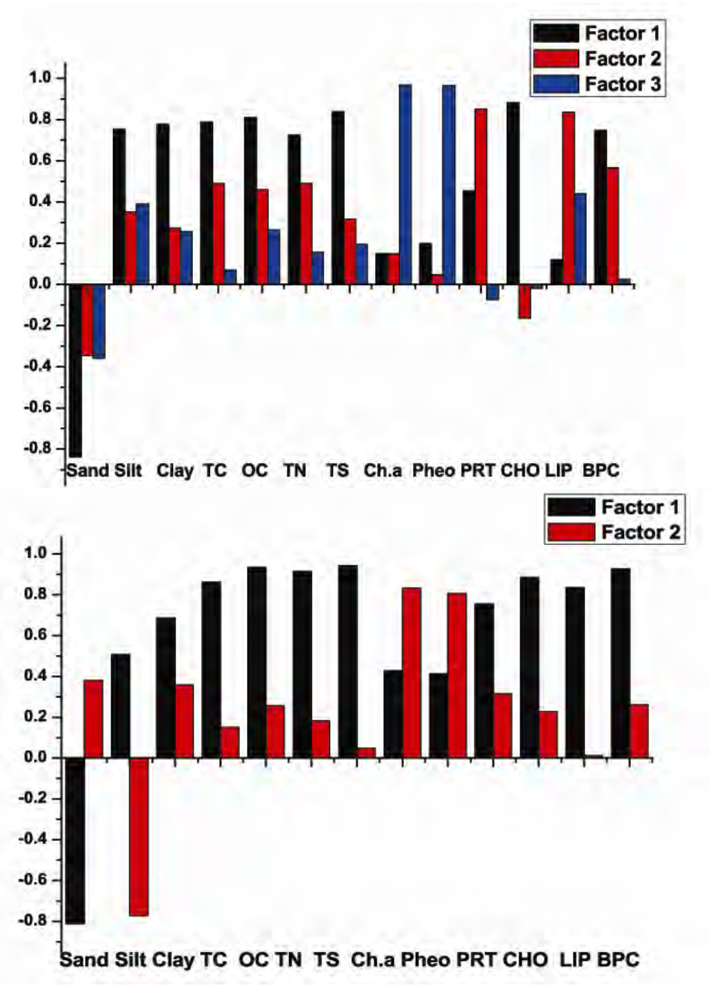
bold gives correlation si significant at the 0.05 level

3.3.3 Principle Component Analysis

The quantity and quality of sedimentary organic matter in aquatic systems is mainly controlled by the biogeochemical processes; and principal component analysis (PCA) was employed to deduce it. The parameters for the PCA were selected in such a way that the component of the analysis can give an indication to the significance of biogeochemical processes. The concentrations of individual chemical species will be thereby fixing with suitable indicators, it will be possible to identify the relative significance of each process to each species.

The PCA revealed three components accounting for total 88% variance in 2007 pre-monsoon (Figure3.15 PCA loading graph during 2007 and table 3.8 PCA loading pattern during 2007). The first component accounted for 46% of the total variance and resulted high values for silt, clay, TC, OC, TN, TS and BPC% and negative values for sand. The redox indicators like OC, TS and TN and fine sediment texture influence the organic matter enrichment in surface sediments of Cochin estuary. The second component accounted for 22% of variance and showed high values for proteins and lipid. The third component accounted for 19.5% of total variance and is only for chlorophyll a and pheopigments confirming the low autotrophically produced organic matter in the Cochin estuarine system. The high positive loading for redox indicators such as TS, TC, OC, and TN infers that the major process that can operate is the diagenesis process.

In 2008 (pre-monsoon) the principal component analysis gave 2 components (Figure 3.16 PCA loading graph during 2008 and table 3.8 loading pattern during 2008). Here eigen values higher than 1 accounting for 81% of the cumulative variance under consideration. Factor 1 accounted for 61.45% of the total variance and is mainly characterised by high levels of clay, TC, OC, TN, TS, protein, lipid, carbohydrates and biopolymeric carbon. This interprets the behaviour of these biochemical compositions. The factor 2 accounted to 19.68% of total variance and mainly consisted of chlorophyll-a and phaeopigments and high negative values for silt and infers the common origin of chlorophyll-a and phaeopigments.



Figures 3.15 and 3.16 Graphs showing loading pattern of Sedimentary parameters for the different component in PCA analysis during 2007 and 2008

Table 3.8 Factor analysis of general sedimentary parameter with biochemical components in 2007 and 2008 (pre-monsoon)

During 2007 pre-monsoon

Parametes	Component		
	1	2	3
Sand%	-0.838	-0.346	-0.359
Silt%	0.753	0.352	0.390
Clay%	0.777	0.273	0.255
TC%	0.787	0.490	0.069
OC(%)	0.810	0.460	0.264
TN%	0.725	0.490	0.155
TS%	0.838	0.316	0.194
Chlo. a	0.149	0.148	0.966
Pheo	0.197	0.046	0.964
PRT	0.454	0.850	-0.074
CHO	0.881	-0.165	-0.019
LIP	0.120	0.836	0.441
BPC(%)	0.747	0.566	0.025
% of variance	46.090	22.322	19.592
Cumulative %	46.090	68.412	88.004

During 2008 pre-monsoon

Parameters	Component	
	1	2
Sand%	-0.812	0.381
Silt%	0.507	-0.773
Clay%	0.685	0.358
TC%	0.862	0.151
OC(%)	0.935	0.257
TN%	0.914	0.183
TS%	0.943	0.048
Chl.a	0.428	0.832
Pheo	0.413	0.806
PRT	0.756	0.315
CHO	0.885	0.228
LIP	0.834	0.012
BPC(%)	0.926	0.261
% of variance	61.445	19.668
Cumulative %	61.445	81.113

3.4 Conclusion

Cochin estuary is one of the major estuarine systems along the west coast of India. The six rivers mainly contribute to anthropogenic loading to these estuarine environments from the surrounding niche. The regular monitoring of the sedimentary parameters will help us to understand the processes that occur in the sedimentary phase and between the sedimentary phase and the water column. The antifouling biocide has high half-life in sediment when compared to the water column. Now a day's biochemical parameters are used to predict the trophic status, decay and source of pollutants in the marine environment. The results of organic carbon content and biochemical components in the surface sediments of Cochin estuary showed comparable concentrations to those reported in highly industrialized and urbanized eutrophic estuaries. Carbohydrates, lipids, proteins and their ratios are used to understand the pollution status of marine environment, especially estuaries and coastal areas. High level of carbohydrates in sediment gave an idea of aged organic detritus prevailing nature along the study location. PRT/CHO ratio, the indicator of organic matter status, showed more degraded organic matter in the studied location during these two consecutive years of study. The high lipid concentration revealed that the accumulation of plankton detritus. The lipid/ carbohydrate ratio is commonly used as indicator of quality of organic content in the sediment and the present study also showed very low values which gave an idea that estuary behave as a detritus trap for organic matter. The C/N ratios predict the sources of organic matter from terrestrial input and their fast utilisation. Other ratios like BPC and autotrophic biomass revealed that eutrophic environment prevailing in this ecosystem. These ratios noted in eutrophic environment and aged organic matter in these estuarine system. To understand the redox condition of the sediment environment of the estuary Rare Earth Elements and their ratios were investigated. Therefore the chapter 4 mainly deals with the distribution and their redox pattern in Cochin Estuarine System.

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Chapter 4

Evaluation of Redox Condition of Sediments using Rare Earth Elements

CONTENTS

4.1 Introduction

Estuaries are commonly described as semi-enclosed bodies of water, situated at the interface between land and ocean, where seawater is measurably diluted by the inflow of freshwater (Hobbie 2000). The term “estuary,” derived from the Latin word aestuarium, means marsh or channel (Merriam-Webster 1979). These dynamic ecosystems have highest biotic diversity and production in the world. Not only do they provide a direct resource for commercially important estuarine species of fishes, but also a shelter and food resources for commercially more useful species that spend their juvenile stages in estuaries.

The Cochin Estuary constitutes a complex estuarine system, characterised by an ox-bow shape with its long axes running parallel to the coast and islands (Soman 1997). It is a bar-built microtidal estuary which receives about 320 cm rainfall annually, of which nearly 60% occurs during the southwest monsoon season (Qasim 2003). The estuary is connected to the Arabian Sea at two locations, Cochin (Latitute 9^{0} 58'N) and Azhikode

(Latitute 10^0 $10^{'}$N) and is divisible into two parts: the southern arm extending from Cochin to the south and the northern arm extending from Cochin to Azhikode. The Cochin inlet is about 450m wide, whereas the Azhikode inlet is relatively narrow. During December to April, a salinity barrier at Thanneermukkom virtually cuts off the tidal propagation further south and modifies the circulation in the remaining part of the estuary. The associated quantity of rainfall contributes to the variability of the estuarine water levels and flow.

Sediments are normally a mixture of several components including different mineral species as well as organic debris. Sediments represent one of the ultimate sinks for heavy metals discharged into the environment (Luoma and Bryan 1981; Bettinetti et al. 2003). In recent years considerable attention has been given for assessing the state of the coast receiving wastewater discharges.

The geochemical properties of the Rare Earth Elements (REEs) make them particularly useful tool for inquiring into processes that mobilise elements during weathering and redistribution between particulate and dissolved phases in rivers and estuaries (Sholkovitz 1995). The REEs make up series of 15 elements from the lightest La (LREE) to the heaviest Lu (HREE). They are predominantly trivalent, with two exceptions. Cerium can exist as Ce (IV) and Europium as Eu (II), as a function of redox potential (Ahrens 1964; Henderson 1984). The concentration and fractionation of dissolved REEs in river and estuarine waters are dependent on pH and on the presence of colloidal particles (Sholkvitz 1995). Waters with a high pH have the lowest REE concentration depleted by sorption onto particles (Sholkovitz 1995). A large proportion of the dissolved REE inventory in river water is associated with colloid material which can undergo large scale coagulation in estuaries. This process results in highly fractionated phases being transported in rivers from the continents to the oceans (Sholkovitz 1995). Light rare earths (LREEs) are preferentially scavenged on the surface of sedimentary particles such as Mn-Fe oxyhydroxides and clay minerals, or are precipitated as REE phosphates (Byrne and Kim 1990). In most natural waters, the REEs form strong complexes with carbonate ions (Millero 1992). The formations of both dissolved inorganic and organic complexes may promote the enrichment of HREEs in sea water. Certain minerals such as aluminosiliactes or apatite tend to have high concentrations of REEs while other minerals such as quartz have very low concentrations (Graf 1977). REEs are considered as useful indicators of anthropogenic inputs. LREEs are enriched in petroleum cracking catalysis, in the production of light weight hydrocarbon such as gasoline and fuel oil (Olmez et al. 1991). High concentration of REEs were found in the Tinto and Odiel estuaries in spain, caused by the waste from the fertilizer industries which use phosphate as a source material (Borrego et al. 2004). Dudas and Pawluk (1977) found high concentrations of REEs in cultivated soils and in cereal crops in Alberta, Canada. Phosphogypsum, a by-product from the production of phosphate fertilizers contains trace elements and rare earth element. Table 4.1 shows the earlier reported works on rare earth elements. REEs are sensitive to environmental changes such as redox condition, salinity, chealtes, pH changes, adsoption-desorption, complexation, precipitations, etc. Present study on REEs are used to understand the distribution profile and redox changes existed in the Cochin estuary with the help of anomalies and other REE ratios.

Table 4.1 Previously reported works on Rare Earth Elements around the world

REEs	Floridabay sediments	East Indonesian sediments	Tinto river sediments, Spain	Odiel river sediments, Spain	Mandovi estuary, India	Mae Klong estuary, Thailand	Continental shelf SW coast, India	Elimbah creek, Australia (water Sample)	Cental indian ocean basin	Corals of Isla de Sacrificios reef, Mexico
Y	30(ng/g)	21.3(μg/g)	9.92	19						
La	15.4(ng/g)	15.1(μg/g)	24.9 (μg/g)	17.9 (μg/g)	32.4(μg/g)	30.05(μg/g)	52(μg/g)	0.72(nmol/kg)	31.75 (μg/g)	12.1 (ng/g)
Ce	28.6(ng/g)	29.4(μg/g)	46.9 (μg/g)	35.7 (μg/g)	74.6(μg/g)	53.54(μg/g)	109(μg/g)	1.54(nmol/kg)	82.45 (μg/g)	12.9(ng/g)
Pr	3.6(ng/g)	3.39(μg/g)	7.1 (μg/g)	5.4 (μg/g)	7.2(μg/g)	5.48(μg/g)	N.A	0.18(nmol/kg)	8.07 (μg/g)	2.5(ng/g)
Nd	14.8(ng/g)	13(μg/g)	27.4 (μg/g)	21.3 (μg/g)	30.5(μg/g)	22.35(μg/g)	45(μg/g)	0.7(nmol/kg)	35.44 (μg/g)	12.4(ng/g)
Sm	3(ng/g)	2.56(μg/g)	6 (μg/g)	4.9 (μg/g)	6.6(μg/g)	4.51(μg/g)	8.93(μg/g)	0.1(nmol/kg)	9.28 (μg/g)	2.8(ng/g)
Eu	0.72(ng/g)	0.63(μg/g)	1.4(μg/g)	1.1(μg/g)	1.7(μg/g)	0.85(μg/g)	1.92(μg/g)	N.A	2.38 (μg/g)	2.5(ng/g)
Gd	2.8(ng/g)	2.43(μg/g)	5.6 (μg/g)	4.7 (μg/g)	5.8(μg/g)	3.41(μg/g)	N.A	.12(nmol/kg)	8.49 (μg/g)	3.7(ng/g)
Tb	0.39(ng/g)	0.35(μg/g)	0.8 (μg/g)	0.7(μg/g)	1.1(μg/g)	0.56(μg/g)	1.5(μg/g)	0.01(nmol/kg)	1.37 (μg/g)	0.6(ng/g)
Dy	2.2(ng/g)	2.39(μg/g)	4.2 (μg/g)	3.7(μg/g)	6.3(μg/g)	2.99(μg/g)	N.A	0.07(nmol/kg)	8.19 (μg/g)	3.5(ng/g)
Ho	0.48(ng/g)	0.51(μg/g)	0.8 (μg/g)	0.7 (μg/g)	1.3(μg/g)	0.56(μg/g)	N.A	0.02(nmol/kg)	1.74 (μg/g)	0.8 (ng/g)
Er	1.3(ng/g)	1.47(μg/g)	2.2 (μg/g)	1.9 (μg/g)	3.8(μg/g)	1.55(μg/g)	N.A	.04(nmol/kg)	4.30 (μg/g)	2.7 (ng/g)
Tm	0.2(ng/g)	0.2(μg/g)	N.A	N.A	0.5(μg/g)	0.23(μg/g)	N.A	.0006(nmol/kg)	0.57 (μg/g)	0.3 (ng/g)
Yb	1(ng/g)	1.39(μg/g)	1.8 (μg/g)	1.5 (μg/g)	3.1(μg/g)	1.33(μg/g)	1.5(μg/g)	0.04(nmol/kg)	3.76 (μg/g)	2.7 (ng/g)
Lu	0.1(ng/g)	0.22(μg/g)	0.3 (μg/g)	0.2 (μg/g)	0.5(μg/g)	.20(μg/g)	0.4(μg/g)	0.0006(nmol/kg)	0.54 (μg/g)	2.7 (ng/g)
Th		9.2(μg/g)	5.7 (μg/g)	4.5 (μg/g)	N.A	N.A	13.1(μg/g)	N.A	10.8 (μg/g)	N.A
U		4.19(μg/g)	N.A	N.A	N.A	N.A	4.13(μg/g)	.024(nmol/kg)	1.03 (μg/g)	N.A
References	Caccia and Millero (2007)	Varoon et al. (1995)	Borrego et al. (2004)	Borrego et al. (2004)	Shynu et al (2011)	Censi et al. (2007)	Nath et al. (2000)	lawrence and Kamber (2006)	Pattan et al. (2005)	Kasper-zubillaga et al. (2010)

4.2 Results

The REEs examined for the present study are; La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, V, Th and U (Tables 4.2 and 4.3). La showed concentrations 28.45-52.37 ppm (average 42.16 ppm) in 2007 (pre-monsoon) and gave 36.15-53.62 ppm (average 47.52 ppm) in 2008. During pre-monsoon 2007, Ce ranged from 50.80 to 102.93 ppm with an average of 84.41 ppm. In pre-monsoon 2008 it gave 75.29- 104.86 ppm, average being 95.70 ppm. Pr is another REE which showed 4.93-10.02 ppm (average 7.96 ppm) during pre-monsoon 2007 and in 2008 it gave 7.13-9.98 ppm with an average of 8.82 ppm. Nd showed concentration from 18.63-38.78 ppm averaging 30.85 ppm in 2007. But in 2008, it ranged from 28.19 to 38.71 ppm. In pre-monsoon 2007 Sm showed concentration from 3.57 to 6.93 ppm with an average of 5.52 ppm and in 2008 it was 4.63 to 6.75 ppm (average 5.83 ppm). The Eu during 2007 showed concentration 1.08-1.65 ppm (average 1.37 ppm) and 0.94- 1.57 ppm (average 1.29 ppm) in 2008. Gd in 2007 ranged from 2.75- 5.67 ppm (average 4.47 ppm) and during 2008 it was 4.16-5.63 ppm (average 4.76 ppm). In 2007, Tb gave 0.44-0.89 ppm (average 0.70 ppm) and 0.57-0.88 ppm (average 0.72 ppm) during 2008. The concentration of Dy during pre-monsoon of 2007 was 2.32-4.94 ppm (average 3.87ppm) and in 2008 it showed 2.87- 5.01 ppm (average 3.84 ppm). Ho has the concentration from 0.46 to 1.03 ppm (average 0.79) during pre-monsoon 2007 and 0.54 to 1.32 ppm (average 0.78) during pre-monsoon 2008. Er showed 1.30 to 2.83 ppm (average 2.17 ppm) in 2007 pre-monsoon and 1.54 to 2.80 ppm (average 2.10 ppm) in 2008. In the case of Tm, it showed the values from 0.18 to 2.69 ppm (average 0.30 ppm) in 2007 pre-monsoon and 0.20 to 0.38 ppm (average 0.29 ppm) during 2008 pre-monsoon. The Yb concentration ranged from 1.08 to 2.34 ppm (average 1.79 ppm) during 2007 pre-monsoon and from 1.23 to 2.32 ppm (average 1.77 ppm) in 2008 pre-monsoon. Lu showed 0.15–0.36 ppm (average 0.28 ppm) in 2007 pre-monsoon and 0.19 – 0.36 ppm (average 0.28 ppm) in 2008 pre-monsoon. Vanadium is a trace metal which gave 5.30 to 73.63 ppm (average 51.94 ppm) in 2007 and 45.19 to 93.09 ppm (average 65.51 ppm) in pre-monsoon 2008. Th, a radioactive element, showed a concentration of 6.13-13.96 ppm (average 9.91ppm) during 2007 and 6.52 to 15.60 ppm (average 10.96 ppm) during 2008. U was found to be in the range 1.09 - 4.27 ppm (average 2.70ppm) in 2007 and 1.99 – 11.09 ppm (average 3.22) during 2008.

4.3 Discussion

4.3.1 Factors Controlling REEs in Cochin Estuarine System

A). Distribution of REEs along the Cochin Estuarine System

The terrigenous input of REE from the continents, authigenic removal of REE from the water and early diagenesis are the major processes that control the enrichment and depletion of metals in the sediments (Shilkovitz 1988). The sediments of Cochin estuary contain high concentration of LREEs than HREEs. It is an expected behaviour since the REE contents of most shales and solid phases are normally enriched in LREEs relative to HREEs (Haskin et al. 1966). Although the absolute concentrations in most of the sediments are similar, it is a common exercise to normalise the concentration of REEs to shale composite such as the North American Shale Composition (NASC) or the Post-Archean Australian Shale (PAAS). The commonly used normalisations are displayed in table 4.4.

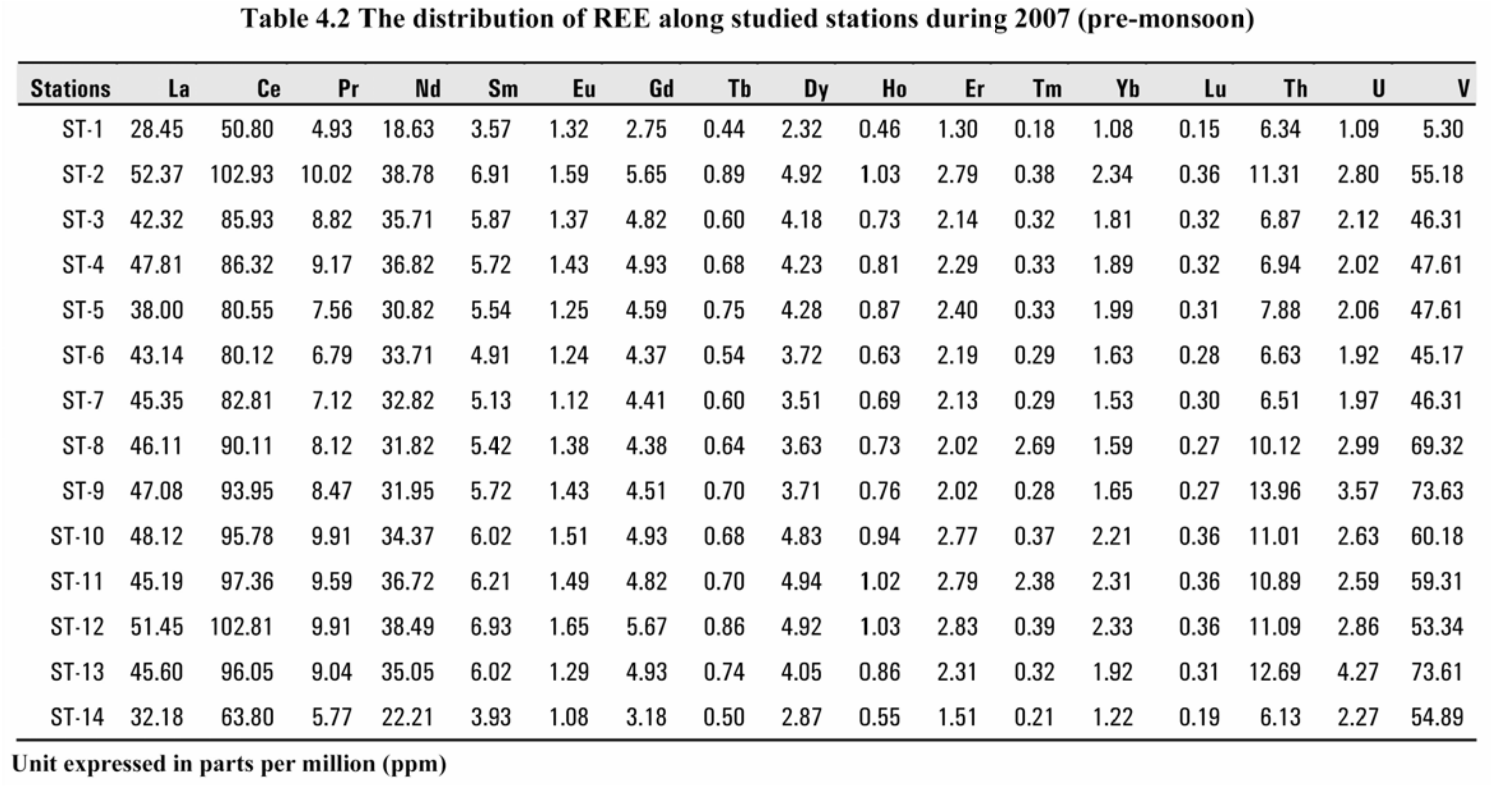


Table 4.2 The distribution of REE along studied stations during 2007 (pre-monsoon)

Stations	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Th	U	V
ST-1	28.45	50.80	4.93	18.63	3.57	1.32	2.75	0.44	2.32	0.46	1.30	0.18	1.08	0.15	6.34	1.09	5.30
ST-2	52.37	102.93	10.02	38.78	6.91	1.59	5.65	0.89	4.92	1.03	2.79	0.38	2.34	0.36	11.31	2.80	55.18
ST-3	42.32	85.93	8.82	35.71	5.87	1.37	4.82	0.60	4.18	0.73	2.14	0.32	1.81	0.32	6.87	2.12	46.31
ST-4	47.81	86.32	9.17	36.82	5.72	1.43	4.93	0.68	4.23	0.81	2.29	0.33	1.89	0.32	6.94	2.02	47.61
ST-5	38.00	80.55	7.56	30.82	5.54	1.25	4.59	0.75	4.28	0.87	2.40	0.33	1.99	0.31	7.88	2.06	47.61
ST-6	43.14	80.12	6.79	33.71	4.91	1.24	4.37	0.54	3.72	0.63	2.19	0.29	1.63	0.28	6.63	1.92	45.17
ST-7	45.35	82.81	7.12	32.82	5.13	1.12	4.41	0.60	3.51	0.69	2.13	0.29	1.53	0.30	6.51	1.97	46.31
ST-8	46.11	90.11	8.12	31.82	5.42	1.38	4.38	0.64	3.63	0.73	2.02	2.69	1.59	0.27	10.12	2.99	69.32
ST-9	47.08	93.95	8.47	31.95	5.72	1.43	4.51	0.70	3.71	0.76	2.02	0.28	1.65	0.27	13.96	3.57	73.63
ST-10	48.12	95.78	9.91	34.37	6.02	1.51	4.93	0.68	4.83	0.94	2.77	0.37	2.21	0.36	11.01	2.63	60.18
ST-11	45.19	97.36	9.59	36.72	6.21	1.49	4.82	0.70	4.94	1.02	2.79	2.38	2.31	0.36	10.89	2.59	59.31
ST-12	51.45	102.81	9.91	38.49	6.93	1.65	5.67	0.86	4.92	1.03	2.83	0.39	2.33	0.36	11.09	2.86	53.34
ST-13	45.60	96.05	9.04	35.05	6.02	1.29	4.93	0.74	4.05	0.86	2.31	0.32	1.92	0.31	12.69	4.27	73.61
ST-14	32.18	63.80	5.77	22.21	3.93	1.08	3.18	0.50	2.87	0.55	1.51	0.21	1.22	0.19	6.13	2.27	54.89

Unit expressed in parts per million (ppm)

Table 4.3 The distribution of REE along studied stations during 2008

Stations	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Th	U	V
ST-1	36.15	75.29	7.13	28.19	5.12	1.21	4.25	0.68	3.84	0.82	2.15	0.31	1.79	0.30	7.18	1.99	57.44
ST-2	51.63	103.78	9.94	38.71	6.75	1.51	5.63	0.87	4.72	1.00	2.71	0.38	2.28	0.33	11.69	3.07	93.09
ST-3	44.38	87.96	8.12	34.91	5.93	1.38	4.53	0.62	4.20	0.79	2.20	0.31	1.81	0.31	6.72	6.72	46.78
ST-4	46.88	86.20	9.98	36.99	5.91	1.41	4.91	0.66	4.28	0.83	2.30	0.33	1.88	0.32	6.91	6.91	47.21
ST-5	45.31	94.88	8.96	36.36	6.33	1.41	5.32	0.88	4.75	0.97	2.54	0.37	2.23	0.35	10.72	2.72	71.39
ST-6	43.82	82.19	7.97	32.82	4.63	1.30	4.44	0.59	3.82	0.69	2.02	0.28	1.69	0.28	6.52	6.52	45.19
ST-7	46.14	80.28	8.11	33.82	5.48	1.28	4.37	0.57	3.71	0.65	2.11	0.29	1.59	0.29	6.67	6.67	46.81
ST-8	45.20	91.82	8.93	31.52	5.76	1.41	4.40	0.65	3.49	0.79	2.12	0.27	1.58	0.26	10.32	10.32	70.11
ST-9	53.38	104.86	9.38	35.40	6.01	1.48	4.81	0.73	3.76	0.77	2.05	0.28	1.73	0.28	10.16	4.06	64.41
ST-10	47.81	96.82	8.78	35.92	6.31	1.57	4.82	0.69	4.92	0.97	2.80	0.37	2.27	0.36	11.09	11.09	61.87
ST-11	45.97	96.41	9.00	37.81	6.02	1.41	4.99	0.70	5.01	1.32	2.80	0.37	2.32	0.36	10.91	10.91	59.93
ST-12	44.55	91.83	8.35	32.31	5.62	1.43	4.61	0.70	3.78	0.81	2.08	0.29	1.77	0.29	8.31	4.33	67.46
ST-13	47.99	99.02	9.29	36.47	5.93	0.94	4.54	0.64	3.17	0.57	1.65	0.23	1.35	0.20	15.60	4.16	57.12
ST-14	53.62	100.24	8.72	32.10	5.07	1.07	4.16	0.58	2.87	0.54	1.54	0.20	1.23	0.19	13.05	2.19	47.66

Unit expressed in parts per million (ppm)

In the present study PAAS values were used to normalise results (Table 4.5 and 4.6).The surface sediments have ∑REE concentration close to shale values and a sample / shale ratio lies very close to 1, which normally indicates a dominant terrigenous source (Piper 1974a; Pattan et al. 2005). The increase of REE concentration indicates that a further supply of REE to the sediments in addition to the detrital source.

La showed higher concentration in all the stations observed except Barmouth than PAAS in both years. The PAAS of La was found to be 38.2 ppm. The PAAS of Ce was found to be 79.6 ppm. The Barmouth (ST-1) in 2007 and 2008 and Panavali (ST-7) in 2007 results much lower values than PAAS. All other stations indicated that higher concentration than PAAS. The PAAS of Pr was 79.6 ppm. The stations ST-2, ST-5, ST-6 in 2007 and ST-2, ST-3, ST-4 and ST-7 in 2008 gave higher concentrations than PAAS. Nd exhibited same distribution as that of Pr, and the PAAS of Nd was 33.9 ppm. Sm showed higher concentration than PAAS in all the stations except in Barmouth (ST-1) in both the years of study and Panavalli (ST-7) in 2007. The PAAS of Sm was found to be 5.55 ppm. Eu displayed slightly higher concentration than PAAS which was 1.08 ppm in all the stations except ST-7 in 2007 and ST-6 & ST-7 in 2008.

Gd is another REE which is present in the studied sites of Cochin Estuary. Higher enrichment was noted when compared to PAAS in stations ST-2 & ST-5 during pre-monsoon, 2007 and ST-2, ST-3, ST-4 and ST-5 during pre-monsoon, 2008. Tb, Dy and Ho are the three REEs which displayed similar behaviour in the study area. They showed slight enrichment in Bolgatty and Bund during 2007 (pre-monsoon) and Bolgatty & Thevara during 2008 (pre-monsoon). The PAAS of Tb, Dy and Ho are the

Table 4.4 Commonly used normaliser for Rare Earth Elements

different shale compositions	North American Shale compositie (NASC)	Australian Post-Archean Shale (PAAS)	Clay from the Russian Platform	World Shale	Upper continental crust	Chondrite
La	32	38.2	37.5	41	30	0.31
Ce	73	79.6	74.8	83	64	0.808
Pr	7.9	8.83	8.6	10.1	7.1	0.122
Nd	33	33.9	32.2	38	26	0.6
Sm	5.7	5.55	6.2	7.5	4.5	0.195
Eu	1.24	1.08	1.3	1.61	0.88	0.0735
Gd	5.2	4.66	5.21	6.35	3.8	0.259
Tb	0.85	0.774	0.79	1.23	0.64	0.0474
Dy	5.2	4.68	4.88	5.5	3.5	0.322
Ho	1.04	0.991	0.96	1.34	0.8	0.0718
Er	3.4	2.85	2.78	3.75	2.3	0.21
Tm	0.5	0.405	0.41	0.63	0.33	0.0324
Yb	3.1	2.82	2.73	3.53	2.2	0.209
Lu	0.48	0.433	0.41	0.61	0.32	0.0322
Reference	Gromet et al. 1984	McLennan 1989	Migdisov et al. 1994	Piper 1974a	Taylor and McLennan 1988	Boynton 1984

Unit expressed in parts per million (ppm)

Table 4.5 Shale normalised (PAAS) values of REEs in study area during 2007 (pre-monsoon)

Season	Station	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Th	U
2007 pre-monsoon	ST-1	0.74	0.64	0.56	0.55	0.64	1.22	0.59	0.57	0.50	0.47	0.46	0.43	0.38	0.35	0.75	0.64
	ST-2	1.37	1.29	1.14	1.14	1.24	1.47	1.21	1.15	1.05	1.04	0.98	0.93	0.83	0.82	1.33	1.65
	ST-3	1.11	1.08	1.00	1.05	1.06	1.27	1.03	0.77	0.89	0.74	0.75	0.79	0.64	0.73	0.81	1.25
	ST-4	1.25	1.08	1.04	1.09	1.03	1.33	1.06	0.88	0.90	0.82	0.80	0.81	0.67	0.74	0.82	1.19
	ST-5	0.99	1.01	0.86	0.91	1.00	1.15	0.98	0.96	0.91	0.88	0.84	0.82	0.70	0.72	0.93	1.21
	ST-6	1.13	1.01	0.77	0.99	0.88	1.15	0.94	0.69	0.80	0.64	0.77	0.72	0.58	0.65	0.78	1.13
	ST-7	1.19	1.04	0.81	0.97	0.92	1.04	0.95	0.77	0.75	0.69	0.75	0.71	0.54	0.69	0.77	1.16
	ST-8	1.21	1.13	0.92	0.94	0.98	1.28	0.94	0.83	0.78	0.74	0.71	6.64	0.56	0.62	1.19	1.76
	ST-9	1.23	1.18	0.96	0.94	1.03	1.32	0.97	0.90	0.79	0.76	0.71	0.69	0.59	0.63	1.64	2.10
	ST-10	1.26	1.20	1.12	1.01	1.08	1.40	1.06	0.88	1.03	0.95	0.97	0.92	0.78	0.83	1.30	1.55
	ST-11	1.18	1.22	1.09	1.08	1.12	1.38	1.03	0.91	1.05	1.03	0.98	5.88	0.82	0.84	1.28	1.52
	ST-12	1.35	1.29	1.12	1.14	1.25	1.53	1.22	1.11	1.05	1.04	0.99	0.95	0.83	0.84	1.31	1.68
	ST-13	1.19	1.21	1.02	1.03	1.09	1.19	1.06	0.96	0.87	0.87	0.81	0.78	0.68	0.72	1.49	2.51
	ST-14	0.84	0.80	0.65	0.66	0.71	1.00	0.68	0.65	0.61	0.55	0.53	0.51	0.43	0.44	0.72	1.34

Table 4.6 Shale normalised (PAAS) values of REEs in study area during 2008 (pre-monsoon)

Season	Station	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Th	U
2008 pre-monsoon	ST-1	0.95	0.95	0.81	0.83	0.92	1.12	0.91	0.88	0.82	0.83	0.75	0.77	0.63	0.70	0.84	1.17
	ST-2	1.35	1.30	1.13	1.14	1.22	1.40	1.21	1.12	1.01	1.01	0.95	0.94	0.81	0.76	1.37	1.81
	ST-3	1.16	1.10	0.92	1.03	1.07	1.28	0.97	0.80	0.90	0.80	0.77	0.77	0.64	0.72	0.79	3.95
	ST-4	1.23	1.08	1.13	1.09	1.07	1.31	1.05	0.85	0.91	0.83	0.81	0.82	0.67	0.73	0.81	4.07
	ST-5	1.19	1.19	1.02	1.07	1.14	1.31	1.14	1.13	1.02	0.97	0.89	0.91	0.79	0.80	1.26	1.60
	ST-6	1.15	1.03	0.90	0.97	0.83	1.20	0.95	0.76	0.82	0.70	0.71	0.70	0.60	0.66	0.77	3.83
	ST-7	1.21	1.01	0.92	1.00	0.99	1.19	0.94	0.73	0.79	0.65	0.74	0.71	0.56	0.67	0.78	3.92
	ST-8	1.18	1.15	1.01	0.93	1.04	1.31	0.94	0.84	0.75	0.80	0.74	0.67	0.56	0.60	1.21	6.07
	ST-9	1.40	1.32	1.06	1.04	1.08	1.37	1.03	0.94	0.80	0.78	0.72	0.69	0.61	0.64	1.20	2.39
	ST-10	1.25	1.22	0.99	1.06	1.14	1.46	1.03	0.89	1.05	0.98	0.98	0.91	0.81	0.83	1.30	6.52
	ST-11	1.20	1.21	1.02	1.12	1.08	1.30	1.07	0.90	1.07	1.33	0.98	0.92	0.82	0.83	1.28	6.42
	ST-12	1.17	1.15	0.95	0.95	1.01	1.32	0.99	0.90	0.81	0.82	0.73	0.72	0.63	0.67	0.98	2.55
	ST-13	1.26	1.24	1.05	1.08	1.07	0.87	0.97	0.82	0.68	0.58	0.58	0.56	0.48	0.45	1.84	2.45
	ST-14	1.40	1.26	0.99	0.95	0.91	0.99	0.89	0.75	0.61	0.54	0.54	0.50	0.43	0.44	1.54	1.29

following; 0.77 ppm, 4.68 ppm and 0.99 ppm. The REEs like Er, Tm, Yb and Lu were present in the study area, but their concentrations were well below the PAAS values and were found to be 2.85 ppm, 0.405 ppm, 2.82 ppm and 0.43 ppm respectively.

The world shale average for V was found to be 98 ppm. It was noticed that the study area has a concentration which lies well below the shale value during both the sampling years.

Th is a radioactive element which gave higher concentration than the world shale average (8.5 ppm) in stations ST-2, ST-8, ST-9, ST10, ST-11, ST-12 and ST-13 during 2007 and ST-2, ST-5, ST-8, ST-9, ST-10, ST-11, ST-13 and ST-14 in 2008. The world average shale value for U is 1.7 ppm. U is another radioactive element which showed higher concentration in all the stations observed in both years, except station ST-1 during pre-monsoon, 2007.

B). Inter-elemental relationship

Pearson correlation coefficients among the granulometry, OC, V, Th, U and REEs were studied. The pre-monsoon (2007 & 2008) data are presented in tables 4.7 and 4.8.

During pre-monsoon 2007, La showed strong positive correlation with other REEs except Eu. REEs with Eu and radioactive element like Th gave positive correlation of 95% confidence. Ce, Pr and Nd showed strong positive correlation with other REEs including Th, whereas they exhibited no correlation with Eu. Ce gave positive correlation with U. From the correlation it is clear that Ce is the only REE that showed positive correlation with U. So the source of U and Ce may be the same and different from other REEs.

Table 4.7 Correlation between REEs and textural characteristics during 2007 (pre-monsoon)

	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	V	Th	U	Sand%	Silt%	Clay%	OC(%)
La	1																				
Ce	***0.98***	1																			
Pr	***0.99***	***0.99***	1																		
Nd	***0.97***	***0.99***	***1.00***	1																	
Sm	***0.97***	***0.98***	***0.99***	***1.00***	1																
Eu	***0.80***	0.70	0.75	0.72	***0.78***	1															
Gd	***0.95***	***0.97***	***0.99***	***1.00***	***1.00***	0.75	1														
Tb	***0.93***	***0.95***	***0.97***	***0.98***	***0.99***	0.73	***0.99***	1													
Dy	***0.90***	***0.92***	***0.94***	***0.97***	***0.97***	0.70	***0.98***	***0.99***	1												
Ho	***0.91***	***0.93***	***0.95***	***0.98***	***0.98***	0.72	***0.99***	***1.00***	***1.00***	1											
Er	***0.89***	***0.91***	***0.94***	***0.96***	***0.97***	0.72	***0.98***	***0.99***	***1.00***	***1.00***	1										
Tm	***0.89***	***0.91***	***0.94***	***0.96***	***0.97***	0.71	***0.98***	***0.99***	***1.00***	***1.00***	***1.00***	1									
Yb	***0.89***	***0.91***	***0.94***	***0.96***	***0.97***	0.72	***0.98***	***0.99***	***1.00***	***1.00***	***1.00***	***1.00***	1								
Lu	***0.90***	***0.94***	***0.96***	***0.98***	***0.98***	0.68	***0.99***	***0.99***	***0.99***	***1.00***	***0.99***	***1.00***	***0.99***	1							
V	0.66	0.74	0.66	0.65	0.59	0.10	0.59	0.56	0.54	0.53	0.49	0.51	0.48	0.57	1						
Th	***0.85***	***0.85***	***0.81***	***0.77***	0.75	0.58	0.72	0.68	0.60	0.63	0.59	0.60	0.59	0.65	0.74	1					
U	0.70	***0.77***	0.71	0.68	0.62	0.22	0.61	0.55	0.50	0.53	0.49	0.50	0.48	0.57	***0.91***	***0.87***	1				
Sand%	-0.49	-0.57	-0.57	-0.61	-0.58	-0.12	-0.61	-0.66	-0.64	-0.64	-0.62	-0.63	-0.64	-0.65	-0.50	-0.38	-0.42	1			
Silt%	0.54	0.60	0.58	0.61	0.59	0.16	0.60	0.66	0.64	0.63	0.60	0.60	0.61	0.63	0.57	0.44	0.43	***-0.96***	1		
Clay%	0.39	0.49	0.50	0.56	0.53	0.06	0.56	0.60	0.60	0.61	0.60	0.60	0.61	0.62	0.37	0.28	0.37	***-0.95***	***0.84***	1	
OC(%)	0.58	0.64	0.63	0.66	0.63	0.20	0.64	0.68	0.65	0.66	0.63	0.63	0.64	0.67	0.58	0.53	0.50	***-0.98***	***0.98***	***0.89***	1

Bold italic underlined gives correlation is significant at 0.01 level
Bold italic gives correlation is significant at 0.05 level

Table 4.8 Correlation between REEs and textural characteristics during 2008 (pre-monsoon)

	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	V	Th	U	Sand%	Silt%	Clay%	OC(%)
La	1																				
Ce	***0.96***	1																			
Pr	***0.82***	***0.94***	1																		
Nd	0.63	***0.81***	***0.95***	1																	
Sm	0.34	0.56	***0.79***	***0.91***	1																
Eu	0.09	0.18	0.25	0.30	0.57	1															
Gd	0.26	0.45	0.67	***0.81***	***0.96***	0.69	1														
Tb	-0.01	0.18	0.41	0.60	***0.85***	***0.76***	***0.94***	1													
Dy	-0.19	-0.02	0.23	0.43	0.74	***0.78***	***0.87***	***0.98***	1												
Ho	-0.25	-0.11	0.12	0.30	0.65	***0.84***	***0.80***	***0.93***	***0.98***	1											
Er	-0.18	-0.03	0.21	0.39	0.72	***0.80***	***0.86***	***0.95***	***0.99***	***0.99***	1										
Tm	-0.27	-0.12	0.14	0.34	0.68	***0.76***	***0.82***	***0.94***	***0.99***	***0.99***	***0.99***	1									
Yb	-0.19	-0.03	0.21	0.39	0.72	***0.81***	***0.86***	***0.97***	***1.00***	***0.99***	***1.00***	***0.99***	1								
Lu	-0.35	-0.22	-0.02	0.17	0.54	***0.83***	0.71	***0.88***	***0.95***	***0.98***	***0.95***	***0.96***	***0.96***	1							
V	0.12	0.29	0.53	0.65	***0.87***	0.75	***0.93***	***0.87***	***0.86***	***0.85***	***0.89***	***0.85***	***0.87***	0.74	1						
Th	0.61	0.64	0.65	0.59	0.24	-0.57	0.06	-0.21	-0.37	-0.52	-0.41	-0.44	-0.42	-0.63	-0.13	1					
U	0.28	0.44	0.44	0.43	0.36	0.18	0.17	0.00	-0.09	-0.09	-0.11	-0.14	-0.08	-0.12	0.17	0.20	**1**				
Sand%	-0.53	-0.65	***-0.79***	***-0.78***	-0.60	0.21	-0.42	-0.14	0.00	0.11	-0.01	0.04	0.03	0.28	-0.35	***-0.82***	-0.33	1			
Silt%	0.36	0.48	0.61	0.62	0.41	-0.44	0.18	-0.07	-0.19	-0.31	-0.20	-0.22	-0.23	-0.44	0.12	***0.83***	0.41	***-0.95***	1		
Clay%	0.63	0.66	0.74	0.69	0.70	0.56	***0.76***	0.63	0.53	0.50	0.58	0.50	0.54	0.37	0.73	0.19	-0.11	-0.45	0.14	1	
OC(%)	0.69	0.72	0.73	0.66	0.68	0.70	0.72	0.62	0.51	0.48	0.52	0.44	0.51	0.41	0.62	0.06	0.08	-0.29	-0.01	***0.89***	1

Bold italic underlined gives correlation is significant at 0.01 level
Bold italic gives correlation is significant at 0.05 level

Sm is another REE which gave strong positive correlation with other REEs and positive correlation with Eu. In the case of Eu, it has no correlation with REEs, V, Th and U. So this gave an idea that the origin of Eu may be different from other REEs. The REEs like Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu showed similar correlation with other REEs. U showed strong positive correlation of 99% confidence with V and 95% confidence level with Th. In the case of texture characteristics, sand strongly shows negative correlation with silt and clay. This is an expected behaviour since mud (silt+ clay) dominates the study area where accumulation gathers more intensively. The organic carbon also displayed strong positive correlation with mud fraction and possess negative correlation with sand fraction, as it is evident that the organic matter is more attached with finer fractions like silt and clay than sand.

Correlation observed during 2008 (pre-monsoon) was entirely different from that of 2007 (pre-monsoon). La, exhibited positive correlation with Ce and Pr. Meanwhile Ce gave positive correlation with Pr and Nd. Similarly Pr positively correlated with Nd and Sm, the similar correlation repeated for Sm with Gd and Tb. A different correlation behaviour was observed for Eu in 2008 when compared with 2007 values. Unlike the year 2007, Eu gave a correlation with 95% confidence. Gd showed positive correlation with REEs like Tb, Dy, Ho, Er, Tm, Yb and transition metal V as well as with the clay fraction. No positive correlation was observed with Th and U for Gd. The REEs like Tb, Dy, Ho, Er, Tm and Yb showed similar correlation trend, besides strong positive correlation with each other. Th and U do not exhibit any significant correlation with REEs and V, while Th showed positive correlation with silt and strong negative correlation with sand. This phenomenon was expected since metals tend to attach with lighter fraction than with the heavier fraction. Clay fraction displays strong positive correlation with organic carbon indicating that the organic matter is bound to fine fraction.

C). Fractionation Indexes

Fractionation indices are represented by (La)n/(Yb)n ie, $\{(La_{sample}/La_{PAAS})/(Yb\ sample\ /Yb_{PAAS})\}$ratio, and it defines the relative behaviour of LREE to the HREEs (Figures 4.1 and 4.2). This ratio was calculated for all the samples during 2007 (pre-monsoon) and 2008 (pre-monsoon) and ratio ranges from 1.411 to 2.101 during 2007 and 1.494 to 3.231 in 2008 (Tables 4.9 and 4.10). This indicated that HREE are very much depleted for these stations during 2007 and 2008. The studies of Nath et al. (2000) supported the above results, whom had found significant LREE enrichment in the sediments from the Vembanad lake and the inner shelf sediments of off Cochin.

I) Ce-Anomaly as a Redox Indicator

The Ce-anomaly is calculated as follows

Ce-anomaly = $\log[2\ Ce_n/(La_n+Pr_n)]$ (Wilde et al. 1996)

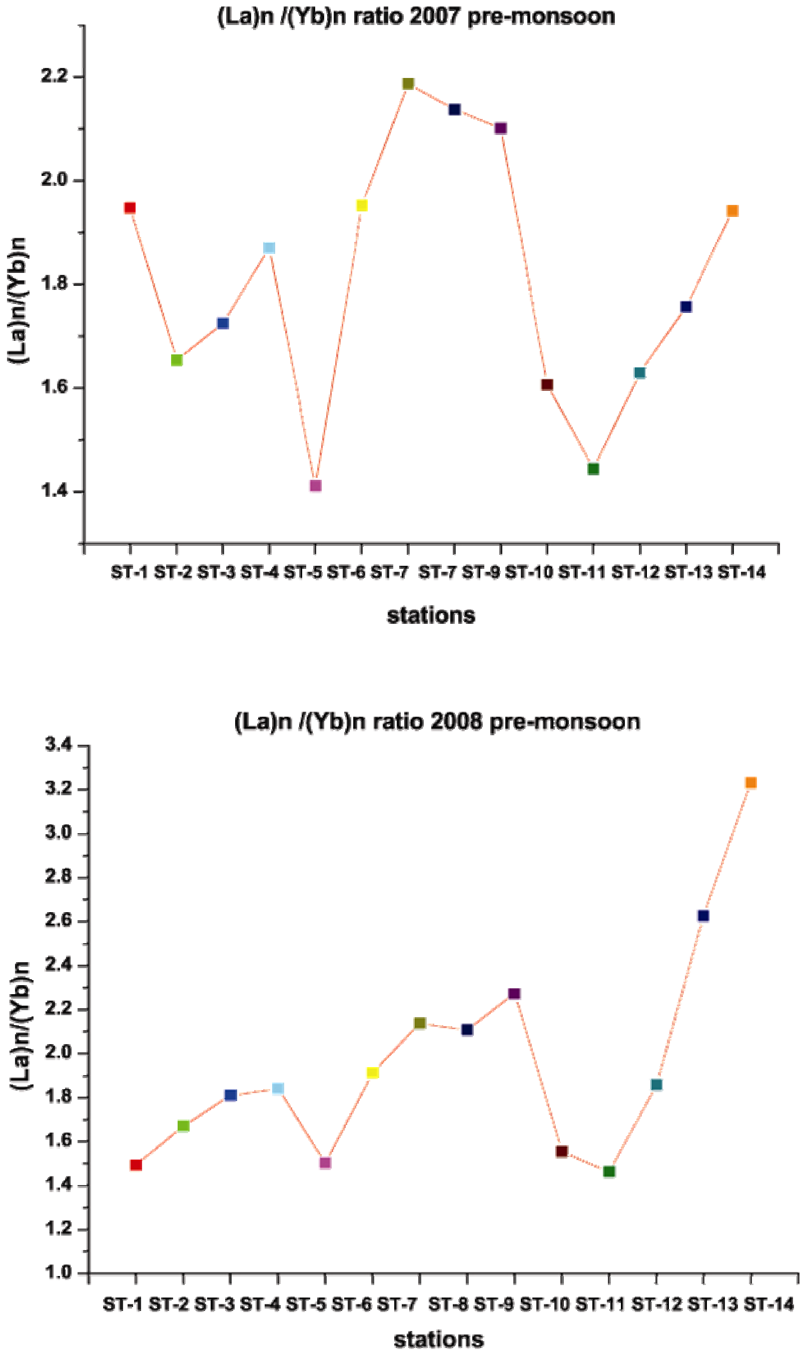
Where 'n' is the shale normalised concentration. The Ce-anomaly represents its enrichment or depletion compared to its neighbouring elements. No Ce-anomaly exists when the normalised Ce concentration falls exactly between La and Pr. In this research findings the REE are generally considered to be of terrigenous origin as reported by Piper (1974a). A depletion of Ce relative to its neighbour REEs give rise to a negative Ce-anomaly and may result from the presence of siliceous organisms or Bementite (Piper 1974 a,b; Tlig and Steinberg 1982; Pattan et al. 2005).

Table 4.9 Parameters indicative of oxygenation condition during 2007 (pre-monsoon)

Season	stations	(La)n/(Yb)n	Ce-Anomaly	Eu-anomaly	TOC	U/Th ratio	Au-U	Cu/Zn	V/Cr
pre-monsoon 2007	ST-1	1.948	-0.009	2.240	0.55	0.172	-1.024	0.124	0.339
	ST-2	1.654	0.014	2.873	2.59	0.247	-0.972	0.079	1.509
	ST-3	1.725	0.011	2.485	2.30	0.308	-0.173	0.211	1.285
	ST-4	1.870	-0.024	2.719	2.16	0.291	-0.291	0.410	1.754
	ST-5	1.411	0.039	2.273	2.21	0.261	-0.570	0.267	1.301
	ST-6	1.952	0.025	2.432	2.48	0.290	-0.289	0.202	1.470
	ST-7	2.187	0.019	2.120	2.51	0.302	-0.202	0.228	0.795
	ST-8	2.137	0.027	2.462	2.32	0.296	-0.378	0.282	1.280
	ST-9	2.101	0.032	2.479	1.80	0.255	-1.087	0.379	1.552
	ST-10	1.607	0.004	2.729	0.47	0.239	-1.039	2.460	0.680
	ST-11	1.444	0.033	2.543	0.12	0.238	-1.039	0.328	0.499
	ST-12	1.630	0.020	2.978	0.98	0.258	-0.841	0.479	2.828
	ST-13	1.757	0.037	2.328	2.00	0.337	0.042	0.307	4.440
	ST-14	1.942	0.030	1.914	0.98	0.370	0.226	0.174	2.342

Table 4.10 Parameters indicative of oxygenation condition during 2008 (pre-monsoon)

Season	stations	(La)n/(Yb)n	Ce-Anomaly	Eu-anomaly	TOC	U/Th ratio	Au-U	Cu/Zn	V/Cr
pre-monsoon 2008	ST-1	1.493	0.033	2.206	0.17	0.277	-0.406	0.220	3.624
	ST-2	1.670	0.022	2.784	1.67	0.263	-0.827	0.051	2.350
	ST-3	1.811	0.026	2.327	1.99	1.000	4.481	0.254	1.213
	ST-4	1.841	-0.037	2.586	0.92	1.000	4.607	0.322	1.300
	ST-5	1.501	0.035	2.612	1.13	0.254	-0.853	0.233	1.894
	ST-6	1.913	0.003	2.752	1.70	1.000	4.345	0.248	1.348
	ST-7	2.138	-0.023	2.253	1.89	1.000	4.447	0.225	0.680
	ST-8	2.108	0.022	2.377	1.61	1.000	6.879	0.976	4.026
	ST-9	2.273	0.030	2.608	1.64	0.400	0.676	0.984	4.726
	ST-10	1.554	0.035	2.648	1.02	1.000	7.394	0.169	0.780
	ST-11	1.462	0.037	2.573	0.72	1.000	7.274	0.782	1.681
	ST-12	1.857	0.038	2.588	0.49	0.521	1.563	0.257	4.142
	ST-13	2.626	0.033	1.593	0.26	0.267	-1.043	0.349	4.179
	ST-14	3.231	0.023	1.930	0.84	0.168	-2.160	0.379	1.526



Figures 4.1 & 4.2 La-Yb ratio during 2007 and 2008 (pre-monsoon)

A positive Ce-anomaly results, where Ce is enriched relative to its neighbours and might have resulted from the presence of Fe-Mn oxyhydroxides (Piper 1974b; Pattan et al. 2005).

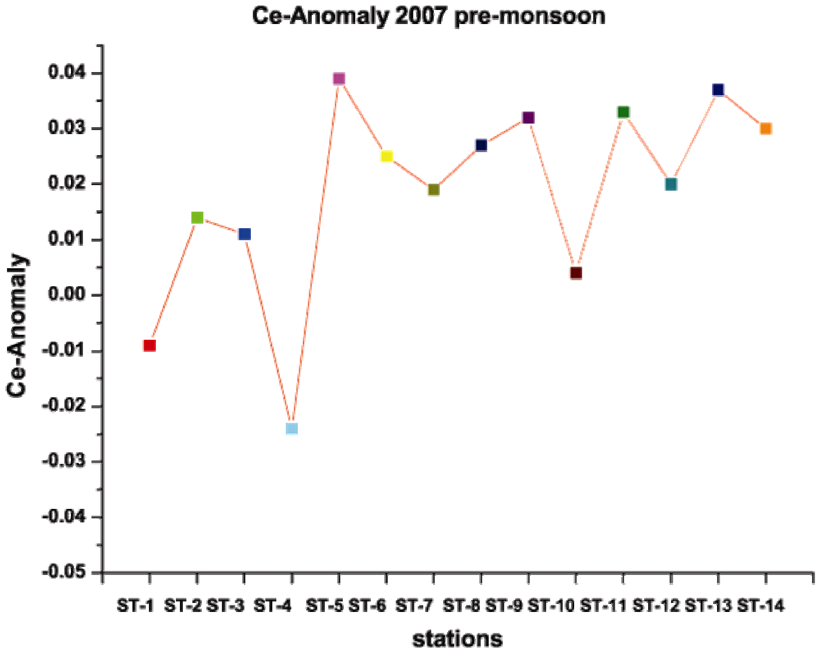
The Ce-anomalies of the study area during 2007 (pre-monsoon) varied from 0.920 to 1.029 and it ranged from 0.988 to 1.027, for 2008 (pre-monsoon) (Figures 4.3 and 4.4). Ce anomaly is positive in stations during the two consecutive years, which suggested that the behaviour of sediment deposition and oxic conditions. The positive Ce-anomaly could be attributed to the oxidation of Ce^{3+} to Ce^{4+} and incorporation into Mn oxyhydroxide phases as CeO_2. Sholkovitz (1995) also reported that the Ce-anomaly in the trophical rivers and estuaries are mainly due to the scavenging of Ce in oxides, principally iron oxyhydroxides. The fact that redox condition is formed from the Ce-anomaly alone in the sediments which could be confirmed by the other depositional parameters as they are sensitive to the redox condition.

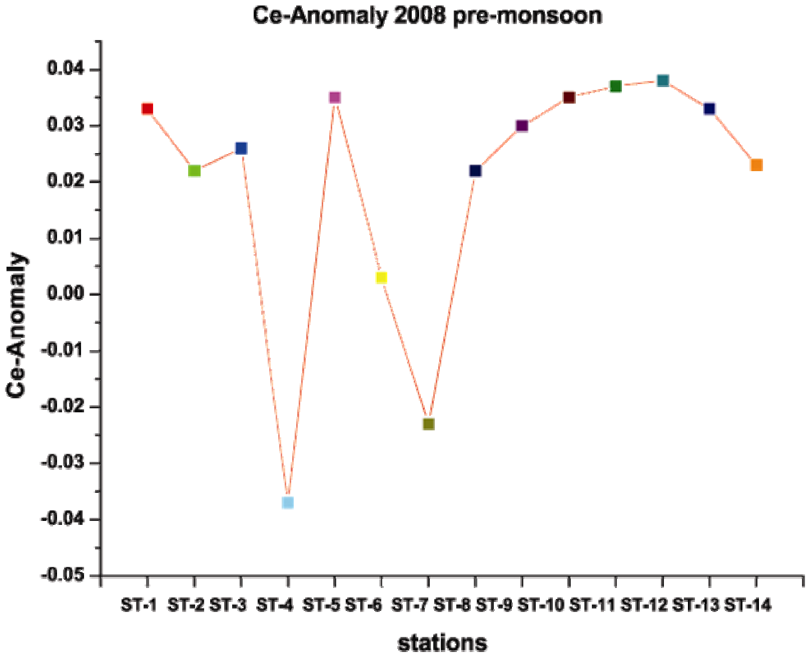
II) Eu-Anomaly

Eu –Anomaly = $Eu_{(normalised)} / Eu^*$ (Mascarenhas-Pereira and Nath 2010)

Where Eu^* is $[Sm_{(normalised)} / Gd_{(normalised)}]/2$

Eu anomaly is represented as Eu/Eu* where, Eu is the concentration of Eu in the sediments normalised with shale value and Eu* is a predicted value obtained by linear interpolation of Sm/Sm* and Gd/Gd*. Value greater than 1 and less than 1 indicate positive and negative anomalies respectively. A value of 1 indicates no anomaly.





Figures 4.3 & 4.4 **Graph showing Ce-anomaly during 2007 and 2008 (pre-monsoon)**

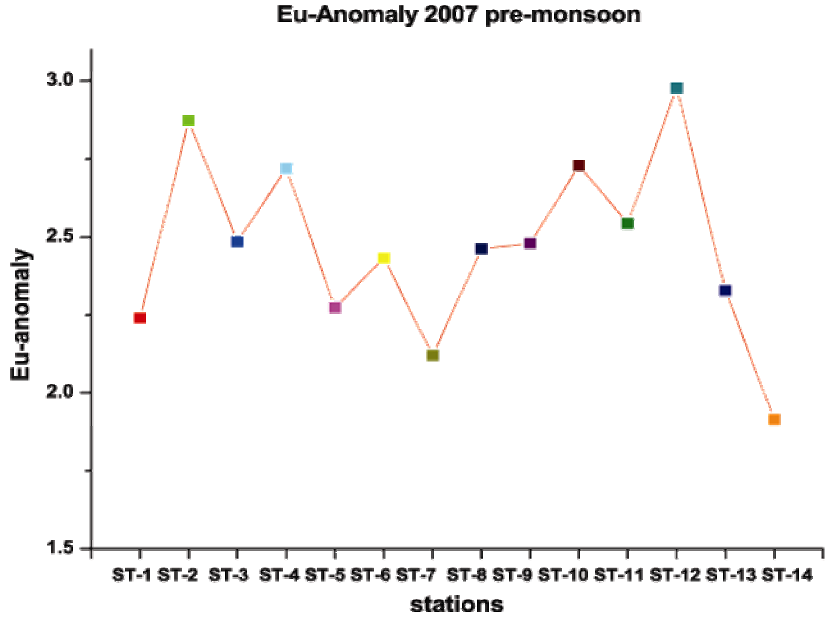
The positive Eu anomaly occurs in areas prominent in hydrothermal vents or due to the effect of hydrothermal vents in the nearby areas means of feldspar origin (Rudick 1991).

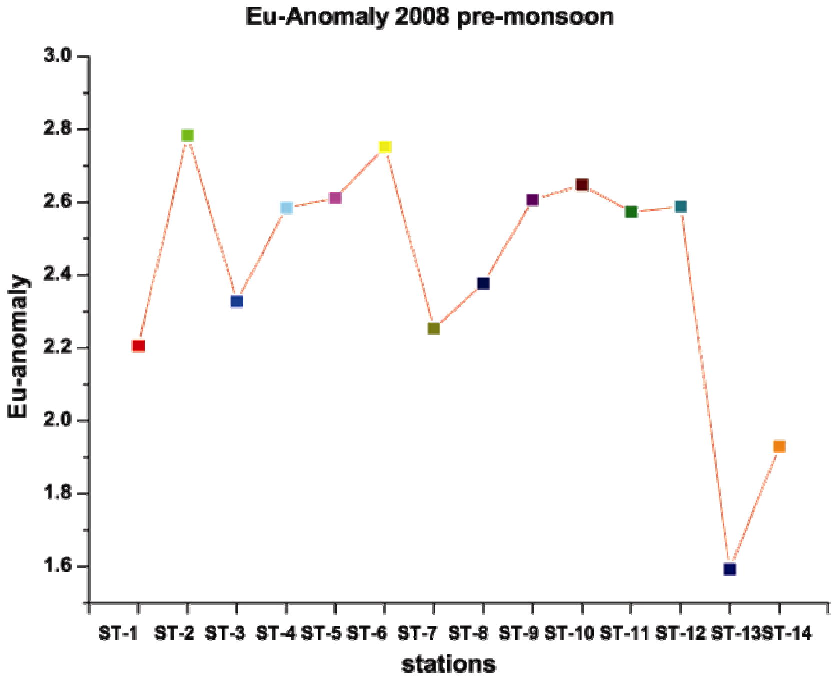
Eu anomaly values varied from 1.914 to 2.978 in 2007 (pre-monsoon) and it ranged from 1.593 to 2.784 during 2008 (pre-monsoon) (Figures 4.5 and 4.6). All the stations showed values greater than 1. This gave an idea that the input may be from hydrothermal vent system or from feldspar origin whereas in the present study area there is no hydrothermal vent system, which meant that it may have originated from feldspar. Mascarenhas-Pereira and Nath (2010) also supports the same trend.

III) Geochemical parameters indicative of oxygenation condition

A number of parameters such as Mn, total organic carbon, U/Th, Authigenic U, Cu/Zn, V/Cr, sulphur to organic carbon, and stable isotopes are all widely used as a constraint on the depositional environments (Kuspert 1982; Jones and Manning 1994; MacLeod and Irving 1996; Pattan et al. 2005). Some of these above mentioned parameters were used in the present study to understand the depositional characteristics.

The total organic carbon content in the study area varied from 0.55% to 2.59% during 2007 and 0.17% to 1.67% during 2008 (Figures 4.7 and 4.8). The carbon /nitrogen ratio in the stations ST-6, ST-7 and ST-8 suggested that the source character was similar to that found in the study of Pattan et al. (2005). These stations showed low organic carbon content, and thus attributed to less sediment accumulation rates, which allows more mineralisation at the sediment-water interface. The estuarine regions may facilitate extensive mineralisation of organic matter during settling through a water column resulting in a low organic carbon content.





Figures 4.5 & 4.6 Graph showing Eu-anomaly during 2007 and 2008 (pre-monsoon)

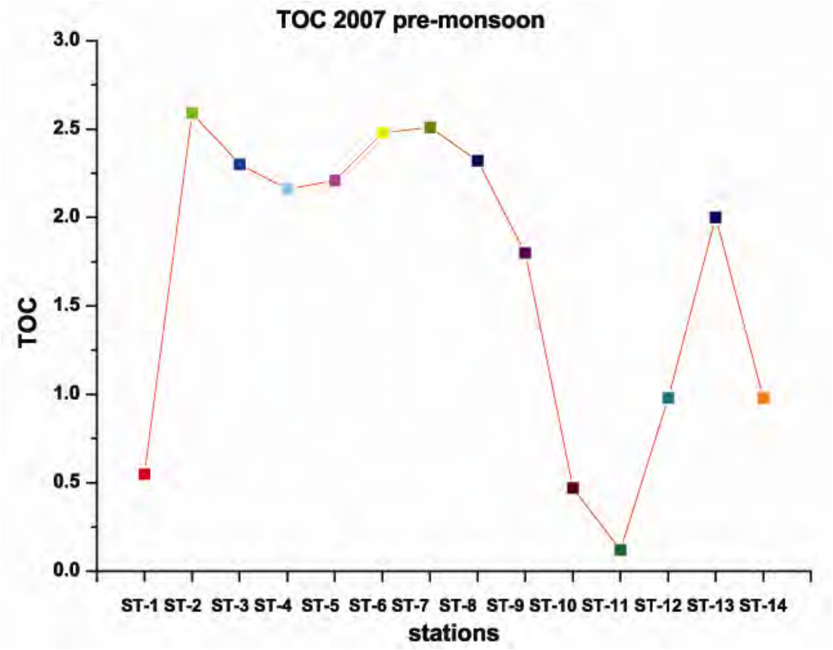
The low and almost constant organic carbon content thus suggests a well oxidised environment and earlier works of Warren (1982) contributes this outcome.

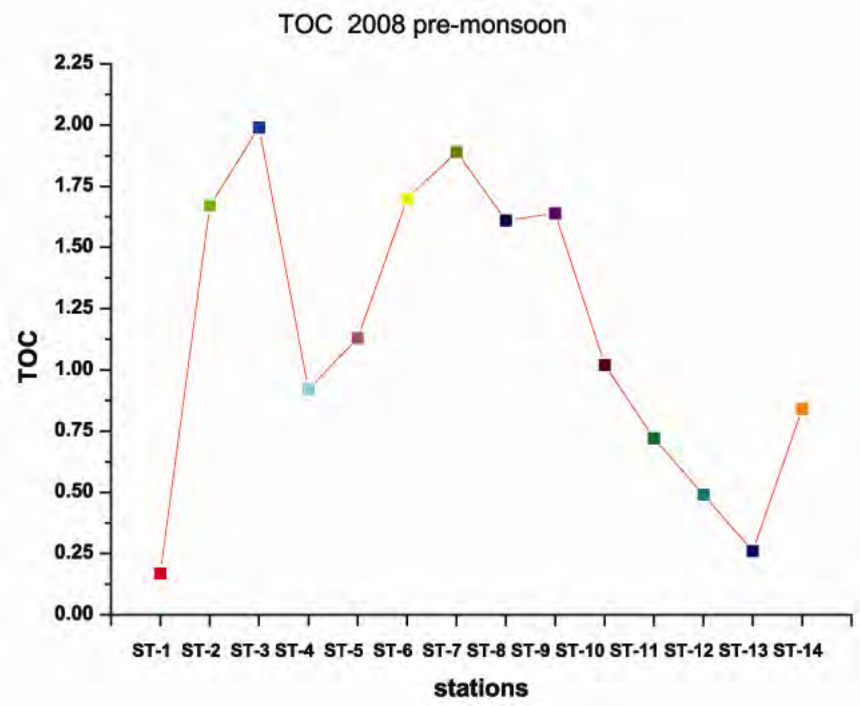
In oxic water U^{6+} is present as Uranyl tricarbonate species, which is highly soluble. In the case of oxygen deficient or reducing environments, U^{6+} is reduced to U^{4+} which is particle-reactive and can become fixed onto sediment (Klinkhammer and Palmer 1991). But in the case of Th, it is relatively immobile (Anderson et al. 1983). The low U contents in stations during 2007 (pre-monsoon) and 2008 (pre-monsoon) indicated that the oxic environment. Station ST-5 showed the presence of Uraninte, a mineral of U and also estimated a higher concentration than PAAS. Because of the difference in behaviour of U and Th as in U/Th ratio, can be valued as a redox index with high ratio (>1.25) and associated with anoxic environment. The low ratio (0.75) associated with oxic environment as supported by Jones and Manning (1994). In 2007, the U/Th ratio ranged from 0.172 to 0.370 and in 2008 it varied from 0.168 to 1.00 which indicated the oxic environment in the studied area (Figures 4.9 and 4.10).

Uranium is one of the interesting proxies of redox condition in marine sediments because it is conservative in the oxygenated waters but becomes enriched in anoxic sediment (Yamada and Tsunogai 1984). Authigenic U content has been proposed as an index of bottom water anoxia (Wignall and Myres 1988) and is calculated as,

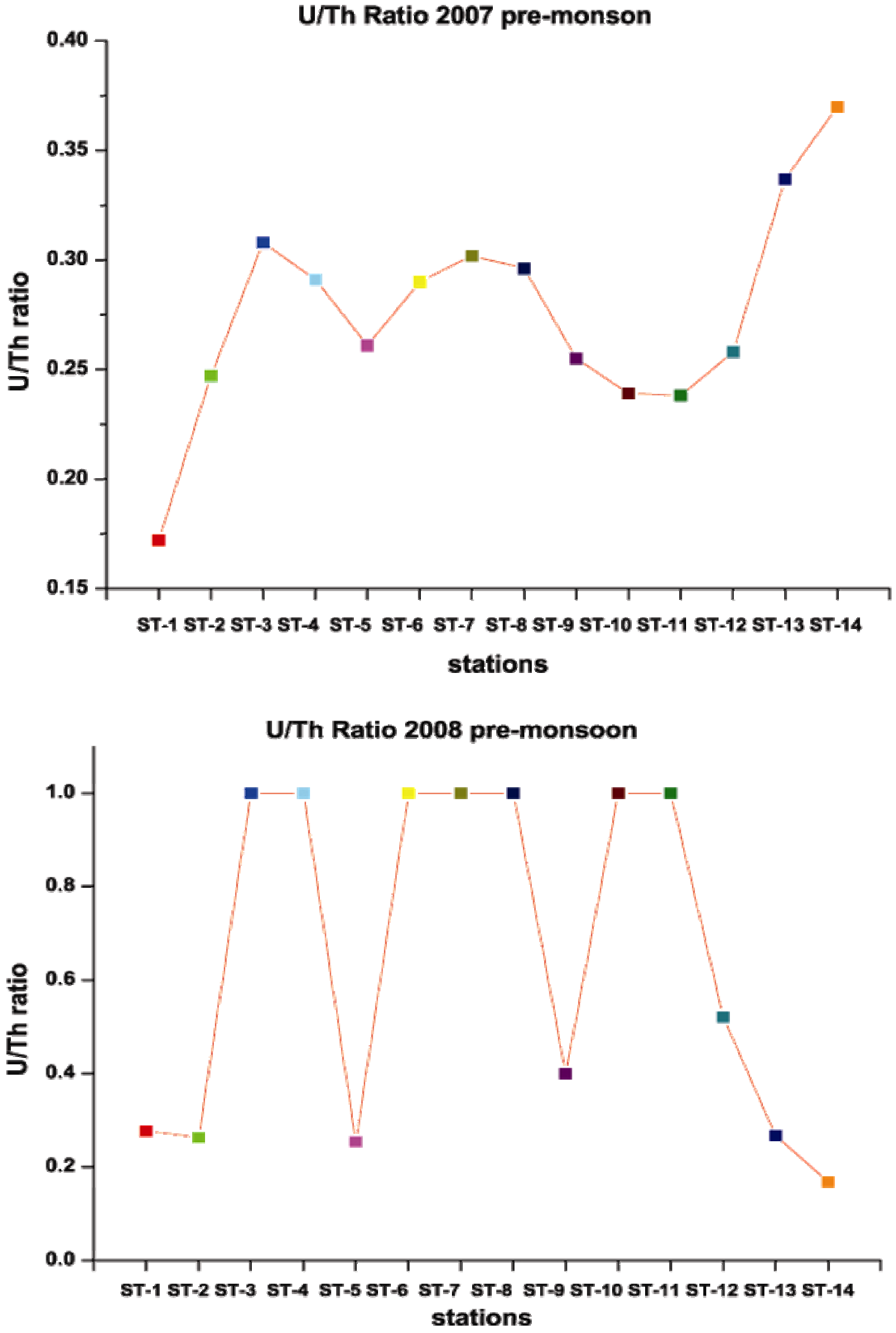
Authigenic U = (total U) - Th/3

Uranium enrichment in sediments tends to be deposited under anoxic conditions which allow large amount of organic matter to accumulate and

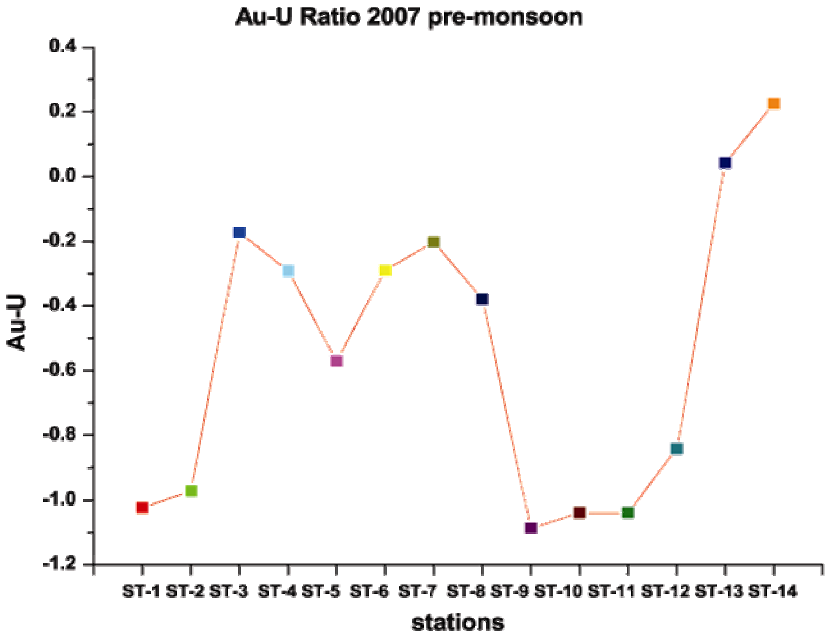


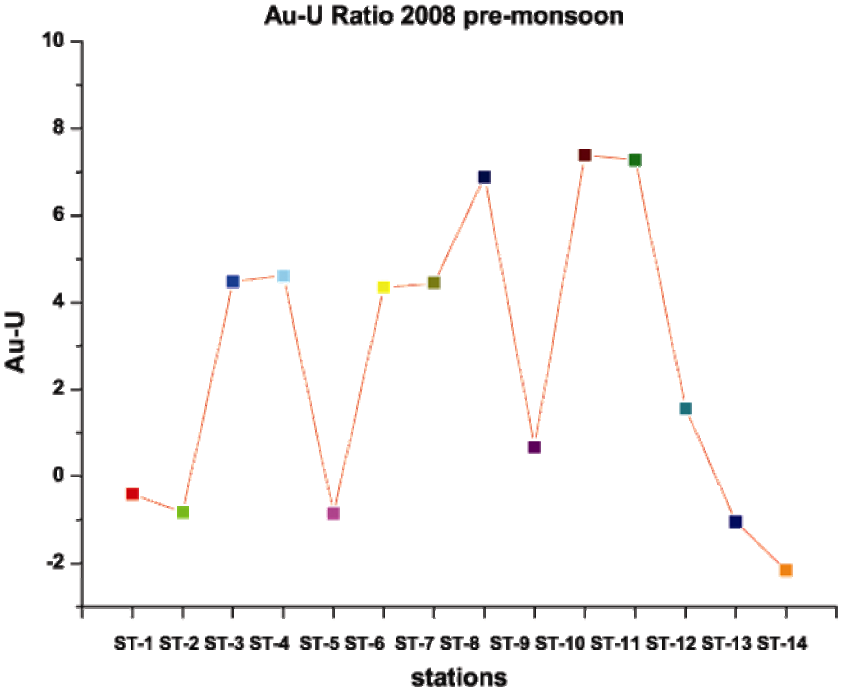


Figures 4.7 & 4.8 **Graphs showing TOC %o during 2007 and 2008 (pre-monsoon)**



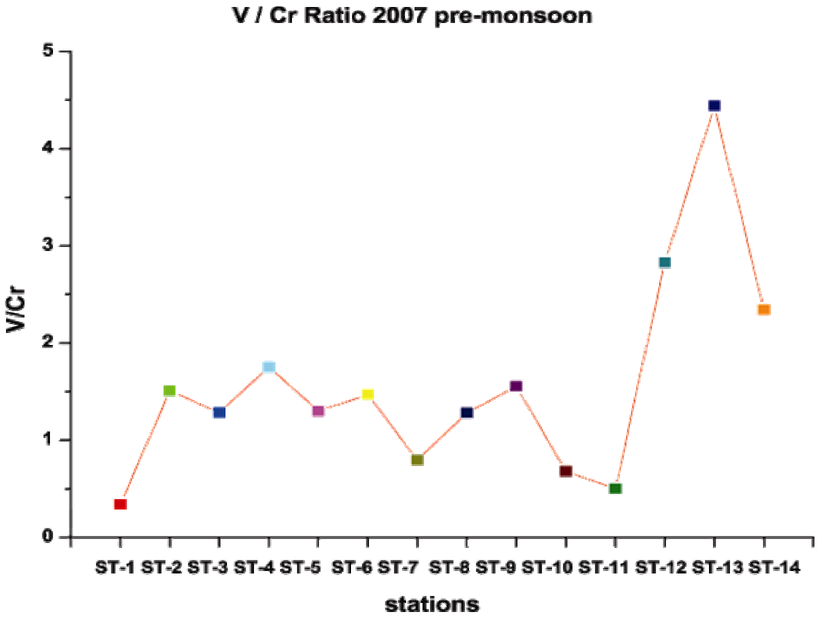
Figures 4.9 & 4.10 Graphs showing U-Th ratio during 2007 and 2008 (pre-monsoon)

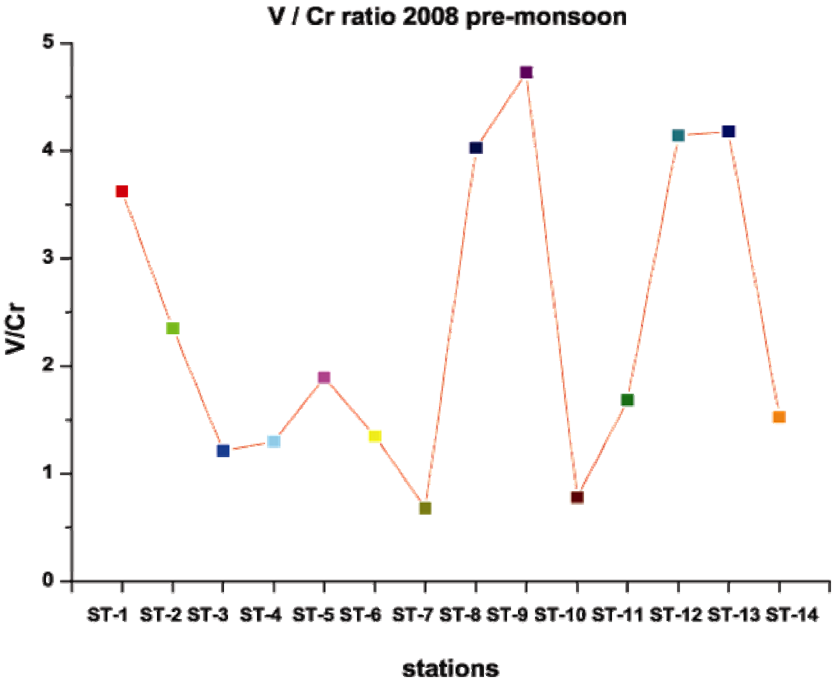




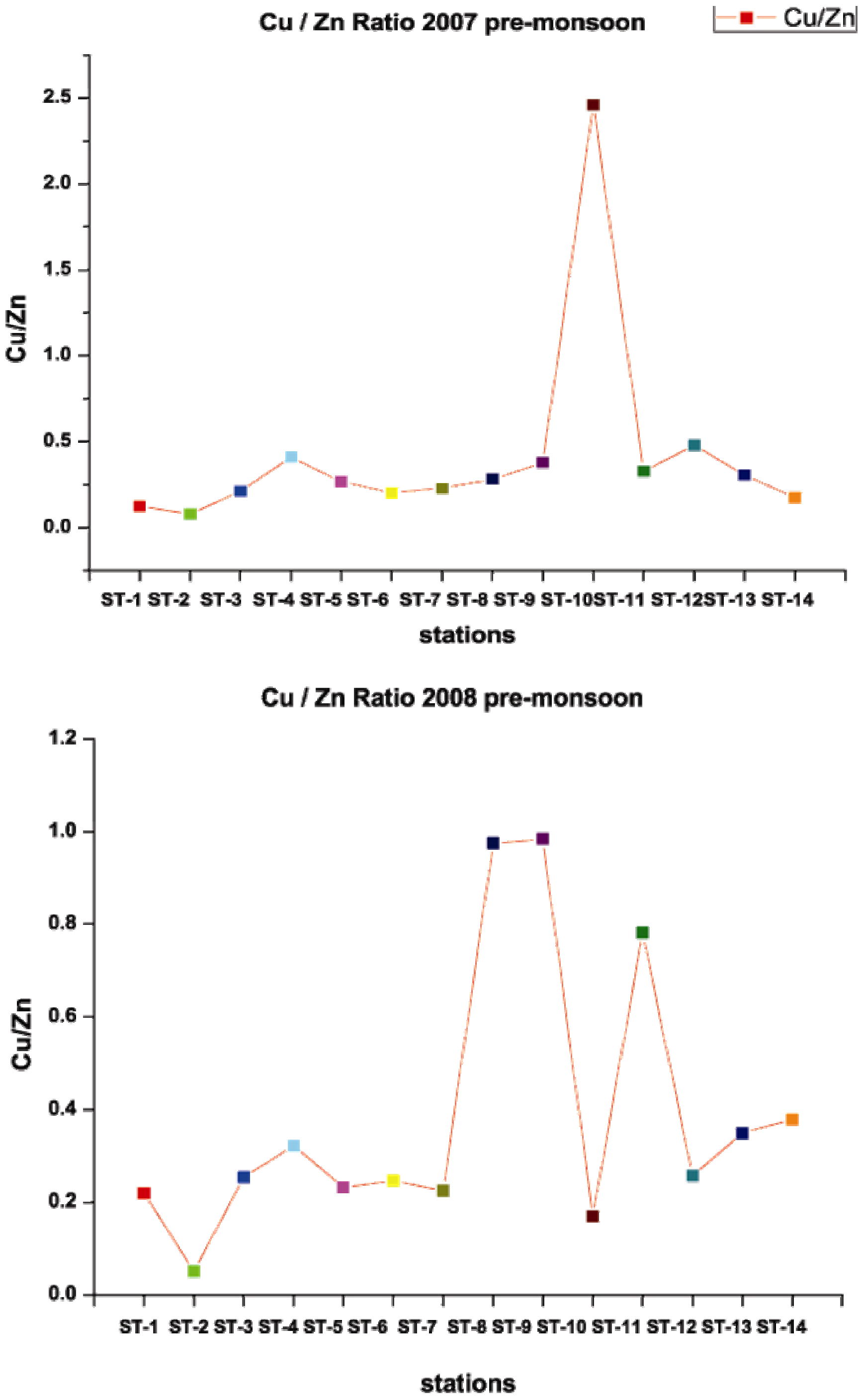
Figures 4.11& 4.12 Graphs showing Au-U ratio during 2007 and 2008 (pre-monsoon)

U to be fixed (Wignall and Myres1988). The absence of any Authigenic U content in the sampled sediment reveals an oxic environment, as evidenced in the study of Jones and Manning (1994), suggested that Authigenic U content of <5 ppm are considered to be indicative of oxic environments. In the present study, all the stations except Perumbalam (ST-13) and Panavali (ST-14) in 2007 and Poothotta (ST-4) and Bund (ST-5) of 2008 showed slight positive values (Figures 4.11 and 4.12). From these fractional indexes, it is concluded that an oxic environment is present in sediments of the study area, except at St-8, ST-10 and ST-11 during 2008. In oxygenated waters the stable form of vanadium is vanadate, and it is adsorbed onto both Fe and Mn oxyhydroxide phases (Wehrli and Stummm 1989). In the absence of oxygen, vanadate is reduced to vanadyl. Chromium, in contrast, is relatively insoluble and in these conditions it is usually held within the detritus fraction. The vanadium varied from 5.303 ppm to 73.628 ppm in 2007 (pre-monsoon) and 10.373 ppm to 93.094 ppm in 2008 (pre-monsoon) and Cr varied from 7.575 ppm to 26.438 ppm in 2007 (pre-monsoon) and 2.888 ppm to 29.813 ppm in 2008 (pre-monsoon) in the study area and are lower than PAAS values (Talyor and McLennan 1988). The V/Cr ratios of sediment (Figures 4.13 and 4.14) have also been suggested as an index of bottom water oxygenation and the ratio > 4.5 and <2 are suggestive of anoxic and oxic conditions respectively (Jones and Manning 1994). In the present study, ratio varied from 0.339 to 4.440 during 2007 (pre-monsoon) indicating an oxic environment. During 2008 pre-monsoon ratios varied from 0.680 to 4.726, suggesting an oxic environment. Hallberg (1976) previously indicated that the Cu/Zn ratio in sediment may reflect redox conditions of the ancient environment and the ratio increased in reduced conditions and decreased in oxidising conditions.





Figures 4.13 & 4.14 Graphs showing V/Cr ratio during 2007 and 2008 (pre-monsoon)



Figures 4.15 & 4.16 Graphs showing Cu/Zn ratio during 2007 and 2008 (pre-monsoon)

The Cu/Zn ratio in the sediments (Figures 4.15 and 4.16) varied from 0.079 to 2.460 during 2007 (pre-monsoon) and 0.051 to 0.984 in 2008 (pre-monsoon) pointing that there is no drastic changes in the depositional environment. So these ratios suggested that an oxic environment is prevailing along the studied locations.

4.4 Conclusion

The Rare Earth Elements and their ratios are usually employed as proxies to understand the redox behaviour and origin of sediments around the world. This study is the first attempt to understand the redox conditions of Cochin estuarine system using rare earth elements. The ratios like Ce-anomaly and Eu-anomaly were used to understand the sedimentary behaviour. In recent years, much attention has been paid to the behaviour of Ce, and particularly the Ce-anomaly in marine environment to understand the redox conditions. The positive Ce-anomaly indicated an oxic environment prevailing in the surface sediments of the area under study. The Eu anomaly is normally used to record the origin of sediment. The study of Eu anomaly indicated that its source is feldspar. The other proxies such as Mn, total organic carbon, U/Th ratio, Authigenic-U, Cu/Zn ratio, V/Cr ratio were also useful to predict the environmental behaviour. The total organic carbon content was very low and almost constant which was an indication of oxidised environment. U/Th ratio is another important proxy in which U concentration varies with change in environment, which in turn affect U/Th ratio. The lower U/Th ratio is recording oxic environment, where U is mostly in the water phase. Th concentration did not vary considerably. The Authigenic-U gave an idea of oxic environment exhibited in the study area. Cu/Zn and V/Cr ratios are the two other proxies to predict the environmental behaviour. Both Cu/Zn and V/Cr ratios indicated the oxic environment prevailing in the studied locations. These results endorse the oxic environment existing in the studied location of the estuary. The trace metals are regarded as major pollutants of the estuaries and coastal systems. Some of these trace metals are also used in antifouling biocides as pigment and co-biocides. Therefore the next chapter viz chapter 5 mainly focus on the major trace metals used in biocide industry and their residues in the sediments of the selected sampling locations of the Cochin estuarine system.

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Chapter 5

Role of Trace Metals as Biocides

Contents

5.1 Introduction

In many aquatic systems, deposition of contaminants, including heavy metals, can lead to elevated sediment concentrations that have the potential to cause toxicity to aquatic biota (Yang and Rose 2003; Heyvart et al. 2000). Because of the importance of sediments to the overall quality of the aquatic systems, their analysis is often included in the environmental assessment and enough literature exists in this field of study (Adekola and Eletta 2007; Li et al. 2006; Jain et al. 2005 Horsfall and Spiff 2002). Sediments and suspended particulate matter (SPM) play an important role in the adsorption of dissolved metals and later releasing them to the water column under changing physical and chemical conditions (Karbassi et al. 2007). The levels of certain trace elements in rivers, lakes, estuaries and other water systems have been found to be moderately polluted or very highly as a result of industrial discharges (Coker et al. 1995; Al-Masri et al. 2002). It is known that sediments play a significant role in controlling the metal concentrations in many of these aquatic environments and therefore the behaviour of metals, including sedimentation and resuspension have attracted the attention of many researchers (Bellucci et al. 2003; Betrolotto et al. 2003). It is generally believed that metals bound in the form of carbonate, sulphides and organic compounds are more toxic due to their high bioavailability and are more critical in the stand point of environmental risk assessment. The overall effects, pathways, bioavailabilities and fates of trace metals in the marine environment are strongly influenced by their aqueous speciation, of particular significance in antifouling formulations is the relative contribution of organometallic or hydrophobic species of the metals.

With the phasing out and ultimate ban on triorganotin (TOT) formulation, most contemporary marine antifouling paints contain a Cu(I)-based biocide pigment (e.g. Cuprous oxide or less commonly, cuprous thiocynate). Zinc oxide is sometime used as the principal, although relatively weak biocide pigment, but is more generally used in combination with Cu(I) as a booster, increasing the toxicity of the latter by 200 fold, and to impart flexibility and facilitate the erosion process of the coating (Waterman et al. 2005). Some diatoms and algae are resistant to inorganic Cu & Zn, the antifouling properties of contemporary formulations are further enhanced by the addition of one or more secondary or booster co-biocides. These include Zinc and Copper Pyrithione, Irgarol 1051, Chlorothalonil, TCMS pyridine, Sea-Nine 211, Ziram, Zineb, Dichlofluanid and Diuron.

In this chapter, the aim is to investigate the enrichment of sediment bounded trace metals (Cu, Cr, Cd, Zn, Pb, Ni & Sn) in the identified sampling sites of Cochin estuary as its contributing role in antifouling biocides extensively used in shipping activities. Table 5.1 gives the previous studies related to metals in various estuaries and coastal regions around the world.

Table 5.1 Previously reported studies on trace metals in estuaries and coasts

Area	Trace Metals							References
	Cd	Cu	Cr	Ni	Pb	Sn	Zn	
Cauvery Estuary, India	-	2.0-46	6.0-85	-	12.0-47	-	14-120	Ramanathan et al. 1993
Vellar estuary, India	4.0-9.0	4.0-20	28-43	15-31	4.0-8.0	-	71-122	Ramanathan et al. 1999
Brisbane and Logan estuaris, Australia	-	0.0-67	1.0-85	6.0-75	7.0-107	-	1.0-246	Cox and Preda 2005
Cochin estuary, India	0.8-1.8	5.0-61	-	-	-	-	116-1385	Paul and Pillai 1983
Cochin estuary, India	0.06-4.2	0.17-1.12	0.50-3.5	-	-	-	0.20-192	Jayasree and Nair 1995
Cochin estuary, India	-	5.4-53.2	15-121	16-66.5	19.3-71.3	-	92-1266	Balachandran et al. 2006
Suez Bay	2.26-4.40	1.84-10.25	-	-	13.90-28.34	-	4.26-23.68	El-Moselhy and Gabal 2004
California, USA	31-81.9	51-229	-	-	55-165	-	276-3193	Muochi et al. 2003
Gulf of Paua	0.02-.011	8.0-29	14-128	10.0-41	4.0-34	-	12-113	Haynes and Kwan 2002
Cadiz and sancti petri channel, spain	0.22 ± .02	34.5 ± 2.5	53.19 ± 1.73	24.2 ± 1.1	34.01 ± 5.14		88.73 ± 2.5	Rodriguez et al. 2009
Nemrut Bay, Aegean Sea	0.161	31.83	69.55	50.41	65.16		196	Esin Esen et al. 2010
Tamaki estuary, New Zealand	0.28	34.5			73.3		207.2	Abrahim and parker 2008
Soil, Malta	0.58 ± 0.4	37.29 ± 11	47 ± 8.2		53.14 ± 35	1.24 ± 0.5	83.86 ± 28	Turner et al. 2009
Tamer estuar, UK	0.42 ± 0.05	145 ± 16		26.4 ± 1.3	152 ± 13.2	66.1 ± 19.7	265 ± 16.5	Anu singh and Turner 2009
Temerton Lake, UK	0.567 ± 0.72	160.59 ± 5.84	31.74 ± 1.43	34.45 ± 2.21	135.63 ± 7.42	16.159 ± 0.66	236.5 ± 19.89	ranjith varma et al. 2011
Pandoh Lake, Lesser Himalaya, India	3.2 ± 1.59	10.1 ± 1.99		36.8 ± 7.18	6.40 ± 2.03		23.2 ± 1.80	Anshumali et al. 2009
Matla, India		24		29	13		45	Debapriya Mukherjee et al. 2009
Saptamu Khi, India		23		30	9		35	Debapriya Mukherjee et al. 2009
Hugli, India		32		33	14		44	Debapriya Mukherjee et al. 2009
NW Black Sea	0.02-3.99	2-108	1-135	1-207	0.5-50	-	1-185	Secrieru and Secrieru 2002

5.2 Results

Seven trace elements (Cu, Zn, Cd, Cr, Ni, Pb and Sn) were analysed in order to understand their toxic role in antifouling paints and they are mainly used in antifouling industries. The table 5.2 shows the results of varying levels in trace metal concentrations in studied location during 2007 (pre-monsoon) and 2008 (pre-monsoon). The concentrations of Cu during pre-monsoon, 2007 varied as 7.58-26.44 ppm (average 18.02 ppm) and 2.89-29.81 ppm (average 15.56 ppm) in 2008. Zn concentrations ranged from 3.13 to 333.75 ppm (average 84.51 ppm) in 2007 and 5.63 to 585.00 ppm (average 88.13 ppm) in pre-monsoon, 2008. The Cd content during 2007 showed 0.16- 4.28 ppm (average 1.26 ppm) and in 2008 it was noted as 0.19 - 7.06 ppm (average 1.15 ppm). Cr concentration was 13.11-64.90 ppm (average 30.80 ppm) and 4.81-76.83 ppm (average 37.34 ppm) during 2007 and 2008 respectively. The Ni concentration ranged from 10.89 to 61.93 ppm (average 38.99 ppm) during 2007 (Pre-monsoon) and 5.91 to 60.74 ppm (average 32.23 ppm) in 2008 (Pre-monsoon). The Pb showed 4.38–26.13 ppm (average 15.30 ppm) in 2007 pre-monsoon and 1.88– 24.63 ppm (average 14.24 ppm) in 2008 (pre-monsoon). In the case of Sn, it resulted as 1.87 to 8.68 ppm (average 4.75 ppm) in 2007 (pre-monsoon) and 0.93 to 10.66 ppm (average 4.76 ppm) during 2008 (pre-monsoon).Fe concentration varied from 2.16% to 8.22% in 2007 (pre-monsoon) and 0.87% to 8.36% in 2008 (pre-monsoon). Present study Fe used as metal normaliser in study location.

Table 5.2 Distribution of trace metals in the sediments of study area during 2007 and 2008 (pre-monsoon)

Season	stations		Cd (ppm)	Cr (ppm)	Cu (ppm)	Ni (ppm)	Pb (ppm)	Zn (ppm)	Sn (ppm)	Fe %
Pre-monsoon 2007	Barmouth	ST-1	0.69	15.66	8.83	19.38	11.50	71.25	3.93	2.84
	Bolgatty	ST-2	4.28	36.58	26.21	49.38	26.13	333.75	6.62	5.93
	Sulphur	ST-3	1.45	36.24	21.38	48.98	20.50	101.25	6.30	5.93
	Shipyard	ST-4	1.05	30.38	23.85	44.49	17.25	58.13	8.68	7.64
	Thevara	ST-5	1.70	36.60	23.18	59.96	16.63	86.88	7.86	6.94
	F.H. mattanchery	ST-6	1.03	29.88	18.90	41.63	16.63	93.75	7.55	4.75
	IOC	ST-7	1.86	64.90	24.38	59.03	19.50	106.88	6.52	7.57
	Kumbalam	ST-8	1.50	48.39	26.44	61.93	20.63	93.75	3.74	8.22
	Poothotta	ST-9	0.79	47.45	20.59	38.61	13.50	54.38	3.74	6.54
	M.P	ST-10	0.16	13.11	7.69	10.89	4.38	3.13	2.08	2.16
	I.P	ST-11	0.46	14.51	7.58	13.64	7.00	23.13	1.87	2.81
	Bund	ST-12	0.65	18.86	10.48	21.84	10.00	21.88	2.22	4.47
	Perumbalam	ST-13	1.06	15.23	21.48	49.83	18.13	70.00	2.15	6.53
	Panavali	ST-14	1.03	23.44	11.30	26.26	12.38	65.00	3.26	4.39
Pre-monsoon 2008	Barmouth	ST-1	0.75	4.81	2.89	5.91	5.75	13.13	0.93	0.87
	Bolgatty	ST-2	7.06	39.61	29.81	49.64	24.63	585.00	5.28	7.66
	Sulphur	ST-3	1.63	40.23	25.90	57.88	21.50	101.88	7.09	1.08
	Shipyard	ST-4	1.55	44.79	21.76	34.04	17.38	67.50	10.66	5.30
	Thevara	ST-5	1.20	37.69	21.10	50.26	20.13	90.63	8.59	5.69
	F.H.Mattanchery	ST-6	1.96	34.86	26.93	60.74	22.13	108.75	8.28	7.69
	IOC	ST-7	1.81	76.83	21.83	56.38	19.63	96.88	5.59	8.36
	Kumbalam	ST-8	0.79	15.78	11.59	19.51	9.75	11.88	4.57	3.27
	Poothotta	ST-9	0.59	8.55	8.61	12.88	9.13	8.75	3.32	2.49
	M.P	ST-10	0.68	13.30	4.44	20.73	1.88	26.25	3.25	4.14
	I.P	ST-11	0.19	7.53	4.40	6.83	3.88	5.63	3.46	1.48
	Bund	ST-12	0.93	16.29	11.74	28.40	15.13	45.63	1.80	4.23
	Perumbalam	ST-13	0.61	11.28	4.15	7.59	6.38	11.88	2.08	1.49
	Panavali	ST-14	1.26	31.23	22.75	40.43	22.13	60.00	1.80	6.94

5.3 Discussion

5.3.1 Spatial variability of trace metal concentrations in sediments and its comparison with average shale values

Very low concentration of copper is essential for organisms and several copper-containing proteins have been identified in biological system. Copper can exist in aquatic environments in three broad categories; in particulate/colloidal, in sediments, and in soluble form. It sorbs rapidly to sediments, and it's desorption into the bulk water depends on pH, salinity and the presence of natural and synthetic chelating agents. Copper is one of the main trace metals which are used in antifouling biocide industry. The world average shale value of Cu is found to be 25 ppm. In the present investigation, the sampling stations, ST-2 and ST-8 in 2007 and ST-2, ST-3 and ST-6 in 2008 showed slightly higher values than world average. Stations ST-2 and ST-8 are located the riverine end, and in the banks of the river, major industries like, petroleum refining, fertilizer plants, insecticide producing companies, Zn manufacturing companies are situated. These stations are further influenced by boating and shipping activities. One of the major contributing factors of Cu is the release from the antifouling paint coating from the ships and boats. Thus, considerable enrichment of Cu residues in the harbour and shipping channel sediment samples exists. It may be attributed to the use of Cu_2O and other Cu based pigment in contemporary and historic antifouling formulations. In the semi-enclosed waters of the harbours, Cu(I) is readily leached from the hulls of resident and visiting boats/ship and, after oxidation, Cu^{2+} ions are able to adsorb to suspended particles which may settle on the harbour bed. Paint fragments and dusts generated during boat maintenance are also a direct source of particulate residual contamination in such settling, besides the shipyard region where boats are repaired and repainted (Turner, 2010).

The shale average of Cd is found to be 100 ppb (Turekian and Wedephol 1961). During two years of study period, Cd showed values higher than shale average, indicating that the study area is highly contaminated with this metal. Station ST-2 was extremely contaminated with Cd during 2007 and 2008. Station ST-2 is located in the lower arms of river Periyar where India’s one of the major industries are located. Greenpeace has considered this as one of the hot spot of contamination in this area. Another major contributor of Cd concentration is from shipyard activities. Because of it antifouling character, Cd is used in biocide industry. In order to understand Cd dynamics in the Cochin estuary, more studies are to be conducted in coming future. The high flocculation observed in this station is also a contributing factor for the elevated concentration of Cd.

The main source of Cr in water and sediments are from electroplating, steel manufacturing, leather tanning, textile industries and from ship hulls as antifouling paints biocides. The hexavalent Chromium is widely known for its toxic effects on humans and animals, within the threshold limit it is an essential trace element. The shale average of Cr is 126 ppm (Turekian and Wedephol 1961). All the studied area showed the range well within the average shale value which implies that it is not causing much pollution threat with Cr during 2007 (pre-monsoon) and 2008 (pre-monsoon).

The average shale value of Ni is found to be 56 ppm (Turekian and Wedephol 1961). Mainly nickel is used for the production of stainless steel and other nickel alloys with a high corrosion and temperature resistance. Nickel can enter surface waters from natural sources such as from particulate matter, through rain water and through the dissolution of bed rock mineral and soil phases (Boyle 1981). It may also be deposited in the sediments by precipitation, complexation and adsorption on clay particles, and via uptake by biota. The release of nickel from sediments may occur as a result of microbial activity or changes in the physical and chemical parameters such as pH, ionic strength and sorption processes (Di Toro et al. 1991). The present, investigation resulted the Ni concentration ranging from 10.85- 61.92 ppm during the first year and 5.913- 60.73 ppm during the second year. Stations ST-5, ST-7 and ST-8 in 2007 and ST-3, ST-6 and ST-7 in 2008 showed slightly higher values than the world averages.

The average shale value of Zn is 65ppm by Turekian and Wedephol (1961). The estuarine values are higher than world average. Stations ST-10, ST-11 and ST-12 during 2007(pre-monsoon) and ST-9, ST-10, ST-11, ST-12 and ST-13 during 2008 (pre-monsoon) were observed and it showed low concentrations. These stations are near to the fresh water regions of the Vembanadu Lake. The station 2 is extremely contaminated with Zn. This station is located near the lower arm of river Periyar; where upper reaches have industries related to the production of Zn. The shipping areas and shipyard mainly showed higher concentration and same does the hub of leisure crafts. Zinc is also employed in antifouling paints as a pigment, erosion facilitator and co-biocide (Turner 2010). Other diffuse sources of Zn related to boating and shipping include leaching of the metal from Zn-based sacrificial anodes and galvanised steel components (Matthiessen et al., 1999). Concentrations similar to present study was reported by Khaled et al. (2006) from the sediments of Gulf of Suez (33.5 to 352.7 ppm). Zn tend to accumulate towards the northern estuary and a clear anthropogenic influence along Bolgatty Island was noted which was situated just downstream of the industrial area of the river Periyar (Balachandran et al. 2005) and it was also near the shipping channel (Deepulal et al. 2012). During the period 1976- 2000, Zn concentration in sediments of Cochin estuary increased from 70 to 1266 mg/kg. This could be expected since there is an annual loading of approximately 80 T of Zn and 63 μm^3 of effluents from 247 chemical industries situated upstream of the northern estuary and high traffic of ships (Shibu et al. 1995, SCMC, 2004; Deepulal et al. 2012). This could lead to high pollution load.

Generally the main sources of lead in the aquatic system arise from the manufacturing industries, smelting and refining of metals, sewage and domestic waste water and from antifouling paints (Furgussion 1990). Pb sorption by sediments is correlated with organic contents, grain size and anthropogenic pollutants (Muniz et al. 2004). In 2007 (pre-monsoon) Pb showed higher concentration in all the stations, except ST-1, ST-10 and ST-11 and resulted in values higher than the world shale average (14.8ppm). In 2008 (pre-monsoon) also, the same trend was observed. Lead is employed in many boat paints, and at dry weight concentrations upto a few percent as a pigment (e.g. white lead and lead chromate), anticorrosion agent and/or dryer (Turner et al. 2009). Quantities of many trace metals have been reported in fresh antifouling formulations (Waterman et al. 2005; Paradas and Amado Filho 2007), suggesting that they are also constituent of additives and non-biocidal pigments used in contemporary antifouling paints. Such pigments include lead antimonate and lead chromate

(Abel 2000). Paints that may contain appreciable quantities of Pb include primers, antifouling formulations and deck, bulkhead and overhead paints. Precipitation may results the very high values observed at station ST-2. Besides station ST-2 is located near the mixing zone of the river waters and highly saline coastal waters. On the banks of the river, one of the largest industrial area in south west coast of India is located and heavily discharges effluents from these industries and accumulates the trace metals residues, Pb in station ST-2.

The world shale average of tin is found to be 2.3ppm. In the present studied area, Sn showed enrichment mainly along the harbour and port regions. During 2007 (pre-monsoon) Sn has higher concentration in stations ST-1 to ST-9 and ST-14 than world shale average. But in the consecutive year (2008), the concentration showed an increase along the study region, i.e. stations ST-2 to ST-11 have higher concentrations. Sn in the Cochin estuarine sediments are generally attributed to its use in antifouling paints as organotin biocides. Organiotin was known to persist for decades as paint fragments (Viglino et al. 2004), although banned outright by the International Maritime Organisation in 2008 (Gipperth 2009). Tin has some additional application in boat paints (Turner 2010), but concentration measured is generally two orders of magnitude lower than those of Cu (Turner et al. 2009). Enrichment of Sn were found spatially in sediments of the Cochin estuary which suggested an anthropogenic source for this metal.

Since antifouling is effected by the slow, controlled leaching of biocides from the painted surface, elevated environmental concentrations of these chemicals are most significant in semi-enclosed marine systems, such as harbours, marinas and estuaries, where the transport, berthing or docking of vessels is tremendous. The aqueous concentrations and environmental effects of these chemicals in situ or under controlled laboratory condition are, therefore to be well documented (Comber et al. 2002; Warnken et al. 2004; Koutsaftis and Aoyama 2007; Cima et al. 2008; Di landa et al. 2009; Lam et al. 2009; Karlsson et al. 2010).

Other than Cu and Zn, the majority of the composite consists of relatively inert elemental constituents of environmentally significant trace metals such as Cd, Cr, Ni, Pb and Sn. The presence of Sn may reflect traces of old, triorganotin formulations in the composite, presumably as historic applications which removed concurrently with newer paint layers (International Maritime Organisation, 2003; 2007). Tin may also exist in some tin-free "silicone-based paints in the form of a curing catalyst at concentrations as high as 0.1% by weight of the formulation (Waterman et al. 2005). Many lead-free cabin and paints with considerable concentrations of Pb as dryers and to provide corrosion resistance (Booher 1988; Zedd et al. 1993). Quantities of many of these trace metals have, however been reported in fresh antifouling formulations (Waterman et al. 2005; Paradas and Amado Filho 2007), suggesting that they are also constituents of additives and non-biocidal pigments, are used in contemporary antifouling paints. Such pigments include lead antimonate, cadmium yellow and lead chromate (Abel 2000). Provided that the concentrations of these metals or pigments are below 1% by weight in a formulation, even though manufactures are not obliged to specify their presence or function (Sandberg et al. 2007; Turner 2010). Therefore it can be regarded as an important, heterogeneous source of a wide range of inorganic contaminants to the marine environment.

5.3.2 Factors Controlling Trace Metals in Sediments

A). Geochemistry and Inter-elemental Relationship

Pearson’s correlation coefficients among the granulometry, organic carbon (OC), total nitrogen (TN), total carbon (TC), total sulphur (TS), total phosphorus (TP), inorganic carbon (IC), total kjeldal nitrogen (TKN) and metals content were studied. The correlation results are presented in tables 5.3 and 5.4. These results showed that metals have negative correlation with percentage of sand and positively correlated with mud (clay + silt) during the pre-monsoon of 2007. This corroborates the affirmation of Haque and Subramanian (1982), according to the reports, the capacity of adsorption of metals are in the increasing order as sand < silt < clay due to the composition of minerals and organic matter present in the sediment under investigation. During pre-monsoon of 2008, the correlation results indicated no closeness with the percentage of sand and metals. Mud gave positive correlation with Cr, Cu, Ni and Pb and showed no significant correlation with Zn, Cd and Sn. The overall correlation indicated that there is enrichment in concentration in the finer fractions.

The metals gave positive correlation with OC, TC, TS and TP during 2007 and only Cu, Cr, Ni and Sn showed positive correlation with OC, TN, TC and TS. The positive correlation revealed that organic matter plays a greater role in the binding capacity of metals and ligands. It is necessary to emphasise that the organic carbon coefficients are predominant, followed by sulphur and phosphorus in 2007 (pre-monsoon) and sulphur followed by organic carbon and total nitrogen during 2008 (pre-monsoon). Year after year the pattern changes which infer the environmental irregularities with the varying conditions.

Table 5.3 Table showing the Pearson correlation of trace metals with general sedimentary parameters during 2007 pre-monsoon

	Cu	Zn	Cd	Cr	Ni	Pb	Sn	Sand%	Silt%	Clay%	OC(%)	TN%	TC%	TS%	IC	TP	TKN
Cu	1.00																
Zn	***0.58***	1.00															
Cd	***0.66***	***0.97***	1.00														
Cr	***0.75***	0.36	0.46	1.00													
Ni	***0.96***	0.48	***0.59***	***0.75***	1.00												
Pb	***0.90***	***0.79***	***0.83***	***0.62***	***0.87***	1.00											
Sn	***0.65***	0.45	0.49	0.51	***0.62***	***0.62***	1.00										
Sand%	***-0.93***	***-0.56***	***-0.63***	***-0.68***	***-0.92***	***-0.90***	***-0.77***	1.00									
Silt%	***0.82***	***0.54***	***0.60***	***0.65***	***0.81***	***0.84***	***0.73***	***-0.93***	1.00								
Clay%	***0.88***	0.48	***0.55***	***0.58***	***0.88***	***0.80***	***0.68***	***-0.90***	***0.66***	1.00							
OC(%)	***0.95***	***0.59***	***0.64***	***0.72***	***0.93***	***0.90***	***0.73***	***-0.97***	***0.89***	***0.88***	1.00						
TN%	0.10	0.03	0.09	0.05	0.14	0.16	0.02	-0.16	0.10	0.19	0.19	1.00					
TC%	***0.83***	***0.58***	***0.55***	***0.59***	***0.74***	***0.87***	***0.57***	***-0.82***	***0.74***	***0.76***	***0.85***	0.09	1.00				
TS%	***0.82***	***0.71***	***0.70***	***0.69***	***0.80***	***0.91***	***0.60***	***-0.86***	***0.83***	***0.74***	***0.88***	0.04	***0.89***	1.00			
IC	-0.25	-0.03	-0.19	-0.24	-0.36	-0.08	-0.30	0.28	-0.28	-0.23	-0.28	-0.19	0.26	0.00	1.00		
TP	***0.76***	0.38	0.38	0.41	***0.78***	***0.75***	0.49	***-0.76***	***0.75***	***0.62***	***0.81***	0.06	0.74	***0.75***	-0.14	1.00	
TKN	-0.40	-0.21	-0.22	-0.28	-0.40	-0.38	-0.34	0.33	-0.27	-0.34	-0.50	-0.34	-0.42	-0.36	0.16	***-0.57***	1.00

bold italics gives correlation is significant at the 0.05 level

bold italics underlines gives correlation is significant at the 0.01 level

Table 5.4 Table showing the Pearson correlation of trace metals with general sedimentary parameters during 2008 pre-monsoon

	Cu	Zn	Cd	Cr	Ni	Pb	Sn	Sand%	Silt%	Clay%	OC(%)	TN%	TC%	TS%	IC	TP	TKN
Cu	1.00																
Zn	***0.63***	1.00															
Cd	***0.66***	***0.99***	1.00														
Cr	***0.78***	0.38	0.42	1.00													
Ni	***0.93***	0.48	0.51	***0.83***	1.00												
Pb	***0.97***	***0.58***	***0.61***	***0.75***	***0.90***	1.00											
Sn	***0.66***	0.23	0.25	***0.61***	***0.64***	***0.54***	1.00										
Sand%	-0.48	-0.30	-0.35	-0.50	-0.46	-0.39	-0.38	1.00									
Silt%	0.08	0.12	0.16	0.05	-0.04	0.02	0.13	***-0.79***	1.00								
Clay%	***0.69***	0.34	0.37	***0.76***	***0.80***	***0.62***	0.46	***-0.63***	0.03	1.00							
OC(%)	***0.64***	0.37	0.40	***0.58***	***0.67***	0.53	0.53	***-0.66***	0.24	***0.77***	1.00						
TN%	***0.54***	0.30	0.32	***0.61***	***0.55***	0.44	***0.59***	***-0.61***	0.34	***0.57***	***0.89***	1.00					
TC%	***0.59***	0.26	0.30	***0.61***	***0.54***	0.48	***0.74***	-0.52	0.26	0.52	***0.81***	***0.92***	1.00				
TS%	***0.74***	0.47	0.52	***0.69***	***0.70***	***0.66***	***0.63***	***-0.72***	0.41	***0.66***	***0.86***	***0.90***	***0.91***	1.00			
IC	0.27	0.02	0.06	0.37	0.16	0.21	***0.65***	-0.13	0.16	0.02	0.24	***0.55***	***0.76***	***0.56***	1.00		
TP	0.43	0.45	0.44	0.39	0.51	0.40	0.31	-0.41	0.00	***0.67***	0.35	0.22	0.21	0.39	-0.03	1.00	
TKN	-0.11	-0.02	-0.04	0.01	0.08	-0.26	0.07	0.08	-0.18	0.09	0.24	0.13	0.02	-0.04	-0.23	-0.13	1.00

bold italics gives correlation is significant at the 0.05 level

bold italics underlines gives correlation is significant at the 0.01 level

B). Principal Component Analysis

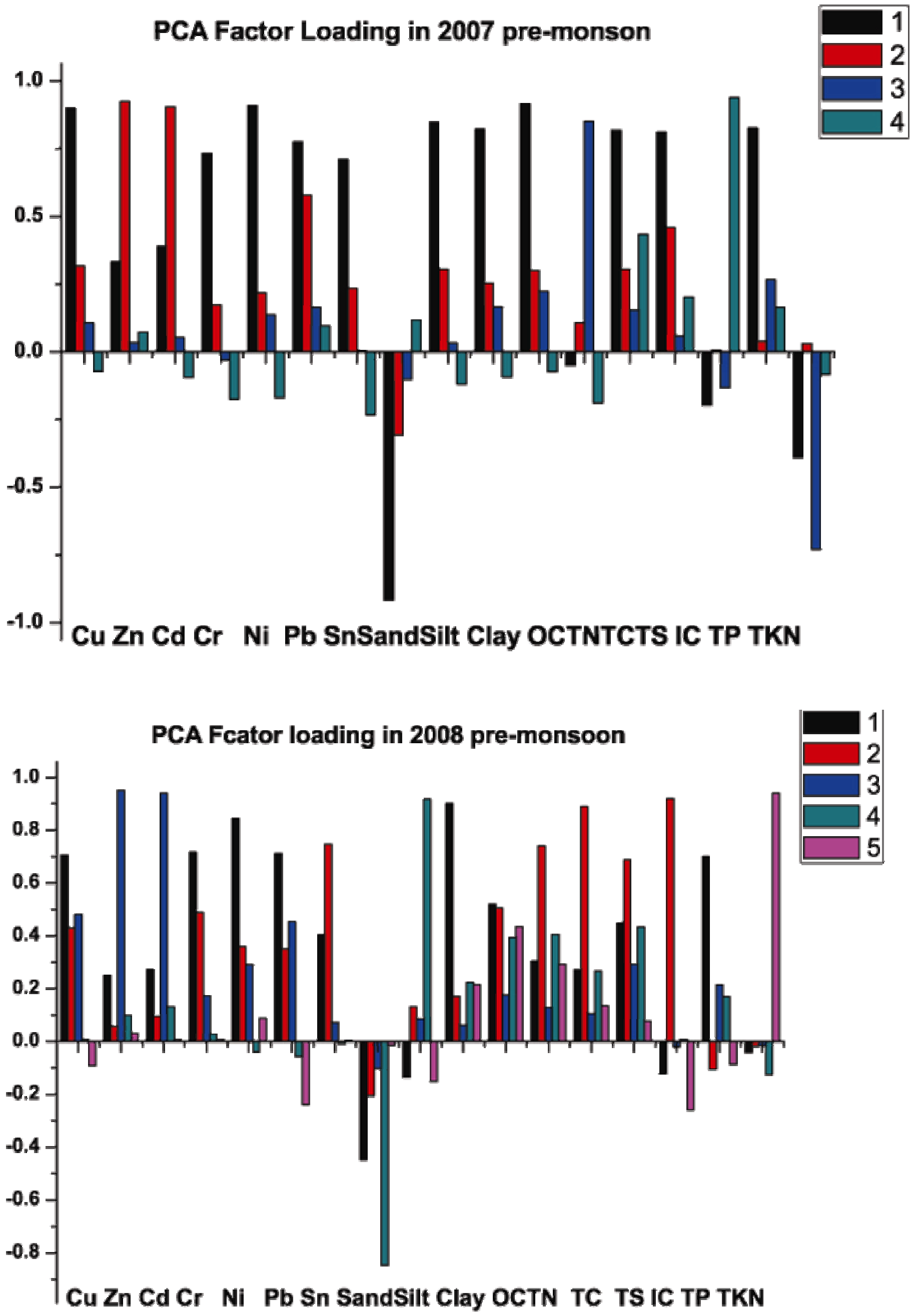
The factor analysis was applied to obtain more reliable information about the relationship among the variables (Bartolomeo et al. 2004; Glasby et al. 2004; Ghrefat and Yusuf 2006). Variables in 2007(pre-monsoon) can be expressed in four components. The two tables give varimax component of four factors for sediments of 2007 (pre-monsoon) and five factors in sediments for 2008 (pre-monsoon) (Table 5.5). The four significant components, whose eigen values are higher than 1 accounting for 85% of the cumulative variance were distinguished for the analytical data in the year 2007 (pre-monsoon). Factor 1 accounted for 51.884% of total variance and is mainly characterised by high levels of Cu, Cr, Ni, Pb, Sn, mud (silt +clay), TC, TS, and TP (Figure 5.1). The high levels of trace metals coupled with mud, TC, TC and TP indicated that organic matter fluxes derived from anthropogenic sources in the Cochin estuary has a higher capacity to bind most metals during its transport and export to the bottom sediment (Murray et al. 1999; Reimann and de Caritat 2005) and also support these factors in their correlation studies. Factor 2 accounted for 17.046% of the total variance, which mainly consists of Cd and Zn. This showed the same sources of Cd and Zn. Factor 3 contributed to 8.954% of total variance and is mainly characterised by TN. The factor 4 showed 8.014% of total variance which is characterised by inorganic carbon only.

In 2008 (pre-monsoon) principal component analysis gave 5 components. Here the eigen values higher than 1 accounting for 89% of the cumulative variance was taken for consideration. Factor 1 accounted for 27.665% of the total variance and is mainly characterised by high levels Cu, Cr, Ni, Pb, clay, OC and TP (Figure 5.2) main contributors to the first factor is similar in the pre-monsoon, 2007.

Table 5.8 Table showing the loading pattern of variable in Factor Analysis during 2007 and 2008 pre-monsoon

	Component			
	1	2	3	4
Cu	0.900	0.317	0.108	-0.072
Zn	0.332	0.924	0.033	0.073
Cd	0.390	0.904	0.053	-0.095
Cr	0.733	0.173	-0.031	-0.174
Ni	0.909	0.218	0.136	-0.169
Pb	0.775	0.578	0.164	0.096
Sn	0.710	0.234	0.003	-0.233
Sand%	-0.917	-0.308	-0.102	0.117
Silt%	0.848	0.305	0.032	-0.120
Clay%	0.822	0.253	0.165	-0.092
OC(%)	0.917	0.300	0.224	-0.073
TN%	-0.049	0.107	0.851	-0.189
TC%	0.817	0.305	0.154	0.434
TS%	0.812	0.459	0.058	0.202
IC	-0.197	0.006	-0.133	0.938
TP	0.826	0.039	0.267	0.164
TKN	-0.391	0.030	-0.729	-0.084
% of Variance	51.884	17.046	8.954	8.014
Cumulative %	51.884	68.930	77.884	85.898

	Component				
	1	**2**	**3**	**4**	**5**
Cu	0.706	0.430	0.480	0.008	-0.092
Zn	0.252	0.058	0.951	0.098	0.030
Cd	0.272	0.095	0.940	0.131	0.008
Cr	0.716	0.488	0.173	0.029	0.008
Ni	0.844	0.359	0.291	-0.040	0.088
Pb	0.712	0.350	0.455	-0.057	-0.239
Sn	0.403	0.746	0.072	-0.009	0.004
Sand%	-0.447	-0.205	-0.103	-0.848	-0.014
Silt%	-0.135	0.130	0.085	0.918	-0.152
Clay%	0.902	0.171	0.062	0.224	0.216
OC(%)	0.519	0.506	0.175	0.393	0.434
TN%	0.304	0.740	0.128	0.406	0.293
TC%	0.271	0.889	0.105	0.266	0.134
TS%	0.448	0.688	0.292	0.433	0.076
IC	-0.124	0.919	-0.020	0.007	-0.258
TP	0.700	-0.105	0.215	0.170	-0.087
TKN	-0.042	-0.018	-0.016	-0.126	0.940
% of Variance	27.665	25.026	15.045	13.335	8.241
Cumulative %	27.665	52.691	67.736	81.071	89.311

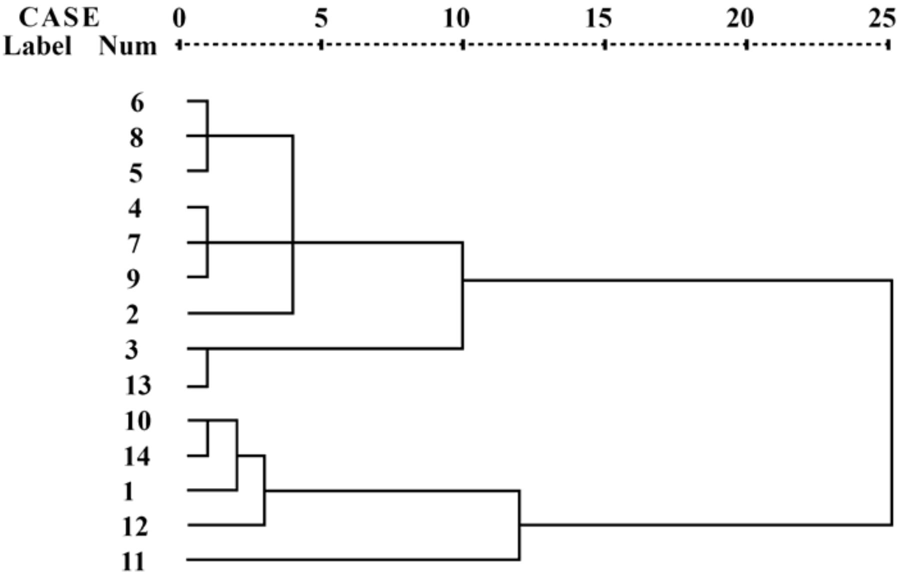


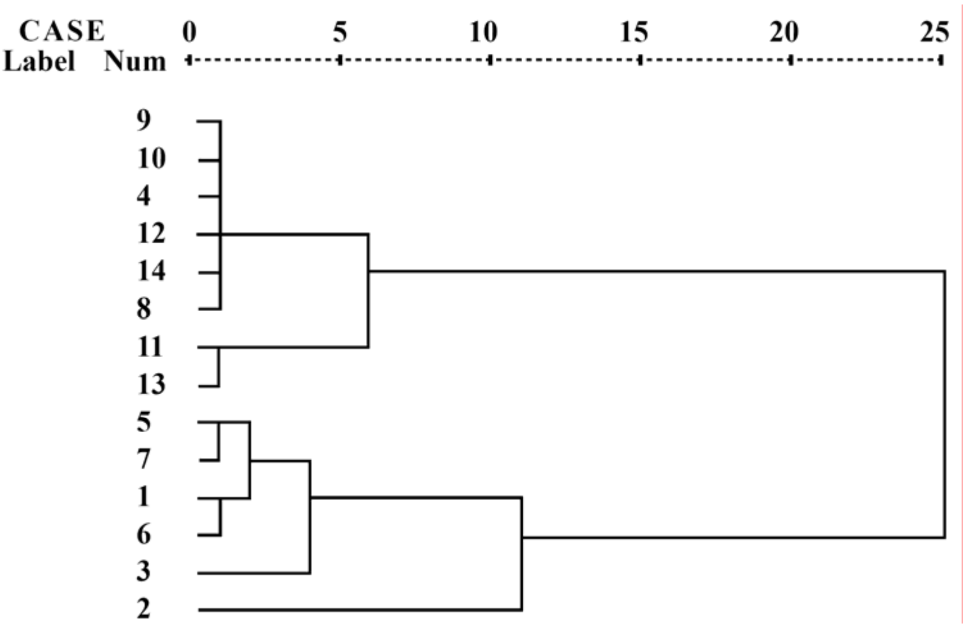
Figures 5.1 & 5.2 Graphs showing the loading of different factors of PCA during 2007 and 2008 pre-monsoon

This confirmed the complicated behaviour of these pollutants, which can be influenced by many factors. Factor 2 contributed to 25.026% of total variance and explained the presence of Sn, TN, TC, TS and inorganic carbon component. Factor three accounted for 15.045% of total variance and mainly consisted of Zn and Cd. This is in good agreement with factor two in pre-monsoon 2007 indicating their persistent common origin. Factor 4 showed 13.335% of total variance and has sand and silt as parameters. The last factor which accounted for 8.241% of total variance is contributed by total kjeldal nitrogen only.

C). Cluster Analysis

During 2007 (pre-monsoon) period, cluster analysis gave two major clusters. The first cluster consisted of three minor groups (Figure 5.3). The first minor group included ST-5, ST-6 & ST-8. These three stations have similar concentration for Cd, Pb & Zn. Next minor group in first cluster was formed by ST-4, ST-7 & ST-9. These stations have similar concentration for Cu, & Cd in 2007 (pre-monsoon). The third minor group in first cluster included stations ST-3 & ST-13. These stations showed similar behaviour for Cu, Ni & Pb. Only Station ST-2, has higher concentration for Cu, Zn & Pb. Second cluster includes two minor groups and independent stations. The first minor group of this cluster consisted of stations ST-10 & ST-14 and second has ST-1 & ST-12. The first minor group has similar behaviour for Sn, TN & TP while the second minor group gave similar character for Cu, Cd, Cr, Ni & Pb in 2007 (pre-monsoon).





Figures 5.3 and 5.4 figures showing the results of cluster analysis in 2007 and 2008 (pre-monsoon)

In 2008 (pre-monsoon) analysis resulted in two major clustering groups, of which first cluster gave two groups and second cluster gave scattered groups (Figure 5.4). In the first cluster the first minor group included stations ST-4, ST-8, ST-9, ST-10, ST-12 & ST-14 and provided similar behaviour for Zn, Cd, Pb, sand, silt, clay, TC, TP & TKN. The second minor group in first cluster formed by stations ST-11 & ST-13 and they showed identical behaviour for Cu, Cd, Cr, Ni, Pb, Sn, TP & TKN. The second cluster formed by ST-5 and ST-7 has similar concentrations for Cu, Zn, Ni, Pb, Sn, OC, TC, IC, TP & TKN while stations ST-6 & ST-1 gave similar Cd & TP concentration. The stations ST-2 & ST-3 have similar concentration for Cu, Cr, Pb, Sn, OC, TN, TC & TP.

The cluster analysis provide the idea of inter elemental relationship between stations.

D). Enrichment Factor

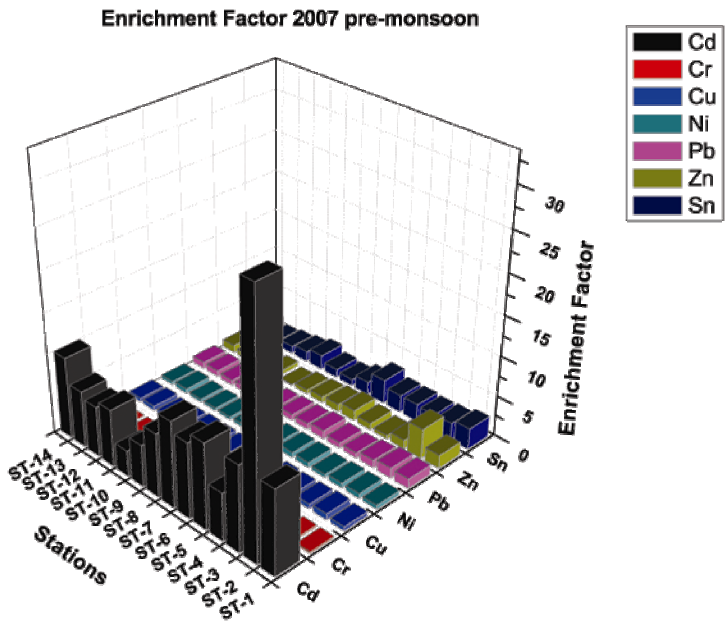
Earlier studies suggested that, when $EF \geq 0.5 \leq EF$, the existence of trace metals are due to the crustal materials or natural weathering processes. But $EF > 1.5$, showed a significant portion of trace metals are executed from other external sources (Zhang et al. 2007). In this study, Cd showed $EF > 1.5$ in all the stations under investigation during the two years of study period (Table 5.6). This inferred the fact that the main sources of Cd may be from anthropogenic input. ST-2, gave very high enrichment of Cd. From the previous discussion mentioned, this station, ST-2 can be represented as an industrial effluent affected area and thereby confirm the main source is from anthropogenic origin.

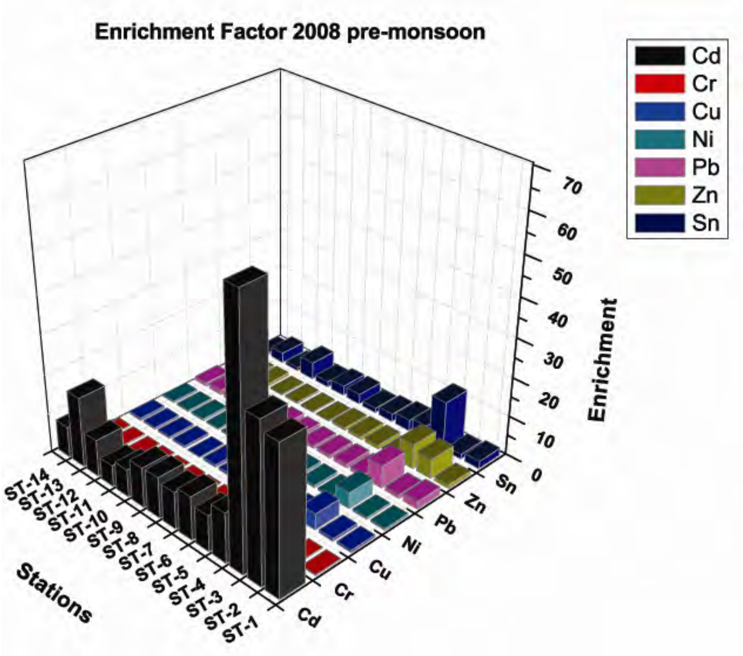
The trace metals like Cr, Cu, Ni and Pb showed enrichment factor lies very well below 1.5 which account for the natural weathering.

Zn gave EF higher than 1.5 in stations ST-1 & ST-2 in 2007 (pre-monsoon) and station ST-2 & ST-3 in 2008 (pre-monsoon) (Figures 5.5 and 5.6). All other stations showed EF values below 1.5. These out writes that the natural weathering dominates in other stations and in station ST-2, the origin is from anthropogenic input during both sampling periods. Zn producing industry is located in the upper reaches of station ST- 2, may be the reason for this enrichment. The high enrichment factor of Cd and Zn suggested anthropogenic sources, mainly the industrial effluents. Many recent studies also support these outcomes. The leaching of antifouling paints from ships, boats and leisure crafts may be the secondary reason for enrichment contribution of Zn in these regions. The difference in EF values of other metals may be due to the difference in the magnitude of input for each metal in the sediment and / or the difference in the removal rate of each metal from the sediment. The previous studies of Esin Esen et al. (2010), Enfeng Liu et al. (2010), Abrahim and Parker (2008) were also showed high enrichment of Cd and Zn in their study regions. The results of Alagar samy and Zhang et al. (2010), Rodriguez et al. (2009), Sundaray (2010), Bintal Amin et al. (2009), Hatje et al. (2001), Fatma Cevik et al. (2009) were also showed moderate enrichment of Cu, Cr, Ni & Pb apart from Cd and Zn.

Table 5.6 Estimated Enrichment Factor for trace metals in sediments of the study area during 2007 and 2008 (pre-monsoon)

Season	Stations	Cd	Cr	Cu	Ni	Pb	Zn	Sn
2007 pre-monsoon	ST-1	10.47	0.19	0.54	0.53	1.18	1.67	2.60
	ST-2	31.14	0.21	0.76	0.64	1.29	3.74	2.09
	ST-3	10.56	0.21	0.62	0.64	1.01	1.13	2.00
	ST-4	5.93	0.14	0.54	0.45	0.66	0.51	2.13
	ST-5	10.59	0.18	0.58	0.67	0.70	0.83	2.13
	ST-6	9.32	0.22	0.69	0.68	1.02	1.31	2.98
	ST-7	10.63	0.29	0.56	0.60	0.75	0.94	1.62
	ST-8	7.88	0.20	0.56	0.58	0.73	0.76	0.85
	ST-9	5.20	0.25	0.54	0.46	0.60	0.55	1.07
	ST-10	3.26	0.21	0.62	0.39	0.59	0.10	1.81
	ST-11	7.12	0.18	0.47	0.37	0.73	0.55	1.25
	ST-12	6.28	0.14	0.41	0.38	0.65	0.33	0.93
	ST-13	7.03	0.08	0.57	0.59	0.81	0.71	0.62
	ST-14	10.09	0.18	0.45	0.46	0.82	0.98	1.39
2008 pre-monsoon	ST-1	37.29	0.19	0.57	0.53	1.93	1.00	2.01
	ST-2	39.82	0.18	0.67	0.50	0.94	5.07	1.29
	ST-3	65.30	1.28	4.16	4.15	5.84	6.30	12.38
	ST-4	12.63	0.29	0.71	0.50	0.96	0.85	3.78
	ST-5	9.10	0.23	0.64	0.68	1.03	1.06	2.83
	ST-6	11.02	0.16	0.60	0.61	0.84	0.94	2.02
	ST-7	9.36	0.31	0.45	0.52	0.69	0.77	1.25
	ST-8	10.41	0.17	0.61	0.46	0.87	0.24	2.62
	ST-9	10.18	0.12	0.60	0.40	1.07	0.23	2.50
	ST-10	7.05	0.11	0.19	0.39	0.13	0.42	1.48
	ST-11	5.47	0.17	0.51	0.36	0.76	0.25	4.39
	ST-12	9.46	0.13	0.48	0.52	1.04	0.72	0.80
	ST-13	17.79	0.26	0.48	0.39	1.25	0.53	2.62
	ST-14	7.85	0.15	0.57	0.45	0.93	0.57	0.49





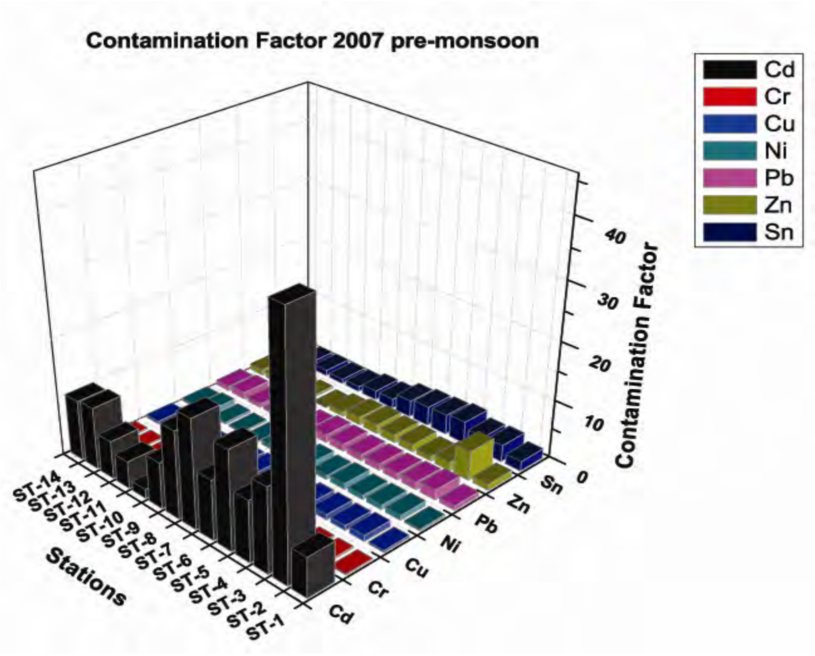
Figures 5.5 and 5.6 Graphs showing the enrichment during 2007 and 2008 (pre-monsoon)

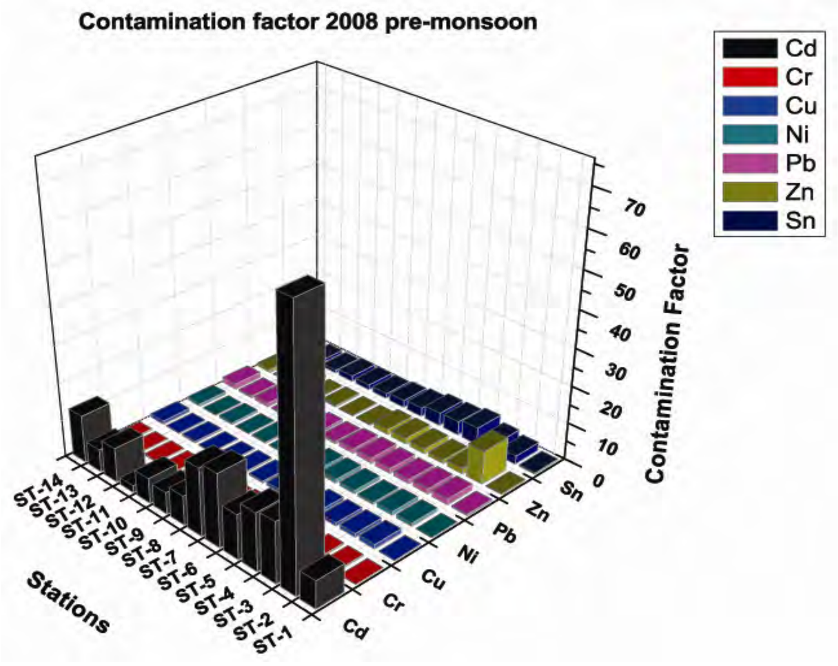
E. Contamination Factor

The contamination factor of the various trace metals in the sediments of Cochin estuarine system are presented in Table 5.7. The metals like Cr, Pb in all stations showed low contamination factor, whereas Cu at stations, ST-2 & ST-8 in pre-monsoon 2007 and at stations ST-2, ST-3 & ST-6 in pre-monsoon 2008 showed moderate contamination (Figures 5.7 and 5.8). Zn showed moderate contamination at stations ST-1, ST-3, ST-5, ST-6, ST-7, ST-8, ST-13 & ST-14 in pre-monsoon 2007 and ST-3, ST-4, ST-5, ST-6 & ST-7 in pre-monsoon 2008. But in station ST-2 during 2007 there was considerable contamination of Zn and in 2008, the status was changed to very high contamination. This indicates that the Zn accumulation is increasing year after year. In the case of Ni, it showed moderate contamination at station ST-5 & ST-8 during 2007 and ST-3, ST-6 & ST-7 during 2008. But trend is different in the case of Cd. It showed very high contamination at all stations (except at station ST-10 & ST-11 in 2007 and at station ST-11 in 2008) during the successive years. Among these stations, ST-2 was highly contaminated when compared with other studied locations and may be due to the flocculation observed at this station and also the leaching from ships, boats and from the outlet of industrial effluents. The previous studies of Esin Esen et al. (2010) and Alessandra Accornero et al. (2008) also showed the high contamination factors for Cd and Zn.

Table 5.7 **Estimated Contamination factor for trace metals in sediments of the study area during 2007 & 2008 (pre-monsoon)**

Season	Stations	Cd	Cr	Cu	Ni	Pb	Zn	Sn
2007 (pre-monsoon)	ST-1	6.88	0.12	0.35	0.35	0.78	1.10	1.71
	ST-2	42.75	0.29	1.05	0.88	1.77	5.13	2.88
	ST-3	14.50	0.29	0.86	0.87	1.39	1.56	2.74
	ST-4	10.50	0.24	0.95	0.79	1.17	0.89	3.78
	ST-5	17.00	0.29	0.93	1.07	1.12	1.34	3.42
	ST-6	10.25	0.24	0.76	0.74	1.12	1.44	3.28
	ST-7	18.63	0.52	0.98	1.05	1.32	1.64	2.83
	ST-8	15.00	0.38	1.06	1.11	1.39	1.44	1.63
	ST-9	7.88	0.38	0.82	0.69	0.91	0.84	1.63
	ST-10	1.63	0.10	0.31	0.19	0.30	0.05	0.90
	ST-11	4.63	0.12	0.30	0.24	0.47	0.36	0.81
	ST-12	6.50	0.15	0.42	0.39	0.68	0.34	0.96
	ST-13	10.63	0.12	0.86	0.89	1.22	1.08	0.93
	ST-14	10.25	0.19	0.45	0.47	0.84	1.00	1.42
2008 (pre-monsoon)	ST-1	7.50	0.04	0.12	0.11	0.39	0.20	0.40
	ST-2	70.63	0.31	1.19	0.89	1.66	9.00	2.29
	ST-3	16.25	0.32	1.04	1.03	1.45	1.57	3.08
	ST-4	15.50	0.36	0.87	0.61	1.17	1.04	4.63
	ST-5	12.00	0.30	0.84	0.90	1.36	1.39	3.73
	ST-6	19.63	0.28	1.08	1.08	1.49	1.67	3.60
	ST-7	18.13	0.61	0.87	1.01	1.33	1.49	2.43
	ST-8	7.88	0.13	0.46	0.35	0.66	0.18	1.99
	ST-9	5.88	0.07	0.34	0.23	0.62	0.13	1.44
	ST-10	6.75	0.11	0.18	0.37	0.13	0.40	1.41
	ST-11	1.88	0.06	0.18	0.12	0.26	0.09	1.50
	ST-12	9.25	0.13	0.47	0.51	1.02	0.70	0.78
	ST-13	6.13	0.09	0.17	0.14	0.43	0.18	0.90
	ST-14	12.63	0.25	0.91	0.72	1.49	0.92	0.78





Figures 5.7 & 5.8 Graphs of Contamination factor during 2007 and 2008 (pre-monsoon)

F). Geoaccumulation Index

Possible sediment accumulation of metals in Cochin estuary was evaluated in terms of the I_{geo} values. The average geoaccumulation index for various metals ranged from 1-6 indicating that the sediment sample fell within an uncontaminated to extremely contaminated category. The trace metals like Cr, Cu, Ni and Pb showed negative geoaccumulation index indicating their pollution free nature in these study area (Table 5.8). Zn which had moderate pollution during 2007 was changed to strongly polluted in 2008 at station ST-29 (Figures 5.9 and 5.10). Thus the accumulation behaviour of Zn along the study area was also observed.

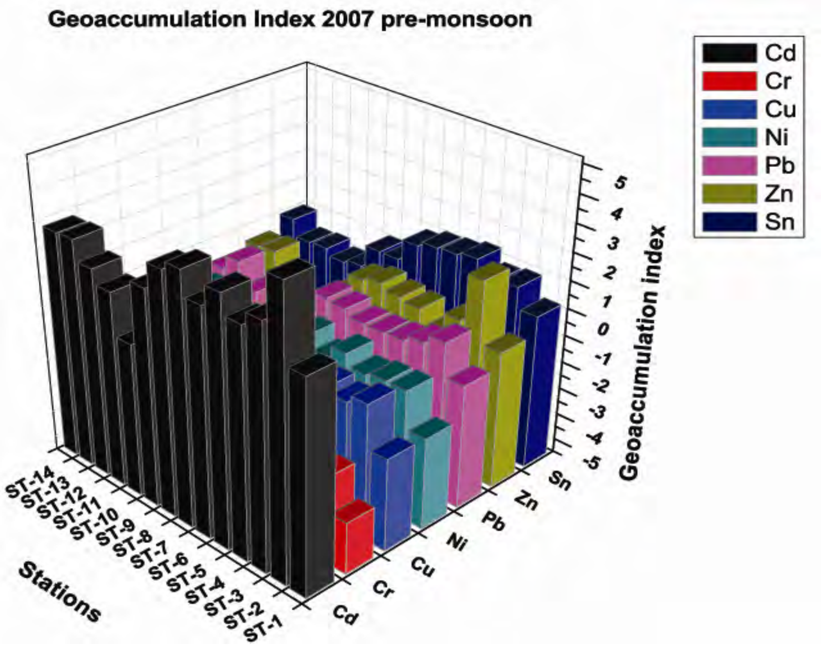
In the case of Cd, it showed moderately strong to very strong pollution during both the sampling period. In station ST-2, Cd was very profoundly polluted which confirmed its accumulation character in this station. These results, substantiated and supported the outcome of both enrichment factor and contamination factor.

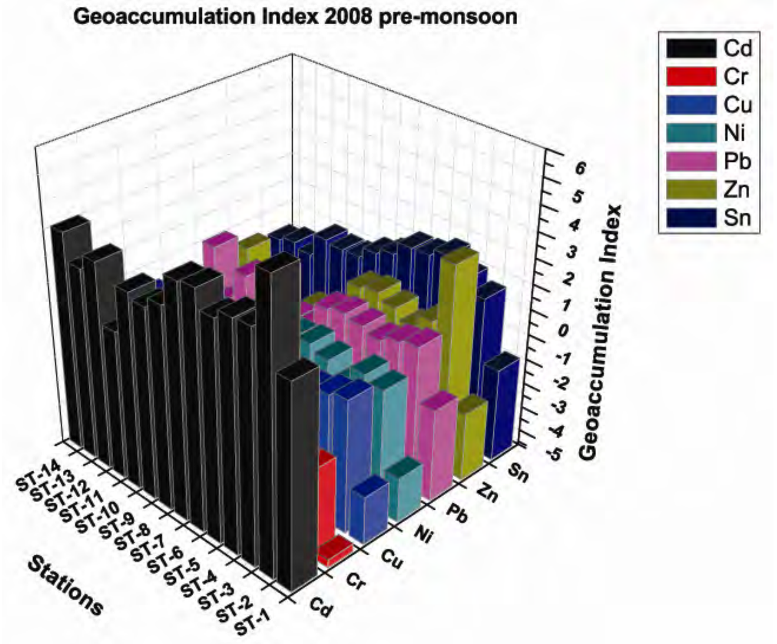
Sn is another trace metal which has significant accumulation along the study area. Stations ST-4, ST-5 & ST-6 during 2007 and ST-3, ST-4, ST-5 & ST-6 during 2008 were found to be moderately polluted with Sn, as these stations are the counterparts residing near to the shipping channel. The accumulation of Sn also may be due to the leaching of antifouling paints used in ships and other vessels.

The I_{geo} values calculated for trace metal concentrations in Cadiz Bay and Sancti Petri Channel by Rodriguez et al. (2009) reveal that the sediments are unpolluted with respect to the total analysed metals.

Table 5.8 Geoaccumulation index for trace metals in sediments of the study area during 2007 & 2008 (pre-monsoon)

Seasons	Stations	Cd	Cr	Cu	Ni	Pb	Zn	Sn
2007pre-monsoon	ST-1	2.20	-3.59	-2.09	-2.12	-0.95	-0.45	0.19
	ST-2	4.83	-2.37	-0.52	-0.77	0.23	1.78	0.94
	ST-3	3.27	-2.38	-0.81	-0.78	-0.11	0.05	0.87
	ST-4	2.81	-2.64	-0.65	-0.92	-0.36	-0.75	1.33
	ST-5	3.50	-2.37	-0.69	-0.49	-0.42	-0.17	1.19
	ST-6	2.77	-2.66	-0.99	-1.01	-0.42	-0.06	1.13
	ST-7	3.63	-1.54	-0.62	-0.51	-0.19	0.13	0.92
	ST-8	3.32	-1.97	-0.50	-0.44	-0.11	-0.06	0.12
	ST-9	2.39	-1.99	-0.87	-1.12	-0.72	-0.84	0.12
	ST-10	0.12	-3.85	-2.29	-2.95	-2.34	-4.96	-0.73
	ST-11	1.62	-3.70	-2.31	-2.62	-1.67	-2.08	-0.89
	ST-12	2.12	-3.32	-1.84	-1.94	-1.15	-2.16	-0.64
	ST-13	2.82	-3.63	-0.80	-0.75	-0.29	-0.48	-0.69
	ST-14	2.77	-3.01	-1.73	-1.68	-0.84	-0.58	-0.08
2008 pre-monsoon	ST-1	2.32	-5.30	-3.70	-3.83	-1.95	-2.89	-1.90
	ST-2	5.56	-2.25	-0.33	-0.76	0.15	2.58	0.61
	ST-3	3.44	-2.23	-0.53	-0.54	-0.05	0.06	1.04
	ST-4	3.37	-2.08	-0.79	-1.30	-0.35	-0.53	1.63
	ST-5	3.00	-2.33	-0.83	-0.74	-0.14	-0.11	1.32
	ST-6	3.71	-2.44	-0.48	-0.47	0.00	0.16	1.26
	ST-7	3.59	-1.30	-0.78	-0.58	-0.18	-0.01	0.69
	ST-8	2.39	-3.58	-1.69	-2.11	-1.19	-3.04	0.40
	ST-9	1.97	-4.47	-2.12	-2.71	-1.28	-3.48	-0.06
	ST-10	2.17	-3.83	-3.08	-2.02	-3.57	-1.89	-0.09
	ST-11	0.32	-4.65	-3.09	-3.62	-2.52	-4.12	0.00
	ST-12	2.62	-3.54	-1.68	-1.56	-0.55	-1.10	-0.94
	ST-13	2.03	-4.07	-3.18	-3.47	-1.80	-3.04	-0.73
	ST-14	3.07	-2.60	-0.72	-1.06	0.00	-0.70	-0.94





Figures 5.9 & 5.10 **Graphs of Geoaccumulation index during 2007 and 2008 (pre-monsoon)**

Earlier authors used geoaccumulation index, to understand sediment pollution at the vicinity of the mouths of major rivers that flow into Kaohsiung harbour, Taiwan (Chen et al. 2007). Ghrefat and Yusuf (2006) also found sediments very strongly polluted with Cd in a dam of Jordan. Rodriguez-Barroso et al. (2009b) detected sediments moderately contaminated with trace metals in rivers from the north of Morocco.

Similar results were reported by Muth Raj and Jayaprakash (2008) in marine sediments of Bay of Bengal off Ennore. The I_{geo} also gave moderate pollution of Cd in Seyhan dam, Turkey (Fatma Cevik et al. 2009).The results indicated very high contamination for Cd followed by Cu, Cr, Pb, Ni & Zn in all stations. The I_{geo} values of Cd, Cu & Cr signifies extremely contaminated condition along the Bay of Bengal. The above finding highlights the role of industrial effluents as a pollution factor.

I_{geo} factor leads to uncontaminated to moderately contaminated sediments in the study of Abrahim and Parker (2008) at Tamaki estuary Auckland, New Zealand. Sediments of Souani and Mghogha also showed values <0, giving lacks of accumulation. I_{geo} values reported by Bintal Amin et al (2009) along the coastal sediment of Dumai, Indonesia showed moderate pollution of Cd and no pollution indication for Cu, Pb, Zn, Ni & Fe.

5.4 Conclusion

This chapter mainly dealt with the distribution pattern of trace metals which are used as non-biocidal pigments in antifouling paints (Cr, Cu, Cd, Zn, Pb, Ni & Sn). These results illustrates the higher enrichment of Cd, Zn,

& Sn along the study area. These trace metals are the major metals used in antifouling paints. In order to understand their role and enrichment in Cochin estuarine system, statistical tools like Pearson correlation, principal component analysis, cluster analysis, enrichment factor, contamination factor and geoaccumulation index were performed. During the two year study period, Cu gave strong correlation with other trace metals and mud fractions resulted its strong affinity to finer fractions. Principal component analysis was done in order to understand the inter-elemental relationships. The results showed that Zn & Cd are originated from the same sources in 2007. Similar trend was observed in 2008 and confirmed the same origin. Sn was always seen the same trend as OC, TN, TKN indicating its binding capacity towards organic fraction and organic matter. Cluster analysis, during 2007, stations ST-2, ST-3, ST-4, ST-5, ST-6, ST-7, ST-8 & ST-9 were in same cluster and showed the similar sedimentary behaviour in these stations. Stations ST-10, ST-11, ST-12 & ST-14 were merged in same cluster referring their behaviour in the study area. In 2008 pre-monsoon, the pattern was almost similar from the previous year. Here stations ST-1 to ST-7 were clubbed in same clusters, with similar behaviour. Stations ST-8 to ST-14 are outside the port area, are mainly influenced by anthropogenic sources. In order to understand sediment pollution and enrichment, the enrichment factor, contamination factor and geoaccumulation index were calculated. The enrichment factor results out weights the significant enrichment of Cd in nearly all stations during 2007 & 2008. Zn and Sn are another trace metal which gave high enrichment factor. The contamination factor showed very high contamination for Cd during both the years. Sn also showed considerable contamination inferring the leaching of paint particles. Cu & Zn also showed moderate contamination in several stations during 2007 & 2008. The geoaccumulation index for Cd & Sn viewed as the major pollutants. These results indicate that the enrichment of Cu, Zn, Cd & Sn may be mainly due to the shipping and boating activities, apart from industrial activities along the banks of the tributaries of the estuary.

The overall effects, pathways, bioavailabilities and fates of trace metals in the marine environment are strongly influenced by their aqueous speciation. Analysis of the sediment sample collected in the vicinity of a boatyard, however, describes a clear enrichment of Cu, Pb, Sn and Zn. The chemical characteristics of sediment highlight the potential for heterogeneous trace metal contamination from small quantities of antifouling residues derived from leisure and commercial boat maintenance. The metallic composition of sediments collected in the shipyard is consistent with such an assertion. So in order to understand the biocide pollution, the main constituent used in biocides as: organotins, Irgarol 1051, Chlorothalonil were to be investigated. Therefore the next chapter deals with the clear picture of organic and organometallic antifouling biocides drastically used in shipping industries recently.

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Chapter 6

Antifouling Biocides- Organic and Organometallics

6.1 Introduction

The need for effective antifoulants for preventing the settlement and growth of marine organisms on submerged structures such as oil rig supports, buoys, fish cages and ship hulls is recognised universally. They are particularly important to the shipping industry. Fouling of a ship’s hull leads to increased friction between the hull and sea water, causing ‘hull roughness. This could combine with the increased weight of fouling organisms which are attached to the hull, leads to considerable increase in fuel consumption. A layer of algal slime, (1mm thick), will increase hull friction by 80% and causes a 15% less in ship speed. A 5% increase in fouling for a tanker weighing 250,000 dwt (dry weight tonnes) will increase fuel usage by 17% (MER 1996b).

Chemicals including organo-mercury compounds, lead, arsenic and DDT were historically used as antifoulants. However, such compounds pose severe environmental and human health risks and were withdrawn voluntarily by the paint industry during the early 1960’s (Bennett 1996). They are replaced largely by organotin compounds (OTC). Initially, organotin compounds were incorporated into so-called ‘free association’ paints, in which the biocide was dispersed in a soluble resinous matrix. However release rates were uncontrolled. The initial rate was high, providing good antifouling performance, later declined so that performance diminished with time. Self-polishing copolymer paints (SPC) were introduced in the mid 1970s. Triorganotin (TOT) was chemically bonded in a copolymer resin system via an organotin-ester link. There was slow and controlled release of the biocide, as the link hydrolysed on contact with sea water, at the paint’s surface. These systems were highly effective. They could provide antifouling cover for five or more years, and have been acclaimed widely as the most effective antifoulants ever devised (Bosselmann 1996). Reduced fuel costs and less frequent need to dry-dock and repaint vessels were estimated to be worth US$5.7 billion per annum to the shipping industry during the mid-1990s (Rouhi 1998). Not surprisingly, they dominated the market and were applied to more than 70% of the world’s commercial shipping fleet. Prior to regulations, they were also used extensively on small craft, such as yachts and fishing boats. Different types of biocides used in antifouling paints are given below.

6.1.1 Organotins

Organotin (OT) molecules are comprised of a tin (Sn) atom which is covalently bound to one or more organic substituents, such as methyl, ethyl, butyl or phenyl groups. Triphenyltin (TPT) compounds are a group of OTs confirming to a general formula $(C_6H_5)_3Sn\text{-}X$, where X is an anion or anionic group, such as chloride, hydroxide and acetate. OTs are hydrophobic their solubility in water is relatively small (Rudel 2003). In the pH range of 6-8, the solubilities of TPT chloride (TPTCl) and TPT oxide

(TPTO) in water are approximately 1mgL^{-1} at 25^{0}C (Inaba et al. 1995). TPT compounds such as TPT acetate (TPTA) and chloride (TPTCl) can be hydrolysed to triphenyltin hydroxide (TPTOH) in the marine environment. Neutral hydroxyl-complexes were accounted for more than 93% of TPT compounds in well-buffered sea water (Veltman et al. 2006). The log K$_{ow}$ values of TPTs such as TPTOH and TPTCl are between 3 and 4 (Tsuda et al. 1990a; WHO 1999), which suggests that these compounds are potentially bioaccumulative.

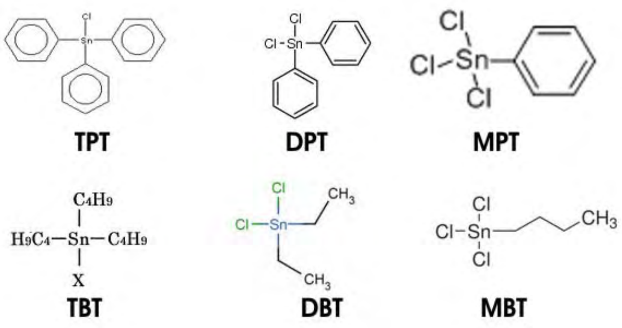

Figure 6.1 Structure of Organotins

TPT are major ingredients of antifouling products and fungicides. OT-based antifouling products used on hull of vessels and submerged mariculture facilities are the main sources of TPT in the marine environment. TPT compounds have also been widely used in agriculture as fungicides thereby it cause TPT in the marine environment by runoff. The total TPT produced annually in china was estimated 200 tonnes (Hu et al. 2009), while in Japan, 140-160 tonnes of TPT were produced for export annually during the period 1994-1996 (WHO 1999).

6.1.1.(a) Chemical and Physical Properties

Organic tin compounds comprise a group of organometallic compounds characterised by a Sn atom, covalently bound to one or more organic substituents (eg: methyl, ethyl, butyl, propyl, phenyl). Chemically these compounds are represented by the general formulas $RSnX_3$, R_2SnX_2, R_3SnX, R_4Sn, in which R is any alkyl or aryl group and X is an anionic species, for example halide, oxide, or hydroxides. The Sn-C bonds are reported to be stable at temperatures upto 200^0C (Zuckerman et al. 1978), hence thermal decomposition has no significance under environmental conditions. UV radiation, strong acids and electrophilic agents readily cleave the Sn-C bonds. The solubility of organotin compounds in water decrease with increasing number and length of the organic substitutes, but it also depends onto the particular X (Blunden and Chapman 1986).

(b) Toxicity

The inorganic form of tin is generally accepted as non-toxic compound, however the organotin compound is toxic. The toxicological pattern of Organotins is very complex. The biological effects of the substances depend on both the nature and the number of the organic groups bound to the Sn cation. In general maximum toxicological activity for organisms is proved for the trisubstituted compounds in any R_3SnX_{4-n} series. The nature of the x-group in R_3SnX derivatives has little or no effect on the biocidic activity except that X itself is a toxic component. In this case the biological activity of the OTC may be enhanced.

Within the class of trialkyltin compounds there are considerable variations in toxicity depending on the nature and the side chain length of the alkyl groups. However, triethyltin acetate (et_3SnOAc) is the most toxic compound of all organotins to mammals (oral LD_{50} rat =4mg/kg; (Smith et al. 1978). An increase in the n-alkyl chain length produces a sharp drop in biocidial activity and the long-chain species, like octyltin derivatives, are essentially non-toxic to all organisms. Thus octyltin compounds can be used as stabilizers in PVC food packaging material. Tetra organotins showed a delayed toxicity in organisms. It is suggested that only after their degradation to trisubstituted compounds, the symptoms of poisoning identified.

Presumably, TPT and TBT are agent showing a high toxic effect to aquatic life. Even at low nano-molar aqueous concentrations (1-2ng/l), they causes chronic and acute poisoning of the most sensitive aquatic organisms, such as algae, zooplankton, molluscs and the larval stage of some fish (Gibbs and Bryan 1996; Maguire 1987). The highest toxicity was shown by the trisustitutued species, but the less toxic derivatives mono and di substituted species are also environmental concern. Triphenyltin is hazardous to aquatic life. Tas et al. (1990) found that guppies (*Poecila reticulate*) when exposed to different concentrations of TPT in water, fish died as the TPT concentration in the body reached upto 2.2µ/l. Early life stages of fish are more sensitive to TPT pollution than adult fish, eg; the larvae of the fathead minnow (*Pimephales promelas*) had a 96h LD_{50} OF 7.1µg/l for TPT hydroxide (Jarvinen et al. 1988).

(c) Source of Environmental Pollution

Organotins shows remarkably various activities in their physical, chemical and biological properties. Therefore, Sn has a large number of derivatives such as organometallic compounds which are in commercial use than any other element and are having divergent industrial applications, and some of them are R_4Sn, R_3SnX, R_2SnX_2, and RSnX3. Tetraorganotin compounds do not have any large-scale commercial outlets, but are important intermediates in the production of less alkylated derivatives.

(i) Manufacture

Organotin compounds can be synthesised by several methods, Grignard route, Wurtz route, alkyl aluminium route and by direct synthesis. These routes to produce organotin halides involve two reaction steps. The first step is a reaction of tin tetrachloride ($SnCl_4$) with suitable reagents to form various tetraalkyltin compounds (R_4Sn). In the second step, RnSn react with $SnCl_4$ in a redistribution reaction to form less alkylated organotin chlorides, like R_3SnCl, R_2SnCl_2 or $RSnCl_3$ (Blunden and Evans 1990). From these organotin chlorides, various Sn derivatives can simply be produced.

The commercial production of organotin compounds by using Grignard reagent (nRMgCl) began in the USA at the Metal & Thermit Corporations plant in Rathway, New Jersey, in the late 1940s (Bennett 1983). The processes give high yields, but the use of high amounts of solvents is required. Na metal together with side reactions and uncompetitive economics this process is disregarded for industrial production. Manufacturing of organotin compounds by the alkyl aluminium route started in Germany at Schering A G, Industrial chemical divition, Bergkamen, in 1962 (Bennett 1996).

(ii) Antifouling coating

Growth of aquatic organisms on vessel hulls creates roughness which give rise to reduced vessel speed per unit energy consumption. To reduce the running shipyard costs, roughness of vessel hulls must be prevented.

Primary marine antifouling paints were based on Cu_2O (Champ and Seligman 1996). But these coating withstand for only a minimum period of 1 year and therefore more effective biocides were needed. A widespread use of organotin-based antifouling paints, which come up to this demand, and introduced in the early 1970s. Antifouling paints consist of a film-forming material with a biocidal ingredient and a pigment. It releases small amounts of the biocide from the painted hull into the water, forming a thin envelope of highly concentrated triorganotins around the boat. The toxic concentration repels the settling stages of fouling organisms, like barnacles, seaweeds or tube worms on the boat’s bottom.

(d) Fate of Organotin compounds in Marine Environment

With the wide industrial application, considerable amounts of toxic OTCs have entered in the aquatic ecosystems. Research is mainly restricted to tributyl- and triphenyltin compounds because these compounds directly enter into the environment by industrially applied OT biocides. Recently evidence was found that municipal and industrial wastewater, sewage sludge and landfill leachates are also important sources of OTs. The persistence of OTC in polluted ecosystems is a function of physical (eg: adsorption to suspended solids and sediments), chemical (eg: chemical and photochemical degradation) and biological (eg: uptake and biological degradation) removal mechanisms. Hence, it is important to study the distribution and the degradation processes of these pollutants under natural conditions.

(i) Degradation

The degradation of the OTs in the environment may be defined as a progressive lose of organic groups from the Sn cation.

$$R_4Sn \rightarrow R_3SnX \rightarrow R_2SnX_2 \rightarrow RSnX_3 \rightarrow SnX_4$$

The removal of the organic groups can be caused by various processes which include;

(1) Ultraviolet (UV) irradiation;

(2) Biological Cleavage;

(3) Chemical Cleavage.

(ii) Bioaccumulation

The criteria for the persistence of OTC in the environment are their lipophillic character. Studies concerning the uptake of these pollutants by aquatic organisms deal with triorganotin (TOT) owing to its extreme toxicity to non-target organisms. It was reported that some marine bacteria display a remarkable ability to accumulate this contaminant. Research on TBT accumulation by aquatic invertebrates has been mostly confined to molluscs (bivalves) and crustaceans (decapods) and demonstrated that marine bivalves are able to accumulate significant amounts of TBT (upto > 5µ/g) (Laughlin 1996). Crustaceans and fish accumulate much lower amounts of this pollutants due to their possession of efficient enzymatic mechanisms that degrade TBT in the body (Laughlin 1996). Uptake of uncharged TBT across epithelia or membrane is one possible mechanism. The accumulation proceeds through either uptake from solutions alone or a combination with diet ingestion. Recent studies have shown that marine mammals and birds also accumulate high levels of toxic butyltins (BTs) in various tissues and organs (Iwata et al. 1995; Kannan et al. 1998a, b).

(iii) Adsorption

A large proportion of the OT contaminants are found to be associated with the clay fraction by adsorption onto particulate matter and controls the distribution and fate of OTs in the environment. Thus, soils and sediments serve as traps for these toxic contaminants. Randall and Weber (1986) reported that between 57 and 97% of TOT in the water column is sorbed under simulated estuarine conditions. The adsorption behaviour of OT species is important in determining the transport processes as well as their bioavailability especially to aquatic organisms. Dissolved species are more likely to be expelled to the sea or directly ingested into the food chain by bioaccumulation.

Laboratory studies under simulated estuarine conditions indicate that the adsorption behaviour of various OTC depends on their molecular structure (Donard and Weber 1985; Randall and Weber 1986). For example, the sequence of adsorption affinity of OTCs on hydrous Fe oxide (MOT > TOT > DOT) suggests that MOT (monoorganotin) is most likely to remain in an estuary, while DOT (diorganotin) preferentially remains in solution. The strong toxic TOT (triorganotin) is likely to be present in the water column as well as in the sediment phase and thus, it might be available to both pelagic and benthic organisms. A variety of other processes, in particular interactions of OTC with organic matter can have a significant effect on the transport, reactivity and bioavailability of the contaminants in natural systems.

Systematic investigation on the remobilisation of OTC from the sorption materials are still few, although this process may determine the future levels of contamination in marine and fresh water systems.

Bueno et al. (1998) reported the sorptive behaviour of TOT on natural quartz sand at low concentration levels to have information regarding the pathway to possible transfer from surface freshwaters to aquifers and concluded that the sorption mechanism is reversible in natural quartz sand.

6.1.2. Irgarol 1051

Irgarol 1051 is an algistatic compound used in copper-based antifouling paints. It is both water soluble and persistent (log K_{OW} 3.95, half-life 23-96 days) (Ciba Speciality Chemicals Corporation 1999; Hall et al. 1999). Environmental monitoring has found that it is a widespread contaminant, with maximum Irgarol concentration of 4.2 µg/L for marinas worldwide, 0.19 µg/L for surface waters around the world and 0.412 µg/L for US surface waters (US EPA 2005). Irgarol 1051 has a predicted environmental concentration (PEC) of 5.59 µg/L (US EPA 2005). Despite, reports of its low affinity for particulate matter, studies have reported the presence in the estuarine sediments upto 1mg/kg (Boxall et al. 2000).

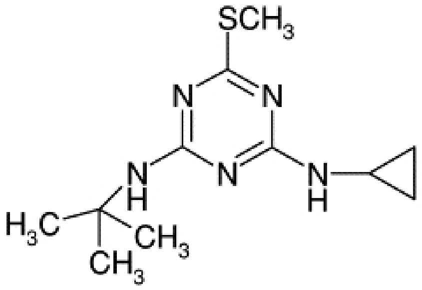

Figure 6.2 Structure of Irgarol 1051

Although Irgarol 1051's primary mode of action involves disrupting electron transport within the photosystem II of the photosynthesis reaction, it has also been shown to affect metabolic reactions within the mitochrondria. Bragadin et al. (2006) investigated that their inhibition of ATP synthesis in mitochondria of rat liver and concluded that the inhibition is as a result of inducing nature of Irgarol 1051.

The use of a marine gastropod would strengthen Irgarol 1051's toxicity assessments, particularly due to its environmental exposure to the contaminants in both water and sediment

6.1.3. Dichlofluanid

Chlorothalonil has been found to degrade after four weeks in natural sea water and even in faster rate, in water supplemented by cultured marine bacteria indicates that biodegradation of the compound can be considerably higher (Callow and Willingham 1996). Davies et al. (1988) has demonstrated that degradation also could occur when the biocide is present in low concentrations. However, Chlorothalonil was found in most of the stations indicating its persistence in sediments. A number of studies have shown that the biocide is more persistent in soil with a half-life of 1-2-months (Davies et al. 1988; Caux et al. 1996).

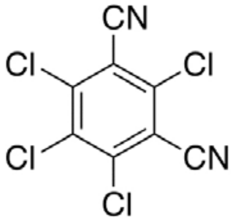

Figure 6.3 Structure of Chlorothalonil

6.1.4. Chlorothalonil

The very low water solubility of Dichlofluanid and the high octane/water partition coefficient (2mg/L at 20^0C and 3.7 respectively) suggest that this biocide probably becomes associated to particulate matter and has the potential to accumulate in sediment. Although Dichlofluanid was used extremely on various crops, scanty literatures were available on the environmental fate and effects. Dichlofluanid has been found to degrade in sea water under natural sunlight as well as in dark indicating that both photodegradation and hydrolysis of the compound taking place (Sakkas et al. 2001). Considerable concentration levels of the biocides were observed in sediments. These levels were higher than that of other biocides, and indicating a tendency to partition to sediments phase.

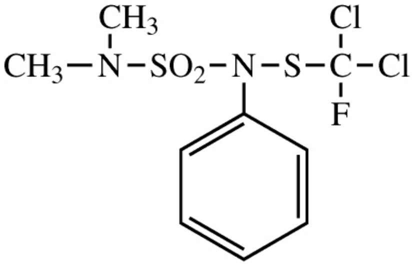

Figure 6.4 Structure of Dichlofluanid

Very few published data were available regarding the presence of Dichlofluanid in the marine environment. Voulvoulis et al. (2000) studied the concentration of Dichlofluanid in the Blackwater estuary, Essex, UK and found a considerable concentration in water and sediments. Table 6.1 previous studies conducted for biocides around the world.

Table 6.1 Previous studies conducted for biocides around the world

		Area	Water	Sediments	References
Organotins	1	USA	0.93 µg/l	0.909 µg/g	Valkirs et al. 1986; Wade et al. 2004
	2	UK	1.06 µg/l	3.9 µg/g	Cleary and Stebbing 1987; Dowson et al.1992
	3	France	1.5 µg/l	8.163 µg/g	Alzieu et al. 1989; Amourx et al. 2000
	4	Hong Kong	1.05 µg/l	53 µg/g	Lau Wong 1991; Ko et al. 1995
	5	Egypt	0.083 µg/l	2.07 µg/g	Abd-Allah 1995; Barakat et al. 2001
	6	Singapore	3.2 µg/l	-	Basheer et al. 2002
	7	Spain	2.8 µg/l	13.3 µg/g	Prego and Cobelo-Garcia 2003; Arambrri et al. 2003
	8	Portugal	0.071 µg/l	0.003 µg/g	Diez et al. 2005
	9	Australia	0.012 µg/l	89 µg/g	Reitsema 2008
	10	India	0.037µg/l	16.82 µg/g	Meena et al. 2009;Bhosle et al. 2006
	11	Korea	0.16 µg/l	9.58 µg/g	Choi et al. 2009
	12	japan	0.002 µg/l	0.011 µg/g	Eguchi et al. 2010
Irgarol 1051	1	UK/English Coast	< 2-500ng/l	< 10-132 ng/g	Gough et al. 1994
	2	France	14-640ng/L		Tolosa et al. 1996
	3	Lake Geneva	< 0.3-145ng/l	2.5-8 ng/g	Toth et al. 1996
	4	UK/Hamber	< 1-39 ng/l		Zhou et al. 1996
	5	Baltic Sea	90 ng/l	220 ng/g	Scarlett et al. 2000
	6	North Sea	11 ng/l	25ng/g	Scarlett et al. 2000
	7	UK		< 10-1011ng/g dry wt	Boxall et al. 2000
	8	UK		3.3-222 ng/g dry wt	Voulvoulis et al. 2000
	9	UK		< 1-110 ng/g dry wt	Thomas et al. 2000
	10	Netherlands	90 ng/l	< 1000 ng/g	Lamoree et al. 2002; Thomas et al. 2000
	11	Spain	3-325 ng/L		Martinez et al. 2001
	12	Greece		3-690 ng/g dry wt	Albanis et al. 2002
	13	UK	< 1-964 ng/L		Bowman et al. 2003
	14	Australia	6 ng/l	1340 ng/g	Reitsema 2008
	15	Japan	18 ng/l	10 ng/g	Eguchi et al. 2010

6.2 Results

Detailed observations were conducted to assess the occurrence of organotin compounds and several other antifoulants in ports and marinas along the Cochin estuary. Apart from organotin compounds, Irgarol 1051, Chlorothalonil and Dichlofluanid were measured in surface sediments collected in the ports and marinas of the Cochin estuary. These locations figure among the biggest and most important port and marinas for leisure boats along the South west coast of India. Throughout the sampling period all the harbour is at full capacity. Vessels of every size are docked either permanently or temporarily coming from all around the world and ensuring a constant environmental pressure. Tables 6.2 and 6.3 give the biocide concentration along the studied location in Cochin estuary. Figures 6.10 (a) to 6. 24 (g) displayed the chromatogram of biocides along studied locations.

Table 6.2 Biocide concentrations of sediments obtained during 2007 (pre-monsoon)

Biocides (ppb)	2007 pre-monsoon						
	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6	ST-7
MPT (μg/kg)	0.000	0.000	0.000	1.667	0.333	14.000	0.333
DPT (μg/kg)	0.000	1.000	1.333	4.333	0.333	0.333	0.000
TPT (μg/kg)	2.667	1.000	16.667	40.333	8.000	2.667	13.000
Irgarol 1051 (μg/kg)	BDL	1.599	12.880	962.560	BDL	BDL	3.162
Chlorothalonil (μg/kg)	BDL	4.688	3.479	11.392	BDL	3.133	2.395

Table 6.3 Biocide concentrations in sediments obtained during 2008 (pre-monsoon)

Biocides (ppb)	2008 pre-monsoon						
	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6	ST-7
MPT(μg/kg)	2.667	0.000	0.000	0.000	0.000	1.000	1.000
DPT(μg/kg)	0.000	0.333	0.000	0.667	0.333	6.000	1.000
TPT(μg/kg)	0.000	0.667	14.667	20.667	6.667	4.667	7.667
Irgarol 1051 (μg/kg)	BDL	1.392	6.997	471.429	BDL	BDL	2.280
Chlorothalonil (μg/kg)	BDL	5.386	5.535	9.702	BDL	4.200	2.696

The phenyltin in the environment is seen as three disintegration products; triphenyltins, diphenyltins and monophenyltins. The triphenyltin in sediment degraded to form diphenyltin, which in turn to form monophenyltin. The concentration of these three components varied spatially.

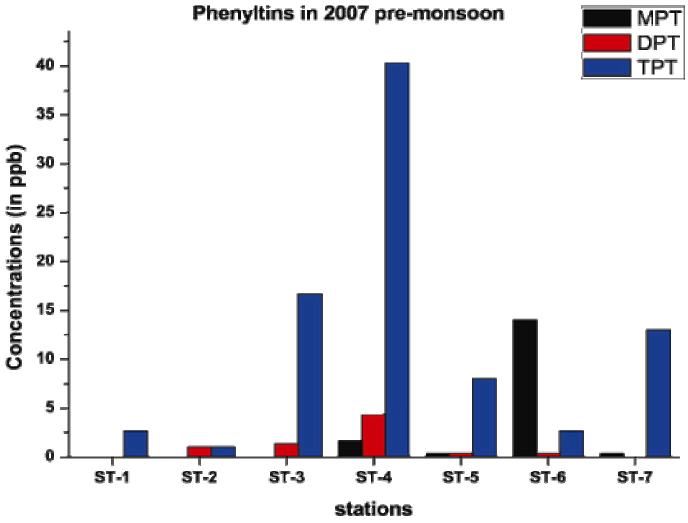

Figure 6.5 Graph Showing the distribution of Phenyltins during 2007 (Pre-Monsoon)

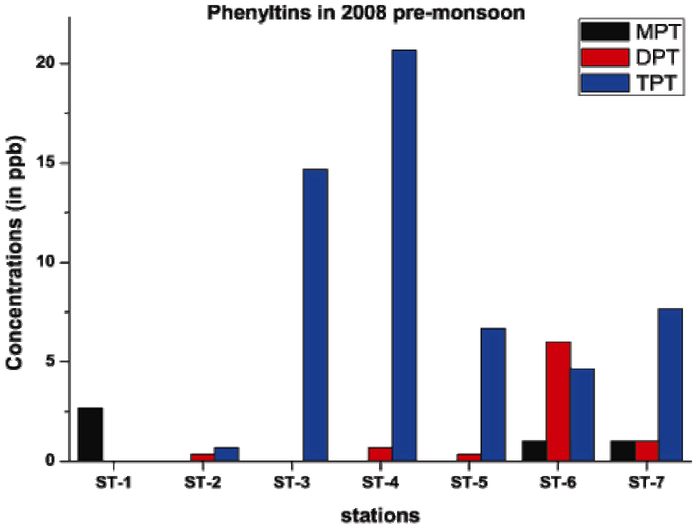

Figure 6.6 Graph Showing the distribution of Phenyltins during 2008 (Pre-monsoon)

The triphenyltin varied from 1 to 40.333 ppb along all the stations during 2007 and from 0.667 to 20.667 ppb during 2008 (Figures 6.5 and 6.6). In 2007, Barmouth (ST-1) showed lower concentration and ST-4, near the shipyard area which exhibited higher concentration. Similar trend was seen on 2008 (pre-monsoon).

The DPT accumulation varied below detection limit to 4.333 ppb along all the sampling locations during 2007 and BDL to 1 ppb during 2008 (pre-monsoon). Higher concentration was found at shipyard in 2007 and at fishing harbour in 2008.

The MPT concentration varied from BDL to 14 ppb among the sampling location during 2007(pre-monsoon) and BDL to 2.667 ppb in 2008 (pre-monsoon). Fishing harbour showed elevated concentration during 2007 and at sulphur jetty station (ST-3), barmouth and bolgatty results concentration below the detection limit. In 2008, the station near to the barmouth (ST-1) showed higher concentration (2.67 ppb). Stations designated nearer to the Shipyard, Sulphur jetty, Thevara and Bolgatty also showed concentration below the detection limit.

Irgarol 1051 is the major biocide used in ship painting industry after the ban of organotin formulations by International Maritime Organisation (IMO). The Irgarol 1051 is widely distributed in sampling locations. The Irgarol 1051 concentration varied from BDL to 962.560 ppb among the studied location during 2007 and from BDL to 471.429 ppb during 2008 (pre-monsoon) (Figure 6.7). In stations Barmouth, Thevara, and Fishing harbour, Irgarol 1051 concentration was below the detection limit during both the sampling period. During 2007, Shipyard showed 962.560 ppb, at Sulphur jetty it was 12.88 ppb, Bolgatty 1.599 ppb and near Indian oil Jetty showed 3.162 ppb of Irgarol1051. But in 2008, the concentration varied as; Shipyard 471.429 ppb, Sulphur Jetty 6.997 ppb, Bolgatty 1.392 ppb and near Indian oil Jetty 2.280 ppb.

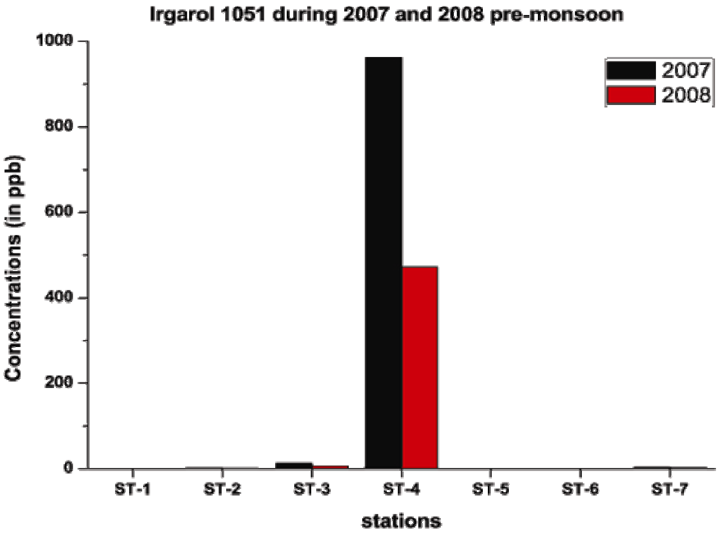

Figure 6.7 Graph Showing the distribution of Irgarol 1051 during 2007 & 2008 (pre-monsoon)

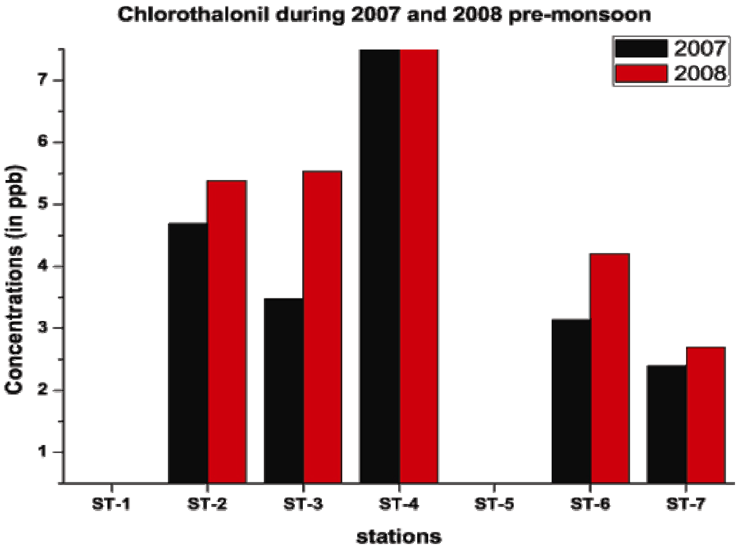

Figure 6.8 Graph Showing the distribution of Chlorothalonil during 2007 & 2008 (pre-monsoon)

The Chlorothalonil concentration varied from 0 to 11.392 ppb (µg/kg) during 2008 and from 0 to 9.702 ppb during 2008 (Figure 6.8). In ST-1, Chlorothalonil was not detected. The stations ST-2, ST-3 and ST-4 showed concentrations 4.688 ppb, 3.479 ppb and 11.392 ppb respectively. In the case of ST-5, Chlorothalonil was not detected, and in ST-6 & ST-7 noted the concentration 3.133 ppb and 2.395 ppb respectively. In 2008 ST-1 & ST-5 showed similar results as: below detection limit and ST-2, ST-3, ST-4, ST-6 & ST-7 showed 5.386 ppb, 5.535 ppb, 9.702 ppb, 4.200 ppb and 2.696 ppb respectively.

Concentration of Dichlofluanid was in below the detection limit in the studied location during the study period.

6.3 Discussion

6.3.1 Orgnaotins

In station-1 (ST-1) only TPT (triphenyltin) was found and its degradation products were below detection limit. This result indicated the presence of freshly leached TPT in the sediments. This station is in Barmouth side, which is highly influenced by heavy tidal effects which results in low concentration of TPT and the absence of MPT (monophenyltin) and DPT (diphenyltin).

The station-2 (ST-2) is near Bolgatty Island, where TPT concentration is 1 ng Sn/g dw and DPT concentration is also 1 ng Sn/g dw. High settling rate is the major contributing factor noted in this station. This influences the degradation of TPT into DPT which may be caused by the greater residence time or may be due to the anaerobic microbial degradation in sediments.

Station 3 (ST-3) located near to the Sulphur jetty, where Sulphur transport is the main activity. Compared to ST-1 and ST-2, higher concentration of TPT and DPT were observed in this location. These results describe the degradation of TPT into DPT.

Station 4 (ST-4) is near to the Cochin Shipyard, a prolonged and high concentration of TPT was found in this site. This influence is mainly due to the heavy ship painting activities in the shipyard. Besides, DPT and MPT were also present in detectable concentration. Therefore concluded that TPT degradation also contributed in certain extent.

Station 5 (ST-5) is near to the Thevara which is far (500m) from ST-4. The presence of TPT is higher, DPT and MPT were also present in but comparatively less than TPT.

Station 6 (ST-6) is the hub for largest fleet of fishing boats and a harbour was built for this purpose. Here the concentration of TPT is lower as compared to other stations. But high concentration (14 ppb) of MPT was detected and the rate of degradation by anaerobic microbial degradation may be one of the main reasons accounted.

The next station (ST-7) is near to the Indian Oil Corporation storage tanks. This place is mainly for storage of petroleum products and large oil tanks are built for this purpose. The TPT concentration is higher (13 ppb) in this station and DPT is below the detection limit. MPT was found in this station with lower concentration (0.33 ppb). This indicated a slow degradation of TPT in this region.

In 2008, the change in concentration of TPT, DPT and MPT was observed in all sampled stations. In ST-1 only MPT were present and this may be due to the high degradation of TPT into MPT. In ST-2, lower concentration of TPT and DPT was detected and it was observed that slow degradation of TPT into DPT. In ST-3, higher concentration of TPT was observed and DPT and MPT were absent in this station. This indicate the fresh input of biocides into the sediments due to ship berthing. Higher concentration during 2008 was found in ST-4, near to the shipyard. Here TPT concentration is 20.67 ng Sn/g dw. Besides, lower concentration of DPT and MPT were noted and it lies below the detection limit. In ST-5, TPT and DPT were present and MPT was below the detection limit. TPT concentration is 6.67 ng Sn/g dw and low level of DPT (0.33 ng Sn/g dw) were observed. In station ST-6; TPT, DPT and MPT were detected. The concentration of TPT is 67 ng Sn/g dw , DPT is 6 ng Sn/g dw and MPT 1 ng Sn/g dw were determined. TPT is degraded into its degradation products as DPT and MPT, which gave meaningful influence of aged TPT in the studied station. In ST-7, all the three phenyl tin were present. Highest concentration was observed for TPT followed by DPT and MPT. The concentration of TPT is found to be 7.67 ng Sn/ g and for DPT and MPT 1 ng Sn/g dw and 1 ng Sn/g respectively.

During the two year study period, it was observed that phenyltin compounds are present in the sediment sampled location of Cochin estuary which is situated in and around the main shipping channel, port and shipyard. The dredging activities and the tidal influence are the major cause of varying concentration of phenyltins along the study area. Compared to all stations, Station-4 recorded higher concentration in both the consecutive years, 2007 and 2008. Since ship building and the concurrent use of phenyltin in this station may be the major source. These elevated concentrations observed in different sampling sites were within the port area which records that the contamination is widespread.

6.3.2. Organotin degradation index

Once released into the environment, BTs (butyltins) and PTs (Phenyltins) are subjected to both biotic and photochemical debutylation and dephenylation from the parent compounds (TBT & TPT) to their degradation products (DBT and MBT for butyltins and DPT and MPT for phenyltins). In the water column, microbial degradation and photolysis are the main processes, whereas anaerobic biodegradation can only occur in sediments (Amouroux et al. 2000). The degradation of TBT in the aquatic phase takes place within days to weeks (Stewart 1996), while the half-life was estimated to be about 2 years in the sediments underlying a seawater margin (de Mora et al. 1989).

Table 6.4 Organotin degradation index during 2007 & 2008 (pre-monsoon)

Degradation Index	Sampling Year	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6	ST-7
	2007	8.081	1.000	12.500	6.722	12.000	0.186	39.000
	2008	0.000	2.000	0.000	31.000	20.000	0.667	3.833

To assess whether the organotin concentration drives from recent origin or not, it is useful to calculate the degradation indexes which defines as the ratios between parent compound and the main metabolites (TBT/ MBT+DBT and TPT/DPT+MPT). These ratios >1 would suggests that the contamination is due to “fresh” or recent inputs of parent compounds. On the contrary, ratios <1 would illustrate that the parent compounds have degraded and thus the contaminants should be considered as “old” (Diez et al. 2002).

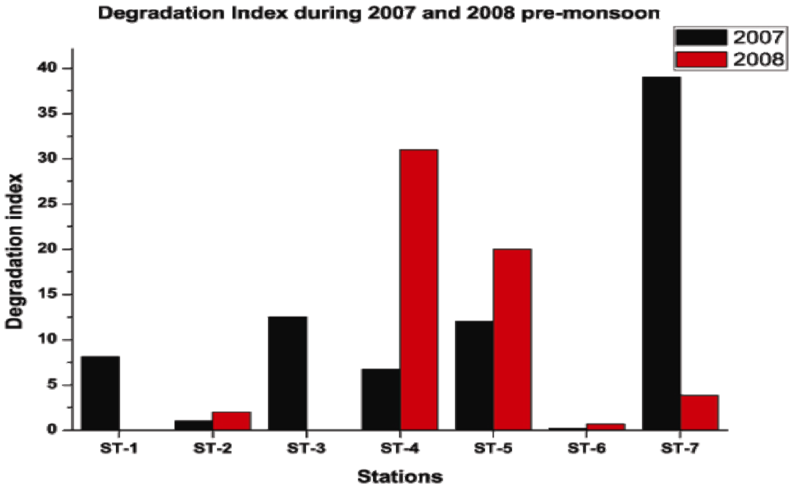

Figure 6.9 Graph Showing the degradation index during 2007 & 2008 (pre-monsoon)

According to the organotin degradation index, the contamination is rather fresh. Similarly, the ratio TPT/ DPT suggests that the contamination is relatively recent in most of the samples. The degradation index > 1 was found in ST-1, ST-2, ST-3, ST-4, ST-5 and ST-7 during 2007 and ST-2, ST-4 and ST-7 during 2008 showing that the contamination is mainly due to fresh or recent input of these compounds (Table6.4 and figure 6.9). Station ST-6 in 2007 and ST-1, ST-3 and ST-6 during 2008 resulted organotin degradation index as <1, denoting that the compounds have degraded and the contaminants are considered as old or as aged.

According to the degradation indices, TPT are still introduced in the environment, most probably through the ongoing use of organotin based antifouling paints.

6.3.3. Irgarol 1051

Little is known about the aquatic partitioning of Irgarol 1051, although its water solubility 7mg/l (Ciba Geigy 1988), which implies a stronger tendency for adsorption to the particles and sediments in comparison to other triazines that exhibit a higher water solubility (i.e., atrozine 33mg/l, prometryne, 40mg/l). In addition, the relatively high octane/water partition coefficient (log K_{OW}=3.95) of Irgarol 1051 suggests an enrichment of the antifouling compound in the sediment.

Irgarol 1051 is the main biocide which is used as a replacement compound after the ban of organotin compounds by IMO (International maritime organisation). The presence of Irgarol 1051 was found in certain studied locations under consideration.

During the year 2007, ST-1 was found to be free zone. This may be due to the high flow and flushing out of water in this area and also the regular dredging taking place in this location for the smooth travelling of larger vessels.

In ST-2, Irgarol 1051 recorded 1.599 µg/kg during 2007. This station is close to hubs for leisure craft which frequently visit this area. The enrichment of Irgarol 1951 may be due to the leaching of these materials from these vessels.

ST-3 which is the main location for sulphur transport and recorded Irgarol 1051 concentration as 12.880 µg/kg in 2007. This station is the main berth for sulphur import, and the transportation causes the higher concentration in this station.

ST-4 recorded higher concentration compared to other station (962.560 µg/kg). This station is near to the shipyard and regular ship painting and frequent repairing activities are taking place in this region.

ST-5 did not show the presence of any Irgarol 1051 because of the absence of shipping activities and established as an Irgarol 1051 free station.

In ST-6 Irgarol 1051 was absent. This may be due to the regular dreading in this area. High tidal variations also affect the occurrence of Irgarol 1051.

The next station is near to the Indian Oil Corporation storage tanks and is situated near to the Barmouth. Regular shipping activities happen in this area. So this station recorded 3.162 ppb of Irgarol 1051.

In 2008, the study area displayed different concentration compared to the previous year 2007. In ST-1, again Irgarol 1051 was not detected. This can be explained by the high water exchange rates based on the direct connection to the sea including the tidal effect. Most of the contaminated surface water is presumably removed from the port prior to the transport of the pollutants to the sediments. The concentration of Irgarol 1051 in ST-2 was 1.392 µg/kg similar to the previous year (2007) data. Moreover, other earlier studies suggested that environmental pollution by antifouling biocides arose largely from pleasure craft activity rather than commercial shipping which occurred mostly in the station, ST-2.

ST-3 showed higher concentration in the year, 2008 and it is recorded as 6.997 µg/kg. In this station, large ships usually berth for sulphur transport. So the higher concentration is due to leaching from antifouling paint coating from these ships hull.

ST-4 showed higher concentration among all the other studied stations and it may be due to the presence of ship painting activities and ship repairing activities. Due to the ban of organotin based paints by IMO, vessels building for outside countries may be coated with formulation other than organotins.

ST-5 is outside the port area and no shipping activities were found in this region. Irgarol 1051 were absent in this location in the year 2008.

The same result of 2007 was observed at ST-6 in 2008 also. So dreadging influence the enrichment of Irgarol 1051 in this area.

ST-7 is near to Indian Oil Corporation Jetty and detected Irgarol 1051 in 2008. This area recorded 2.696 ppb of Irgarol. Regular shiiping activities may be the reason behind this enrichment.

These results showed that during the two consecutive years of study, the presence of Irgarol 1051 was observed in the studied locations around the Cochin estuary. The exact concentration of Irgarol 1051 is very difficult to determine because of regular dredging and intense tidal effects.

6.3.4. Chlorothalonil

Chlorothalonil is one of the major pesticides used in agriculture field around the world. After the ban of organotin formulation as antifouling paints, Chlorothalonil was started to use as a substituent in most of the antifouling biocides. Chlorothalonil has been found to degrade after four weeks in natural sea water and even faster in water supplemented by cultured marine bacteria indicating the strong biodegradation of the compound (Callow and Willingham 1996). Davies et al. (1988) has demonstrated that degradation also occur when the biocide is present in low concentrations. However, chlorothalonil was found in most stations indicating the persistence in sediments. A number of studies have shown that the biocide is more persistent in soil with a half-life of 1-2-months (Davies et al. 1988; Caux et al. 1996).

In ST-1 during 2007, Chlorothalonil was not detected and the main reason is similar to that of Irgarol 1051, i.e. the dredging activities and high water currents movements.

ST-2 showed Chlorothalonil (4.688 µg/kg) during 2007. The presence of Chlorothalonil in this station is mainly due to frequent visit of leisure crafts in this region. A new marina for yachts was built in this place. High concentration of Chlorothalonil can be expected in the station.

ST-3 near the sulphur jetty is mainly for sulphur transport. The Chlorothalonil in this station recorded 3.479 µg/kg during 2007 and it may be due to prevailing of the shipping activities in this sampling location. The leaching of biocides is the main cause for the accumulation in these stations.

ST-4 recorded high concentration among all the stations during 2007. The ship building activities are the main cause of this high enrichment in this location. Also the berthing of warships is near to this location. These points out the higher concentration of Chlorothalonil.

ST-5 is placed near to the Thevara which is outside the port area and shipping activities are less in this location as compared to the main area. Therefore this site was noted as free stations with respect to Chlorothalonil during 2007.

ST-6 is situated near to the fishing harbour and could expect the accumulation of Chlorthalonil in this location. The Chlorothalonil concentration in this sampling station is 3.133 µg/kg during 2007. Leaching from fishing boats may be the main reason for the accumulation.

ST-7 is situated near to the IOC petroleum storage plant and is also a location of passenger ship berthing. The Chlorothalonil concentration was 2.395 µg/kg. Leaching and runoff is the main sources for this contamination.

In 2008, the Chlorothalonil concentration varied slightly higher than the previous year (2007). ST-1 showed Chlorothalonil free zone. As already discussed the high water flush out through narrow outlet may prevent the suspended particles to settle in the aquatic phase.

ST-2 showed higher concentration than previous year. Increase leisure craft visiting this area may contribute this enrichment. The runoff from agricultural field may be another reason for this type of accumulation.

ST-3 also showed higher concentration than the previous year (5.535 µg/kg). The increased shipping activities enhance the accumulation of Chlorothalonil.

ST-4 showed higher concentration than all the sampled stations in 2008, than that of the previous year 2007, Chlorothalonil concentrations recorded were 9.702 µg/kg. Along the west coast of India, the ship building industry in this location is largest. Simultaneously the out coming vessels are increasing year by year thereby the usage of antifouling formulation also increases concurrently. Therefore, higher concentration of Chlorothalonil was observed in this location.

The lack of shipping activity in the region denotes the absence of Chlorothalonil at ST-5 in 2008.

ST-6 near to the fishing harbour showed higher accumulation of Chlorothalonil than the previous year and showed 4.2 µg/kg in this region. The leaching may be the main cause supported by the agricultural runoff.

ST-7 recorded 2.696 µg/kg Chlorothalonil in this region. The frequency of passenger ships from outside the country increases year after year. The ban on organotin based formulation by International Maritime Organisation and the alternative biocide also may be the reason for the accumulation of this compound.

In general, the Chlorothalonil concentration increases year after year. Increased shipping activities and increased use of Chlorothalonil as one of the main antifouling agents in ship painting industry to contribute to this trend.

Therefore to understand the harmful effect of these biocides in the Cochin estuary, ecotoxicological evaluation with indigenously available indicator organisms were needed and the next chapter 7 *"Ecotoxicological Studies of Antifouling biocides"* infers some of the salient features observed in the ecotoxicological studies conducted during the study period.

6.4. Conclusion

The present study gave an insight into the knowledge of antifouling biocides used in shipping industry. The pollution caused by antifouling biocides in the Cochin estuary is high when compared to other shipping channels and ports around the world. These elevation may be due to the use of organotin compounds in paints, while in other countries, these were banned from 2003 onwards and completely adopted different approach of painting from 2008. Ecotoxicological studies were needed to understand more about the harmful effect of these antifouling biocides in the Cochin Estuarine system. Therefore the Chapter 7 substantiates the harmful effect of these biocides and their role on the inhibition rate of Acetylcholine esterase enzyme in a black Clam *"Villoritta"* species available indigenously in the Cochin estuarine system.

Figures 6.10 (a) to Fig. 24 (g) Chromatogram of biocides along the studied area during 2007 & 2008 (pre-monsoon)

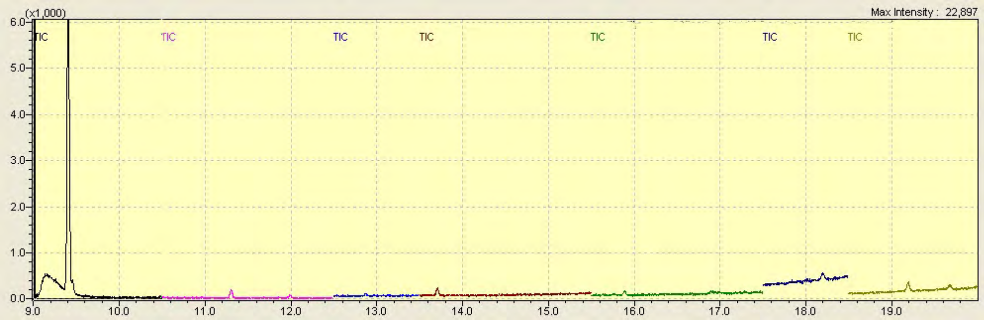

Figure 6.10 (a) PT at ST-1 in 2007 (pre-monsoon)

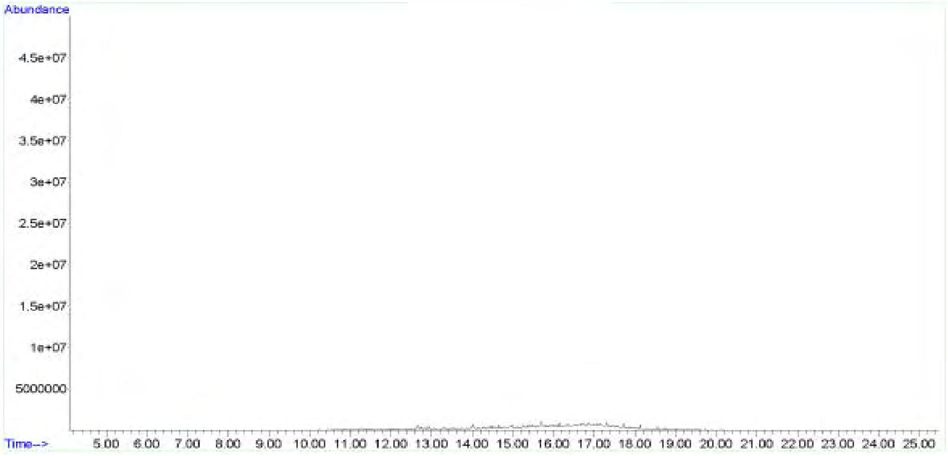

Figure 6.10 (b) Irgarol 1051 at ST-1 in 2007 (pre-monsoon)

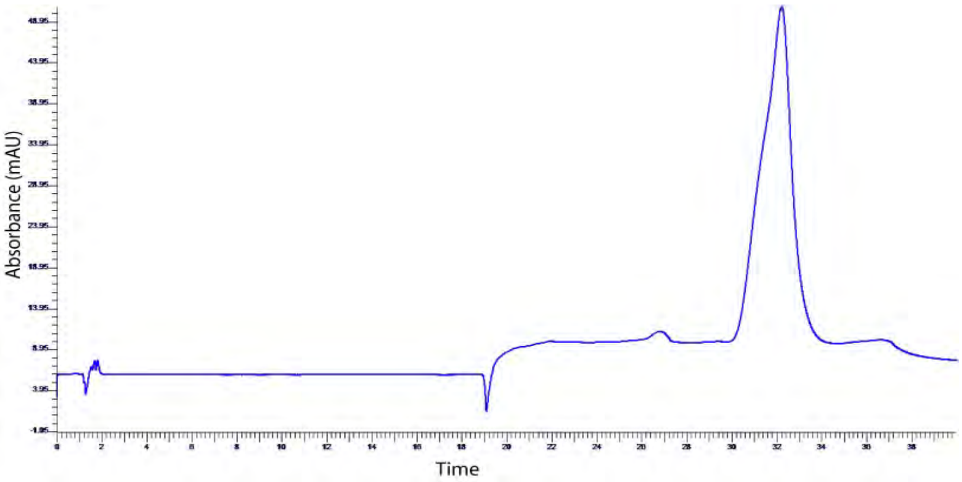

Figure 6.10 (c) Chlorothalonil at ST-1in 2007 (pre-monsoon)

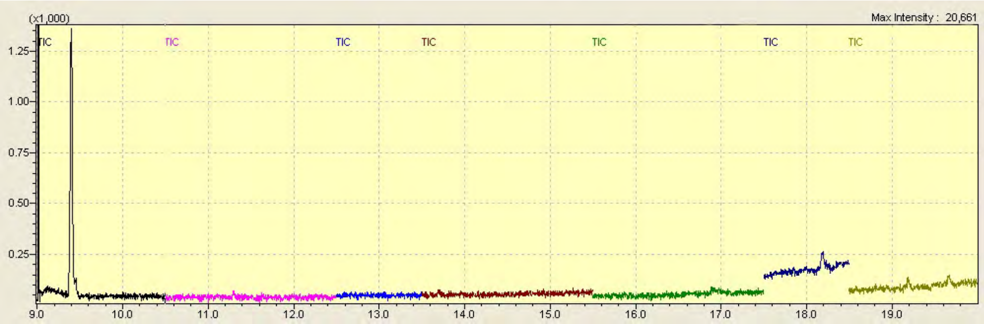

Figure 6.11 (a) PT at ST-2 in 2007 (pre-monsoon)

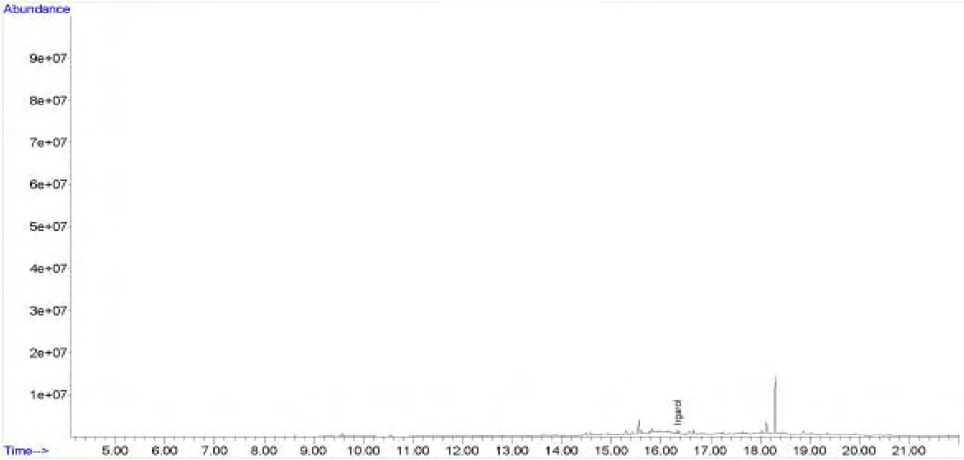

Figure 6.11 (b) Irgarol 1051 at ST-2 in 2007 (pre-monsoon)

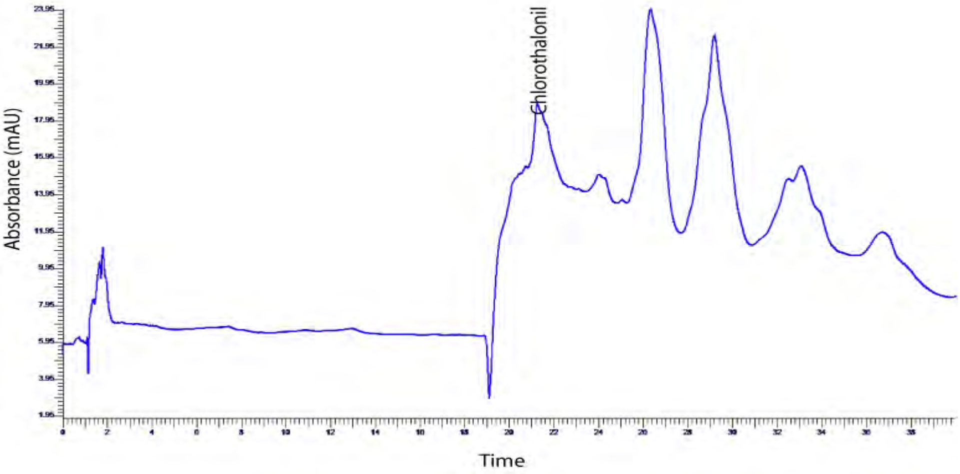

Figure 6.11 (c) Chlorothalonil at ST-2 in 2007 (pre-monsoon)

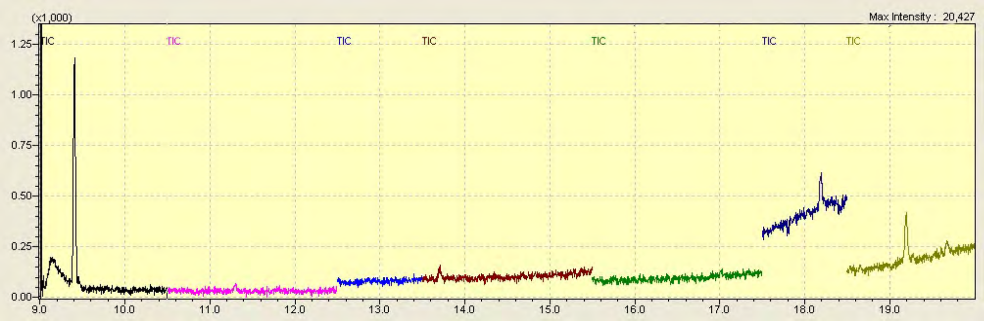

Figure 6.12 (a) PT at ST-3 in 2007 (pre-monsoon)

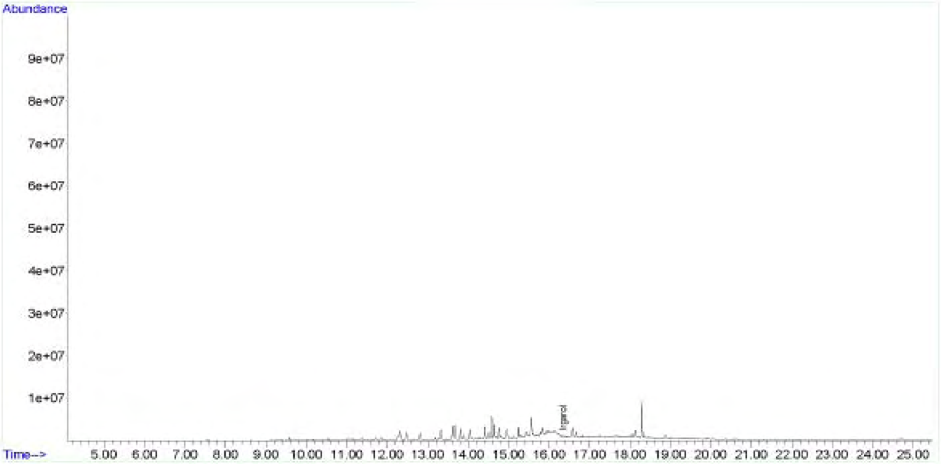

Figure 6.12 (b) Irgarol 1051 at ST-3 in 2007 (pre-monsoon)

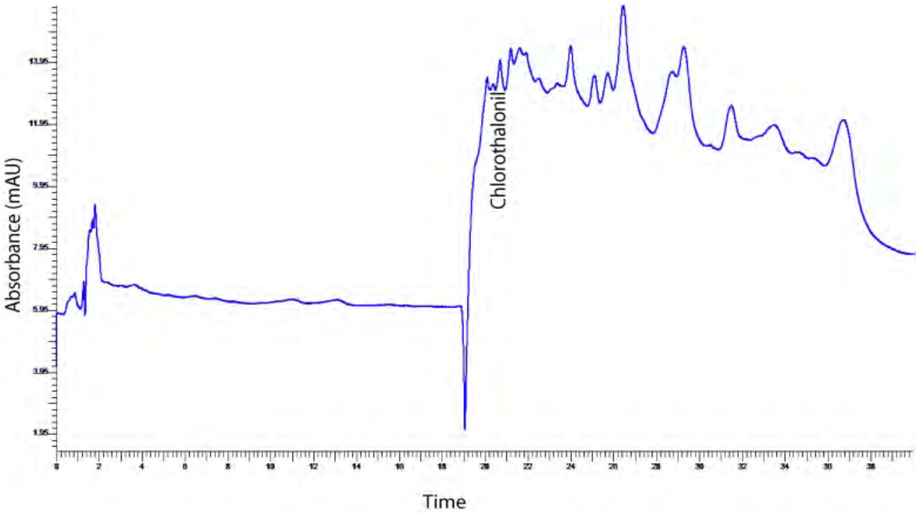

Figure 6.12 (c) Chlorothalonil at ST-3 in 2007 (pre-monsoon)

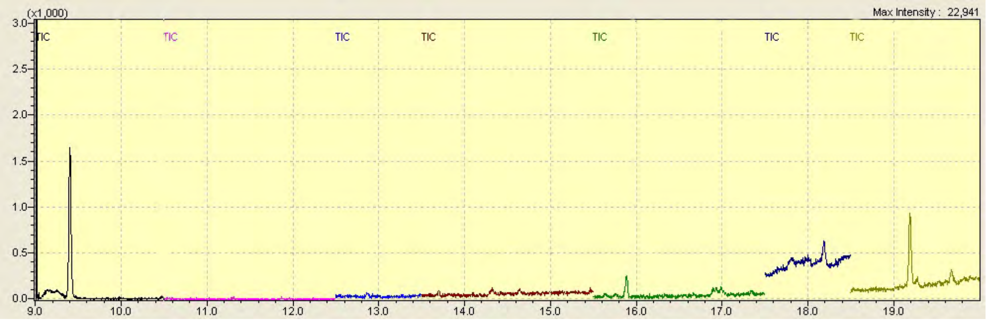

Figure 6.13 (a) PT at ST-4 in 2007 (pre-monsoon)

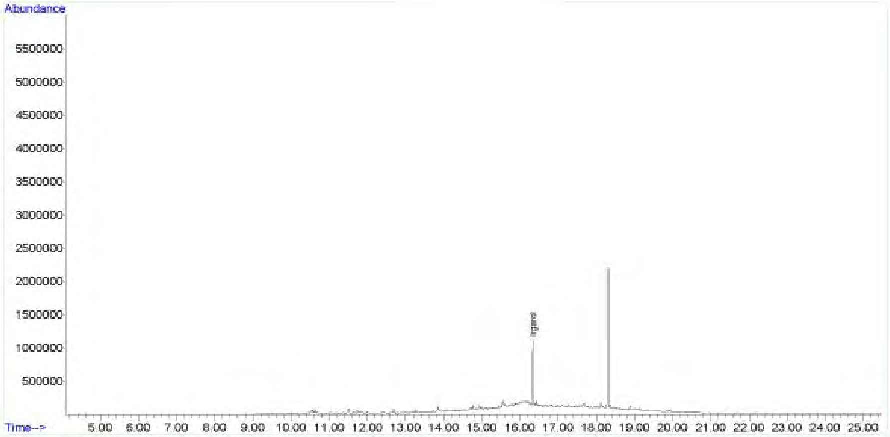

Figure 6.13 (b) Irgarol 1051at ST-4 in 2007 (pre-monsoon)

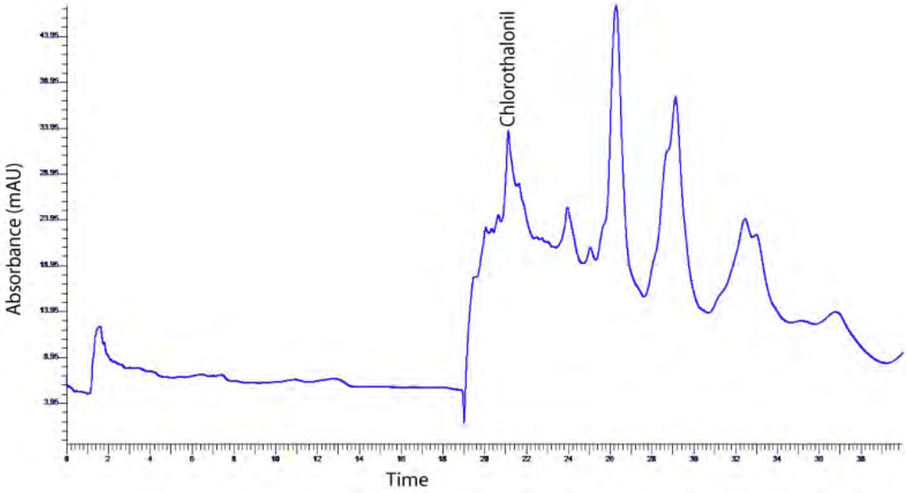

Figure 6.13 (c) Chlorothalonil at ST-4 in 2007 (pre-monsoon)

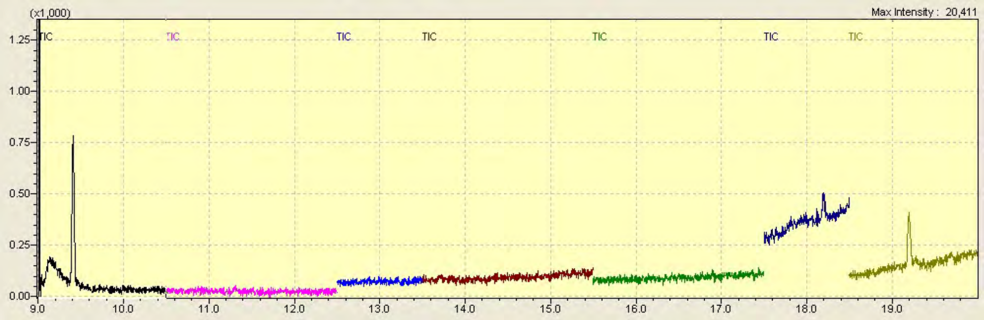

Figure 6.14 (a) PT at ST-5 in 2007 (pre-monsoon)

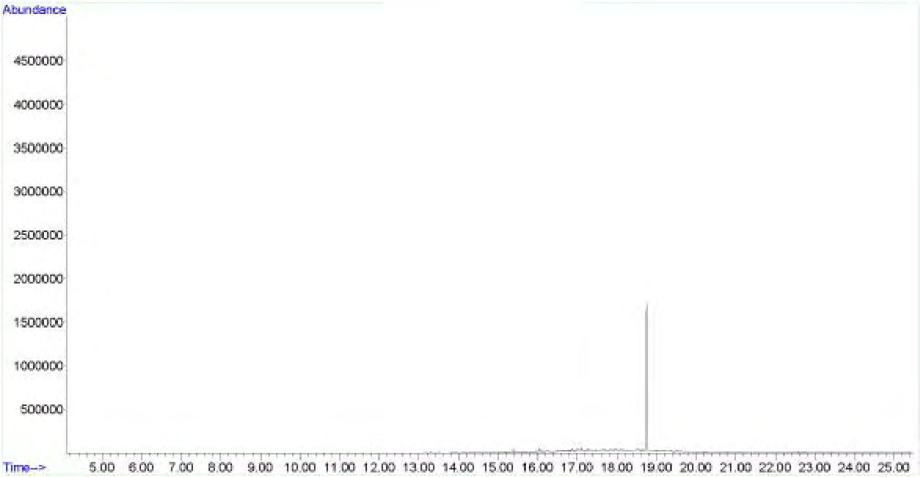

Figure 6.14 (b) Irgarol 1051 at ST-5 in 2007 (pre-monsoon)

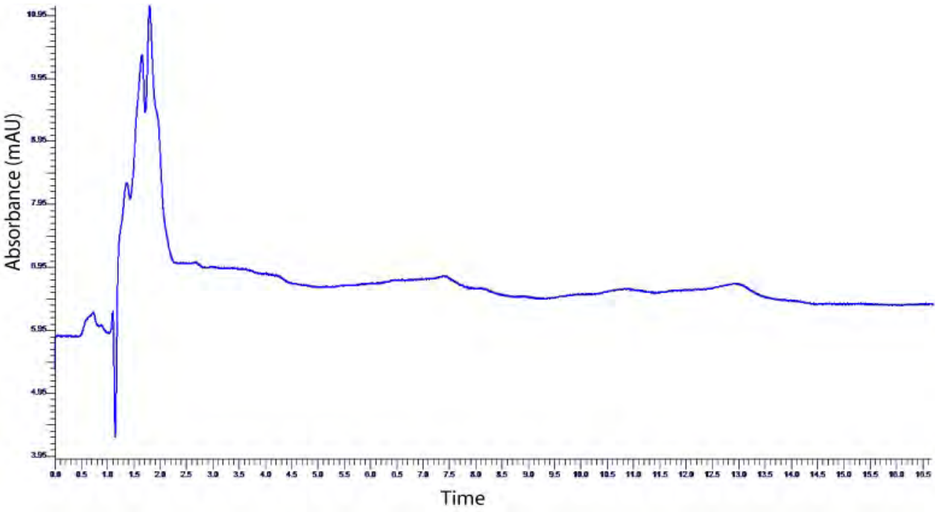

Figure 6.14 (c) Chlorothalonil at ST-5 2007 (pre-monsoon)

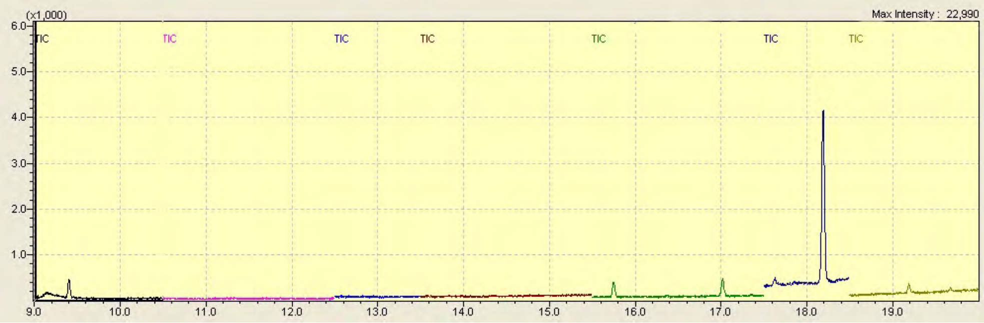

Figure 6.15 (a) PT at ST-6 in 2007 (pre-monsoon)

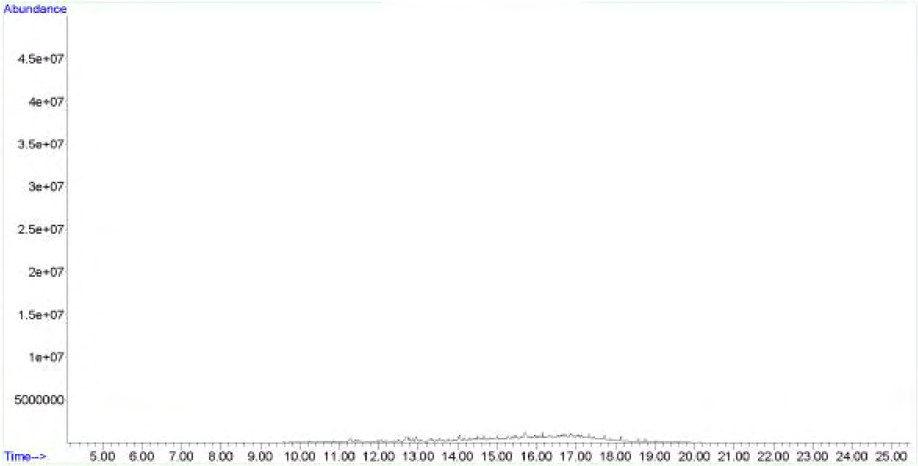

Figure 6.15 (b) Irgarol 1051 at ST-6 in 2007 (pre-monsoon)

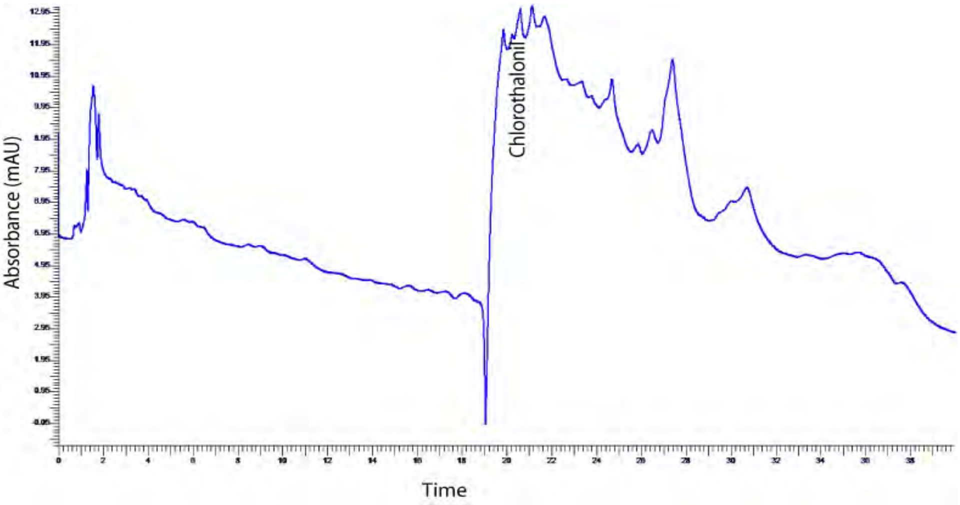

Figure 6.15 (c) Chlorothalonil at ST-6 in 2007 (pre-monsoon)

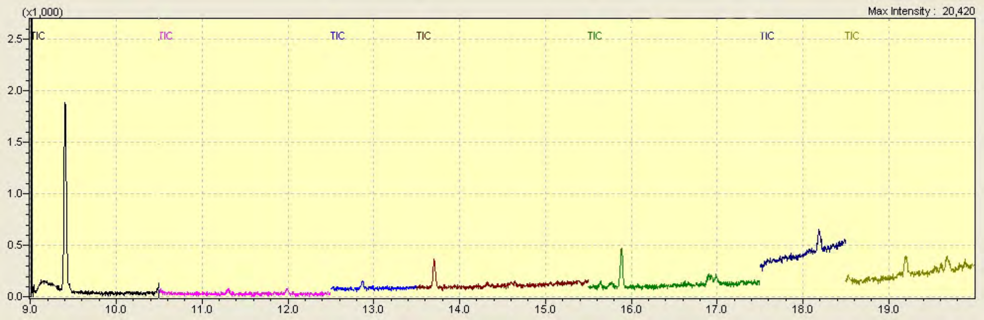

Figure 6.16 (a) PT at ST-7 in 2007 (pre-monsoon)

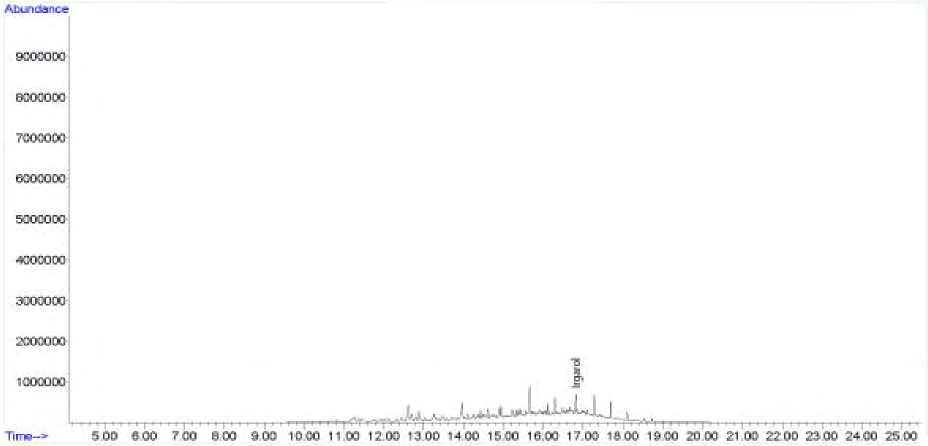

Figure 6.16 (b) Irgarol 1051 at ST-7 in 2007 (pre-monsoon)

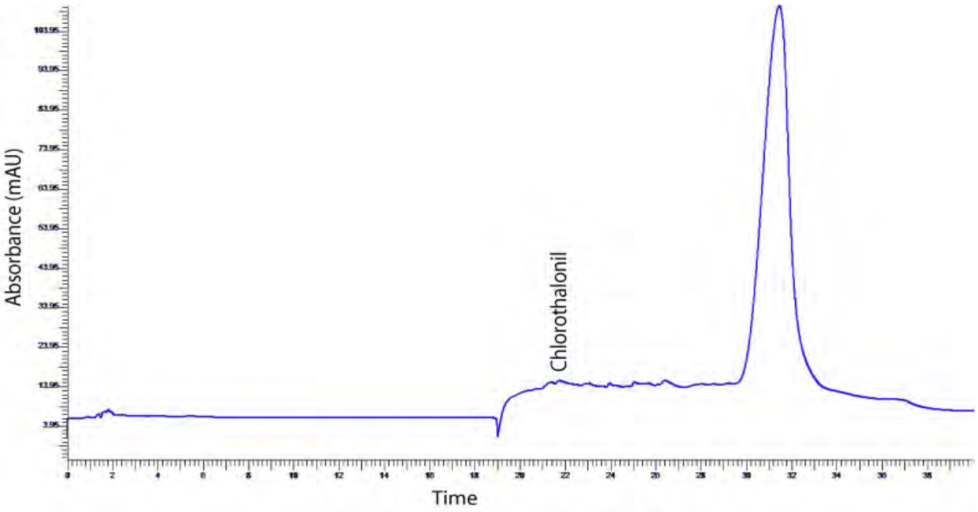

Figure 6.16 (c) Chlorothalonil at ST-7 in 2007 (pre-monsoon)

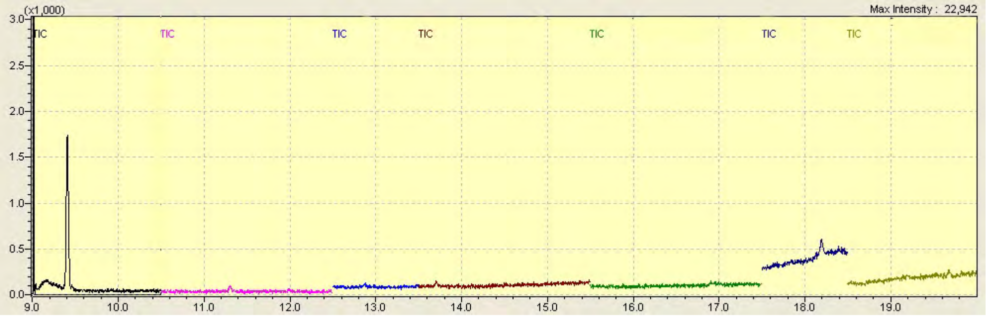

Figure 6.17 (a) PT at ST-1 in 2008 (pre-monsoon)

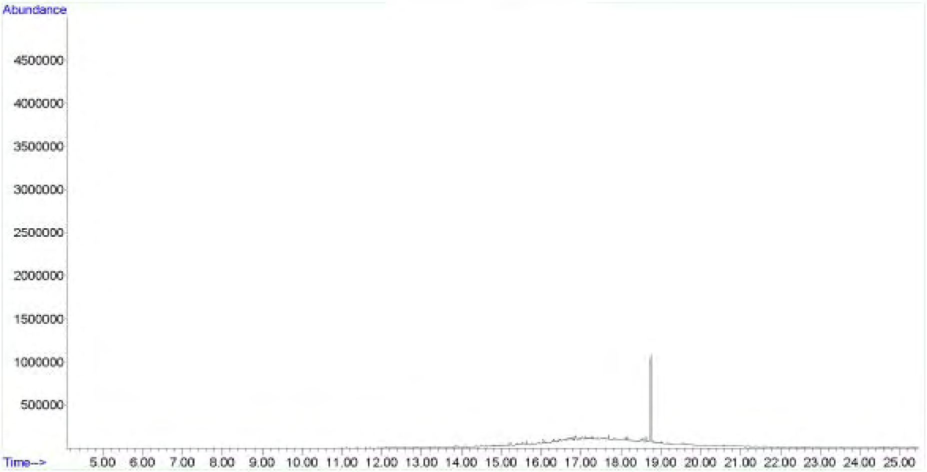

Figure 6.17 (b) Irgarol 1051 at ST-1in 2008 (pre-monsoon)

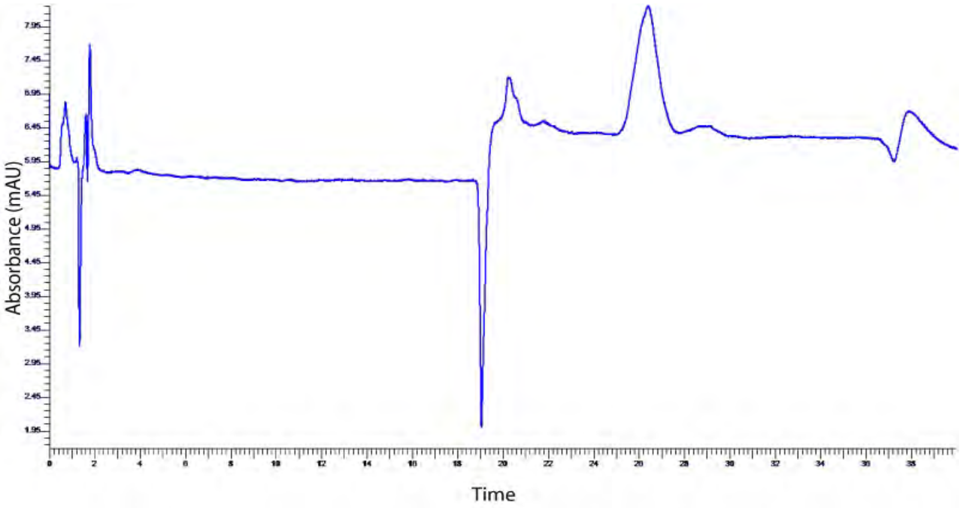

Figure 6.17 (c) Chlorothalonil at ST-1 in 2008 (pre-monsoon)

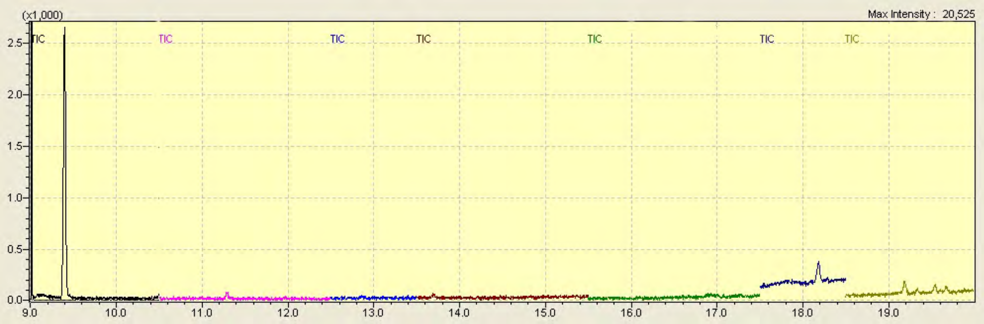

Figure 6.18 (a) PT at ST-2 in 2008 (pre-monsoon)

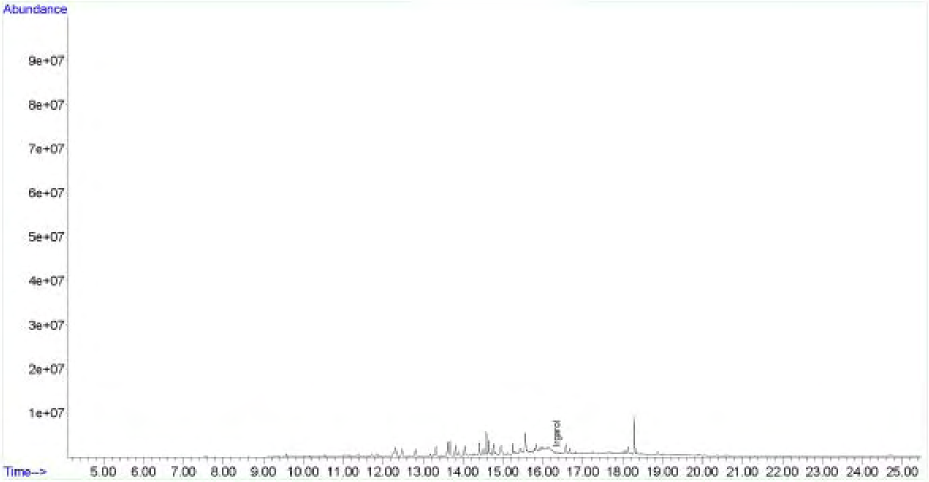

Figure 6.18 (b) Irgarol 1051 at ST-2 in 2008 (pre-monsoon)

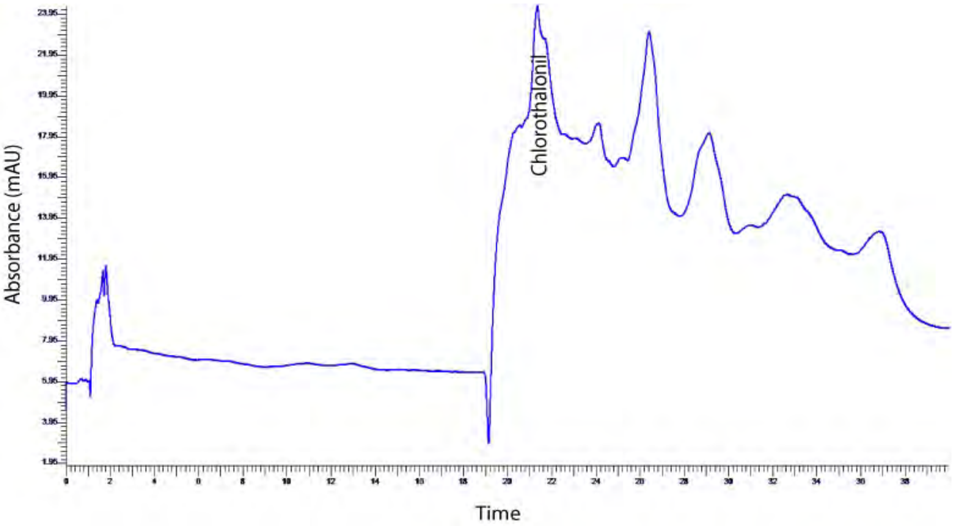

Figure 6.18 (c) Chlorothalonil at ST-2 in 2008 (pre-monsoon)

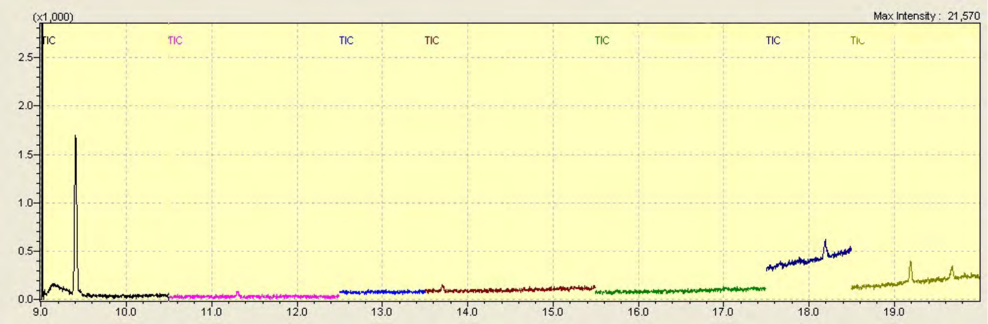

Figure 6.19 (a) PT at ST-3 in 2008 (pre-monsoon)

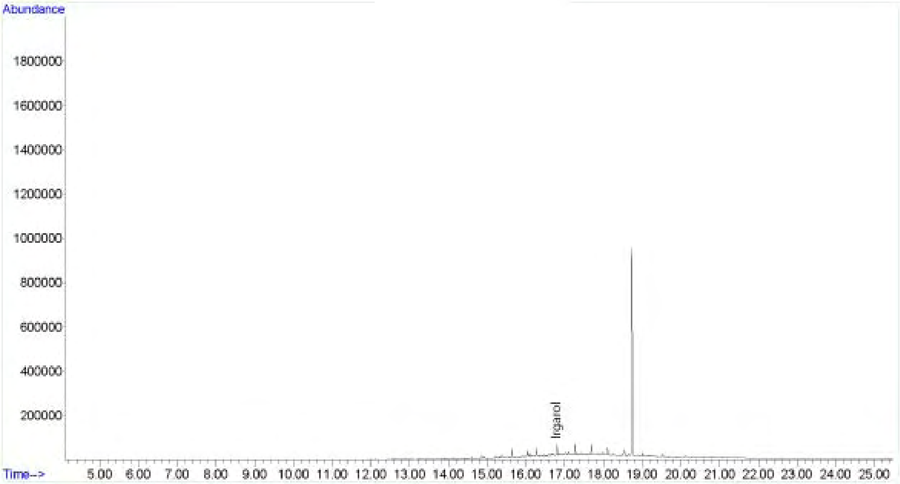

Figure 6.19 (b) Irgarol 1051 at ST-3 in 2008 (pre-monsoon)

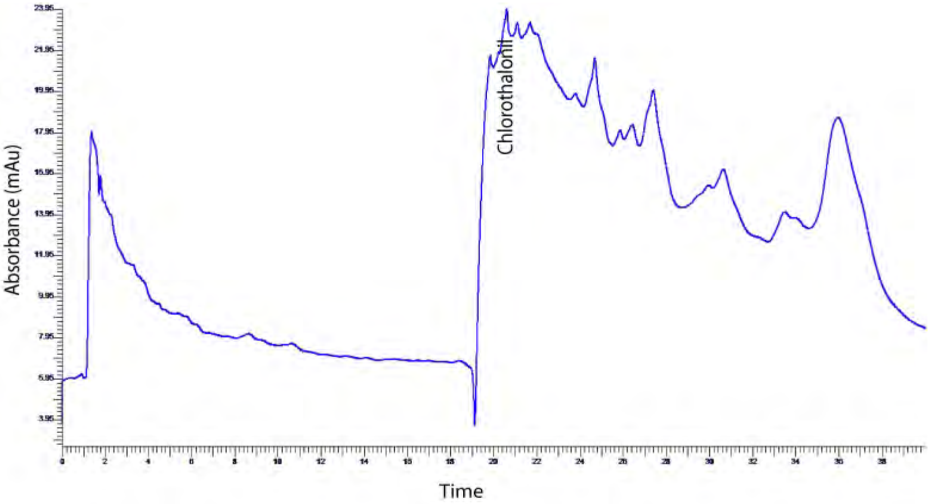

Figure 6.19 (c) Chlorothalonil at ST-3 in 2008 (pre-monsoon)

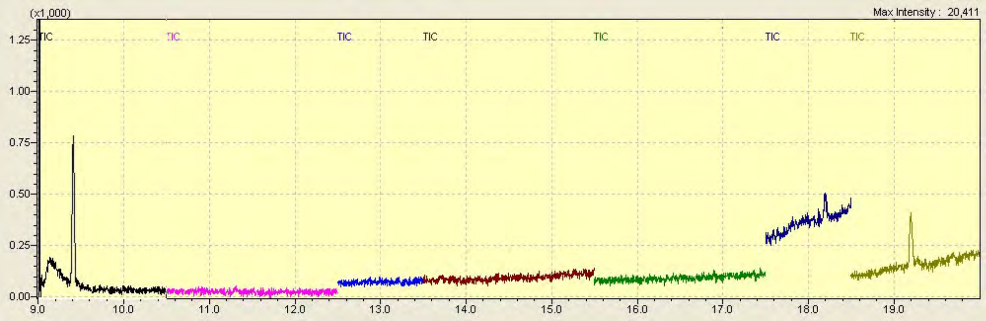

Figure 6.20 (a) PT at ST-4 in 2008 (pre-monsoon)

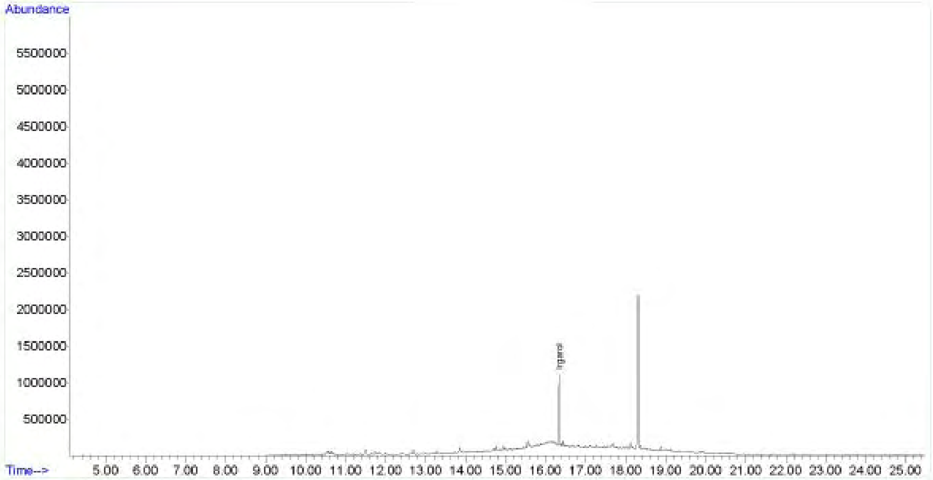

Figure 6.20 (b) Irgarol 1051 at ST-4 in 2008 (pre-monsoon)

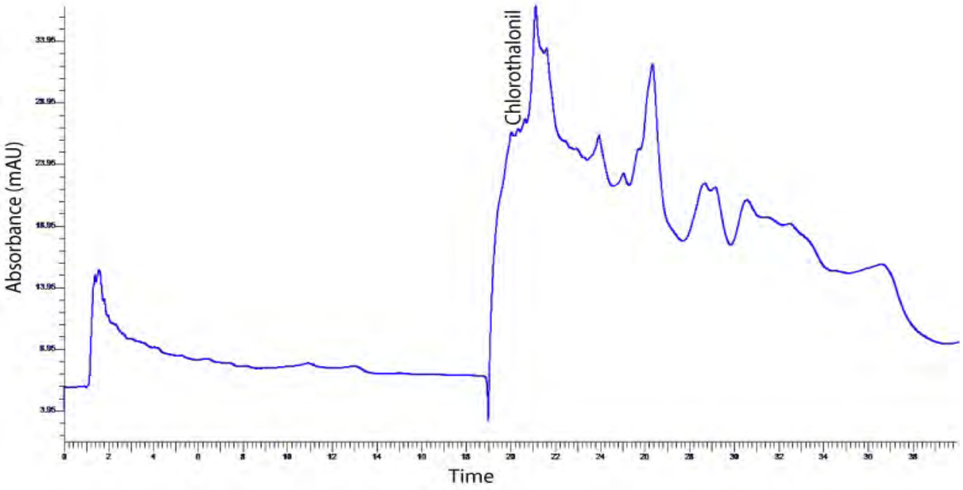

Figure 6.20 (c) Chlorothalonil at ST-4 in 2008 (pre-monsoon)

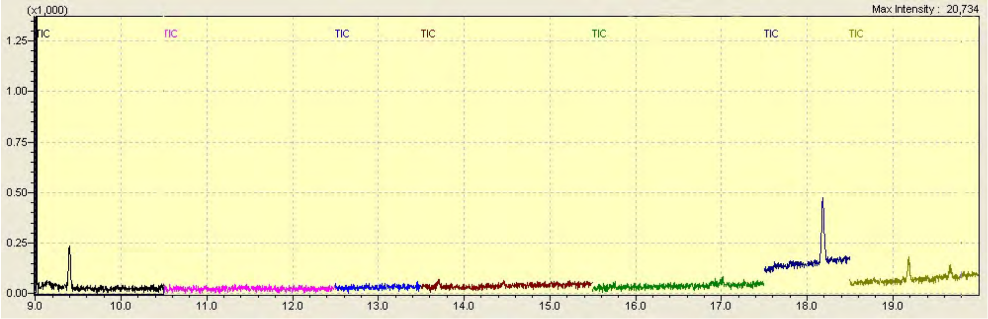

Figure 6.21 (a) PT at ST-5 in 2008 (pre-monsoon)

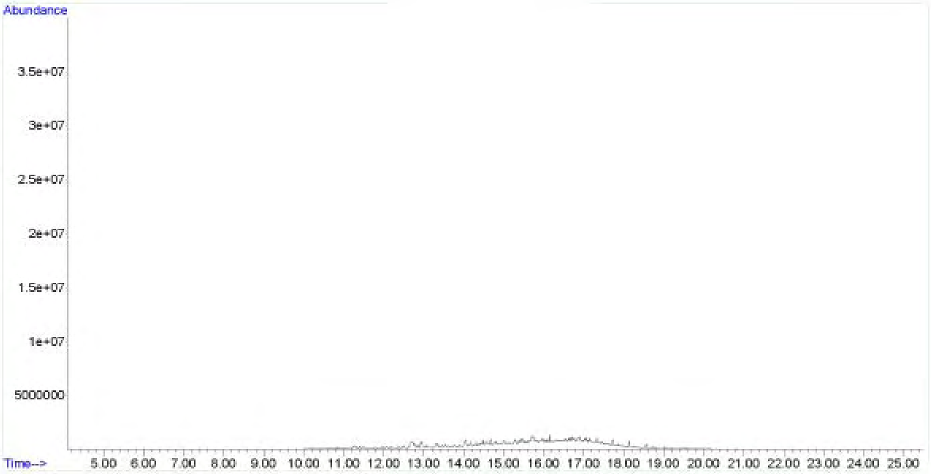

Figure 6.21 (b) Irgarol 1051 at ST-5 in 2008 (pre-monsoon)

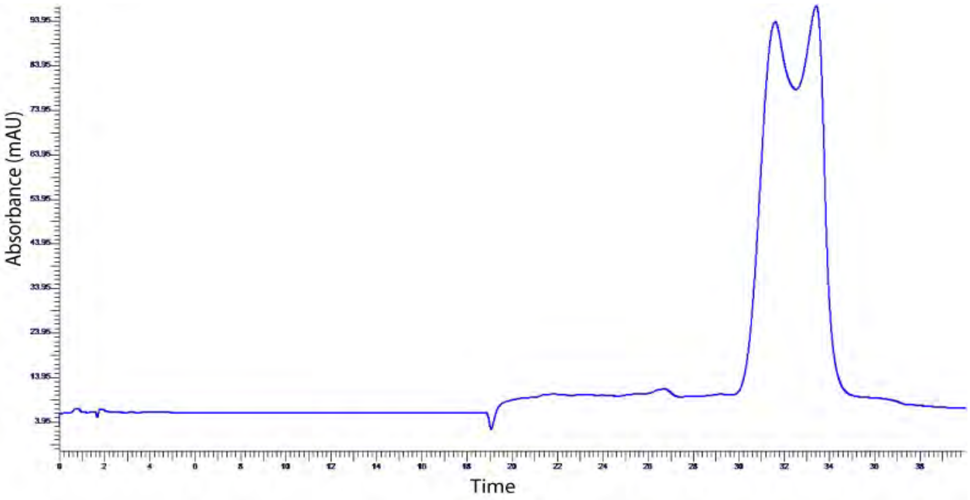

Figure 6.21 (c) Chlorothalonil at ST-5 in 2008 (pre-monsoon)

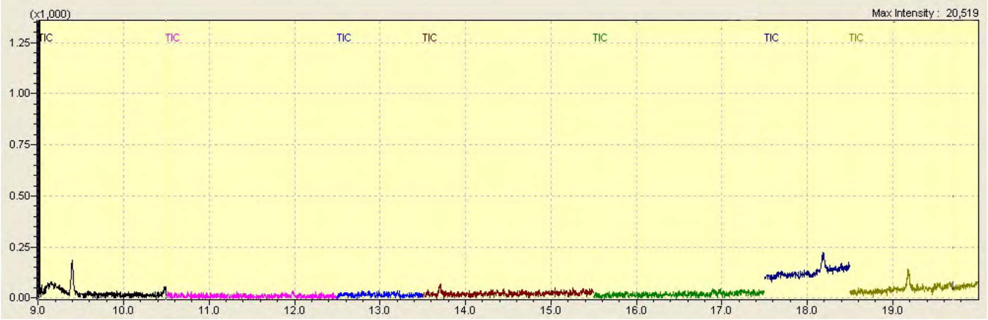

Figure 6.22 (a) PT at ST-6 in 2008 (pre-monsoon)

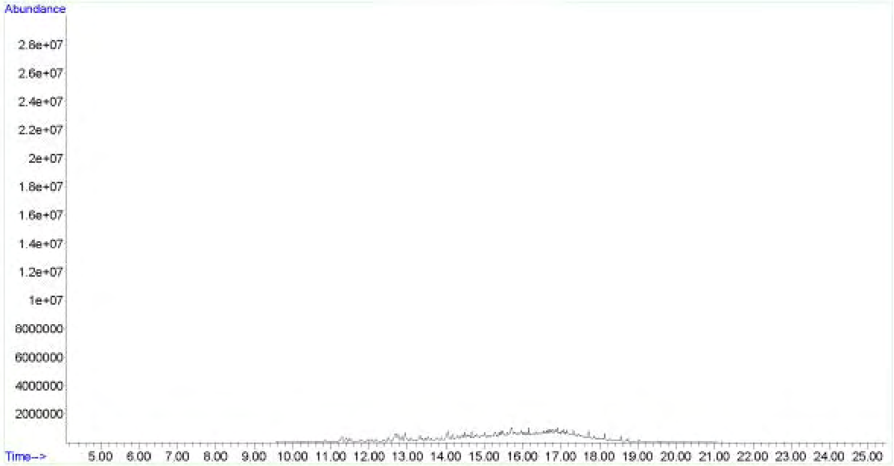

Figure 6.22 (b) Irgarol 1051 at ST-6 in 2008 (pre-monsoon)

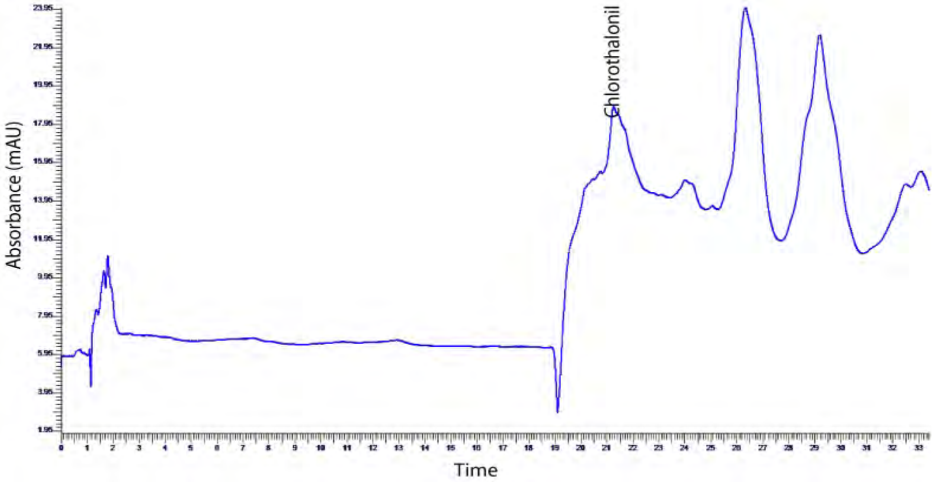

Figure 6.22 (c) Chlorothalonil at ST-6 in 2008 (pre-monsoon)

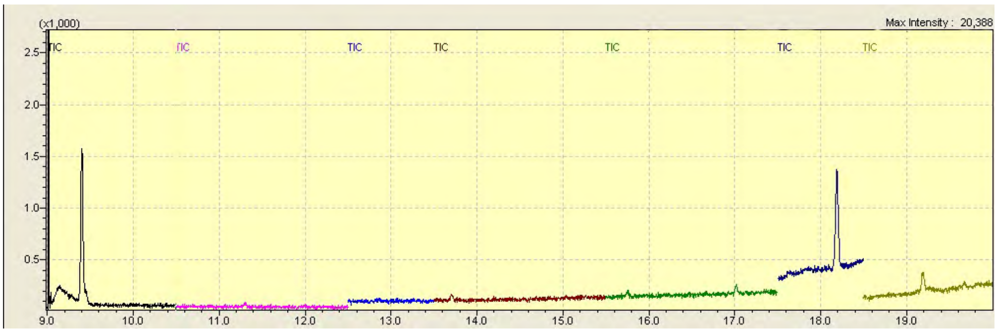

Figure 6.23 (a) PT at ST-7 in 2008 (pre-monsoon)

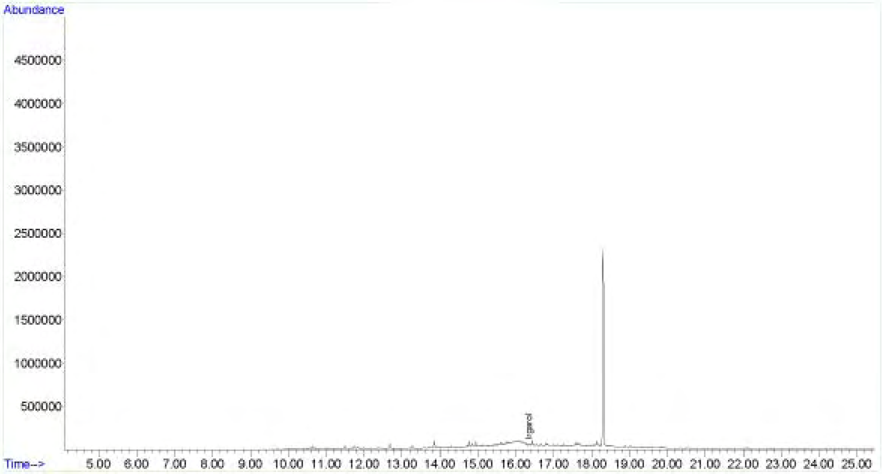

Figure 6.23 (b) Irgarol 1051 at ST-7 in 2008 (pre-monsoon)

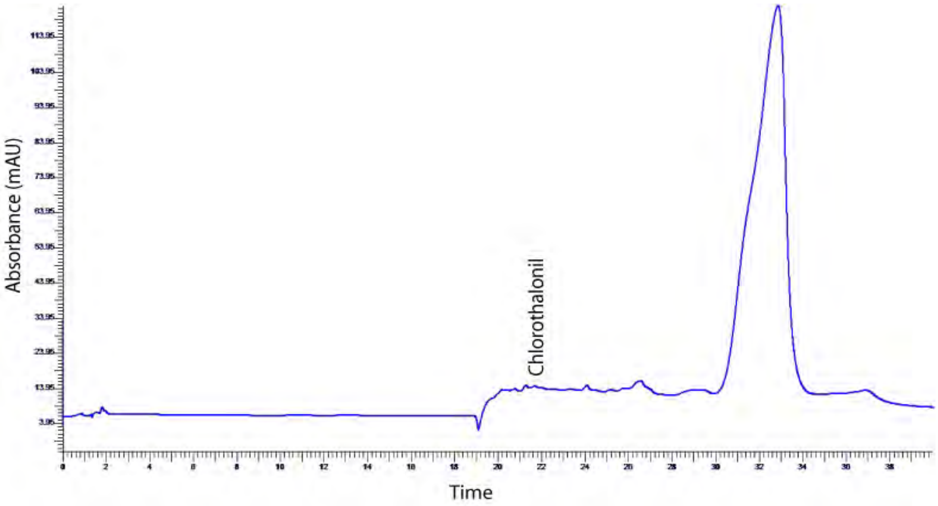

Figure 6.23 (c) Chlorothalonil at ST-7 in 2008 (pre-monsoon)

Figure 6.24 (a) PT Standards

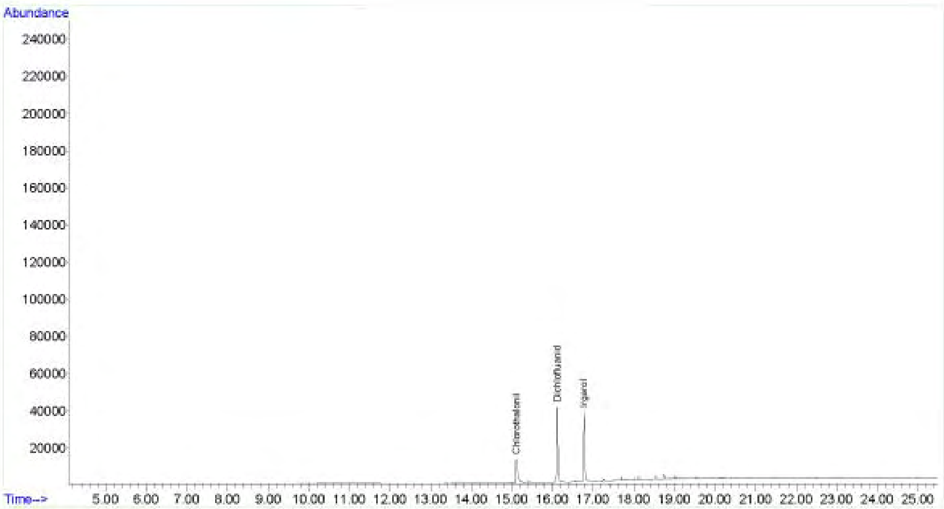

Figure 6.24 (b) Irgarol 1051 Standard

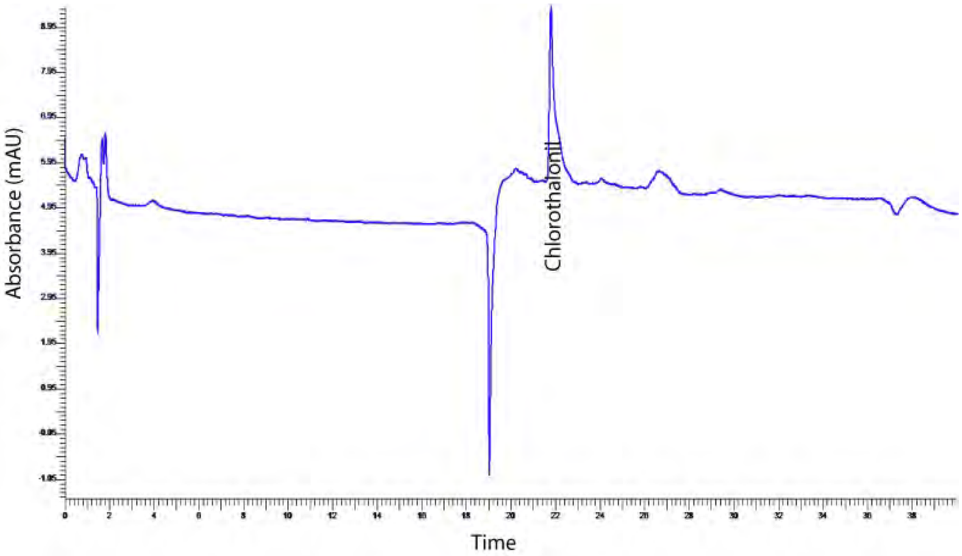

Figure 6.24 (c) Chlorothalonil Standard 1ppm

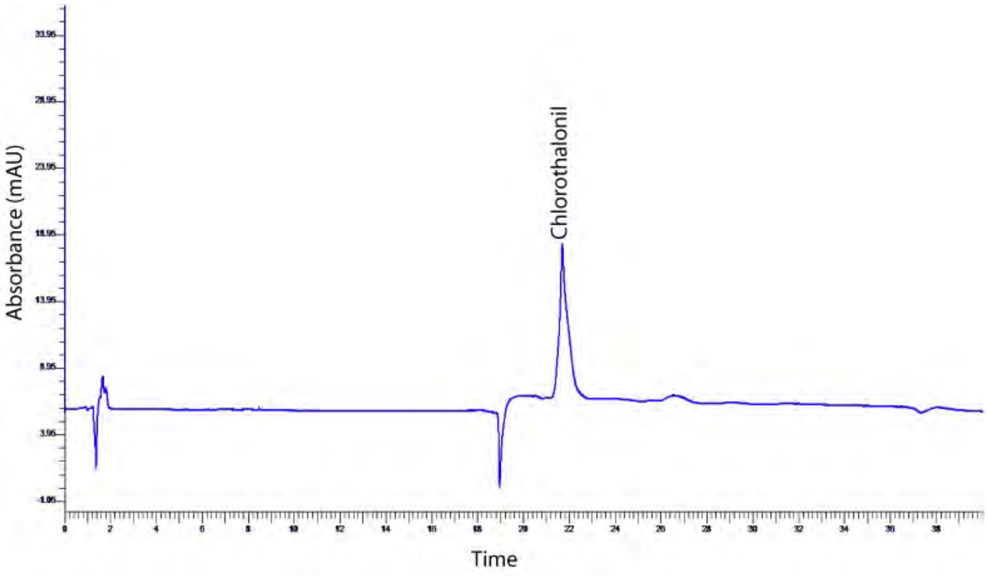

Figure 6.24 (d) Chlorothalonil Standard 2ppm

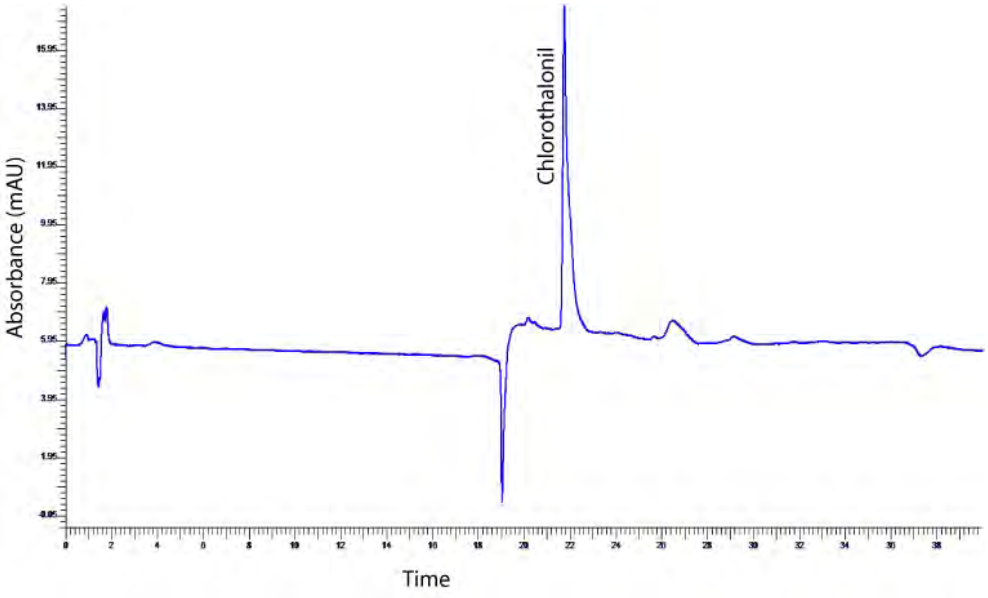

Figure 6.24 (e) Chlorothalonil Standard 3ppm

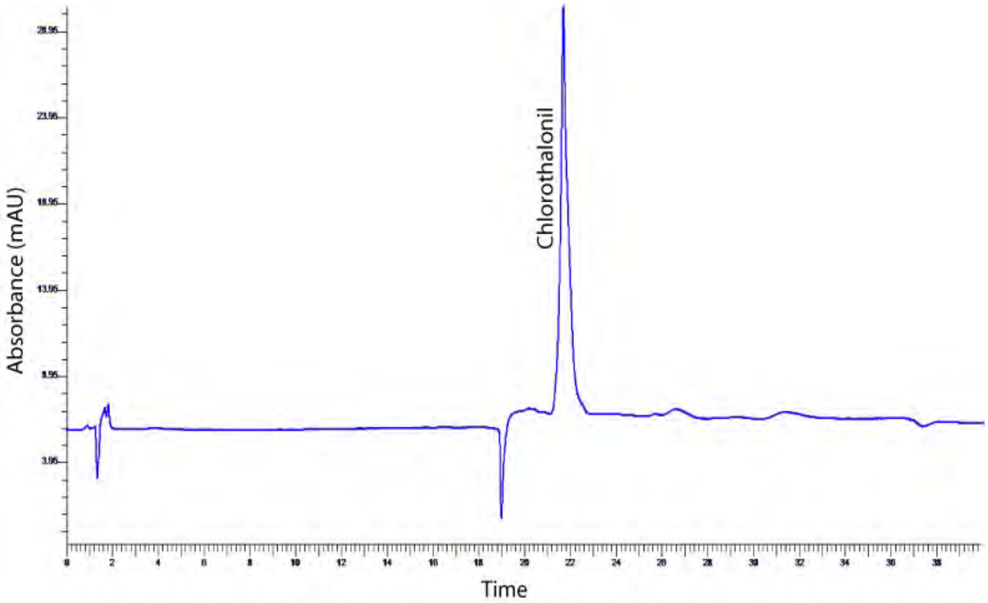

Figure 6.24 (f) Chlorothalonil Standard 4ppm

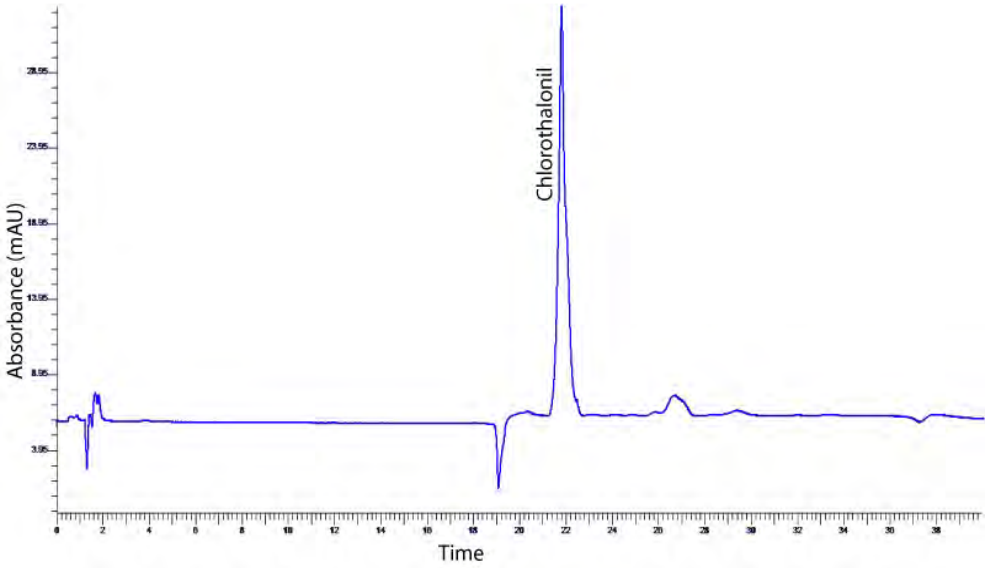

Figure 6.24 (g) Chlorothalonil Standard 5ppm

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Chapter 7

Ecotoxicological studies of Antifouling Biocides

Contents

7.1 Introduction

The attachment and establishment of sessile organisms, ranging from single proteins to bacteria, algae and marine animals, on artificial structures submerged in the sea such as offshore platforms, jetties, harbours and ships generally designated as the biofouling (WHOI 1952; Abarzua and Jacobowsky 1995; Olsen 2007). It demands substantial financial implications to the marine engineering constructions. The problem is so critical that the worldwide expenditure obtained on antifouling measures alone is approximately US $ 6.5 billion a year (Bhadury and Wright 2004). Antifouling coatings have been a widely employed strategy for controlling fouling on underwater marine structures. Among the various coatings, Self- Polishing Copolymer antifouling paints (SPCs) with triorganotin (TOT) as biocide was mainly preferred (Chambers et al. 2006). Unfortunately, this most popular antifouling coating, having a life time up to five years, also turned out to be the most toxic. Heightened establishment of this biocide into the environment lead to shell thickening in oyster population and imposex in gastropods (His and Robert 1987; Alzieu 1991). Their build-up in the marine food chain during bioaccumulation and biomagnifications engrossed supreme concern. The subsequent total global ban imposed on TOT-based coatings by the International Maritime Organization (IMO) led the antifouling paint industry into a very precarious situation (Champ 2000). After the ban of TOT compounds in antifouling formulation, shipping industry introduced Irgarol 1051, Chlorothalonil, Dichlofluanid etc. as new biocides. Irgarol 1051 is basically a triazine compound and the degradation of Irgarol 1051 is slow and expected to accumulate in the ecosystem (Callow and Willingham 1996). Irgarol 1051, like other triazinic herbicides, acts by blocking electron transport in chloroplasts during photosynthesis (Holts et al. 1993) and thus it is toxic to aquatic lives at low concentrations. Sediment contamination by Irgarol 1051 was also found to be relatively high than that in water column (Gough et al. 1994; Thomas et al. 2002). Dichlofluanid is relatively insoluble in water and Thomas et al (2002) suggested that it is easy to accumulate by the association with particulate matter. The global market share of non- organo tin products increased from 2% to 16% between 1988 and 1993 (Voulvoulis et al. 1999). In the ecosystem some booster biocides are present at measurable levels. The reported concentrations of such biocides in sediments are sufficient to damage microalgal communities (Dahl and Blank 1996), macroalgae (Scarlett et al. 1997), endosymbiotic corals, sea grasses and indirectly but ultimately to herbivorous mammals, such as dugongs (Scarlett et al. 1999).

Biomarkers allow an integrated measurement of contaminant’s bioavailability that can cause biochemical responses, providing early indicators of potential pollution. For coastal and marine pollution biomarkers have many advantages when compared to chemical analysis. Among the biological effects of pollutants, biochemical changes occur more quickly than physiological responses and provide information on the sensitivity of organisms with regard to uptake, biotransformation and detoxification patterns (Galloway et al. 2002). The toxicity of a compound depends ultimately on its concentration, exposure duration and its bioaccumulation. The rate of degradation is important and can be influenced by many factors. The degradation depends on the chemical and physical properties of compounds and environmental parameters, such as the nature and concentration of microbial populations, dissolved and suspended material, temperature and light. However, persistence does not necessarily equate to a compound being toxic because it may not be bio available. Laboratory studies suggest that there are considerable differences in the degradation pattern of biocides. Acetylcholinesterase (AChE) has been reported as a responsive biomarker to neurotoxic compounds in biomonitoring studies that assess negative effects on aquatic organisms and environmental quality (Escartín and Porte 1997; Radenac et al. 1998; Mora et al. 1999; Galgani and Bocquené 2000; Dailianis et al. 2003). The enzyme acetylcholinesterase which catalyses the hydrolysis of acetylcholine is omnipresent in the animal kingdom. It is a well-characterized enzyme in the vertebrates because of its critical catalytic function at the cholinergic synapses. The enzyme acetylcholinesterase (EC. 3.1.1.7) hydrolyzes the neurotransmitter acetylcholine to acetic acid and choline at the cholinergic synapses, terminating nerve impulse transmission. AChE as a potential cell membrane marker enzyme was already approved (Severson et al. 1972; Steck et al. 1974; Watts et al. 1978).

Bivalves are often used for ecotoxicological monitoring because they are filter-feeding organisms living in sandy-mud bottoms and can accumulate contaminants in their tissues to relatively high levels. Bivalve enzyme activity including AChE has already been widely used in monitoring environmental pollution in laboratory and field conditions (Le Bris et al. 1995; Dellali et al. 2001; Doran et al. 2001; Alves et al. 2002; Mohamed et al. 2003). AChE activity of invertebrates, in particular bivalves, differs from vertebrates. The classifications, characteristics and tissue localization of cholinesterase (ChEs) in vertebrates are generally valid for invertebrates, but there are differences. ChE may exhibit broad substrate specificities and are able to cleave among acetylthiocholine (ATC), butarylthiocholine (BTC) or propionylthiocholine (PrTC) (Kristoff et al. 2006). The ChE activity of sample organisms can vary with biotic (class or species of organism, age, size, reproductive stage, and physiological conditions) and abiotic factors like temperature, pH, salinity, etc related to the habitat (Varela and Augspurger 1996; Fairbrother et al. 1989). Variations in the ChE activity of aquatic organisms may reflect various contaminants including heavy metals, PAHs, hydrocarbons, detergents, phytotoxin, and other industrial pollutants (Payne et al. 1996; Flammarion et al. 1996; Magni et al. 2006; Senger et al. 2006; Linde-Arias et al. 2008; Bervoets et al. 2009).

7.1.1 Bivalves as Pollution Indicators

Bioindicators are organisms, which are used to monitor environmental pollution. Bivalves are mainly used as bioindicators, because of its filter feeding habit. Najimi et al. (1997) and Bainy et al. (2006), evaluated the effect of heavy metal exposure on AChE activity in mussel. Most studies on acetyl cholinesterase inhibition in marine molluscs have focused on whole organism or muscle extracts. (Bocquene 1997, Radenac et al.1998; Bainy et al. 2006). *Villorita cyprinoides var Cochinensis* is a bivalve species, which is commonly found in the Cochin estuary. The bivalves *Villorita cyprinoides* var *cochinensis* were sampled from freshwater region, near Vikkom an area deprived from industrial activities. It is a purely brackish water species and is capable of tolerating a wide range of salinity up to a maximum of 34 ppt. It is a cheap source of protein rich food for the habitants and used as a raw material for the manufacture of cement and lime. The animal abundant in those parts of the backwaters wherever the bottom deposit consists of sand and silt. Large organisms were obtained during December to April and young one during August and September when the salinity was comparatively low. The optimum habitat salinity of the clam has been reported to be parts per thousand.

7.1.2 Acetylcholine esterase as Indicator of Toxicity

Previous studies mainly concentrate on the effects of pollutants on individual organisms which have direct toxic effects. The classical toxicological approach of the toxic effects on individual animals is highly relevant. The most obvious effect of exposure to a pollutant is acute toxicity which leads to a rapid death and it is common practice to assess this type of toxicity studies by the LD_{50}, LC_{50} measurements. These measures are used to assess the ecological impact of pollutants.

Earlier reports on Organophosphorus (OP) compounds which inhibit a whole range of esterase enzymes, and it is the consequences of inhibition of one of these enzymes, acetylcholinesterase (AChE), that produce the symptoms of acute poisoning. Acetylcholine (Ach), the natural substrate for AChE, is one of the principal known transmitters of impulses across synapses between adjacent nerve ending, and across neuromuscular junctions. Nerve impulses stimulate the release of Ach, which transmits the stimulus across the gap to the adjacent nerve or muscle cell. The Ach is normally broken down rapidly, by hydrolysis, catalysed by the enzyme, AChE. Inhibition of AChE means that Ach persists much longer, normal nerve functions are grossly disturbed, and sufficiently serve disturbance ends in death (Eto 1974). In brief, the lethal lesion disturbs impulse transmission across synapses and neuromuscular junctions, many physiological processes are interrupted in consequence, and death, in vertebrates at least, usually results from paralysis of the respiratory system. Figure 7.1 shows the action of Acetylcholine esterase enzyme in nerves and table 7.1 displayed previous studies on toxicological studies around the world.

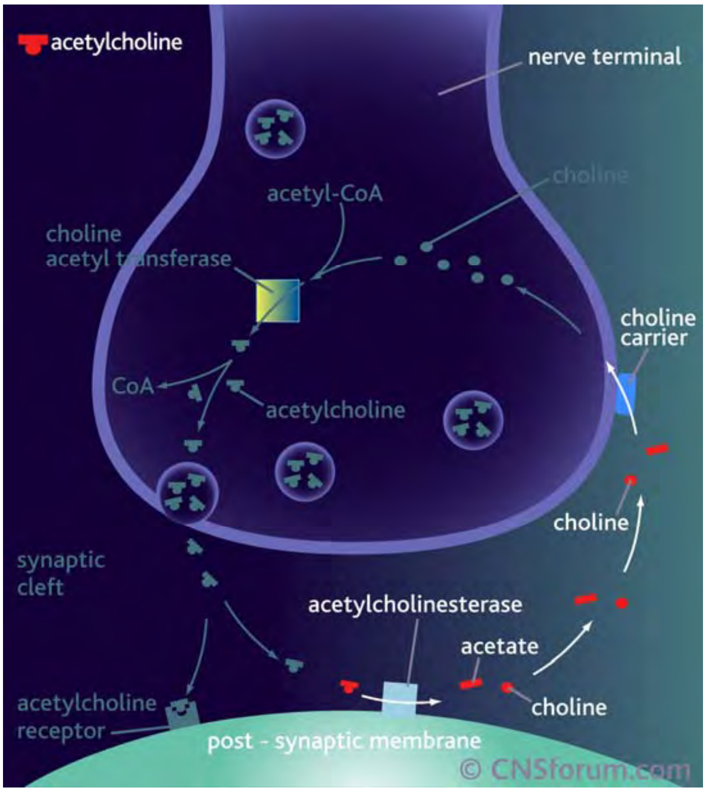


Figure 7.1 Action of Acetylcholine esterase enzyme in nerves

Table 7.1 Previous studies on toxicological studies around the world

	Sl.No	Area	Organotin	References
Organotin in bivalves	1	South and East Coast, Korea	42-2993 (ng/g dry wt)	Hong et al. 2002
	2	Port of Osaka, Japan	49-953 (ng/g dry wt)	Harino et al. 1998
	3	US coast	32-269 (ng/g dry wt)	O'connor and Lauenstein 2006
	4	Port of Geniva, Italy	1784-14151 (ng/g dry wt)	Rivaro et al. 1999
	5	Atlantic coast, Portugal	< 14-1195 (ng/g dry wt)	Diez et al. 2005
	6	Bay of Prian, Slovenia	1222-8554 (ng/g dry wt)	Nemanic et al. 2002
Irgarol-1051	1	Daphnia sp. (Crustaceans)	66mg/l (24 h EC50)	Ciba-Geigy 1988
	2	Daphnia species (Algae)	2.63mg/l (48h LC 50)	Roger et al. 1996
	3	Fish (fresh water)	2.13 mg/l (96h LC50)	Roger et al. 1996
	4	Fish (saline water)	1.33 mg/l (96 h LC50)	Roger et al. 1996
	5	Oncorhynchus mykiss (fish)	0.86 mg/l (96 h LC50)	Toth et al. 1996
	6	Daphnia magna (Crustaceans)	16 mg/l (24 h LC50)	Toth et al. 1996
	7	Reduced growth of E. interstinalis	< 500ng/l (EC 50)	Scarlett et al. 1997
	8	Artemia salina (Crustaceans)	> 40mg/L (24 h LC50)	Okamura et al. 2000a
	9	Daphnia pulex (Crustaceans)	5.7 mg/l (24 h LC50)	Okamura et al. 2000a
	10	Thamnocepharus platyurus (Crustaceans)	12 mg/l (214 h LC50)	Okamura et al. 2000a
Chlorothalonil		Crustaceans		
	1	Cancer magister	560μg/l	Armstrong et al. 1976
	2	Penaeus Duorarum	0.162 mg/l	Montforts 1999
		Molluscs		
	3	Mytilus edulis	5.94 mg/l	Ernst et al. 1991
	4	Crassostea virginica	0.026mg/l	Montforts 1999
		Teleosts		
	5	Cyprinodon variegatus	0.033mg/l	Montforts 1999; U.S.EPA Office of Pesticide Programs (2000)
	6	Galaxias auratus	0.029mg/l	Davis and White 1985
	7	Galaxias maculates	23.7 μg/l	Davis and White 1985
	8	Galaxias truttaceus	0.019 mg/l	Davis and White,1985
	9	Leiostimus xanthurus	0.032 mg/l	Mayer 1987
	10	Pseudaphritis urvillii	8.2 μg/l	Davies et al. 1994

7.2 Result and Discussion

7.2.1 LC_{50} experiment and enzyme assays of Biocides

In order to understand the toxic effect of organotin in the Cochin estuary, ecotoxicological studies were carried out. *Villorita Cyprinoides var Cochinensis* were selected as indicator organism for this investigation. The *Villoritta* species were collected an area which was considered as less human intervention in the Vembanadu lake and been used for the toxicity study. The bivalve were acclimatised for a week in the laboratory with appropriate suitable environment- habitat condition. For the feeding of bivalves, chlorella species were cultured in the laboratory and stock is maintained for this purpose. The LC_{50} of each biocides were seperately determined. The sublethal concentration considered from the LC_{50} values (1/5th, 1/7th and 1/10th of LC_{50}) and the inhibition rate of acetylcholinesterase studies were conducted at 96hr intervel. The bivalves were not fed during the study period. On each day, water was changed and fresh toxins were added to maintain the same concentration throughout the experimental period. In the final day, enzyme inhibition activity experiments were conducted. Table 7.2 shows the LC_{50} concentrations and its sublethal concentrations of biocides.

Table 7.2 LC_{50} values and sublethal concentrations of Biocides

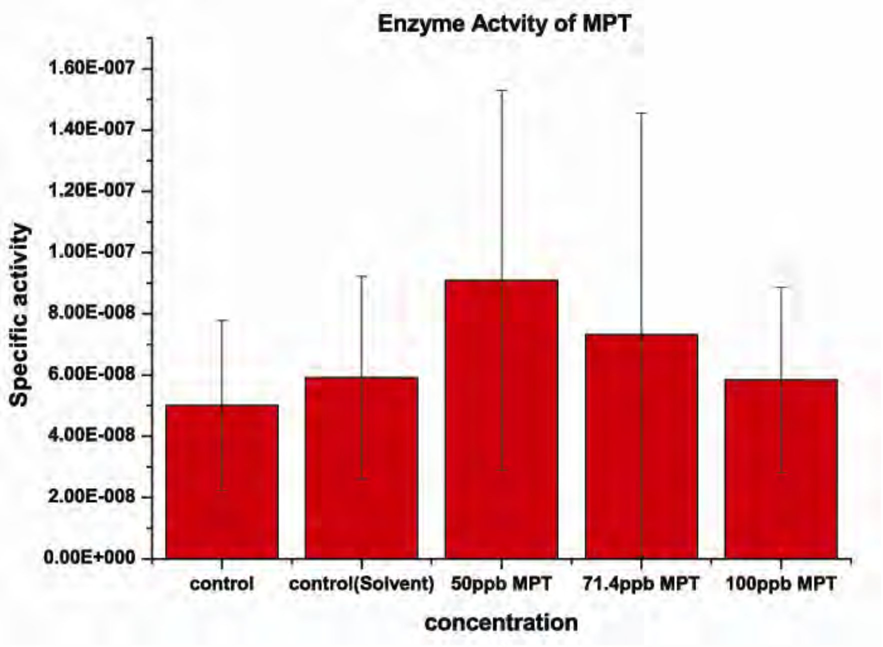
Toxine	LC-50	1/5th of LC-50	1/7th of LC-50	1/10th of LC-50
TPT	200 ppb	40 ppb	28.57 ppb	20 ppb
DPT	500 ppb	100 ppb	71.4 ppb	50 ppb
MPT	300ppb	60 ppb	42.86 ppb	30 ppb
Irgarol-1051	200 ppb	40 ppb	28.57 ppb	20 ppb
Chlorothalonil	400 ppb	80 ppb	57.14 ppb	40 ppb

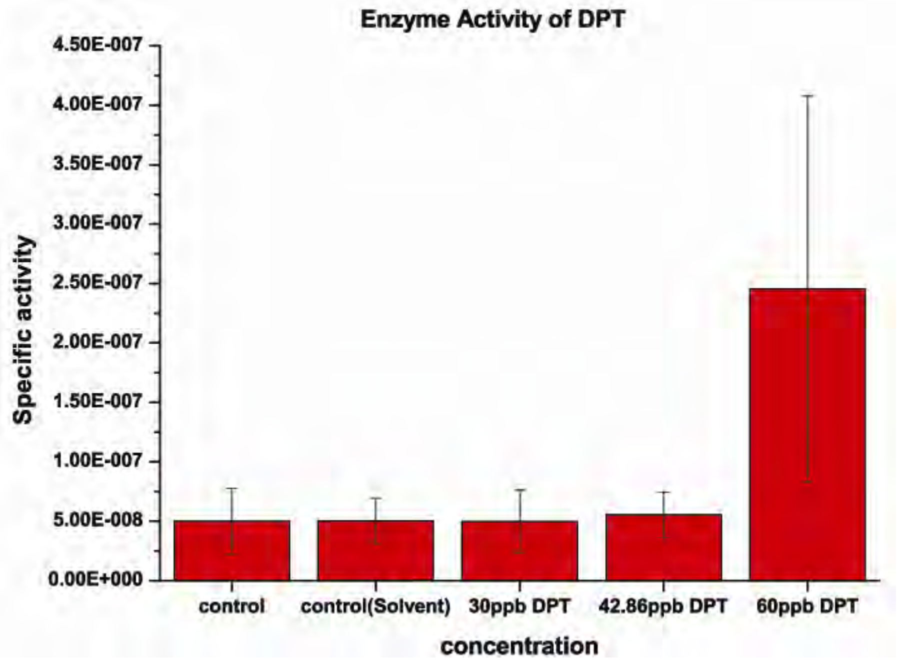
The LC_{50} concentration of Monophenyltin (MPT) from the experiment is 300 ppb. The three sublethal concentrations of the above LC_{50} were selected and experimental concentrations are 60 ppb, 42.86 ppb and 30 ppb. Acetone was used as solvent to dissolve MPT. Five tanks were maintained in the order; control, solvent control (acetone) and three experimental toxicant (30 ppb, 42.86 ppb and 60 ppb). The solvent control influenced to gives a little more activity than control (Figure 7.2). The three sublethal concentrations showed inhibition of acetylcholine esterase enzyme. So acetylcholine is unable to split at the nerve ganglion which later leads to death of organism. In the present study, a linear relationship between dose-response has been observed. The dose-response relationship established between monophenyl tin trichloride and acetylcholine esterase can be taken as an indicator for the extent of pollution.

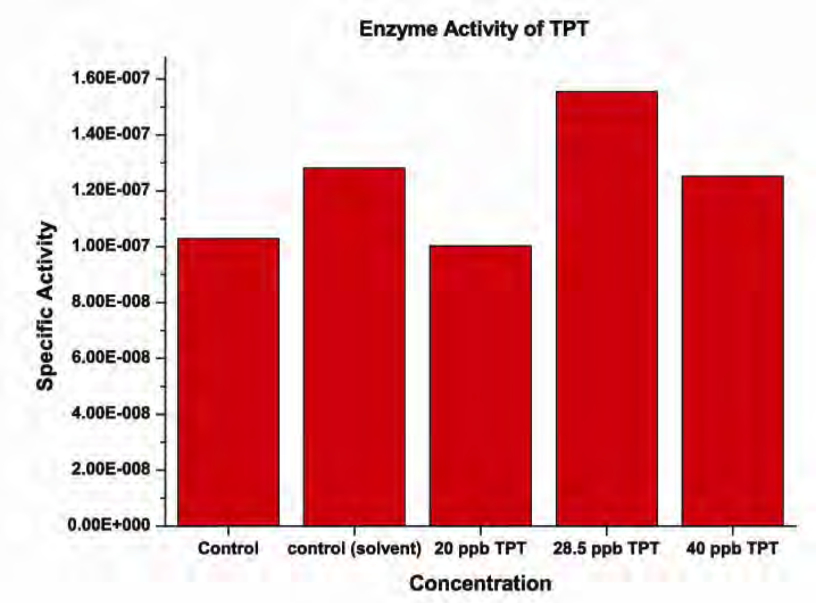
In the case of Diphenyltin (DPT), the LC_{50} concentration was found to be 500 ppb and the sublethal concentrations selected for the study were 100 ppb, 71.4 ppb and 50 ppb. The toxins were dissolved in milli-Q water which is used as solvent control and total five tanks were maintained for the experiment. The control and solvent control samples were showed normal physiological activity of the enzymes. The 50 ppb and 71.4 ppb also showed high enzyme activity when compared to control and solvent control (Figure 7.3). Whereas at 100 ppb, it was found that the inhibition of acetylcholine esterase enzyme. This is due to the activation of enzyme synthesis to nullify the effect of stress by increasing the neurotransmission. Hence the acetylcholine regeneration at the nerve ganglions start to diminish in 100ppb and this inhibition increases as the concentration increases and finally it leads to paralysis and death of the organism.

Triphenyltin (TPT) belongs to a class of chemicals (organotins) known to be immunotoxic. TPT is considered to be a toxicant, although it is not considered to have mutagenic/genetic toxicity properties. It was carcinogenic both in the rat (inducing pituitary and testicular tumors) and in the mouse (inducing liver tumors). It is classified as a B2, possible human carcinogen by all routes of exposure (oral, dermal and inhalation).`

The LC_{50} of TPT from present experiment was found as 200 ppb. In order to understand the enzyme activity, three sublethal concentration (1/5th, 1/7th, and 1/10th of LC_{50}). They are 40 ppb, 28.5 ppb and 20 ppb respectively. In the present study, the solvent, ethanol was used as diluent for TPT and it activate the acetylcholin esterase at a very low rate (Figure 7.4). From the graph at 20 ppb (1/10th) sublethal concentration, the biocide has almost no specific effect on acetylcholinesterase and the enzyme activity has been increased at 1/7th subletehal concentration. At 28.5 ppb (1/7th) the inhibition of acetylcholine esterase is prominent. At higher concentration of biocide 40 ppb (1/5th of LC50) showed the minimum enzyme actvity and gives an idea that the excess acetylcholine released from the ganglion were not split into acetyl and choline groups. Without the enzyme, muscles would continue to contract causing spasms in bivalve.


Enzyme Actvity of MPT
Specific activity
1.60E-007
1.40E-007
1.20E-007
1.00E-007
8.00E-008
6.00E-008
4.00E-008
2.00E-008
0.00E+000
control
control(Solvent)
50ppb MPT
71.4ppb MPT
100ppb MPT
concentration


Enzyme Activity of DPT
Specific activity
4.50E-007
4.00E-007
3.50E-007
3.00E-007
2.50E-007
2.00E-007
1.50E-007
1.00E-007
5.00E-008
0.00E+000
control
control(Solvent)
30ppb DPT
42.86ppb DPT
60ppb DPT
concentration



Figures 7.2 to 7.4 Graphs showing the AChE action against MPT, DPT, and TPT

Similar to OT's the acetylcholine esterase study were conducted for Chlorothalonil and Irgarol 1051. From the toxicity study conducted the LC_{50} value for Chlorothalonil was 400 ppb. It's mode of action involves its combination with a molecule called glutathione. When these glutathione-Chlorothalonil derivatives form, they tie up all of the cells' available glutathione, leaving enzymes glutathione-dependent unable to function. Several enzymes that are important in cellular respiration, the process by which large molecules are broken down and provide the cell with energy, are glutathione dependent (Tillman et al. 1973). In order to determine the effect of Chlorothalonil on acetylcholine esterase enzyme, sublethal concentration of the LC_{50} values were taken. These are 1/5th - 80 ppb, 1/7th - 57.14 ppb and 1/10th -40 ppb. 96 hr experiments were conducted and experimental tanks were maintained for the three sublethal concentrations and one control and one solvent control. The solvent, acetone itself has and inhibitory effect on the enzyme and the combined action with Chlorothalonil and acetone increased the inhibitory action in the toxicity experiment (Figure 7.5). All the sublethal concentrations showed a decline in acetyl choline activity. According to EPA reports Chlorothalonil's acute toxicity through ingestion is low and the median lethal dose (LD_{50}) for laboratory animals is found between 5 and 10 grams of Chlorothalonil per kilogram of body weight (US EPA 1993). The control without toxin and solvent showed high normal physiological activity as compared to other samples and the experimental animals were found health. Solvent control and three sub lethal concentrations showed less physiological activity when compared to control organisms. Although Chlorothalonil proponents refer to Chlorothalonil as "not genotoxic," (toxic to genetic material) (Wilkinson and Killeen 1996), this fungicide has caused genetic damage in mammals in studies of both live animals and cell cultures (Lodovici et al. 1994) earlier.

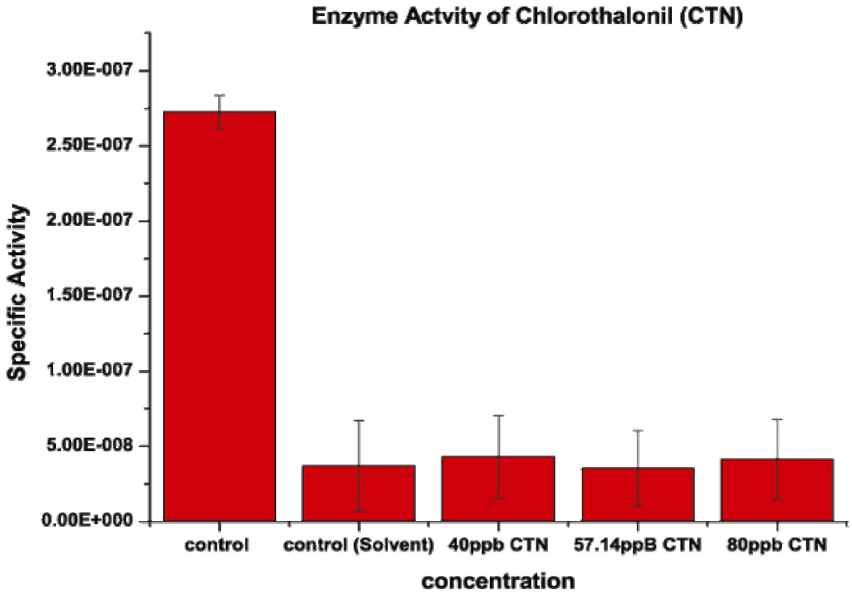


Figure 7.5 Graphs showing the AChE action against Chlorothalonil

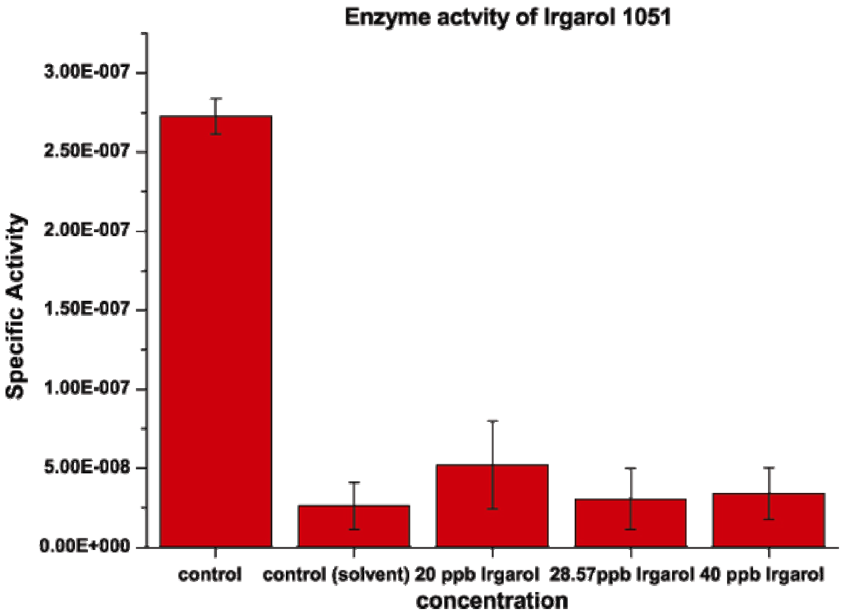


Figure 7.6 Graphs showing the AChE action against Irgarol 1051

Irgarol 1051 has been formulated as a more hydrophobic analogue of triazine herbicides (Ciba-Geigy 1995).The present toxicity study of Irgarol 1051 on marine organism is the first experiment performed in India. similar to other biocides, the LC_{50} and toxicity experiments were conducted. The LC_{50} value obtained for Irgarol 1051 is 200 ppb. The sub lethal concentrations were 80 ppb, 57.14 ppb and 40 ppb. Irgarol 1051possesses a combined inhibitory effect on acetylcholinesterase activity and due to the solvent acetone. A slight increase in the enzyme activity was observed in the concentration 1/10th (20 ppb) due to the synthesis of enzyme to overcome the stress caused by Irgarol 1051 (Figure 7.6). The control without toxins and solvent showed the normal high acetylcholinesterase activity showing the healthy condition of the organism. The solvent control showed less activity than the control. The three sub lethal concentrations showed very less activity than to the solvent control and control, and thus well illustrates the rate inhibition of choline formation in the ganglions. Irgarol 1051 significantly inhibited periphyton photosynthetic activity at 0.81 mg/l in short-term (h) tests, and with long-term (weeks) exposure, produced significant changes in the community structure at 0.25 mg/l (Dahl and Blanck. 1996). It has also been shown by Scarlett et al. (1997) that Irgarol significantly inhibited the growth of *Enteromorpha intestinalis* zoospores, a key reproductive stage of the early colonizing alga at its maximum concentration of 127 ng/l that found at Sutton Harbour marina. These toxic levels are within the threshold limit concentration reported for environmental samples and may cause damage to the biota in later stages. The minimum inhibition concentration reported for algae (i.e. *Enteromorpha intestinalis*) and several diatoms (i.e. *Navicula, Nitzschia, Amphora* and *Achnanthes*) is 10 mg/l (Readman et al.1993). In addition, it is toxic to several microalgae with 50% effective concentration (EC_{50}) values ranging from 0.45 to 2.12 mg/l (Toth et al. 1996). Its phytotoxicity to *Selenastrum capricornutum* and *Skeletonema costarum* has been reported at 1.26 mg/L (120 h EC_{50}) and 0.45 mg/l, respectively. Acute toxicity to the *crustacean mysid shrimp* is 400 mg/l (96 h LC_{50}) and 3200 mg/l to oyster larvae *Crassostrea virginica* (48 h EC_{50},).Theoretically, Irgarol 1051 action mechanism should not affect organisms that consume algae. Nevertheless, some of the long-lived herbivores, dugong and green turtle, are prone to show toxicity (Hall et al. 1999 and Scarlett et al. 1997).

7.3 Conclusion

The present study gives an insight the study of antifouling biocides used in shipping industry and its toxicological effects on bivalves. The results outweighs that the pollution caused by antifouling biocides in the

Cochin estuary is intense when compared to other shipping channels and ports. This may be due to the indiscriminate use of organotin compounds in paints. Many countries banned paints with organotin compounds from the year 2003 onwards. Lack of legislation is the major reason behind this high concentration. The toxicological studies confirm the toxic effects of organotin. Acetylcholinseterse inhibition occurs at different concentration of three phenyltin derivatives. The residual levels of organotin estimated in the various samples collected from the Cochin estuary is found below the LC_{50} values. But the long term exposure of this system may cause lethality to the aquatic organisms life in the estuary. A reduction in the density of bivalves in and around the Cochin estuary clearly give this evidence. The residues of Irgarol 1051 and Chlorothalonil were also estimated in the study area which points the intense use of these three compounds in the shipping industry. Detailed study with the biota residing in the estuary would give a clear picture of the extensive use of antifouling biocide in the Cochin estuary.

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Summary

Marine ecosystems are well known preservator and nursery for large number of fishes and other aquatic organisms. Estuaries are the transition zone between fresh water rivers and saline sea water.Estuaries are considered as the life line of these ecosystem, which is affected by tidal and salinity variations. Major sources of these are from antifouling biocides, trace metals, pesticides and also due to eutrophication, hence causes fatal effect to the aquatic organisms. Large varieties of fishes were already extinct from these marine environment due to ill effects of the xenobiotic compounds. In order to assess the comprehensive evaluation of the estuary .

Cochin estuary is one of the major estuarine systems along the west coast of India; six rivers mainly contribute to Cochin estuary. Which is home to many industrial activities, i.e. India's largest ship building facility is located here, the Cochin port and newly build International transition terminal is also reside the estuary. A marina for leisure crafts is being build and also is home to southern naval command of Indian Navy. 14 sampling locations were identified, of which seven stations are adjacent to the main shipping channel and the rest of the stations are away from these estuarine region are highly influenced by large volume of fresh water input.

General sedimentary characteristics were analysed initially and resulted the anthropogenic loading to estuary from the surrounding environment. The regular monitoring of these sedimentary parameters would help us to understand the processes that occur between sedimentary phase and the water column. Texture, organic carbon, total nitrogen, total phosphorus, total sulphur, chlorophyll, pheopigments and biochemical parameters like proteins, lipids and carbohydrates were the parameter identified. The proteins, lipids, carbohydrates and their ratios were used to understand the decay and pollution status of sediment and its environment. Comparatively high concentration of carbohydrates revealed, the aged organic detritus matter along the sampling period in both the consecutive years. The PRT: CHO ratio also indicated the presence of degraded organic matter. The relatively high concentration of lipid along the study was confirmed the presence of planktonic detritus. The LIP: CHO ratio was mainly used to assess the quality of organic matter and it gave low values in the present study, which indicate the estuary behaved as a detritus trap for organic matter. The C/N ratios were helped to predict the source of organic matter and it resulted that the organic matter was mainly from terrestrially originated. Thus the results of sedimentary behaviour suggested the presence of aged organic matter existing in the sediments of studied locations.

The redox condition of the study area, were investigated by the distribution pattern of rare earth elements and their proxies. The proxies are Ce-anomaly, Eu-anomaly and ratios like C/N ratio, Au-U ratio, U/Th ratio, Cu/Zn ratio and V/Cr ratio and well clearly understood the environmental and depositional behaviour. The positive Ce-anomaly derived from the study leads to the oxic environment persisting in the Cochin estuary. From the Eu-anomaly was to understand the origin of the sediment and the positive Eu-anomaly indicated the sediment was originated from the feldspar or hydrothermal source. Earlier reports also support this inference. The proxies like Mn, total organic carbon, U/Th ratio, Au-U, Cu/Zn ratio and V/Cr ratios derived could be useful for understanding these sedimentary behaviour. The very low total organic carbon indicated the oxic environment along the study area. The present results of low U/Th ratio indicates assessing the prevailing oxic environment along the studied location. Authigenic-U the another proxy indicated the oxic behaviour of the environment. Cu/Zn and V/Cr ratios were useful for predicting the redox environment and these ratios gave oxic environment. Thus these proxies outweighed the similar results which confirmed the studied locations are oxic in nature.

The trace metals are considered as essential element, but they are highly toxic when exceeds their threshold limits. They are considered as one of the major pollutant in estuaries and coastal areas. Some trace metals are used as co-biocides or pigments in antifouling paints. The results revealed that higher concentration of Cd, Sn and Zn are observed along the study area in the two consecutive years. In order to understand the trace metals pollution status. Statistical tools like pearson correlation, principal component, cluster analysis and sediment pollution indexes like enrichment factor, geoaccumulation index and contamination factor were used. The enrichment factor showed that high enrichment of Cd is found in all the stations. Especially very high at ST-2 in both the sampling periods. Zn also showed high enrichment at ST-2 on both sampling years. Sn also showed significant enrichment along the study area. The contamination factor of Cd showed high during 2007 (pre-monsoon) and 2008 (pre-monsoon). Sn also showed significant enrichment indicating the presence of leaching of antifouling paints from marine structures and vessels. Cu and Zn showed moderate contamination on both these years. These results indicated high concentration of Cd, Zn, Cu and Sn in the sediments of Cochin estuary. Which contribute as the main source for antifouling paint.

Irgarol 1051, Chlorothalonil and Dichlofluanid are the alternative for organotin paint in recent years and their presences were observed in the seven stations along the Cochin estuary. High concentrations were recorded along the shipyard region and harbour area inferring the leaching of these biocides from ships and boats. Irgarol 1051 and Chlorothalonil were absent in stations ST-1, ST-5 and in these stations, sand dominates in the sediments texture. Generally the adsorption capacity of sand is less when compared to mud fraction. The high concentrations were recorded in shipyard regions, indicating the intense usage of these compounds in ship building facilities. The mussel *"Villorita cyprinoides* var *cochinensis"* was used as an indicator organism to understanding the pollution status in Cochin estuary. The acetylcholine esterase enzyme inhibition was used to understand the effect of biocides on indigenously available organism. 96 hrs LC_{50} were conducted and revealed the sublethal concentrations of the toxicant and conducted the toxicity studies. From the toxicity experiments, understood the acetylcholine esterase enzyme inhibition rate of major three biocides. The inhibition concentration was found different for each biocides.

From the present study it is evident that the biocides are one the major toxicity contributor to aquatic life in the Cochin estuary. New antifouling paint has to introduce which must have less toxic effect. This is the first baseline toxicity study on organotin. Other alternative biocides, are needed for conducting enough research on this subject to generate better understanding of the aquatic realm with respect to contaminants.

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Publications

List of Publications in pear reviewed Journals.

1. “Chemometric study on the trace metal accumulation in the sediments of the Cochin Estuary south west coast of India”. **Deepulal. P.M**., Gireesh kumar. T.R., Sujatha. C.H., Rejomone George. Environmental Monitoring and Assessment. 2012, 184, 6261-6279.
2. “Behaviour of REEs in a tropical estuary and adjacent continental shelf of southwest coast of India: Evidence from anomalies”. **Deepulal. P.M**., Gireesh kumar.T.R., Sujatha.C.H. Journal of Earth System Sciences. 2012, 121, 1215-1227.
3. “Phosphorous speciation in surface sediments of the Cochin estuary”. Gireesh kumar.T.R., **Deepulal.P.M.**, Chandramohanakumar.N. Environmental Monitoring and Assessment**.** 2012. Doi: 10.1007s/10661-012-2729-3.
4. “Distribution and sources of sedimentary organic matter in a tropical estuary, south west coast of India (cochin estuary): A baseline study”. Gireesh kumar.T.R., **Deepulal.P.M.**, Chandramohanakumar.N. Marine Pollution Bulletin. 2012, http://dx.doi.org/ 10.1016/j.marpolbul.2012.10.002.
5. “Distribution of REEs along continental shelf of south west India”. **Deepulal P.M**., Sujatha C.H., Gireesh Kumar T.R”. Indian Journal of Marine Sciences. (Acepted).
6. “Risk assessment of rooftop collected rainwater for individual household and community use in central Kerala, India”. Jesmi Y, Mujeeb Rahiman K.M, Hatha, A.A.M, ***Deepu Lal*** and Jyothi S. Journal of Environmental Health. (**Accepted**).

7. "Ecotoxicological evaluation of biocides on the *Villorita cyprinoides var Cochinensis* as pollution indicator in the Cochin estuary-Southwest coast of India. Deepulal, P.M., Harishanker, S.H., Gireesh Kumar, T.R., Sujatha, C.H. Environmental Science and Technology, (Communicated).
8. "Organic matter enrichment and Benthic Trophic status of the Cochin estuary, Tropical estuary South West coast of India". Gireesh Kumar, T.R., Deepulal.P.M., Chandramohanakumar.N, Ratheesh Kumar, C.S., Renjith, K.R., Rejomon George. Environmental Earth Sciences. (Under Review)

Papers presented on Seminars

1. ***Deepualal.P.M***., Gireesh Kumar.T.R, Sujatha.C.H. Distribution of Total organic matter along South west coast of India. ***(Presented at National Conference of Ocean Society of India- OSICON -09)***
2. Gireesh Kumar T.R., Ratheesh Kumar C.S., ***Deepulal P.M.***, Chandramohanakumar, N. Monitoring of water and sediment quality around Cochin Harbor area, with a special emphasis on metal contamination ***(National Conference of Ocean Society of India- OSICON -09)***
3. ***Deepulal P.M***., Sujatha C.H., Gireesh Kumar T.R. distribution of REEs along continental shelf of south west India ***(Presented at National Conference on Coastal Processes, Resources and Management at CESS-Feb 5-7, 2010)***.
4. Hatha, A.A.M., Mujeeb Rahiman, K.M., Dhanya Warrier, Nishana, K.I., Nikhil, S., Neethu, S, ***Deepu Lal*** and A.V. Saramma.. Culturable diversity of heterotrophic bacteria in the Kongsfjord, Norwegian Arctic and their contribution to Phosphorus cycle**. *(Presented at intertnational seminar conducted by assemption college, kottayam 2010)***.
5. ***Deepulal, P.M.,*** SujathaC.H., Gireesh Kumar, T.R. The Distribution of REE's Along South Coast of INDIA (***Presented at National Conference of Ocean Society of India- OSICON-2011***)

Achievements….

1. Participated in the IVth Indian Scientific Expedition to Southern Ocean from 12th Jan to 26th March 2010 in Sagar Nidhi, MoES, Govt. of INDIA.
2. Participated in the cruise No.255 of FORV Sagar Sampada for collecting samples for MoES, Govt. of INDIA funded project related to Benthic Productivity Studies.
3. Participated in the cruise No.260 of FORV Sagar Sampada to study "Studies on Benthic Productivity, Harmful Algal Blooms, Gelatinous Zooplankton, Environmental Productivity, Survey of Marine Mammals along the Continental Shelf and Slope of Indian EEZ from 8th Dec to 27th Dec2008.

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