RACKING OFF MARINE ASSEMBLAGES FOR THE ASSESSMENT OF HEAVY METALS AND ORGANOCHLORINE INSECTICIDES- A DIAGNOSTIC APPROACH

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By

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Racking off Marine Assemblages for the Assessment of Heavy Metals and Organochlorine Insecticides- A Diagnostic Approach

Ph.D. Thesis under the Faculty of Marine Sciences

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Certificate

This is to certify that the thesis entitled **"Racking off Marine Assemblages for the Assessment of Heavy Metals and Organochlorine Insecticides- A Diagnostic Approach"** is an authentic record of the research carried out by Mrs. Shibini Mol PA under my supervision and guidance at the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Cochin 682016, for the 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. All the relevant corrections and modifications suggested by the audience during the pre-synopsis seminar and recommended by the Doctoral Committee of the candidate have been incorporated in the thesis.

Cochin 682016
June 2019

Dr. Sujatha C. H.
(Research Supervisor)

Declaration

I hereby declare that the thesis **"Racking off Marine Assemblages for the Assessment of Heavy Metals and Organochlorine Insecticides- A Diagnostic Approach"** is an authentic record of the research carried out by me under the supervision and guidance of Dr. Sujatha C.H, Professor, Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology for the partial fulfilment of the requirements for Ph.D. degree of Cochin University of Science and Technology and no part of this has previously formed the basis of the award of any degree, diploma, associateship, fellowship or any other similar title or recognition from any University/Institution.

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Dedicated to my loving husband

Preface

The natural resources are under extreme pressure due to the increasing industrialization and rapid urbanization across the world. The minimum requirement for sustaining the quality of the ecosystem is not maintained due to its improper exploitation by humans. Anthropogenic pollution is one of the major stress factors that influence the quality and health of the environment. The toxic pollutants are the major category of stress factor on the natural environment and enter into the aquatic environment through atmospheric fall out, surface run-off or through leaching. Agriculture and public health activities contributed to a widespread alteration of the global ecosystem which mainly deals with heavy metals and organochlorine insecticide compounds (OCIs). Both this type of pollutants was ubiquitous and persistent, and as a consequence, they exist with a high concentration in the different environmental phases. It caused the rapid degradation of water quality and thereby altered the associated environmental niche, as well as its impact on biodiversity, and hence pose a great threat for human existence. This environmental problem has therefore led to the development of different national and international directives in order to regulate, mitigate and restore the contaminant effects on aquatic systems.

The increase of human population has increased the need for food supply which has increased the demand for fishery products. However, the levels of contaminants in fish and poor management of fisheries are of particular interest due to the potential risk to humans who consume them. The consumption of contaminated marine food has been associated with various toxic effects in the human population and wildlife community such as endocrine disruption, food poisoning, nervous system illness and even leads to death. The thesis entitled "**Racking off Marine Assemblages for the Assessment of Heavy Metals and Organochlorine Insecticides- A**

Diagnostic Approach" is an attempt to assess the burden of heavy metals and organochlorine insecticides in order to establish a better picture of how far the environment has been affected by these toxicants. This research further extends to focus on the bioaccumulation pattern in bivalves and their toxicological risk on the human population owing to their health concerns and for sustaining a better food safety and healthy life. To fulfill the objective of the research theme, it is categorized into seven chapters and is briefly discussed hereunder.

Chapter one, Introduction deals with the sources, fate, and toxicity of persistent and non-biodegradable toxic contaminants such as heavy metals and organochlorine insecticides. Also discussed on the effect of the focused contaminant in the marine ecosystem. This chapter also describes the scope and purpose as well as the objectives of the study.

Chapter two, Materials and Methods encompasses the methodology and the types of materials adopted to achieve the objectives of the study. This chapter also describes the study area, sample collection, storage, various methods performed and quality control employed for the research programme.

Chapter 3 entitled "Quality aspects of the fishing zones of Central Kerala" presents the spatial distributional characteristics of various hydrographical parameters such as salinity, temperature, pH, dissolved oxygen, nutrients and also the distribution and phytoplankton diversity of the studied area. It covers the findings on the sediment characteristics including the textural attributes and the elemental composition. It also describes the proximate profile of bivalves. In addition to this, this chapter also includes the potential influence of environmental parameters on the biochemical composition of bivalves

Chapter four, "Distribution profile and risk status of Heavy Metals in sediments" focuses on the discussion of the spatial distribution pattern of heavy metals viz., cadmium (Cd), nickel (Ni), lead (Pb), manganese (Mn),

copper (Cu), iron (Fe), cobalt (Co) and zinc (Zn) to get a comprehensive picture of the current concentration status. This chapter details the potential level of environmental risk associated with the metal using available Sediment Quality Guidelines (SQGs) and evaluated the overall pollution status of sediments using pollution load index, enrichment factor, geo-accumulation index, and contamination factor. This chapter also identified the sources and differentiate the anthropogenic versus natural contribution of metals in sediments using multivariate statistical techniques. Also assessed, the mode of occurrence of heavy metals in different geochemical fractions of sediment and the Risk Assessment Code (RAC) based on the geochemical fractionation is also employed for assessing mobility and toxicity of heavy metals.

Chapter 5, "Concentration of OCI residues in the surface water and sediments" depicts the information on the distribution of OCIs in sediment and water samples. The potential sources and the contamination levels of OCIs in the study area were also analyzed concurrently using multivariate statistical analysis. The impacts of sediments on aquatic species were assessed using sediment quality guidelines with the view of deducing ecotoxicological effects on aquatic species due to organochlorine pollution in the sediment compartments.

Chapter 6 entitled "Status of anthropocenes in the bivalves" deals with the heavy metal and OCI concentration profile in the bivalves and their bioaccumulation pattern using BCF and BSAF. It also highlighted the potential human's health risks associated with the consumption of edible bivalves by comparing with National and International standards.

Chapter seven gives a concluding remark of the present study. It highlights the outcomes that were inferred from the research endeavor and also few recommendations being fulfilled in the future.

References are provided at the end of each chapter.

Contents

List of Tables

List of Figures

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Chapter 1
INTRODUCTION

Contents

Marine ecosystems are the most productive ecosystems in the world, with immense resources of great economic value (UNEP, 2006). Healthy marine ecosystems provide several valuable services like fishery resources for economic growth and marine origin pharmaceuticals. It also provides transportation and aquatic recreational opportunities for tourists (Mehvar et al., 2018). These ecosystems also perform a number of significant environmental functions like climate regulation, prevent erosion, accumulate and distribute solar energy, absorb carbon dioxide and maintain the biological pump in oceanic realm. They support divergent species which could serve as animal feed, fertilizers, additives in food and cosmetics. Marine ecosystems and aquatic habitats face a number of threats from humans. In the course of various human activities, a large amount of pollutants enters into the water bodies through various channels. In India, with the development and utilization of marine resources, they have been continuously deteriorating the aquatic system due to the increase of coastal development, pollution from habitat

destruction, agriculture, land-based sources, the voyage of ships, public health, industrial activities and atmospheric fallout. Besides, complex mixtures of xenobiotics are derived from multiple human activities, such as waste disposal (Poynton and Vulpe, 2009), from dredged spoil dumping yard and by oil spills and leakages. When the toxic contaminants enter into the aquatic environment, they accumulate in the sediment, move down to deeper layer, finally to the benthic fauna, affects the physicochemical properties of both water and sediment, and alter the abiotic and biotic components of the environmental niche. Consequently, marine pollution is a problematic issue and its impacts are numerous with critical dangerous effects on both marine resources and the ecosystems (Leoni and Sartori, 1996; Mendil and Uluözlü, 2007; Odeku and Paulos, 2017). Proper management of this fragile marine ecosystem depends on the knowledge about the extent and impact of pressures from various human activities and it end up in different environmental compartments such as air, water, sediments and shoreline to different ecological niches such as microorganisms as well as from primary producers to fish, marine mammals and seabirds.

1.1 Environmental Contamination

Contaminants are persistent worldwide concern and threaten the ecosystem services upon which billions of humans depend. Anthropogenic stressors have altered marine habitat through, eutrophication, sedimentation, declined oxygen levels, nuisance algae blooms, excessive weed growths, deteriorating fisheries, sediment infilling and shoreline erosion, ultimately leading to changes in aquatic biodiversity. According to the “International

register of potentially toxic chemicals" of United Nations Environment Programme, nearly four million known chemicals in the world today and another 30,000 new compounds are added to the list every year and many of them are potentially toxic (Khoiyangbam and Gupta, 2012). Numerous pollutants (heavy metals, pesticides, hydrocarbons, organic and inorganic wastes) are being produced from the terrestrial environment. Due to the long half-life, bioaccumulative nature and persistivity, these pollutants including heavy metals and organochlorine insecticides have a great concern to the public (Singh et al., 2017).

1.1.1 Heavy metals

Heavy metals occur both naturally and anthropogenically in the marine environment. Altogether have spoiled the environment by elevating the metal level and create pollution and hence there is a significant need to understand the toxicity of metals to the aquatic organisms. The current alarm regarding metal pollution was started with the tragedy of 'Minimata' disease caused by the consumption of fish and shell fish contaminated with methyl mercury taken from Minimata Bay in Japan in the year 1956 (Eto, 1997) and continuing with the affliction known as 'Itai Itai' disease caused by the consumption of foods contaminated by cadmium from Niigata again in Japan (Ui, 1972).

1.1.1.1 Fate of Heavy Metals in the Marine Environment

Unlike organic pollutants, natural decomposition does not remove metals and the chemical aspect of metal may change but the element itself remains. In aquatic systems, heavy metals can be found in different forms, mainly in the dissolved (e.g. free element ion, complexed by

inorganic ions such as chloride and sulphide, and complexed by dissolved organic matter) and in the particulate state (adsorbed onto surfaces of clays, element oxides such as sorption onto mainly iron and manganese oxides, organic material, coprecipitated with sediment phases and incorporated into organic matter) (Williamson and Wilcock, 1994; Al-Naimi, 2013). Before they are released back into the dissolved phase, they are rapidly sorbed onto suspended particulate matter (Kennish, 1997) and enter into the sediment porewater. In the course of time, they are accumulated in sediments by several processes like sorption onto particle surfaces and coprecipitation with solid phases through sedimentation (Wijaya et al., 2016). After the deposition onto sediment surfaces, metals are immobilized through adsorption, coagulation or flocculation. They incorporate into the mineral lattice structure (e.g., Fe-Mn oxides) and precipitate by forming insoluble fractionation (such as metal sulphides) (Bryan and Langston, 1992; Du Laing et al., 2009; Lin et al., 2013). Once heavy metals are deposited in sediments, they undergo diagenesis which determine new chemical phases in the sediment (Williamson and Wilcock, 1994). Due to low solubility, heavy metals would be highly accumulated in sediments (Yacoub et al., 2014; Jia et al., 2018 (a)) and the process of sediment resuspension could release heavy metals from the sediments into the water column where they pose a threat to water quality of the aquatic environment (Ip et al., 2007; Tang et al., 2010). Therefore, the mobility, bioavailability and subsequent toxicity of metals have been a major research area to focus in detail. Owing to the stress effect hampered in the environment and focussing on the health perspective, heavy metal accumulation creates a critical influence on our aquatic environment and

accounts its quality for the health benefits of human. A brief description of controlling mechanism of metal speciation in aquatic environment is depicted in Fig.1.1.

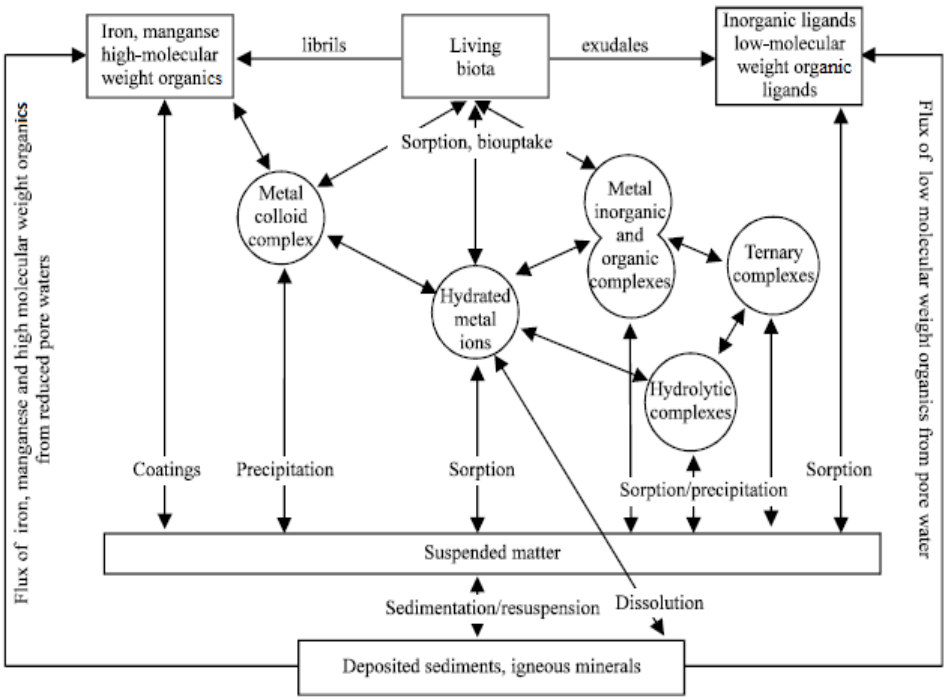


Fig. 1.1: Speciation of metal ions in seawater and the main controlling mechanisms (Ohman, 1988; Ansari et al., 2004)

1.1.1.2 Sources of Metals and their Toxicity

Heavy metals occur naturally, form part of the lithosphere and are often get into the environment through volcanism and weathering of rocks (Fergusson, 1990). Heavy metal contamination is also derived from anthropogenic sources including mining and smelting activities, fuel combustion, waste incineration, foundry, smelter, traffic and from industrial effluents (Jia et al., 2018 (b)). Besides, domestic effluent,

sewage sludge, runoff from urban area and leachate from municipal solid waste disposal sites are also the source input of heavy metals into the marine environment (Drozdova et al., 2015). Other potential sources include ports, harbours, boating, and shipping activities (Denton et al., 1997). Agricultural activities contribute to the overall heavy metal loadings into the environment through the spreading of fertilizers, pesticides and wood preservatives (Bradl, 2005).

Heavy metal toxicity can be categorized into various ways, here under classified into three viz., i) non-carcinogenic effects ii) carcinogenic effects iii) endocrine disruption. Some heavy metals may create harmful effects on the environment as well as potential risk to human health depending upon their bioavailability and toxicity in various environmental compartments. Generally, most metals have a long residence time and hence exert their toxic effects over a long time periods after being released to the ecosystem. A brief explanation about the sources and toxicity of metals under the present research investigation are given below.

Cadmium

Cd is one of the heavy metals prioritized at the top of the toxicity list (Volesky and Prasetyo, 1994) and has been reported no essential biological function and is toxic to plants and animals. Cd is produced mainly as a by-product from mining and smelting of Zn and also occurring from materials containing Cd as impurity like phosphatic fertilizers. It is extensively used in various industrial products and processes including electroplating, plastics industries and the most

significant use is in Nickel/Cadmium batteries. Cadmium is also released from cadmium fungicides, cadmium-based enamel, coal (Fulkerson and Goeller, 1973), pigments, stabilizers, and alloys (ATSDR, 2008 (a)). Smoking tobacco adds an additional burden of Cd and one packet of cigarettes deposits approximately 2-4 µg of cadmium, but levels may vary widely (HSDB, 1996). Cadmium was potentially harmful to human health, depending upon the form of cadmium present and the amount consumed. Cadmium accumulates in the human body and its long-term exposure impairs kidney's normal functioning (Jinadasa et al., 2010; Mehouel et al., 2019) and may cause fragility of bones (Tirkey et al., 2012). It causes a painful disease called Itai-Itai (Rajappa et al., 2010) to humans and high exposure leads to obstructive lung diseases.

Lead

Pb is a non-essential element to the human body and arises from both natural and anthropogenic sources in the environment. Approximately 96% of all Pb emissions originates from anthropogenic sources (Nriagu et al., 1996; Pacyna and Pacyna, 2001). Due to its severe toxicity and great potential hazards to the environment and organisms, Pb is regarded as one of "the big three" toxic metals with great hazards (others are cadmium and mercury) (Shanab et al., 2012). The main sources of Pb in natural waters include burning of lead-based petroleum fuels, organic and inorganic lead compounds and industrial materials including plastics, storage batteries, bearing alloys, insecticides, ceramics, cable covering, radiation shields and even in some paints (Bytyçi et al., 2018). Excess metal intake can damage the skeletal, circulatory, enzymatic, endocrine

and immune systems (Zhang et al., 2012). Even a very low amount of Pb exposure results in a wide range of metabolic disorders and neuropsychological deficits (Nriagu, 1988; Silvany-Neto et al., 1989). Lead affecting the brain (Sanders et al., 2009), especially causes brain retardation in children (Lidsky and Schneider, 2003). Lead exposure may cause weakness in fingers, wrists, or ankles and miscarriage for pregnant women (Finkelman, 2005). High levels of exposure may also lead to the biochemical effects in humans which in turn cause problems in the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints, reproductive system and acute or chronic damage to the nervous system (Tirkey et al., 2012; Bytyçi et al., 2018).

Copper

Cu is a moderately abundant and is an essential element for living systems (Nicholas et al., 1998; Osredkar and Sustar, 2011). Cu is widely utilized in many industries including metal cleaning and plating baths, paints and pigments, fertilizers, paper board, wood pulp, etc. (Zhu et al., 2009; Aksu and Isoglu, 2005). Algaecides and antifouling paints are identified as major contributors of copper to harbour areas (Denton et al., 1997). However, it becomes toxic to aquatic organisms at levels as low as 10 µg/g (Callender, 2003) and causes hypertension and produces pathological changes in brain tissues. Long term exposure can lead to nose, mouth and eyes irritation, headache, dizziness, nausea, and diarrhoea (Finkelman, 2005). Exposure to excess Cu can result in anaemia, developmental problems, immunotoxicity, liver and kidney damage, stomach and intestinal irritation (ATSDR, 2004; Tirkey et al.,

2012) and is also responsible for the specific disease of the bone (Krishnamurthy and Pushpa, 1995). Also, excessive contact to copper has been linked to cellular damage resulting Wilson disease in humans (Tchounwou et al., 2012). The co-carcinogenic character of Cu is also implicated in stomach and lung cancer (Ahmad et al., 2009).

Zinc

Zn is considered as an essential element for life, but at elevated as well as insufficient concentrations, Zn may be harmful on human health (Tapiero and Tew, 2003; Maret and Sandstead, 2006). Approximately one-third of all atmospheric Zn emissions are from natural sources, the rest results from nonferrous metals, burning of fossil fuels, municipal wastes, pesticides, zinc fertilizer and cement production (Denton et al., 2001; Callender, 2003; Bradi, 2005; Damodharan, 2013). It is also utilized in paints, plastics, cosmetics and pharmaceuticals (Weng and Huang, 2004; Sen and Khoo, 2013). The other sources of Zn pollution in the environment are from coatings to prevent rust, in the manufacture of dyes, wood preservatives, in the dry cell batteries and production of alloys such as brass or bronze (Nwankwoala et al., 2016). Taking excess Zn into the body through food, water as well as through diet can have several adverse effects on health like stomach cramps, nausea and vomiting (Damodharan, 2013). Ingesting high levels of zinc for several months can result in anaemia, damage to the pancreas and decreased the levels of HDL cholesterol (Finkelman, 2005) and its deficiency affects the skin, gastrointestinal tract, brain, central nervous system, skeletal and reproductive systems.

Cobalt

The International Agency for Research on Cancer classifies cobalt as a 'possibly carcinogenic to human'. The major anthropogenic sources include mining and processing of cobalt-bearing ores, the use of cobalt-containing sludge or phosphate fertilizers to soil, the disposal of cobalt-containing waste, atmospheric deposition from activities such as the burning of fossil fuels, smelting and refining of metals (Smith and Carson, 1981; Wafi, 2015). It is extensively used in alloys, nuclear power plants, tool industry, manufacture of blue pigments for glass, tungsten carbide and in ghee manufacturing. Hazards related to the exposure are lung diseases, damage to the heart causing heart failure, action on blood vessels, rapid change in catecholamine metabolism, increased blood and urine monochrome level, increased glycogen level and decreased lactate dehydrogenase activity.

Manganese

Mn in combination with other elements is widely distributed in the Earth's crust and found in rock, soil, water and food. Mn is an essential element, involved in the reduction of nitrates in green plants and algae and an excess of this element becomes toxic in plants and animals. It is used for the production of ferromanganese steels, electrolytic manganese dioxide for use in batteries, glass industries, alloys, catalysts, antiknock agents, pigments, dryers, wood preservatives and coating welding rods (Bradi, 2005). It is also used as an oxidant for cleaning, bleaching and disinfection (as potassium permanganate) and as an ingredient in various products (ATSDR, 2008 (b); Muhammed et al., 2018). The high

concentration of Mn causes liver cirrhosis and also produces a poisoning called Manganese or Parkinson disease (Bradi, 2005). Symptoms of the Manganese toxicity in man include dullness, weak muscles, headaches and insomnia.

Nickel

Ni is a common metal and is an essential micronutrient for animals and takes part in the synthesis of vitamin B12. Ni is moderately toxic to most species of aquatic plants, though it is one of the least toxic inorganic agents to invertebrates and fish. The major discharge source of Ni is the urban wastewater followed by smelting and refining of non-ferrous metals (Nriagu and Pacyna, 1988; Denton et al., 2001) and use of fossil fuels, particularly coal. Ni is also introduced as solid waste from metallurgical industries, or as deposition of atmospheric emissions. It is frequently used in different industries such as in electroplating, storage batteries, porcelain enamelling, pigment and steel manufacturing, etc. (Xu et al., 2006; Ewecharoen et al., 2008). Nickel dermatitis, consisting of itching of the fingers, hands and forearms, is the most common effect in humans from chronic skin contact with nickel. The high concentration of Ni causes pneumonia, lung and nose cancer, giddiness, headache, nausea and vomiting (Rathor et al., 2014). Higher levels may also cause rapid respiration, cyanosis and dry cough (Periasamy and Namasivayam, 1995; Malkoc and Nuhoglu, 2006). Some of the most serious health effects due to exposure to Ni includes reduced lung function and some Ni compounds are reported to be carcinogenic to humans (Finkelman, 2005).

Iron

Fe is the fourth most common element in the earth's crust and is an essential nutrient for human life. Concentrations of Fe in most waters do not cause any significant hazards to human life. Fe is used mainly for the production of steel. It is also used as an iron alloy and in manufacturing and equipment industries. Excess exposure causes skin sensitivity to light. High iron concentrations affect vital organs in humans including the liver, cardiovascular system and kidneys (Wafi, 2015). Fe overload in man is not common but may occur due to a genetic defect. Such overload results in oxidative degradation of lipids, destruction of intercellular and extracellular proteins and DNA damage (Tirkey et al., 2012). The shortage of iron causes a disease called "anaemia" and prolonged consumption of drinking water with a high concentration of iron may lead to liver disease called as hemosiderosis (Rajappa et al., 2010).

1.1.2 Pesticides

Pesticides are widely used in agricultural production to prevent or reduce losses by pests for improving the crop yield as well as the quality of the products. According to Damalas and Eleftherohorinos, (2011), pesticides can also improve the nutritional value of food and sometimes its safety. The common type of pesticides includes herbicides to control weeds, insecticides to control insects, fungicides to control a certain type of plant disease, and antifouling agents to inhibit the growth of fouling organisms on the boat hull. The illegal activities have caused damages to the nontarget organism, and even the routine use of pesticides can pose major health risks to farmers both in the short and the long run and could

degrade the environment (Jallow et al., 2017). About 3 million humans are poisoned and 200,000 died every year around the world from pesticide poisoning (WHO/UNEP, 1990; Hulse et al., 2014).

One of the most common pesticides in our environment is organochlorine insecticides (OCIs). These class of compounds are known to be persistent organic pollutant (POPs), can distribute in all the phases of the ecosystem including air, water, soil and animals. OCIs are a large class of multipurpose chlorinated hydrocarbon chemicals and are decomposed slowly in the environment and the bioaccumulation is linked to the lipophilic nature of this labile organic material (Gopalan and Chenicherry, 2018). The organochlorine compounds possess unique physical and chemical properties which could influence their persistence, fate and transport in the environment. Many organochlorine insecticides are endocrine disrupting chemicals (Lemaire et al., 2004) since at extreme low concentrations, they are having the ability to immobilize or kill the aquatic organisms (Jayaraj et al., 2016). Within this context, pesticide use has raised serious concerns, not only of the potential effects on human health but also regarding the impacts on wildlife and sensitive ecosystems.

1.1.2.1 Fate of Pesticides in the Marine System

Pesticides differ from many other environmental substances of concern in such a way that they enter the environment through intentional use for specific purposes. Pesticides enter the aquatic ecosystems from various pathways, including the atmosphere, industrial and municipal effluents as well as from agricultural and urban non-point source and land run-off. There exist point sources of pesticides from non-agricultural use,

e.g. from the application on roadside, wastewater treatment plants, railways or urban sealed surfaces such as parking lots and residential areas (Sujatha et al., 1993; Reichenberger et al., 2007). Organochlorine insecticides can move from the soil surface to the atmosphere and migrate with the air currents to distant areas and then later fall back to land with precipitation, and carry pesticide out into streams, lakes, and rivers (Maurya and Malik, 2016). Surface runoff is considered as one of the most important pathways for pesticides to enter into the marine water (Larson et al., 1995). Subsequently, pesticides may distribute among the various components of the river ecosystem. The behaviour and fate of chemicals in different environmental compartments is dependent on both their physical and chemical properties and on the nature of the environment. In water, the residues and their degradation products are distributed in truly dissolved form and finally incorporated into sediments, aquatic plants, plankton and suspended detritus. Under favourable conditions, the sediments, which are excellent temporary or long-term sinks of OCIs (Akhil, 2014), can be resuspended and then later results to enter into the water column (Vagi et al., 2007; Ahmed et al., 2015). OCIs are highly hydrophobic and once reached into the water column, tend to adsorb to fine particulates or are bioaccumulated into aquatic biota (Maurya and Malik, 2016). These organisms are then consumed by fish and birds, which can result in the transfer to higher trophic communities through aquatic and terrestrial food chains. Fig.1.2 shows the fate and movement of pesticides in the environment.

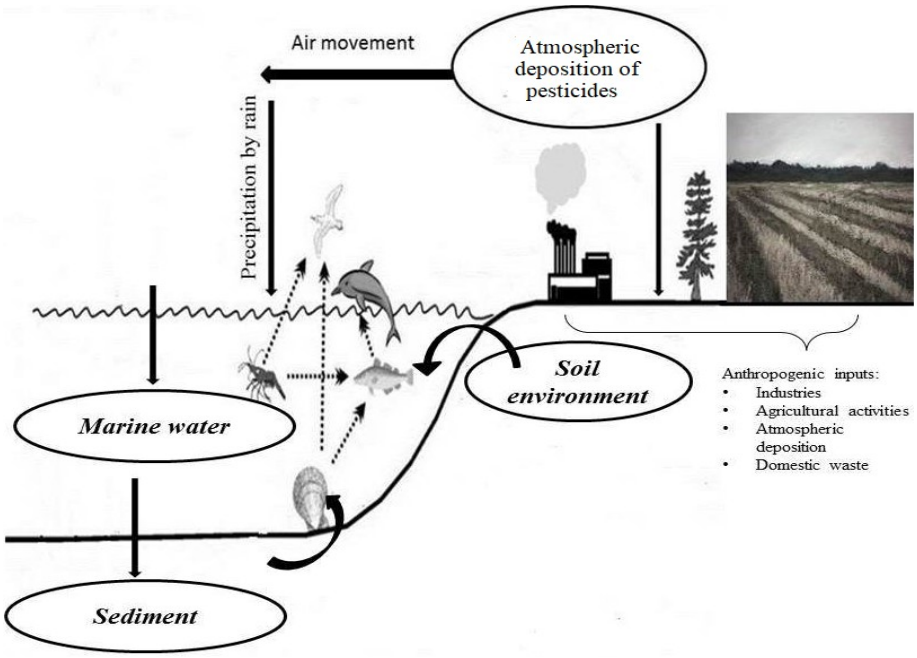


Fig. 1.2: Fate and movement of pesticides in the environment

1.1.2.2 Sources of OCIs and their Toxicity

A pioneer warning signal about pesticides pollution was assessed in 1962, by Rachel Carson, courageous American scientist, wrote down her nature observation and pointed out sudden dying of birds caused by indiscriminate spraying of pesticides (DDT) in her influential book "Silent Spring". After publishing "Silent Spring" (Carson, 1962), a much wider audience was warned about the environmental effects and issues of the widespread use of pesticides. The use of organochlorine residues has been restricted in most of the countries (FAO/WHO, 1978) and international treaties, e.g. the United Nations Aarhus Protocol and the Stockholm Convention aimed towards elimination or restriction in the

manufacturing and use of POPs (Grimalt et al., 2004). Twelve of the known persistent organic pollutants, referred to as the "dirty dozen", has been officially registered by the United Nations Environmental Programme (UNEP, 2002) under the Stockholm convention in 2001 as priority pollutants. Among these, nine of the Stockholm convention priority pollutant compounds for the regulation (DDT, dieldrin, toxaphene, endrin, aldrin, hexachlorobenzene, chlordane, heptachlor and mirex) are insecticides.

Depending on the chemical structure of OCIs, many of these compounds are considered toxic to marine biota. Some of the OCIs like DDT, DDE and heptachlor are suspected to be as carcinogens and have been propagated to POPs or endocrine disrupters (Willett et al., 1998; Fatoki and Awofolu, 2003). Researches have also proven that each isomer of hexachlorocyclohexane may also fairly be anticipated to cause most cancers in human beings (USEPA, 2002; ATSDR, 2005).

There are three main types of organochlorine insecticides (Agbeve, 2011). These are

1) Dichloro-diphenyl-ethanes such as DDT, DDE, DDD.
2) Chlorinated cyclodienes such as aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide.
3) Chlorinated cyclohexanes such as α-HCH, β-HCH, γ-HCH (Table 1.1).

1. Dichloro-diphenyl-ethanes

The most popular and most extensively used dichloro-diphenyl-ethane is the dichloro-diphenyl-trichloro-ethane (DDT). DDT is the broad-spectrum insecticide and it was discovered in 1939 by a Swiss scientist, Paul Muller. It is used in agriculture to control the pest of cotton, soybean and peanut etc. Large amounts of DDT were released into the atmospheric realm and on soil or water after it was sprayed on crops and wild application on forests to control insects. DDT was also sprayed in the environment for the control on vector mosquitoes. It became the most widely used pesticide in the world because it was effective against body lice that transmit typhus, against plague-carrying fleas and mosquitoes that carry vectors of malaria and yellow fever (Baird, 1999; Tren and Bate, 2000.). WHO estimated that DDT saved the lives of more than five million people in its malaria reduction programmes (Baird, 1997). As a result of its application, environmental concentration rose rapidly and began to affect various ecotoxicological effects such as reproductive abilities of birds. DDT breaks down over time into dichloro-diphenyl-dichloro-ethylene (DDE) and dichloro-diphenyl-dichloro-ethane (DDD). Technical-grade DDT however, is a mixture of p,p'-DDT isomer (85%), o,p'-DDT (15%), and o,o'-DDT (trace amounts). DDE and DDD were also occurring as contaminants (Faroon et al., 2002). By 1962, DDT was being called an "elixir of death" by Rachel Carson in the above quoted book, "Silent Spring". It was subsequently banned for agricultural use worldwide in 2004 under the Stockholm Convention, though it is still being used in some underdeveloped countries for controlling vector borne diseases. In humans, over exploitation causes prickling sensation of the mouth,

nausea, dizziness, headache, lethargy, vomiting, fatigue, tremors in the extremities, anorexia, anaemia and muscular weakness (Klaassen and Watkins, 1996; Jayaraj et al., 2016).

2. Chlorinated cyclodienes

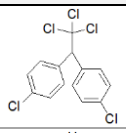
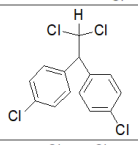
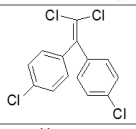
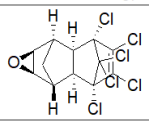
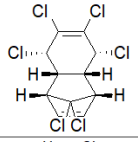
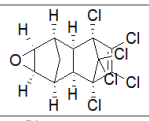
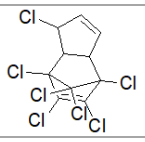
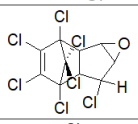
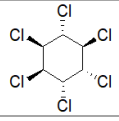
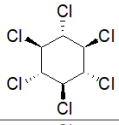
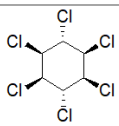
Aldrin and dieldrin are chemicals that are widely applied to control soil insects such as termites, corn rootworm, etc. in agriculture and in public health (Baird, 1997; Nollet, 2000; Afful, 2015). Dieldrin is mainly used to control disease-carrying vectors, mainly mosquitoes. Additionally, it has also been used to control vectors of tropical diseases, including malaria, yellow fever and also used for industrial purposes to protect electric and telephone cables and to preserve timber materials from the attack of termites (de Jong, 1991). These two insecticides have a similar structure (Table 1.1) and therefore show similar chemical properties and toxicity (Baird, 1997). Aldrin does break down to dieldrin in living systems, whereas dieldrin is known to resist bacterial and chemical breakdown processes in the environment (Doyle et al., 1994; Orris et al., 2000). These toxicants have been linked to various health problems such as Parkinson's, breast cancer, reproductive failure and nervous system damage. Aldrin and dieldrin are responsible for neurotoxic, developmental, immunological, genotoxic, tumorogenic effects, nausea, vomiting, muscle twitching and aplastic anaemia in humans (USEPA, 2003).

Endrin is a stereoisomer of dieldrin. It is hydrophobic and thus adsorbs strongly to soil particles and tends to be immobile. Endrin has been primarily used in agriculture to control soil insects, mice, termites and armyworms. Used as an insecticide on crops like cotton, citrus, potatoes, wheat, apples and flowers. Although it is very persistent, its

half-life is well over 10 years and when exposed to sunlight, it partially decomposes to endrin ketone and endrin aldehyde (Nollet, 2000). Endrin poisoning in humans affects the nervous system (Nollet, 2000; USEPA, 2006). Food contaminated with endrin caused several clusters of poisoning, especially in children. It is very toxic to aquatic organisms such as fish, aquatic invertebrates and phytoplankton.

Heptachlor is broad-spectrum insecticide used in agriculture for controlling soil insects and are used extensively as a pesticide on food crops like corn. It also has non-agricultural uses including seed treatment, garden uses and for termite control. It is also used for public health purposes to control disease causing vectors like mosquitoes and their use is suspended due to its potential carcinogenic property. Heptachlor epoxide is the main break down product of heptachlor and is more likely to be found in the environment than its parent compound. The epoxide also dissolves more easily in water than its parent compound and is more persistent. Heptachlor and its epoxide can adsorb to soil particles and in the later stage gets evaporated (ATSDR, 2007). Animals exposed to heptachlor epoxide during gestation and infancy is found to have changes in the nervous system and immune dysfunction (California EPA, 1999). Also, new born animals exposed to higher doses of heptachlor decrease in body weight and cause eventual death (California EPA, 1999).

Table 1.1: Chemical structure and molecular formula of OCIs analysed

OCIs	Structure	Molecular formula
DDT		$C_{14}H_9Cl_5$ M.W. 354.49
DDD		$C_{14}H_{10}Cl_4$ M.W. 320.05
DDE		$C_{14}H_8Cl_4$ M.W. 318.03
Dieldrin		$C_{12}H_8Cl_6O$ M.W.380.91
Aldrin		$C_{12}H_8Cl_6$ M.W. 364.91
Endrin		$C_{12}H_8Cl_6O$ M.W. 380.91
Heptachlor		$C_{10}H_5Cl_7$ M.W. 373.32
Heptachlor epoxide		$C_{10}H_5Cl_7O$ M.W. 389.32
α-HCH		$C_6H_6Cl_6$ M.W. 290.83
β-HCH		
γ-HCH		

3. Chlorinated cyclohexanes

Hexachlorocyclohexane is a synthetic chemical that exists in eight isomeric forms. The structures of the three isomeric forms are presented in Table 1.1. Technical HCH consists of four major isomers viz. α-HCH, β-HCH, γ-HCH and δ-HCH in a mixture of 50–70, 5–14, 10–18 and 6–10 %, respectively (Wang et al., 2014). The gamma isomer of HCH has insecticidal properties and is currently sold as insecticide under the trade name lindane (Baird, 1997; Nollet, 2000). Lindane is a persistent organic pollutant with a relatively long half-life in the environment. USEPA and WHO has classified lindane as moderately hazardous or acutely toxic. The major utility as an insecticide is for food crops and in seed treatments for controlling rodents. Lindane is an active ingredient in several commercial medical preparations, formulated as a shampoo or lotion to get rid of children from lice and scabies (Baird, 1997; USEPA, 2003). It has been used to treat forestry products such as in the treatment of wood and wooden structures, furniture, toys, sporting goods, seed grains and also for providing better health for the livestock and pets. According to ATSDR (2005), exposure to lindane causes adverse health effects in humans such as neurological effects, liver toxicity, reproductive and developmental effects. Exposure to high doses can cause symptoms such as vomiting, nausea, diarrhoea, muscle weakness, seizures, blood disorders and immune deficiencies and eventual death (US CDC, 2005). Prenatal exposure to β-HCH, an isomer of lindane, has been associated with altered thyroid hormone levels and could affect brain development. Previous studies have shown that all isomers of HCH may reasonably be anticipated to cause cancer in humans (USEPA, 2002; ATSDR, 2005). An

international ban on the use of lindane in agriculture is therefore implemented in 2009 under the Stockholm Convention on POPs (UNEP, 2009).

1.2 Environmental hazard assessment using bioindicator

The presence of human-derived inorganic and organic pollutants into the water could affect environmental health effects. Chemical analysis of the environment matrix such as water and sediment are the most direct approach to reveal the pollution due to heavy metal and organochlorine insecticide present in the ecosystem. But these cannot afford the powerful evidence on the influence and possible toxicity of these contaminants on the organisms and ecosystem. The biomonitoring studies provide information on the amount of natural and man-made chemical moieties that have entered and retained in the biota and the respective ill effects induced. The assessment of the levels of heavy metals and organochlorines in the aquatic organisms, known as "bioindicators or sentinel organism" become an important task in preventing health risks to public.

The use of bioindicators for monitoring heavy metals and organochlorine residues is very common for the past and the present (Sujatha et al., 1995 (a,b); George et al., 2013; Krishnakumar et al., 2018). As reported by Salanki (1986), a good bioindicator is one which shows an early response to the pollutants and predicts the consequences of undesirable anthropogenic effects. Among aquatic organisms suitable for biological monitoring, bivalve molluscs including oyster, mussels and clams are often used as sentinel organisms. Bivalve molluscs are filter

feeders, which selectively filter small particles, phytoplankton, zooplankton and inorganic matter from the surrounding water. Their worldwide distribution, sedentary mode of life, abundance, tolerance to environmental alterations, high bioconcentration factor for pollutants, etc. makes them excellent bioindicators for the assessment of environmental pollution (Wade et al., 1998; Wootton et al., 2003). Besides, these are frequently used to assess the toxicity of a large variety of contaminants in marine water or sediments (Rickwood and Galloway, 2004). Earlier studies have demonstrated that marine bivalves readily bioaccumulate both organic and inorganic pollutants (Cajaraville et al., 2000). A major advantage in using bivalves over other animal groups is that they will accumulate chemicals in their tissues at concentrations higher than those found in the environment (Viarengo and Nott, 1993) and have a high affinity for some contaminants for the lipid-rich tissues in bivalves (Stout and Beezhold, 1981). In addition, bivalves act as vector transfer of contaminants to higher trophic levels of the aquatic and sediment food web. In some cases, the chemical may not be detectable by chemical analysis of environmental samples like water or sediments but is still accumulated by the bivalves and is detectable in their tissues. Therefore, a single tissue measurement can provide more useful information about the system under investigation than enough number of water or sediment samples.

1.3 Scope and Purpose of the Study

For sustainable fishing and aquaculture activities in the coastal areas, maintenance of water quality is very important and crucial. Proper understanding of the environmental parameters and their effects on biota

is a pre-requisite in the healthy management of any ecosystem. Sediments are indicators of the quality of water overlying them and hence their study is useful in the assessment of environmental pollution. In recent years, development in the coastal zones due to urbanization and industrialization, Central Kerala, a segment of Southwest coast of India is receiving a large amount of different types of effluent containing numerous pollutants such as organic compounds and heavy metals. In this context, the present investigation aims to assess the concentration levels of heavy metals (Cd, Pb, Cu, Zn, Co, Ni, Mn and Fe) and organochlorine insecticides (aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, α-HCH, β-HCH, γ-HCH, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT) in the environmental compartments. This study on the knowledge of water quality is paramount for understanding the toxic effect of heavy metals and OCIs. It is, therefore, necessary to monitor the habitat characteristics such as hydrography, nutrients, primary productivity and phytoplankton abundance. Any environmental exposure analysis would be meaningful and realistic only if it captures the contribution from different compartments of the aquatic environment.

The present investigation involves such a comprehensive approach, in which the water, the sediment and the biota (represented by bivalves) of Central Kerala, Southwest coast of India were examined and accounted the contamination levels. A good understanding of the sources of metals and pesticides released into the different aquatic phases and their baseline levels in water, bivalves and sediments is necessary for identifying and suggesting the appropriate measures for pollution control. In the present scenario, accumulation of OCIs and heavy metals in the local food web

and to what extent, are always important and crucial in environmental monitoring. Such studies are very useful because of its environmental risk, as well as the enhanced future risk for human health, has monitored and assessed to a certain extent. Hence in the efficient management of aquaculture activities and also for the effective utilization of marine resources, this research work would contribute a valuable input on the residual levels of persistent pollutants.

1.4 Objectives of the study

1) To investigate the nutrient dynamics, phytoplankton abundance and species diversity, sediment characteristics and proximate profile of bivalves for determining the quality of the study area.

2) To elucidate the spatial distribution pattern of heavy metals and to assess the potential level of environmental risk associated with the metal contents in sediments

3) To investigate the distribution profile of OCIs in sediment and surface water from the prominent fishing zones of Central Kerala.

4) To understand the environmental levels of heavy metals and organochlorine insecticides in bivalves (*Meretrix casta*, *Villorita cyprinoides* and *Crassostrea madrasensis*).

5) To determine the potential human's health risks associated with the consumption of edible bivalves.

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Chapter 2
MATERIALS AND METHODS

Contents

2.1 Study Area

Kerala's coastal area lies within latitudes 8°17'30" N and 12°47'40" N and longitudes 74°27'47" E and 77°37'12" E, extending from north to south as a barrier strip of land which includes a chain of lagoons and backwaters in connection to the Sea at various points. The present investigation was carried out around prominent fishing zones of Central Kerala mainly at Vypin, Munambam and Chettuva, along the Southwest coast of India. A total of 12 stations were selected, in which Vypin zone includes sampling stations V1-V4, Munambam zone with sampling stations M1-M4 and Chettuva zone comprising sampling stations C1-C4 (Table 2.1 and Fig. 2.4). The study site selection was based on the viewpoint of focusing the major fishing centres which played a pivotal role in the socioeconomic status of the fishery community of this Central Kerala region. Besides, the location has chosen noting the inflow of pollutants from different discharge point/nonpoint sources (Table 2.1).

Vypin

The Vypin (latitude 9°974'N, longitude 76°244'E) zone (Fig. 2.1) is situated in the central part of the Cochin estuary and banks the perennial opening of Cochin backwaters to the Arabian Sea. This zone has been experiencing extreme adverse impact mainly from the industrial hub area, the Eloor, one of the world's 'top toxic hot spots' (Green Peace, 2003) which resides along the banks of Periyar river. More than 247 industrial units are built upon this stretch of the river, created a cesspool of chemical pollutants. A range of products manufactured by these industries includes petrochemical products, pesticides, rare-earth elements, heavy metal processing, rubber processing chemicals, fertilizers, zinc/ chrome products, leather products, etc. These industries discharge directly ≈17, 35, 00,000 litres of highly polluted effluents per day into the river Periyar from where it is emptied into the Cochin backwaters (Menon et al., 2000; Green Peace, 2003). The effluents from Ambalamugal located 16 km east of Cochin, which is an industrial complex with a giant fertilizer plant and an oil refinery, are discharged into Chitrapuzha river which ultimately flows into the Cochin backwaters. Moreover, untreated effluents from the sewage treatment plant of Cochin metropolitan city and the pollutant wastes from the agricultural lands (paddy fields) located at Muvattupuzha and Kuttanad regions are finally discharged into the Cochin backwaters. Further, the sampling zone has close proximity to the International Container Transhipment Terminal. The area also experiences intense fishing activities, inland water transport and dredging activities. In addition to this, the release of waste oil, paints and paint scrapings from the Cochin shipyard is also drained into the central part of the Cochin

backwater which finally flushed out through the perennial opening to the Arabian Sea.

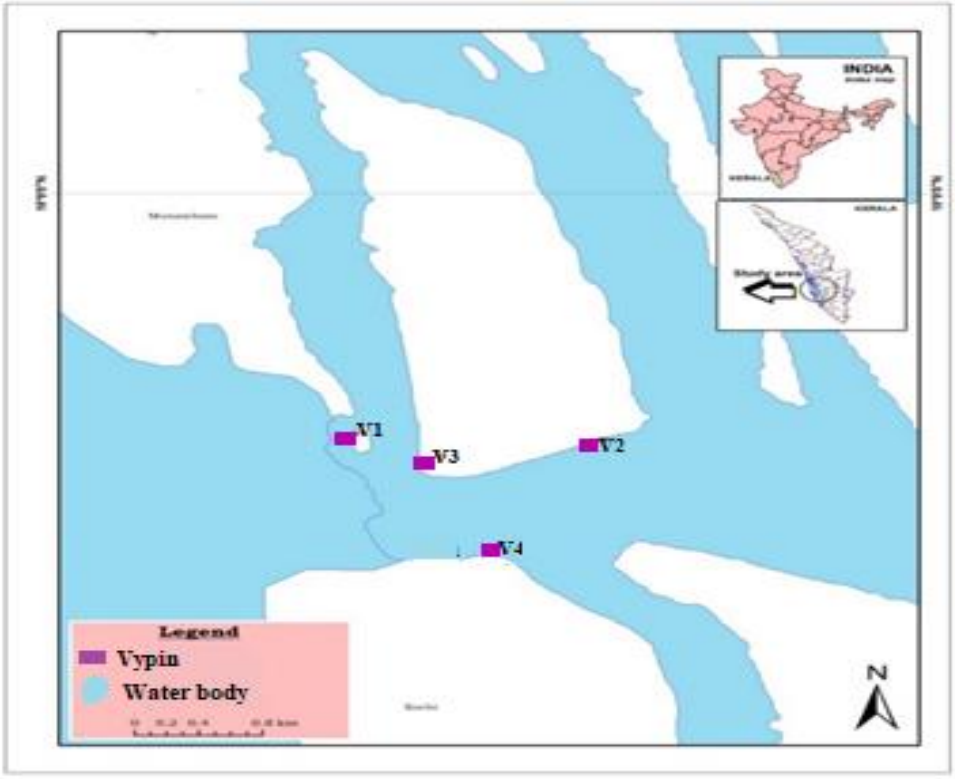

Fig. 2.1: Location map of the Vypin sampling zone

Munambam

The Munambam region is part of the Kodungallur-Azhikode estuary (latitude 10°10'N, longitude 76°10'E) (Fig. 2.2) situated at the northern arm of the Cochin backwaters. This zone is located at the point where the branch of Periyar river joins the Arabian Sea. Chalakudy and Pullut rivers also join this branch of the Periyar river. It is an area of robust fishing and tourism-related activities. The site is connected to intense mariculture establishments and close proximity for human habitation.

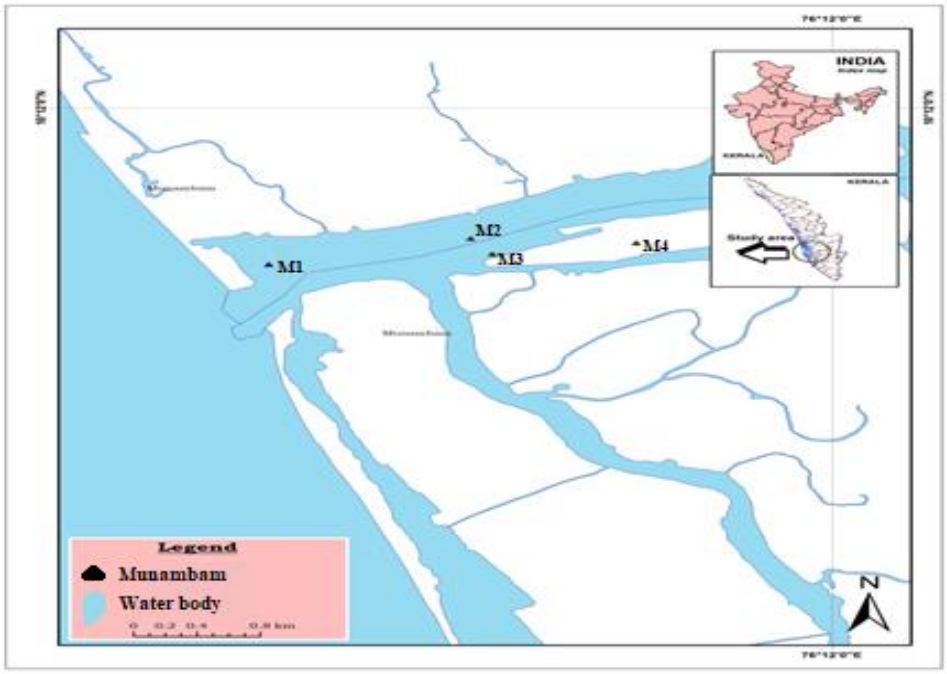

Fig. 2.2: Location map of the Munambam sampling zone

Chettuva

Chettuva estuary (latitude 10°32' N and longitude 76°02' E), (Fig. 2.3) is part of Vembanad – Kol wetlands and belong to an important fish landing centre situated along the Southwest coast of India. Karuvannur, Kecheri and Puzhakkal are finally discharging into the Arabian Sea through the Chettuva. There are numerous canals, with untreated industrial (7800 registered small-scale units in the district) discharges mainly through the outfalls of metal plating and leather tanning from the locality, and finally empties into the boundaries of Arabian Sea via the nearby outlet at Chettuva (Udayakumar, 2012).

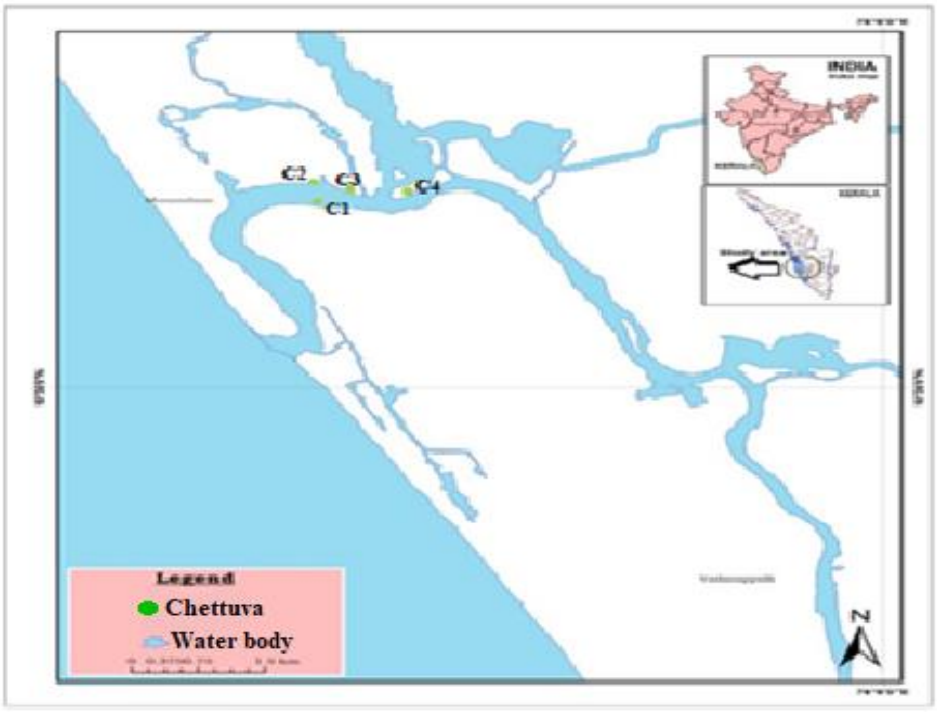

Fig. 2.3: Location map of the Chettuva sampling zone

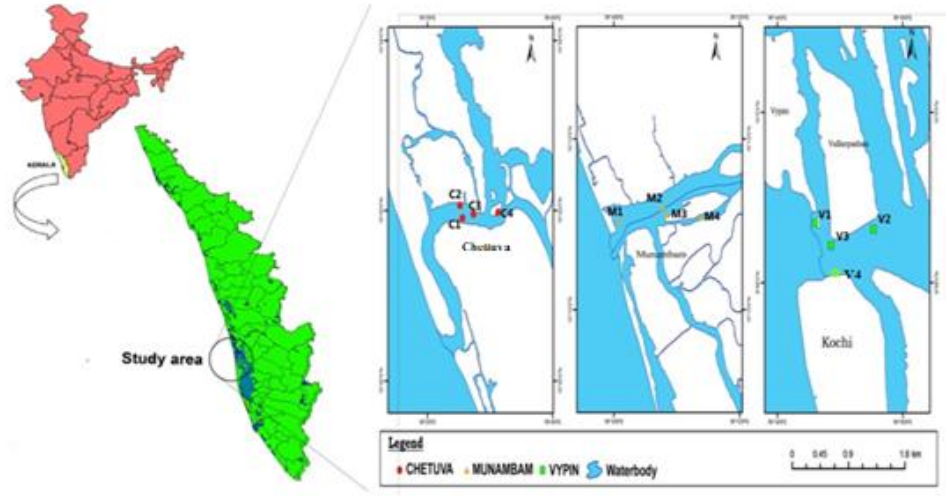

Fig. 2.4: Map of the study area and location of sampling sites from the Central Kerala

Table 2.1: Details of sampling zones selected for the study

Zones	Stations	Latitude	Longitude	Sources of pollution
Chettuva	C1	10°31'911"N	076°02'564"E	Agricultural region, fishing zone
	C2	10°32'107"N	076°02'539"E	
	C3	10°32'030"N	076°02'761"E	
	C4	10°31'995"N	076°03'192"E	
Munambam	M1	10°11'049"N	076°10'084"E	Fishing harbour, densely populated area, agricultural region, small scale industries
	M2	10°11'206"N	076°10'771"E	
	M3	10°11'113"N	076°10'845"E	
	M4	10°11'180"N	076°11'334"E	
Vypin	V1	09°58'701"N	076°14'608"E	Major port, fishing harbours, densely populated zone, busy waterways, tourist destination, major industrial centre.
	V2	09°58'668"N	076°15'426"E	
	V3	09°58'575"N	076°14'871"E	
	V4	09°58'489"N	076°14'997"E	

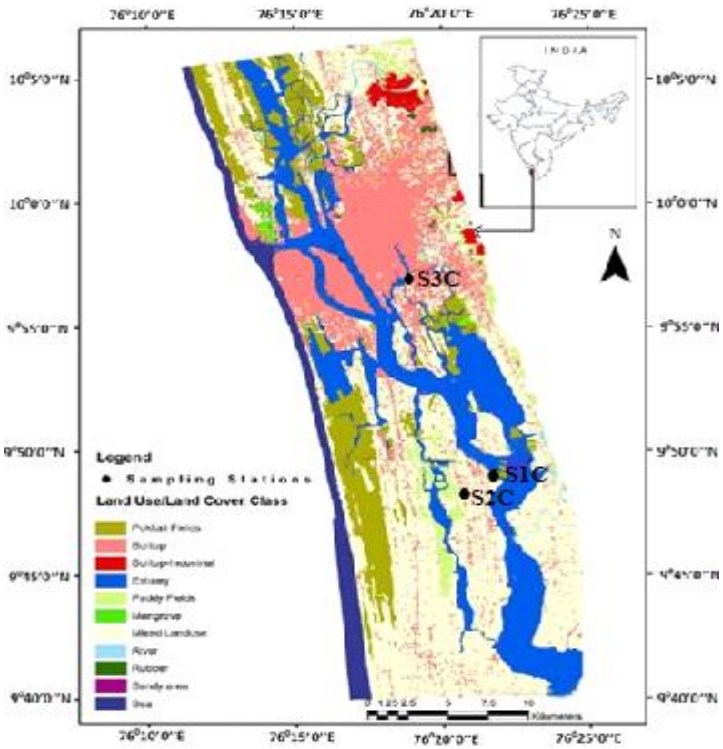

Fig. 2.5: Sampling location from Vembanad Lake

The evaluation of heavy metal and OCI content in organisms inhabiting in the polluted aquatic environment is a prerequisite in environmental toxicological investigations. In connection with toxicological aspects, the samples of organisms were also collected from Vembanad Lake. The present study made an attempt to compare the concentration of heavy metals and OCI in *Villorita cyprinoides* of the study area with the same sample collected from Vemband Lake (Fig. 2.5), in order to assess the contamination status. Samples of *Villorita cyprinoides* were collected from Vembanad Lake viz., Chambakara Canal (S1C) (9°57'64"N 76°19'53"E) is located at the confluence of the Chithrapuzha River, Perumbalam (S2C) (9°49' 43"N& 76°21' 41"E) is located within the central part of Vembanad Lake and Anchuthuruthu (S3C) (9°49'30"N &76°22'15"E) is located at the confluence point of the Muvattupuzha River (Fig. 2.5).

2.2 Sampling

Samples of water, sediments and bivalves were collected from these three prominent fishing zones of Central Kerala and the sampling was conducted on March 2014.

2.2.1 Collection of water samples

Surface water samples were collected using a clean bucket and stored in previously acid washed plastic bottles and in glass bottles of 1 L, which were rinsed with the sample at the collection site. The samples were acidified to pH 2.5 with hydrochloric acid (HCl, 6 M) to inhibit the biological activity, filtered through 0.45µm glass fibre filters (Whatman

GF/F) to remove sand and debris and stored in the dark at temperatures between 0°C and 4°C prior to extraction.

2.2.2 Collection of sediment samples

Surface sediment samples were collected with clean polythene scoop. The sediment samples were subsampled in polyethene bags. All visible marine organisms, coarse shell fragments, and seagrass leaves and roots, when present were removed manually with the help of stainless-steel forceps.

2.2.3 Collection of bivalve samples

The bivalves were collected with the help of a diver at respective sampling sites. All samples were kept in ice chest boxes and transported to the laboratory. Shells were forced open with a knife, washed with a minimum quantity of distilled water and blotted dry. The flesh was removed from the shell and dried in an air oven at 80°C. The dried samples were crushed using a mortar and pestle and were shaken vigorously to produce homogeneity (Yap et al., 2002). The wet samples were frozen at −20°C and stored until analysis.

2.2.3.1 Bivalve under investigation

In the present investigation, Oyster (*Crassostrea madrasensis*) and Clam (*Meretrix casta* and *Villorita cyprinoides*) were plentifully available in the study area was selected as experimental monitoring organisms (Table 2.2 and Fig.2.6) for evaluating the impact of anthropocene.

Crassostrea madrasensis

Meretrix casta

Villorita cyprinoides

Fig. 2.6. Species collected and its sampling for the study

In the research work, species collected include the bivalves, an edible oyster *C. madrasensis* from all the three zones, the clam species namely, *M. casta* from M1, V1 and C1, and *V. cyprinoides* from Chettuva zone.

Table 2.2: General characteristics of bivalves and their habitat

Scientific Name	Common name	Taxonomic hierarchy	Habitat in the study area	Distribution and habitat in India	Uses
Meretrix casta	Backwater clam	Kingdom: Animalia Phylum: Mollusca Class: Bivalvia Order: Veneroida Family: Veneridae Genus: *Meretrix* Species: *casta*	Mud-flats or sandy bottom and are picked by hand	Estuaries and backwaters of east and west coasts, mudflats or sandy bottom, midlittoral	Food for humans, lime production and poultry feed
Villorita cyprinoides	Black clam	Kingdom: Animalia Phylum: Mollusca Class: Bivalvia Order: Veneroida Family: Corbiculidae Genus: *Villorita* Species: *cyprinoides*	It is collected from shallow water, through direct searching using hands or feet	West coast backwaters and estuaries	Food for humans, lime production and poultry feed
Crassostrea madrasensis	Oyster	Kingdom: Animalia Phylum: Mollusca Class: Bivalvia Order: Ostreoida Family: Ostreidae Genus: *Crassostrea* Species: *madrasensis*	It forms beds on the mud-flats mixed with sand and shell fragments and also attach to the inter-tidal rocks	East and west coast estuaries, and backwaters	Food for humans, lime production fertilizer and poultry feed

2.3 Adopted Analytical Methodology

2.3.1 General Hydrography

General hydrographical parameters were analysed and estimated using standard methods. pH of the surface water samples was measured in situ using portable pH meter (Eutech, pH Tester 10) along with temperature using a sensitive thermometer. The salinity of water samples was estimated by Mohr-Knudsen method (Muller, 1979). Modified Winkler method was used for the estimation of dissolved

oxygen (Hansen, 1999). The analyses of nutrients (nitrite, nitrate, ammonia, silicate and phosphate) were performed in the laboratory adopting standard procedure (Grasshoff et al., 1999) within the prescribed time limits and estimated spectrophotometrically (Analytik Jena Specord 200 plus). Phytopigment analyses were carried out according to Lorenzen and Jeffrey (1980). Pigments were extracted with 90% acetone. In the extracts, chlorophyll-*a* (Chl-*a*), chlorophyll-*b* (Chl-*b*) and chlorophyll-*c* (Chl-*c*) were determined spectrophotometrically using standard equations. Their degradation products, the phaeopigments (Phaeo) were measured after acidification with 0.1N HCl (Plante-Cuny, 1974).

Samples for micro phytoplankton were collected by filtering 2 liters of surface water through 20µm sieve (Tait, 1998). Samples were concentrated to 200 mL for further studies and samples were preserved using 4% formalin (Utermohl, 1958). For counting the requisite volume, the sample was transferred into a Sedgewick- Rafter counting cell. The enumeration and identification of plankton were done using a binocular microscope-Magnus (MS13/MS24). Repeated the counting thrice and the average was accounted. The total number of planktonic algal species present in the water sample was calculated using the formula,

$$N = m \times v/V$$

N = total number of phytoplankton cell per litre of water filtered;
m = average number of phytoplankton cells in 1 mL of plankton sample;
v = volume of plankton concentrates (mL);
V = volume of total water filtered (L).

2.3.2 General Sedimentary Parameters

The sediment textural characteristics (sand, silt, and clay) were determined by pipette analysis, based on Stoke's law (Krumbein and Pettijohn, 1938) after removing the inorganic carbonates using 10% HCl and organic matter using 30% H_2O_2 (Folk, 1974). Sediment samples dispersed in sodium hexametaphosphate were wet sieved using a 63µm sieve to determine the sand fraction. The remaining silt and clay fractions were estimated by the gravimetric separation of dispersed sediments.

Sediment samples were freeze-dried and finely powdered using agate mortar and pestle for further analyses. Total carbon (TC), total nitrogen (TN) and total sulphur (TS) were determined with a Vario EL III CHNS Analyzer. Total organic carbon (TOC) was evaluated after treating the sediments with 1M HCl to remove carbonates and analyzed using Elementar Vario Select TOC analyser. Samples were run with blank cups in order to correct the carbon and nitrogen associated with tin/silver cups. Standard sediment supplied by Elementar Vario Select (Germany) was used for calibration in the TOC analyser.

2.3.3 Proximate Composition of Bivalves

2.3.3.1 Estimation of Biochemical Constituents

Total carbohydrates (CHO) were quantified colorimetrically by phenol-sulphuric acid method (Dubois et al., 1956). The concentration was measured at 480 nm and expressed as glucose equivalents. Total protein (PRT) analysis was carried out following the bivalve sample extraction with NaOH and expressed as bovine serum albumin (BSA) equivalents (Lowry et al., 1951). Total lipids (LPD) from bivalves were extracted by direct

elution with chloroform and methanol (Barnes and Blackstock, 1973). Absorbance was measured at 440 nm and determinations were done by spectrophotometric techniques (Analytik Jena Specord 200 plus).

2.3.3.2 Estimation of Water Content

Water content (moisture content) of the bivalve mollusc were determined by calculating the difference between the wet tissue weight and its weight after drying at 60ºC and the results were expressed in percentage. The formula used for the calculation is as follows:

$$\text{Water (moisture) content (\%)} = \frac{Wet\ weight\ of\ flesh - Dry\ weight\ of\ flesh}{Wet\ weight\ of\ flesh} x100$$

2.3.3.3 Estimation of Ash Content

Ash content was found out by heating the sample for12 h in a silica crucible at 525ºC (AOAC, 2000) and subsequently cooled in a desiccator and weighed. Ash content values were expressed in percentage on dry weight basis (Appukuttan and Aravindan, 1995).

$$\text{Total ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} x100$$

2.3.3.4 Condition Index

The gravimetric formula was used for measuring condition indices (CI) values of bivalves. Condition Index of C. *madrasensis*, M. *casta* and V. *cyprinoides* were calculated according to the method proposed by Walne and Mann (1975), outlined in Quayle and Newkirk (1989), and represented as follows:

$$\text{Condition index, CI} = \frac{Weight\ of\ dry\ meat(g)}{Weight\ of\ dry\ shell(g)} x1000$$

2.3.4 Heavy Metals

2.3.4.1 Total metals in the sediment

For metal analysis, the sediments were dried at 60°C in an oven and disaggregated in an agate mortar. The sediment was digested with a solution of di-acid mixture 1:5 $HClO_4$:HNO_3 nearly to dryness in a microwave digester (Anton Paar Multiwave 3000). The residue was diluted to 25 mL (Loring and Rantala, 1992). Blank acid mixtures were digested in the same manner. Heavy metal concentrations (Cd, Pb, Cu, Zn, Mn, Co, Ni, and Fe) were measured using an Atomic Absorption Spectrophotometer (AAS) at specific wavelengths. Appropriate hollow cathode lamps for each metal were used as light sources (Perkin Elmer 3110).

2.3.4.2 Fractionation of heavy metals in sediments

International Union of Pure and Applied Chemistry (IUPAC) technical report suggests that sequential chemical extraction method as the least difficult and most convenient technique obtainable for speciation assessment (Martin et al., 1987). The sequential extraction procedures were based on the principle that heavy metals are in different chemical fractions and the metals bound in the solid phases is converted into soluble forms using appropriate reagents (Tokalioglu et al., 2000; Morera et al., 2001; Veeresh et al., 2003). A large number of sequential extraction method have been reported and the five step Tessier et al. (1979) scheme are most widely accepted (Table 2.3 and Fig. 2.7). The major mechanism of accumulation of heavy metals in sediments leads to the existence of five categories including exchangeable, bound to carbonates (acid-soluble), bound to Fe-Mn oxides (reducible), bound to organic matter (oxidisable) and residual fraction (silicate). These

categories have different behaviour with respect to remobilization under changing environmental conditions. Sequential extraction scheme also provides information on the potential bioavailability and mobility of sediment-bound metal (Stone and Marsalek, 1996).

Table 2.3: Five-step fractionation procedure.

Fraction	Extraction procedure
(F1) Exchangeable (EXC)	8ml of 1M $MgCl_2$(pH=7) was added to 1g sample at room temperature for 1h with continuous agitation
(F2) Bound to Carbonates (CA)	8 mL of 1M NaOAc was added to residue from step 1 and adjusted to pH 5.0 with HOAc and continuous agitation for 5 h.
(F3) Bound to Fe-Mn oxides (FMO)	Residue from step 2 was extracted with 20 mL 0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc at 96 ± 3°C with occasional agitation for 5 h.
(F4) Bound to Organic Matter (OM)	3 mL of 0.02 M HN03 and 5 mL of 30% H_2O_2 adjusted to pH 2 with HNO_3 were added to residue from step 3 and the mixture was heated to 85 ± 3°C for 2 h with occasional agitation. Then 3 mL of 30% H_20_2 (pH 2 with HNO_3) was added to the mixture and the sample was heated again to 85 ± 3°C for 3 h. After cooling, 5 mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added and the sample was diluted to 20 mL and shaken for 30 min.
(F5) Residual (RES)	Residue from step 4 was digested with a 1:5 mixture of $HClO_4$- HNO_3.

The selective extractions were conducted in centrifuge tubes (polypropylene, 25 mL) to minimize the loss of solid materials. Between each successive extraction, separation was affected by centrifuging at 5000 rpm for an hour. The supernatant was removed with a pipette and

analysed for heavy metals, whereas the residue is then washed with 8mL of DDW, centrifuged for an hour and supernatant was discarded.

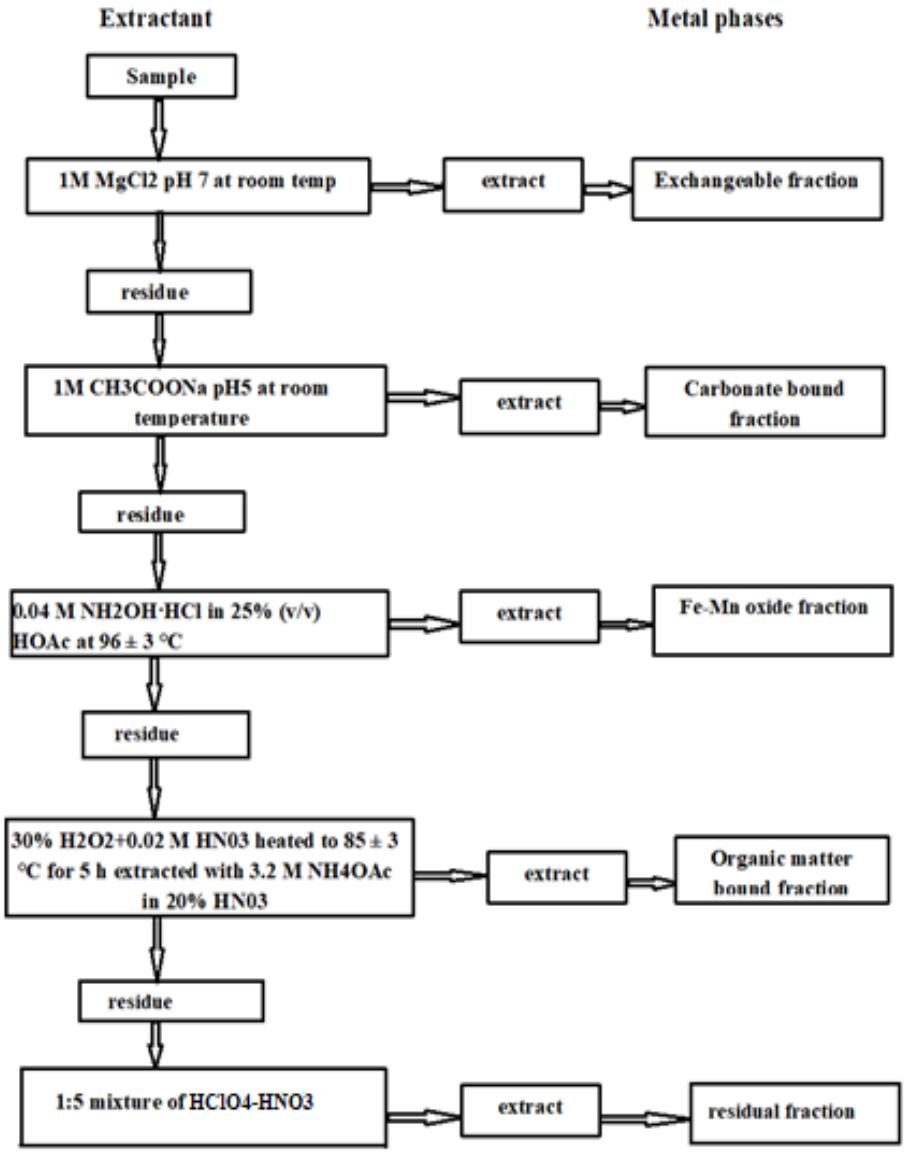

Fig 2.7: Flow chart of the sequential extraction of heavy metals

2.3.4.3 Total metals in the bivalve tissues

Approximately 2 g of dried samples were digested with di-acid mixture 1:5 $HClO_4$:HNO_3. The samples were heated in a hot-block digester first at low temperature (40 °C) for 1 h and then fully digested at 140°C for at least 3 h. After digestion, the samples were evaporated and the residue was washed with milli-Q water and filtered through Whatman 40 filter paper (Yap et al., 2002, 2003). The sample volume was adjusted to 25 mL, and the samples were then analysed for heavy metals by Atomic Absorption Spectrophotometry (Perkin Elmer 3110).

2.3.4.4 QA/QC

Quality assurance and quality control were implemented by sample duplicates. All glassware and plastic were pre-cleaned by soaking in 10% HNO_3 (v/v) for overnight. The quality of analytical data in sediments was assessed by using certified reference material BCSS-1 from National Research Council of Canada. Triplicate analysis of BCSS-1 showed good accuracy and the recovery rate (Table 2.4). Blanks were also used for background correction and random error calculation.

Table 2.4: Analysis of standard reference material (BCSS-1) for heavy metals (mg/kg except Fe in %).

Metal	Co	Cu	Mn	Ni	Pb	Zn	Cd	Fe
Certified values	11.4	18.5	229	55.3	22.7	119	0.25	4.7
Measured values (n=3)	10.21	18.2	213	50.4	21.6	120	0.23	4.5

The sequential extraction of different fractions was cross checked by comparing the total metal concentration with the sum of the five

phases of the sequential extraction in sediments. This comparison reveals a very good agreement between total metal and sum of the fractions. Besides, the accuracy and precision of metal analysis in bivalves were checked with TORT-2, Lobster Hepatopancreas, National Research Council, Canada (Table 2.5).

Table 2.5: Analysis of Lobster Hepatopancreas (TORT-2) for heavy metals (mg/kg)

Metal	Fe	Co	Ni	Cu	Zn	Cd	Pb	Mn
Certified values	105	0.51	2.5	106	180	26.7	0.35	15.6
Measured values (n=3)	106	0.49	2.53	103	179.8	26.4	0.33	15.3

2.3.5 Organochlorine Insecticide

2.3.5.1 Extraction of OCIs in water samples

Organochlorine insecticides in the water samples were determined by using the USA EPA 608 method (EPA, 1984). One litre of previously acidified water sample was used for liquid extraction with 50 mL methylene chloride. Then the sample was transferred to a separating funnel (US EPA Method 3510) and the two layers were separated after 10–15 min. The organic layer was transferred to a 250 mL round bottom flask. After the separation of the organic layer, the water layer was placed back into the separating funnel and the extraction was repeated thrice with fresh portions of 30 mL of methylene chloride. The three methylene chloride extracts were combined and the whole extract was concentrated in a rotary evaporator (Heidolph) and exchanged to hexane during concentration to a volume of 5 mL.

2.3.5.2 Extraction of OCIs in sediment samples

Determination of Organochlorine insecticides in the sediments was conducted using the modified EPA 8081A method (EPA, 1996). The accurately weighed sediment samples were extracted twice with 50 mL of a 1:1 mixture of hexane and acetone (V/V) and 20 mL of methanol mixture. Activated copper granules were added to each collection flask in order to remove potential elemental sulphur (Sarkar et al., 1997; Lino et al., 2007).

The extract was purified using a Florisil column (Florisil for column chromatography 0.150–0.250 mm) in accordance with the EPA 3620B method after the activation of Florisil at 130°C for 12 h. After loading the extract, three fractions were collected, eluted with suitable solvents, to leave interfering compounds on the column:

- The first fraction eluted with 100 mL of hexane containing mainly p,p'-DDE, p,p'-DDT, heptachlor, heptachlor epoxide and aldrin.
- The second fraction eluted with 100 mL mixture of hexane/ dichloromethane (75/25, V/V) containing all OCIs including other DDT compounds (o,p'-DDT, p,p'-DDD, and p,p'-DDT), and HCH isomers.
- The third fraction eluted with 100 mL mixture of acetone/hexane (10/90, V/V) containing endrin and dieldrin.

The three fractions were combined and concentrated in a vacuum rotary evaporator with a gentle stream of nitrogen (Aceves et al., 1988) and reconstructed with 5 mL hexane.

2.3.5.3 Extraction of OCIs in bivalve samples

The homogenised wet bivalve tissues were quantitatively transferred to a stoppered conical flask and extracted with 50 mL of hexane–acetone (1:1, V/V) and 20 mL methanol. Repeated thrice and the total extract obtained was concentrated to 10 mL in a rotary evaporator. The 1mL aliquot extract was used to gravimetrically determine the lipid content. For determining the percentage of lipid content, the solvent was evaporated under a nitrogen stream and the residue was dried to a constant temperature (80°C) and then weighed (Wells and Hess, 2000).

An alkaline saponification step prior to clean up, for remaining extract using the mixture of KOH containing methanol. It was done to release OCI bound to matrix component or to remove some lipid components. For saponification, the extract was transferred to a round bottom flask and 100 mL aqueous 6% methanolic KOH was added (Horwitz et al., 1970). The mixture was refluxed at 75°C for 6 hours. Saponified material was transferred to a separating funnel and 100 mL of n-hexane was added and the funnel was shaken for 3 minutes. Layers were allowed to separate and the organic layer was collected in a beaker. The extraction of the aqueous layer was repeated with 50 mL n-hexane and the layers were allowed to separate and the aqueous layer discarded. The organic layer was collected and treated with activated copper granules (Conc.HCl treated and solvent-rinsed) to remove sulphur and carefully evaporated in a rotary evaporator to about 5mL. The reconstructed extract in 5mL of n-hexane was then purified with an activated Florisil column (Florisil clean up, EPA3620B method) topped

with anhydrous sodium sulphate, producing the three fractions as described above. All the fractions were pooled and further concentrated before analysis (UNEP/IOC/IAEA, 1988) and reconstructed with 5 mL hexane.

2.3.5.4 Gas chromatography with an electron capture detector

Separation and analysis of the OCIs were performed on a gas chromatograph (GC) (model 7890A, Agilent, Waldbronn, Germany) with a ^{63}Ni ECD and equipped with a capillary column (HP-35, 30m × 0.320mm ×0.5 mm) using nitrogen as carrier gas (1.5 mLmin^{-1}). The GC was calibrated with a standard solution of a pesticide mixture (Supelco, USA) prepared in HPLC grade n-hexane. Solvent blanks were used to confirm the absence of any pesticide residues. Analytical reproducibility was checked by replicate measurements. 1 µL of aliquot samples were injected onto the column. The following GC conditions are maintained: injection port temperature 250°C, detector temperature 350°C, oven temperature program: 110° C (5 min) at 5°C min^{-1} to 190°C (2 min) at 15°C min^{-1} to 280°C (10 min).

2.3.5.5 Analytical Quality Controls

Identification and quantification of OCIs were accomplished by using standard reference solution supplied by Supelco (USA). For every set of 10 samples, blank and standard (10 ng of pesticide mix standard) consisting of all reagents were run to check interference and cross contamination. Recovery studies of OCIs in water were performed by adding a known amount of standard mixture of pesticides in Millipore

water. The average percent-recovery of OCIs were in the range of 69.81 to 97.24%. Also, the quality of the analytical data of OCIs in sediment was assured using the CRM 804-050 (Sigma-Aldrich). The recoveries of OCIs ranged from 85% to 116%. Moreover, the recoveries of the analysed concentrations of OCIs in CRM samples were from 78% to 109.8% (Table 2.6).

Table 2.6: The recoveries and the analysed results of CRM standards (840-050) of OCIs in sediments

Insecticides	Recovery (%)	CRM standard (mg/kg)	
		Certified value	Analysed value
α-HCH	85	-*	
β-HCH	87	-	
γ-HCH	88	491.6	470.6
Heptachlor	92	-	
Heptachlor epoxide(B)	90	-	
Aldrin	116	18.04	19.1
Dieldrin	98	1862.5	1693.9
Endrin	103	62.2	68.3
p,p′-DDE	89	1519.6	1269.6
p,p′-DDD	103	1530.6	1193.9
p,p′-DDT	96	1060.1	1067.7
o,p′-DDD	92	-	-
o,p′-DDT	90	-	-

*Not available

The bivalve samples were spiked with standard pesticide mixture and kept for 45 minutes to make proper interactions of pesticides with

shellfish matrix. Along with this one control (without pesticide) and one reagent blank (without fish and pesticide) were maintained. However, the recovery percentages of 95.4, 92.4, 103.0, 94.7, 97.8, 95.8, 104.5, 96.9, 92.4, 92.9, 71.3, 92.4, 80.8, were evaluated for α-HCH, β-HCH, γ-HCH, p,p'-DDD, p,p'-DDE, p,p'-DDT, o,p'-DDD, o,p'-DDT heptachlor, heptachlor epoxide, aldrin, dieldrin and endrin.

2.4 Geo Chemical Indices

2.4.1 Enrichment Factor (EF)

Enrichment factor (EF) is an important tool to assess metal enrichment and here, it is utilized for determining the anthropogenic and detrital metal evolution in the sediments and to predict the pollution status of the environment. The following equation is used to estimate the EF of metals from each sediment samples using Fe as a normalizer.

$$EF = (Ms/Ns)/(Mb/Nb)$$

where Ms and Mb represent the metal concentration in the studied sample and the background respectively, and Ns and Nb are the metal concentration used for normalization in the studied sample and the background respectively. The classification used to denote the degree of enrichment factor is given by Chen et al. (2007). If EF<1 no enrichment, 1-3 minor, 3-5 moderate, 5-10 moderately severe, 10-25 severe, 25-50 very severe, >50 is extremely severe enrichment.

2.4.2 Geo-accumulation index (I_{geo})

Igeo, introduced by Muller (1969) to quantify and assess the extent of heavy metal contamination in sediment. It consist of seven classes: <0 = unpolluted (UP), 0–1 = unpolluted to moderately polluted (UP–MP), 1–2 = MP, 2–3 = MP to strongly polluted (MP–SP), 3–4 = SP, 4–5 = SP to very strongly polluted (SP–VSP), and > 5 = VSP. The I_{geo} is calculated with the equation

$$I_{geo}=\log_2[Cn/(1.5Bn)]$$

where Cn is the concentration of metal analysed, Bn is the background level of the metal and 1.5 is the background matrix correction factor due to lithogenic effects.

2.4.3 Contamination Factor (CF)

The levels of contamination expressed by the CF (Pekey et al., 2004)

$$CF = \frac{Ms}{Mb}$$

where Ms and Mb represent metal concentration in the sediment analysed and background concentration of the respective metal. The CF was classified into four groups, CF < 1 refers to low contamination, 1 ≥ CF ≥ 3 means moderate contamination, 3 ≥ CF ≥ 6 indicates considerable contamination and CF > 6 indicates very high contamination.

2.4.4 Pollution Load Index (PLI)

The assessment of contamination extent of metals in the sediments (Tomlinson et al., 1980) is further calculated using the pollution load

index (PLI). It provides a summative indication of the overall level of heavy metal pollution in a particular sample. PLI is calculated using the equation,

$$PLI=\sqrt[n]{CF_1 X CF_2 X CF_3 \ldots \ldots CF_n}$$

In the above equation, n is the number of metals for which the contamination factor (CF) is calculated as described above. The PLI provides simple but comparative means for assessing a site quality, where a value of PLI < 1 denotes unpolluted area; PLI = 1 presents baseline level of pollutants and PLI > 1 would indicate deterioration of site quality (Tomilinson et al., 1980).

2.5 Species diversity; richness and eveness index

Species diversity of the phytoplankton community was analysed by calculating different diversity indices. Species diversity index (H'), species richness (d), eveness index (j') were calculated using the formula of Shannon and Weaver (1949), Gleason (1922) and Pielou (1966).

2.5.1 Shannon and Weavers Diversity Index (H')

$$H'=[-\sum_{i=1}^{s} Pi \log_2 Pi]$$

Where H' = species diversity in the bits of information per individual (Shannon diversity index)

Pi = Proportion of the sample belongs to the species (fraction of the entire population made up of species i).

S = number of species encountered

$\sum$ = sum from species 1 to species s.

2.5.2 Species richness index (d)

It is calculated as described by Gleason (1922)

$$d=\left[\frac{S-1}{\log_0 N}\right]$$

Where S = the number of species of particular sample and

N = the total number of individuals of all the s species in the sample.

2.5.3 Eveness index(j') (equitability)

It is calculated by the formula of Pielou (1966)

$$J'=\left[\frac{H'}{\log_2 S}\right]$$

H' = Shannon weaver species diversity

S = number of species

2.6 Data treatment and Statistical analysis

Spatial variations were assessed by One-way analysis of variance (ANOVA). The $\log_{10}(x+1)$ transformation was used to reduce the biasing effect of extremely low or high element concentrations (Chapman, 1996). Censored data (below the detection limit) were substituted with zero values prior to all statistical analyses. Correlation analysis and factor analysis were done using Statistical Package for Social Sciences (SPSS), version-13'. Pearson correlation analysis was used to investigate the degree of relationship within the analysed parameters for a better interpretation. Principal Component Analysis (PCA) was used to reduce

the number of variables into fewer linear latent factors of similar characteristics on the basis of correlations or covariances between them (Micó et al., 2006) and factor loadings were considered significant if the components were greater than 0.40. Hierarchical cluster analysis (HCA) creates subclusters for finding easier inter-relationship than individual datum (Rencher, 2001) and results were presented in a two-dimensional plot called Dendrogram. Canonical correspondence analysis (CCA) is a multivariate method to elucidate the relationships between biological assemblages of species and their environment (Ter Braak, 1987), running using XLSTAT statistical software.

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Chapter 3

QUALITY ASPECTS OF THE FISHING ZONES OF CENTRAL KERALA

Contents

3.1 Water Quality Aspects and General Sedimentary Characteristics

3.1.1 Introduction

Quality assessment of the marine environment generally involves measuring and monitoring of several types of aquatic ecosystem health indicators. These include the evaluation of physical, chemical and biological indicators of water. Hence, the evaluation of different indicators provides information about the overall health of the aquatic environment. Water quality is a very sensitive issue for the sustenance of life. Both natural processes such as precipitation inputs, erosion, weathering of crustal materials as well as the anthropogenic sources like urban, industrial and agricultural activities are the deciding factor for fixing the water quality (Chakrapani and Subramanian, 1993; Kumarasamy et al.,

2014). The pollution caused by the various discharged effluents into the water body drastically affect the health of the whole aquatic system (Pai and Reddy, 1981; Edokpayi et al., 2017). The effluents from fertilizer plants contain large quantities of nutrient elements like nitrogen and phosphorus mainly in the form of inorganic salts such as nitrate, nitrite, ammonia, phosphate and related compounds. One of the most harmful effects of human activities on surface waters is nutrient pollution by means of overloading the residues particularly of detergents and it causes several alternations in the water quality (Sujatha et al., 2009). These alternations can lead to ecological consequences such as changes in the phytoplankton composition, decrease in the dissolved oxygen levels and in turn change the ecosystem structural pattern and function as reported decades back (FAO,1992; Alexander et al., 2017).

Phytoplankton abundance and their composition vary with environmental conditional changes, such as temperature, salinity, light and nutrient levels (Muniz et al., 2018). Major interest in phytoplankton investigation was raised to understand the environmental factors that influence their diversity and density (Ye et al., 2017), as phytoplankton occupies the starting point of the food chain. The biota of an aquatic system directly reflects the condition existing in the environment (Pathani and Upadhyay, 2006), since occurrence and abundance of phytoplankton indicate water quality in terms of pollution **(**Venkataraman et al., 1994**)** and thus arises a significant contribution in the exploitation of fishery potential. Therefore, the derived result could be useful added information for future ecological assessment of the marine environment of Kerala.

Furthermore, both inorganic and organic pollutant concentration in sediments varies largely with sediment parameters such as grain-size and organic carbon content. The sources of anthropogenic inputs and the large variations of these contaminants in different phases cannot be explained without the knowledge of hydrographic parameters such as salinity and pH. Thus, assessment of hydrographical and sediment characteristics form an important part of the study.

3.1.2 Results and Discussion

3.1.2.1 Hydrographical parameters

pH: pH is the master variable in water since many properties and reaction are pH dependent. Various scientific organizations have given a permissible pH range in aquatic systems (for example, BIS: 6.5-8.5, WHO: 6.5-8.5, US EPA: 6.5-8.5 and ICMR: 7.0-8.5). Due to the buffering capacity of the sea water, generally, the pH ranges from 7.8 to 8.3 in estuaries (Millero, 1986; George et al., 2012; Vijayakumar et al., 2014). In the case of pollution by acidic and alkaline wastes, the pH serves as an index to denote the extent of pollution. In the present investigation, the pH varied from 7.61 to 8.24. Among the stations, V3 recorded the lowest pH (7.61) whereas the highest (8.24) was observed at station C2. The pH remains alkaline in all the studied stations, which could be attributed to the existence of high saline water.

Temperature: The temperature variation is one of the factors, which regulates various abiotic characteristics and activities of an aquatic ecosystem (Kataria et al., 1995; Prabu et al., 2008) and it influences the

life of organisms and physicochemical parameters (Soundarapandian et al., 2009; Sukumaran et al., 2013; Vajravelu et al., 2018). The temperature was more or less constant in all the three study zones and during the present study, it has fluctuated from 30℃ to 31.5℃ (Table 3.1). Overall, the statistical analysis (ANOVA) in this study showed that there was no significant difference observed in temperature between stations ($p>0.05$).

Salinity: Salinity is one of the most important controlling factors and maintains the abundance and distribution of the organisms in the estuarine environment. Generally, the maximum salinity value was recorded in the Vypin zone with an average value of 32.13 psu and the low values were found at Chettuva zone with an average concentration of 24.87 psu (Table 3.1). A significant fluctuation was recorded in salinity among the stations, maximum salinity of 33.71 psu was recorded at V3 in the Vypin zone and the minimum (23.96 psu) was recorded at C1 from Chettuva zone. A statistically significant difference ($p<0.05$) with respect to salinity was observed between stations.

Dissolved oxygen: DO is the measure of the amount of oxygen freely available in the water. DO is a key factor in determining water quality. In the present study, DO values ranged between 0.50 to 5.76 mg/L, with the highest value at M2 and the lowest value was recorded at V3. Dissolved oxygen concentration fluctuated spatially and varied from 2.45 to 5.76 mg/L (lowest at M3 and highest at M2); 1.12 to 4.80 mg/L (lowest at C4 and highest at C3); and 0.50 to 3.20 mg/L (lowest at V3 and highest at V1) from the Munambam, Chettuva and Vypin zone respectively. No significant difference in the DO value between stations (ANOVA, $p>0.05$)

was recorded. DO concentrations below 5 mg/L may adversely affect the functioning and survival of biological communities and below 2 mg/L may lead to fish mortality (Ramachandra and Solanki, 2007; Pant et al., 2017). Very low DO values were observed at V2 (0.90 mg/L) and at V3 (0.50 mg/L) stations fromVypin zone; C4 (1.12 mg/L) from Chettuva zone and this low DO value is indication of either anthropogenic pollution or natural organic material that enhance the microbial activities, thereafter deplete the oxygen content. On the basis of DO content; Royal Commission (1898) has classified rivers into four groups, viz., (1) very clean (DO value 7 mg/L or more), (2) clean (DO value 6 mg/L) (3) doubtful (DO value 5 mg/L and (4) bad (DO content 4 mg/L). According to this classification, M2 falls under clean; C3 under doubtful and all other stations fall under the bad category.

Table 3.1: Physicochemical properties in the study area(µm/L for nutrients, psu for salinity, mg/L for DO; ℃ for temperature)

Stations	Ammonia	Silicate	Phosphate	Nitrite	Nitrate	pH	Temp	Salinity	DO
M1	59.17	3.92	2.37	0.47	2.41	8.15	31.00	33.54	4.48
M2	57.78	3.95	8.70	0.34	1.58	8.10	30.50	31.61	5.76
M3	58.06	2.59	5.30	0.32	3.01	8.14	31.00	31.25	2.45
M4	59.72	2.84	2.87	0.24	0.98	8.20	31.00	30.94	4.48
C1	39.44	6.31	9.68	1.21	1.24	8.10	30.00	23.96	3.52
C2	59.44	3.55	3.63	0.67	0.56	8.24	31.00	25.78	4.48
C3	59.44	7.14	7.73	0.33	0.67	8.20	31.00	24.75	4.80
C4	60.00	2.90	12.49	0.15	0.44	8.05	30.00	25.00	1.12
V1	46.94	1.84	3.86	9.83	2.78	7.65	31.50	30.06	3.20
V2	46.39	12.50	7.94	7.30	3.33	7.84	31.50	32.61	0.90
V3	53.61	0.79	3.56	20.20	2.91	7.61	31.00	33.71	0.50

3.1.2.2 Nutrient dynamics

Nitrate (NO_3^-_N): Nitrate is one of the most important indicators of water pollution which represents the highest oxidized form of nitrogen. In the present study, the nitrate concentration was observed greater at Vypin zone (3.01 µM/L) as compared to Munambam (2.00 µM/L) and Chettuva zones (0.73 µM/L) (Fig. 3.1, Table 3.1) and significant differences in nitrate were observed in spatial scale (ANOVA, $p<0.005$). Unpolluted freshwaters usually contain a very small quantity of nitrate, WHO has given 50 mg/L of nitrate as the permissible limit in water; BIS and ICMR specify this limit only up to 45 mg/L; ICMR specify this limit to 20 mg/L while USEPA has further reduced the permissible limit to 10 mg/L. In the present study, nitrate content varied from 0.44 (C4) to 3.33 µM/L (V2), which fell within the permissible limits.

Nitrite (NO_2^-_N): Among the three forms of nitrogenous nutrients, nitrite is considered to be a very unstable form being an intermediate in the nitrogen cycle. The result shows a significant differences in spatial scale (ANOVA, $p<0.005$). The lowest nitrite concentration was found at Munambam zone (ranging from 0.24 to 0.47 µM/L), while maximum values have prevailed at Vypin zone (ranging from 7.30 to 20.20 µM/L).

Ammonium (NH_4^+_N):. Ammonia is one of the important nutrients often that regulates the productivity in the aquatic environment. The dissolved ammonia concentrations in the surface waters varied from 39.44 to 60.00 µM/L, 46.39 to 53.61 µM/L and 57.78 to 59.72 µM/L in the Chettuva, Vypin and Munambam zone respectively. Among the three, ammonium concentration was higher than that of nitrate and nitrite

concentrations. There was no significant spatial difference in the ammonium concentration as proven by one way ANOVA statistical analysis that $p>0.05$.

Phosphate (PO_4^-_P): Phosphates are essential for the growth of organisms when present in low concentrations. In Munambam zone, it was noted within the range of 2.37 to 8.70 µM/L at M1 and M2 respectively; in the Chettuva zone fluctuated between 3.63 to 12.49 µM/L (lowest at C2 and highest at C4); and in the Vypin zone varied from 3.56 to 7.94 µM/L (lowest at V3 and highest at V2); respectively. Highest average phosphate content (8.38 µM/L) was observed in the Chettuva zone; domestic wastes containing detergents as well as wastes from agro field rich in phosphate-phosphorous fertilizers along with pesticides were released in and around Chettuva niche and it may contribute the phosphate content into the estuarine environment in elevated level.

Silicate (SiO_4^-_Si): Silicate is different from nitrogen and phosphate and in the strictest sense it is not considered as a nutrient but its usefulness for the formation of skeleton of diatoms. The lowest average silicate concentration was recorded at Munambam zone (3.33 µM/L) while the highest silicate concentration (5.04 µM/L) was observed at Vypin zone (Fig. 3.1). The silicate at Munambam varied from 2.59 (M3) to 3.95 µM/L (M2). Whereas at Chettuva zone, minimum (2.90 µM/L) and maximum (7.14 µM/L) concentration were recorded at C4 and C3 stations respectively. Furthermore, at the Vypin zone, silicate concentration ranged between 0.79 to 12.50 µM/L at V3 and V2 respectively. The one

way ANOVA showed non-significant variations between the stations (ANOVA; p>0.05).

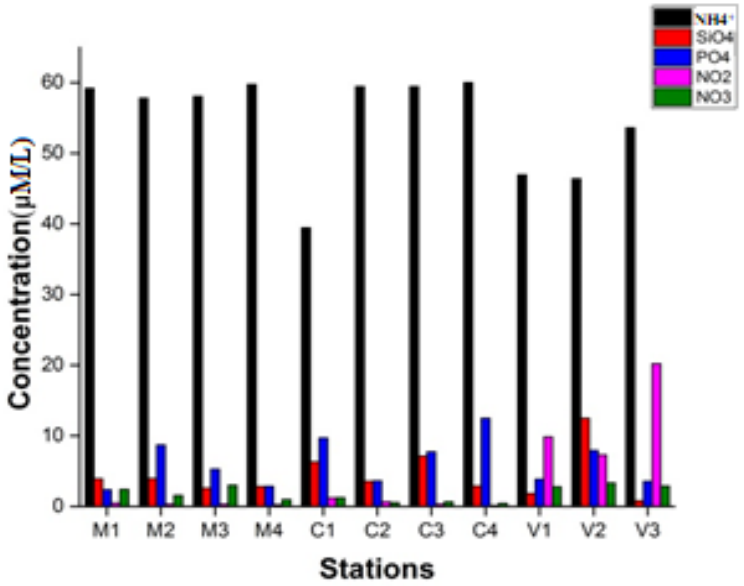

Fig. 3.1: Nutrient profile in the surface water

3.1.2.3 Biological monitoring of water

3.1.2.3.1 Photosynthetic pigments

Chlorophyll is considered as the most reliable index of phytoplankton biomass. The photosynthetic pigment follows the order Pheo> Chl-*a*> Chl-*c*> Chl-*b*. The highest concentration (10.74 µg/L) of Chl-*a* was recorded at C4, whereas the lowest (0.60 µg/L) value was noted at C3 from Chettuva zone and its concentration exhibited considerable spatial variability (Fig. 3.2). The phaeophytin concentration varied between 0.72 (C3) to 14.77 µg/L (V3). A higher concentration of Phaeo compared to Chl-*a* indicated the presence of more detritus matter

in this environment, which could be attributed to the decomposition of organic matter (Tripathy et al., 2005). The predominance of Phaeo might also be ascribed to high turbidity, chemical contamination or other factors affecting the photosynthetic potential of the primary producers (Renjith et al., 2013). The inter-relationship among pigments in the surface water was expressed in terms of ratios, Chl-*b*/Chl-*a* and Phaeo/Chl-*a* and it can be seen that the ratio Chl-*b*/Chl-*a* was considerably lower, ranged from -0.77 to 0.83. While the ratio between Phaeo/Chl-*a* was slightly higher, ranged from 0.57 to 2.68. The higher Phaeo/Chl-*a* ratio suggested the predominance of degraded chlorophyll in the study area and could be associated to the decomposition of sedimentary organic matter and community structure harbouring the surrounding water (Tripathy et al., 2005; Manju et al., 2012).

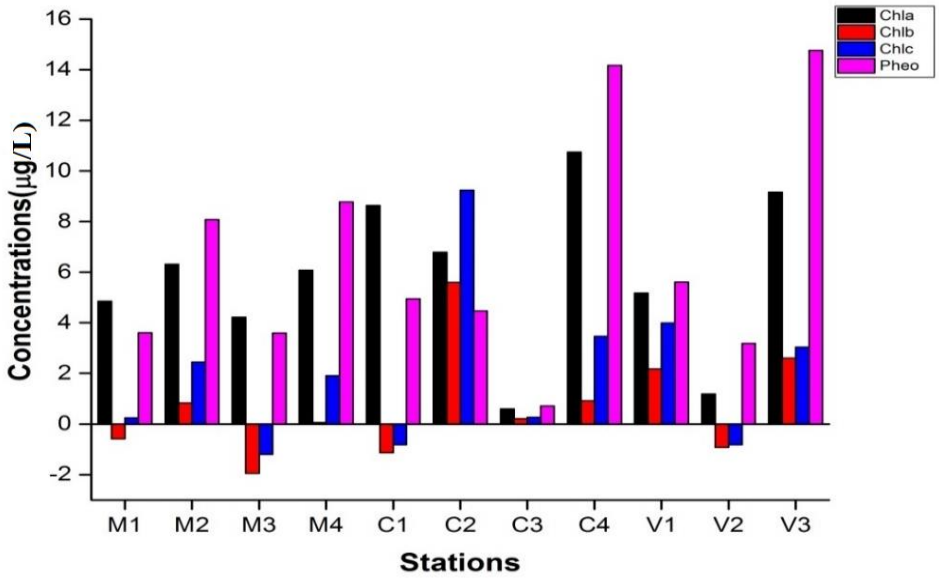

Fig. 3.2: Concentration of phytopigments in the surface water

3.1.2.3.2 Phytoplankton Spectrum

The present work recorded 46 species of phytoplankton comprising diatoms, dinoflagellates cyanophytes, chlorophytes and dictyochophytes. Surface phytoplankton list together with spatial presence (+) or absence (-) indexes encountered in the fishing areas of Central Kerala and are presented in Table 3.2. As far as the average phytoplankton cell density were viewed, dinoflagellates constituted the maximum (426.82 cells/L) with 44.17% to the total, bacillariophyceae found to be next dominant group (421.25 cells/L) with 43.6%. Cyanophyceae (54.55 cells/L), chlorophyceae (45.46 cells/L) and dictyochophyceae (18.18 cells/L) contributed 5.65% , 4.7% and 1.88 % to the total phytoplankton species respectively (Fig. 3.3). The phytoplankton community structures in the study area were found significantly dynamic with respect to spatial level. In general, dinoflagellates were dominated at M1, M2, M4, C1, V1 and V2. But at M3, C2, C3, C4 and V3, diatoms are predominant. These variations in species composition suggest that the local hydrological conditions determine the status of phytoplankton community structure at each site. The coastal waters off Kochi receive a considerable portion of the organic matter loadings from the estuary (Menon et al., 2000; Nair, 2002; Akhil et al., 2013; Renjith et al., 2013; Thomas et al., 2013; Salas et al., 2015). This input could be a reason for the greater dinoflagellate assemblage in the Vypin region.

Table 3.2: Phytoplankton assemblage in the study area

Sl.No	Species	C1	C2	C3	C4	M1	M2	M3	M4	V1	V2	V3
Bacilariophyceae												
1	Acanthus spp	-	+	-	-	-	+	-	-	-	-	+
2	Actinoptychus spp	+	-	-	-	-	-	-	-	-	-	-
3	Asterionella spp	-	+	-	-	-	-	-	+	+	+	-
4	Bascillaria spp	-	+	-	-	-	-	-	-	-	-	-
5	Biddulphia aurita	-	-	-	-	-	-	+	+	-	-	-
6	Biddulphia granulata	+	+	+	-	+	+	+	+	-	-	+
7	Biddulphia mobiliensis	-	-	+	-	-	-	-	-	-	-	-
8	Biddulphia obtusa	-	-	-	-	+	-	+	+	-	-	+
9	Biddulphia pulchella	-	-	-	-	-	-	-	-	-	-	+
10	Biddulphia rhombus	-	-	-	-	-	-	-	+	-	-	-
11	Chaetoceros spp	-	+	-	-	+	-	+	-	+	-	-
12	Coscinodiscus spp	+	+	+	-	-	-	-	+	+	-	+
13	Craticula spp	-	-	-	-	-	-	-	-	-	-	+
14	Cyclotella spp	+	-	+	-	-	-	-	+	+	+	+
15	Cylindrotheca closterium	+	-	+	+	-	-	+	+	+	+	-
16	Diploneis spp	+	+	+	+	-	-	+	+	+	+	+
17	Ditylum brightwellii	-	-	-	-	+	-	-	-	-	-	-
18	Fragilaria spp	+	+	+	+	-	+	-	-	+	-	-
19	Grammatophora spp	+	-	-	-	-	-	-	-	-	-	+
20	Guinardia spp	-	+	+	-	+	-	-	-	-	-	-
21	Gyrosigma spp	-	-	+	-	+	+	+	+	-	+	-
22	Licmophora spp	+	+	-	-	-	-	-	-	-	+	+
23	Navicula spp	-	+	+	-	-	-	-	-	-	+	-
24	Nitzschia spp	-	+	+	-	+	-	+	+	-	+	+
25	Plagiograma spp	-	-	+	+	-	-	+	-	-	-	-
26	Pleurosigma spp	+	+	+	+	+	+	+	+	-	+	+
27	Pseudonitzschia spp	-	-	-	+	+	+	+	-	+	+	-
28	Risozolenia spp	-	+	+	-	+	+	+	-	-	-	+
29	Skeletonema costatum	+	+	-	+	-	-	-	+	+	+	+
30	Surirella spp	+	+	+	-	+	-	+	-	+	+	+
31	Synedra	+	-	-	-	-	-	-	-	-	-	-
32	Thalassionema	-	-	-	-	-	-	-	-	-	+	-
33	Thalassiosira	-	-	-	-	-	-	-	-	+	-	-
Dinophyceae												
1	Ceratium furca	-	+	-	-	+	+	-	+	+	+	+
2	Ceratium fusus	-	+	+	-	+	+	+	+	-	+	+
3	Ceratium platycorne	-	-	-	-	+	-	+	-	-	-	-
4	Dinophysis acuminate	-	-	-	-	-	-	-	+	-	+	-
5	Heterocapsa spp	-	-	-	-	-	-	+	+	+	-	-
6	Prorocentrum gracile	-	-	-	-	-	-	+	+	-	-	-
7	Prorocentrum micans	-	-	-	-	-	-	-	-	+	+	-
8	Protoperidinium spp	+	-	-	-	-	-	-	+	+	+	-
Chlorophyceae												
1	Scenedesmus spp	-	-	-	-	-	-	-	-	+	+	-
2	Staurastrum	-	-	-	-	-	-	-	-	+	-	-
3	Tetrastrum	-	-	-	-	-	-	-	-	+	-	-
Dictychophyceae												
1	Dictyocha fibula	-	-	-	-	+	-	+	-	-	-	-
Cyanophyceae												
1	Nostoc	-	-	-	-	+	-	+	+	+	+	+

In the marine environment, phytoplankton occupies the functional and basic significance in the overall food web (Kalavati et al., 1997) and due to the pollution load, plankton population is affected and leading to drastic changes in the food chain of the marine environment (Verloencar and D'Silva, 1977). Some phytoplankton species are often used as a good indicator for water quality aspects and infer the pollution status. Previous reports revealed that outburst of *Skeletonema costatum* was due to the pollution in Visakhapatnam harbour (Ganapati and Raman, 1979; Periyanayagi et al., 2007). *Skeletonema costatum* is an abundant species in the study area, especially in V1 and V2 (4500, 2400 cells/L respectively). Its abundance is considered as an indicator of eutrophication and pollution (Mihnea, 1985; El Din et al., 2008; Gaballa, 2014) and outweighs as an indicator of environmental stress condition.

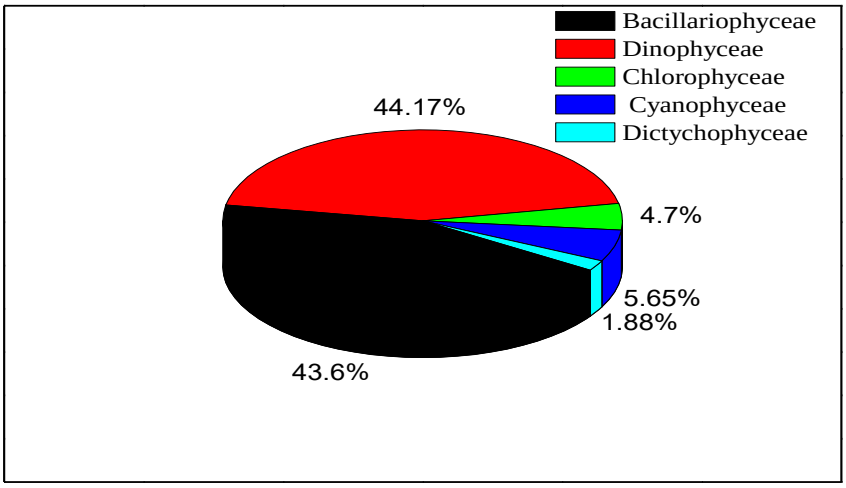

Fig. 3.3: Percentage composition of phytoplankton recorded in Central Kerala

3.1.2.4 Phytoplankton community in relation to water quality

The phytoplankton composition fluctuated according to the ecological changes in the physicochemical parameters. The function of nutrients should not be compartmentalised, it could be considered as a primary triggering factor for the phytoplankton distribution (Dayala et al. 2014). Therefore in this work, the relationship between environmental variables and phytoplankton distribution were analysed using Canonical Correspondence Analysis (CCA) (Lira et al., 2014). CCA was conducted between 10 dominant phytoplankton species and all of the 9 measured physicochemical parameters (Fig. 3.4). The length of the environmental variable arrows represented the relative explanatory note of each variable in relation to individual sample positions within the ordination and shows a positive or negative correlation with the axis. In the CCA plot, axis 1 and 2 explained 69.77% of the variability in the species environment biplot. Temperature, phosphate and nitrite exhibited positive correlations with axis1. At the same time, several phytoplankton species such as *protoperidinium* spp.*, Skeletonema costatum, Cylindrotheca closterium, Chaetoceros* spp.*, Ceratium furca, Diploneis* spp. *and Gyrosigma* spp. were distributed at the right side of axis1. The result pointed out that the increased concentration of above said parameters were responsible for the abundance of these species. In axis 2, *Pleurosigma* spp. was positively correlated with nitrate and salinity. This may be due to the favourable saline conditions and nitrate concentrations which might have positively increased their abundance. Nevertheless, some phytoplankton species such as *Ceratium fusus* and *Biddulphia* spp. are distributed at the left side of axis 2 and were negatively correlated with pH and ammonia which

indicates the decreasing growth and population of phytoplankton with increasing pH and ammonia levels. The overall observations of the present study remarked the strong influence of the above said hydrographical parameters with the phytoplankton composition.

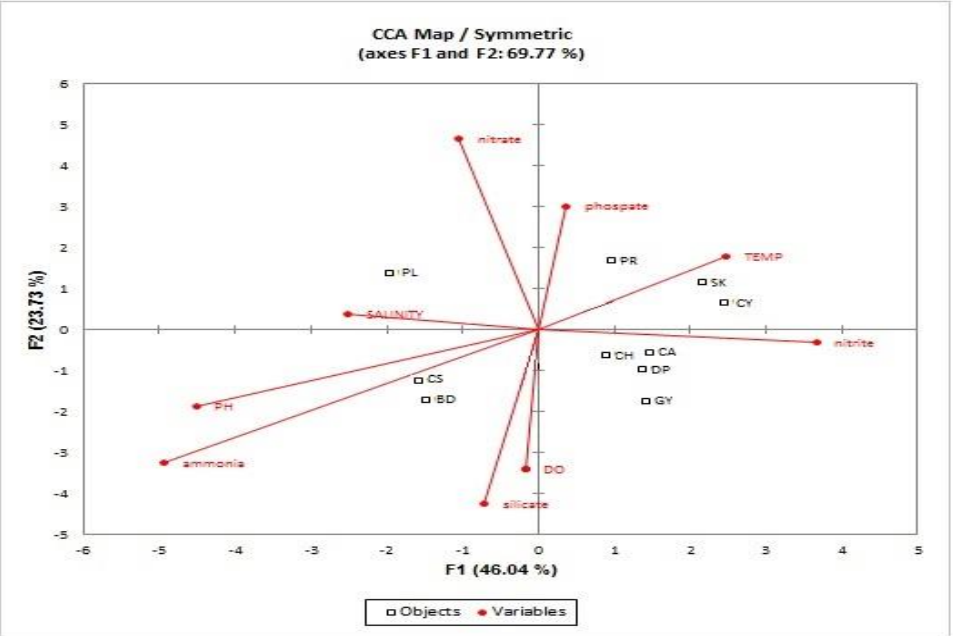

Figure 3.4: CCA biplot showing relationship between the environmental parameters and phytoplankton composition. Environmental variables are depicted by long arrows and species are given in code words. (PL:*Pleurosigma* spp., GY:*Gyrosigma* spp., CH: *Chaetoceros* spp., DP:*Diploneis* spp., CY: *Cylindrotheca closterium*, SK: *Skeletonema costatum*, BD: *Biddulphia* spp., CA: *Ceratium furca*, CS: *Ceratium fusus*, PR: *Protoperidinium* spp.)

3.1.2.5 Species diversity

Three indices were used to estimate the community structure: diversity (H') (Shannon and Weaver, 1949), dominance/richness (d) (Simpson, 1949) and evenness or equitability (J') (Pielou, 1975). Species diversity is a key indicator of water quality as it is closely related to the trophic state of the water body (Borics et al., 2007). In the present study, species diversity index, richness index and eveness index values showed a spatial variation and are illustrated in Table 3.3. Mean diversity index was highest in Vypin zone. At Vypin it ranged between 3.29 and 3.93 (lowest at V1 and highest at V3); at Munambam it fluctuated between 2.26 and 3.44 (lowest at M3 and highest at M1); at Chettuva, it varied from 2.17 to 4.13 (lowest at C4 and highest at C2). The species richness (d) ranged between 0.82 to 2.46 with the higher value at V2 and lower value at C4. Species evenness (J') varied from 0.51 to 0.93; minimum at M3 and maximum at C1 and C2 stations whereas the richness index was altered from 0.82 (C4) to 2.46 (V2). In order to evaluate the environment stress, biological community biodiversity index, Shannon-Weaver species diversity index (H') is commonly used as the indicator. In the aquatic ecosystems, when H' value is more than 3, it implies minor water pollution or the water is pollution-free, when H' value is 1-3, it shows moderate water pollution and H' value between 0-1 meant for serious water pollution (Shen et al., 1990; Bai et al., 2016). From this point of view, the H' values of phytoplankton communities at C4 and M3 was 2.17 and 2.26; indicating moderate pollution, whereas in all other stations falls in the range over 3 (Table 3.3) showing less environmental stress on the macro-benthic molluscan species inhabiting the study area. Meanwhile, it was interesting to note that some stations with

higher pollutant contents (discussed in incoming chapters) had higher H', ie., Vypin zone had an H' value over 3, exhibited to show a contradiction between the higher H' values and the pollutant content.

Table 3.3: Mean values of phytoplankton species diversity in the study zones.

Sampling Zones	Item	Quantity (average)	Range of Variation
Chettuva	Total Species(S)	16.50	
	Abundance/density(N)	5200 cell/L	1500-8000 cell/L
	Diversity index(H')	3.49	2.17-4.13
	Richness index(d)	1.80	0.82-2.34
	Eveness index(J')	0.88	0.77-0.93
Munambam	Total Species(S)	18.50	
	Abundance/density(N)	11875 cell/L	1800-29100 cell/L
	Diversity index(H')	3.08	2.26-3.44
	Richness index(d)	1.93	1.33-2.27
	Eveness index(J')	0.75	0.51-0.92
Vypin	Total Species(S)	23.00	
	Abundance/density(N)	10000 cell/L	7700 -12800 cell/L
	Diversity index(H')	3.56	3.29-3.93
	Richness index(d)	2.40	2.33-2.46
	Eveness index(J')	0.79	0.73-0.89

3.1.2.6 General sedimentary characteristics

3.1.2.6.1 Textural characteristics

In the present study, the maximum mean value for sand was observed (73.68%) at Chettuva zone and minimum at Munambam with a contribution of 67.01% (Table 3.4). Clay content ranged between 17.82% and 26.99% with the minimum and maximum content observed at Vypin and Munambam zone respectively. Textural characteristics showed that

V1, V2 and V4 were more sandy; whereas V3 exhibited clayey nature in the Vypin zone. The sand, silt and clay varied between 9.28 to 95.74%, 0 to 27.08% and 0.66 to 63.64% in the sediments of Vypin zone respectively. While in the Chettuva zone, C1, C2, C3 were shifted to sandy nature and the sediments of C4 displayed clayey in character and the fraction ranged between 12.60 to 91.94%, 4.32 to 21.01% and 1.12 to 66.39% of sand, silt and clay content respectively. In the Munambam zone, M1, M2 and M4 again shifted to the sandy texture while M3 shifted to clayey silt. The sand, silt and clay content of these samples fluctuated between 18.96 to 89.11%, 0 to 11.75% and 6.39 to 69.29% respectively. Sediment texture did not exhibit any significant spatial variation (ANOVA, $P>0.05$) and the granulometric composition disclosed the sandy nature of sediments, except at a few locations (C4, M3 and V3) where clay dominates.

Table 3.4: General sediment properties in the study area

Stations	Sand	Silt	Clay	TC	TOC	TN	TS
V1	94.49	2.81	2.70	0.29	0.23	0.15	ND
V2	95.74	0.00	4.26	1.66	0.18	0.05	0.13
V3	9.28	27.08	63.64	4.77	4.60	2.29	1.87
V4	95.20	4.14	0.66	0.19	0.17	0.89	ND
C1	90.07	4.32	5.61	0.45	0.26	0.96	0.14
C2	91.94	6.94	1.12	0.66	0.54	0.99	0.15
C3	90.37	8.22	1.41	0.25	0.16	0.05	ND
C4	12.60	21.01	66.39	4.34	4.14	2.04	ND
M1	79.80	0.00	20.20	3.68	3.63	1.09	1.24
M2	89.11	4.50	6.39	1.02	0.56	1.05	0.09
M3	18.96	11.75	69.29	5.83	5.15	3.35	2.09
M4	80.16	7.75	12.09	2.64	2.23	1.69	0.38

ND- not detected

3.1.2.6.2 Elemental composition

Organic matter is usually quantified based on its total organic carbon (TOC) content (Stein and Rack, 1992), and have potential significance in aquatic productivity. Estimation of organic carbon could serve as an important tool in determining the status of food availability to benthic fauna and indicates that the bottom is fertile for its nourishment (Manjappa et al., 2003). The value of TOC, TN and TS are shown in Table 3.4. The total carbon present in sediments varied from 0.19 to 4.77% with an average of 1.73% at Vypin zone. At Chettuva zone, it fluctuated from 0.25 to 4.34% and recorded an average content of 1.43%; at Munambam zone, the observed total carbon content found to vary from 1.02 to 5.83% (average: 3.29%). Content of TOC in sediments altered from 0.17 to 4.60% and recorded an average of 1.30% at Vypin zone. At Chettuva zone, TOC varied from 0.16 to 4.14% with average: 1.28% and at Munambam zone, it ranged from 0.56 to 5.15% and the average value was found to be 2.89%. The total nitrogen in sediments ranged from 0.05 to 3.35% (average: 1.22%) whereas TS ranged from ND to 2.09% (average: 0.51%). TS concentrations were not detected for the sediment samples collected from V1 and V4 in the Vypin zone, and C3 and C4 from Chettuva zone. Alagarsamy (1991) reported that the sediment with TOC< 5%, indicated the site as unpolluted. In the present work, TOC values were greater than 5 in sediments of M3 and this data support the polluting nature of these sediments, whereas the other sites are represented as in the unpolluted state. In the present study, the stations C4, V3 and M3 displayed a higher concentration of TOC and the corresponding textural composition was clayey in nature. Furthermore from the Pearson

correlation, TOC showed a positive correlation with silt and clay (r =0.847 and 0.779, p<0.01) and negative correlation with sand (r =-0.818, p<0.01) (Table 3.5). This significant relationship was recognized that the organic carbon in the sediment is controlled by textural attributes and largely associated with finer particles than the coarser (Sarkar et al., 2016).

Table 3.5: Pearson correlation analysis of texture and elemental composition

	Sand	Silt	Clay	TC	TOC	TN	TS
Sand	1						
Silt	-0.788**	1					
Clay	-0.850**	0.854**	1				
TC	-0.808**	0.847**	0.865**	1			
TOC	-0.818**	0.847**	0.779**	0.949**	1		
TN	-0.670*	0.581*	0.553	0.751**	0.837**	1	
TS	-0.611*	0.737**	0.557	0.767**	0.781**	0.664*	1

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

3.2 Proximate Profile of Bivalves

3.2.1 Introduction

Seafood is an important contributor to the diets of many individuals because of their unique nutritional composition. Among shellfishes, bivalves are considered to be a valuable nutritional food source as they constitute a rich amount of essential elements, which are necessary for providing a balanced diet. Rattan (1994) reports that the quality of the biochemical composition of a whole body is an indication

of animal quality and provide information on its nutritional value and health status (Gressler et al., 2010). Generally proximate or percentage of biochemical composition means quantum of five basic constituents namely protein, carbohydrate, lipid, ash and water. For bivalves, condition index (CI) is considered as a standard criterion to select the best product and also serves as a useful biomarker, reflecting the ability of bivalves to withstand natural and anthropogenic stress (Bressan and Marin, 1985; Aníbal et al., 2011; Nagvenkar, 2014). The biochemical constituents and nutritive values of the aquatic organism are required to be analysed from time to time as drastic changes are taking place in the marine environment due to the dumping of pollutants and undergo bioaccumulation which may result in variation of biochemical constituents of the aquatic organism.

3.2.2 Results and Discussion

The human diet requires nutritious source rich in protein as well as other constituents. Any information on the biochemical composition is helpful in assessing the nutritive and edible value of organisms. Proteins are fundamental macro biomolecules in all aspects of cell structure and function, while carbohydrates are major sources of energy for the survival of living beings. The major organic constituent found in the three species, C. *madrasensis*, M. *casta* as well as V. *cyprinoides* was protein. The present study showed that protein content in three bivalves is significantly different from each other ($P<0.05$; $P=0.04$; $df=2$). Protein is the major biochemical constituent of C. *madrasensis* forming 21.28 to 68.47%; M. *casta* varies between 46.83 to 66.82% and V. *cyprinoides* fluctuates

between 33.23 to 48.96% (Table 3.6). The average protein values were observed to be 55.02% in M. *casta*, 41.46% in V. *cyprinoides* and 35.68% in C. *madrasensis*. Lipids serve as an energy reserve in conditions of either imposed or natural nutritional stress and have special importance to the animal body, being primarily a source of energy in the diet. The lipid is the second major biochemical content in C. *madrasensis* and V. *cyprinoides*, varied between 3.11 and 48.51% and 20.00 and 38.82% respectively. While in the M. *casta*, it is the third component and the content was 12.20 to 18.11%. There was a significant difference ($P<0.05$; $P=0.006$; df=2) were found in the lipid content between the species.

The concentration of carbohydrate was less as compared to proteins and lipids and the average carbohydrate content was 21.36%. It is evident from the result that the carbohydrate stands third in percentage of composition of V. *cyprinoides* and C. *madrasensis*. It varied from 2.84 to 36.04% in C. *madrasensis* and in V. *cyprinoides*, it ranged from 19.86 to 27.52%, whereas in M. *casta*, it is the second component and fluctuated between 13.57 to 36.23% (Table 3.6). The inferred results elucidated that was significant variation in the total carbohydrate among species ($P<0.05$; $P=0.01$;df=2). The ash content of any sample is a measure of the likely mineral content (Lee et al., 1993). The total ash content in the M. *casta* and V. *cyprinoides* varied from 0.37 to 0.55% and 0.05 to 0.18% respectively. The ash content was high in the C. *madrasensis* sample with values ranging from 0.99 to 4.26%, recorded significant variation among species ($P<0.05$; P=4.6x10-12; df=2;F=70.44). The high ash content in the C. *madrasensis* was due to increased inorganic content in the body constituents (Mohite, 2010).

For bivalves, condition index (CI) is considered as a standard criterion to select the best product. Besides, it also serves as a useful biomarker reflecting the ability of bivalves to withstand natural and anthropogenic stress (Bressan and Marin, 1985; Nagvenkar and Jagtap., 2013). Highest CI was recorded for V. *cyprinoides* (353.88) collected from the Chettuva zone. It varied from 244.32 to 488.51 with maximum and the minimum value obtained from C2 and C4 stations respectively. Whereas, CI in the C. *madrasensis* ranged from 9.23 to 131.00, and *M. casta* from Munambam showed high CI (400.93) while, low CI of 130.98 was observed in the same species, which were sampled from Vypin zone. Though moisture content is not so important from the nutritional point of view it plays a crucial role in understanding the texture of food material. The evaluation of the moisture content examined hereunder demonstrated that the value ranged between 36.17% for C. *madrasensis* from M2 station to 91.28% for M. *casta* from Vypin zone and this outweighs a good mark. ANOVA suggested a significant ($P<0.05$;$P=0.001$;$df=2$) variation among the bivalves and these variations in the moisture content of molluscs could be due to the effect of environmental characteristics. The findings of Osibona et al. (2006) also supported these inferences.

Table 3.6: Biochemical composition of bivalves (%)

Bivalves	Sample code	CHO	PRT	LPD	Ash content	Moisture content	CI
V. *cyprinoides*	C1C	24.31	33.23	35.12	0.05	79.30	328.28
	C2C	24.73	44.96	38.82	0.12	70.59	488.51
	C3C	19.86	48.96	29.86	0.17	84.53	354.43
	C4C	27.52	38.68	20.00	0.18	83.83	244.32
C. *madrasensis*	C1M	21.24	36.88	3.11	0.99	68.92	131.00
	C2M	29.53	31.04	9.29	1.58	86.07	9.23
	C3M	13.85	25.92	38.11	4.21	80.00	9.56
	C4M	26.92	30.17	48.51	3.35	83.33	25.08
	M1M	18.20	36.37	40.07	2.54	76.35	86.04
	M2M	13.75	21.28	10.37	2.75	36.17	85.71
	M3M	2.84	28.07	17.62	3.25	81.08	10.26
	M4M	15.17	28.22	31.47	1.82	74.26	122.12
	V1M	36.04	60.65	40.39	4.26	81.18	55.41
	V2M	16.81	68.47	14.19	3.95	91.12	33.77
	V3M	24.09	25.37	10.97	3.55	76.45	68.21
M. *casta*	VAC	19.86	46.83	18.11	0.46	91.28	130.98
	CAC	36.23	51.40	14.06	0.37	89.58	223.88
	MAC	13.57	66.82	12.20	0.55	82.49	400.93

3.3 Interrelationship between biochemical composition of bivalves and environmental parameters

The relationships between the biochemical pools and environmental conditions were determined by Principal Component Analysis. The eigenvalues and their contribution to the total variance indicated five components that could adequately explain the observed correlations among the variables (Table 3.7). The first master component termed physicochemical properties dependent accounted for 35.07% of the

environmental variations. This component primarily consisted of nitrate, nitrite, salinity, temperature, phytoplankton abundance (positive loadings), and silicate and phosphate (negative loadings). Temperature recorded a significant correlation with phytoplankton abundance because higher water temperatures promote phytoplankton growth. Nutrient-rich water supports an increase in the phytoplankton biomass, which is the cause for the mineral composition of bivalves. The derived result in PC1 was evident in the correlation of nitrite and nitrate with phytoplankton abundance and ash percentage. The effect of salinity on the biochemical composition was proven in the correlation between salinity and ash percentage. Higher salinity affects the increase of inorganic matter. Component 2 accounted for 20.27% of the environmental variation and was termed phytoplankton dependent. The present data indicated that phytoplankton is a major food source for bivalves. The influence of phytoplankton on the proximate composition of bivalves was evident in the correlation of phytoplankton abundance with ash percentage and protein content. Furthermore, CI was the parameter showing the quality and physiological condition of shellfish, and hence the best time for their consumption. In this study, carbohydrate coincided with Chl-*a*. Although, an observation was noted that a statistical significant correlation between CI and Chl-*a* levels in component 3 with 16.65% of variance. Component 4 accounted for 10.17% with a negative effect of CHO with pH. The loading values of parameters in component 5 are not affecting the proximate composition of bivalves.

Table 3.7: Result of Principal Component Analysis

	Component				
	1	**2**	**3**	**4**	**5**
Eigen value	6.31	3.65	3	1.83	1.26
% of variance	35.07	20.27	16.65	10.17	7
Cumulative % of variance	35.07	55.38	71.99	82.16	89.16
Correlation coefficient					
CHO	-0.400		0.509	-0.539	
PRT		0.706			
ASH	0.622		-0.573		
CI			0.772		
Chla		-0.468	0.622		
SiO4	-0.598				0.556
PO4	-0.524	-0.445			0.615
NO2	0.510	0.469		-0.631	
NO3	0.776	0.435			
pH	-0.599			0.620	
Temp	0.558	0.652			
Salinity	0.887				
Phyto abundance	0.709	0.628			
Sand	-0.550	0.679			
Silt	0.802				
Clay	0.802				
TOC	0.697	-0.553			

Note: Only factor loadings greater than ±0.40 are shown

3.4 Conclusions

Water quality analysis, general sediment characteristics and proximate analysis of bivalves were carried out in the prominent fishing zones of Central Kerala. The hydrographical parameters showed a spatial variation

and the significant difference in most physico-chemical parameters between stations indicated a continuous changing pattern due to the anthropogenic impacts on the study area. This research outcome provides baseline information of the phytoplankton distribution and abundance, and the evidence of pollution as suggested by the presence of pollution indicator species such as *Skeletonema costatum*. Phytoplankton diversity is highly dynamic depending on the nutrient availability which is clearly explained by Canonical Correspondence Analysis. Texture analysis provided an insight into the grain size of the sediments in the study area. Higher concentration for organic carbon reported at C4, V3 and M3 and the relative texture is clayey nature. Data on the biochemical composition of bivalves obtained in this study revealed that it is a quality seafood product that is essential for human consumption and provides a major source of nutritive elements. Comparing the biochemical composition of bivalves, the protein exhibited enriched level compared to lipid and carbohydrate. The mean values of lipid, carbohydrate and condition index for V. *cyprinoides* was significantly higher than those of M. *casta* and C. *madrasensis*. Besides, protein and moisture content were found to be significantly higher for M. *casta*, whereas ash content was higher in C. *madrasensis*. This indicated nutritive superiority of M. *casta* and V. *cyprinoides* over C. *madrasensis.* Principal component analysis pointed out that temperature, phytoplankton abundance, salinity, nitrate, nitrite and Chl-*a* are the main environmental factors influencing the shellfish biochemical composition.

Despite focusing on the nutritive importance of bivalves, there is a high risk of the possibility of contamination in the aquatic system

through various distributional pathways. In this context, little research attempt has been contributed to the toxicological aspects of the marine assemblages. Therefore, the incoming chapter dealt with the bioaccumulation of heavy metals and organochlorine insecticide levels in the edible aquatic organism to ensure the toxicological safety of the food. Furthermore, in the marine environment, sediment provides a habitat for many aquatic organisms and is a major repository for the more persistent chemicals that are introduced into surface waters. Hence, the distribution of heavy metals in sediment can contribute much for the evidence of anthropogenic influence on the aquatic system and helps to control and reduce sediment-bound pollution. Therefore, the analysis of total metal content and its fractions in the surface sediment from prominent fishing zones were carried out to find the distributional pattern of these toxic metals. All the details are described in detail in Chapter 4.

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Chapter 4

DISTRIBUTION PROFILE AND RISK STATUS OF HEAVY METALS IN SEDIMENTS

Contents

4.1 Introduction

Heavy metals are stable and persistent environmental contaminants owing to their non-degrading character. Therefore, they tend to accumulate in the soils, sediments and living organisms. Heavy metals in sediment yield valuable geologic and environmental information and can serve as good indicators of regional pollution status. Natural calamities like flood, earthquake, Tsunami, etc. (Sujatha and Benny, 2010) and various man maid activities have been found to be responsible for the elevation of heavy metal concentrations in the environment (Saleem et al., 2015). Human activity derived metals, different from those that are lithogenic, are highly mobile and bioavailable, hence they are more likely to affect adversely on aquatic species (Tessier and Campbell, 1987). Therefore, it is very important for any monitoring programme to distinguish the source of metals, ie., whether it from natural source or from anthropogenic activities.

Examining the distribution pattern of heavy metals in the sediment leads to a better understanding of their behaviour and is crucial for detecting pollution source to take alarming steps (Hatje et al., 2003; Al-Juboury, 2009). More than 90% of heavy metals load in aquatic systems have been found to be related to suspended particles and sediments (Zheng et al., 2008; Amin et al., 2009; Zahra et al., 2014; Ali et al., 2016) and while comparing only a small portion of free metal ions were dissolved in water (Hou et al., 2013). Therefore, the analysis of heavy metals in the sediments permits detection of contaminants that may be either absent or present in low concentrations in the water column. In the bottom sediments, the transport of heavy metals is affected by mineralogical and chemical composition of suspended material, anthropogenic influences and in-situ processes such as deposition, sorption, enrichment in organism, etc. (Jain et al., 2005; Jain et al., 2008; Shakeri et al., 2014). Several sediment quality indices have been established to evaluate the contamination level of heavy metal in sediments (Sany et al., 2013; Caeiro et al., 2005), such as pollution load index, enrichment factor and geo-accumulation index (Brady et al., 2015; Luo et al., 2014; Manoj and Padhy, 2014; Cheriyan et al., 2015; Sreekanth et al., 2015; Duodu et al., 2016). Thus, evaluating the different sources of heavy metals in sediment helps to control pollution to a certain extent. The common methods for distinguishing the pollution sources include elemental speciation, profile distribution and spatial coverage (Borůvka et al., 2005).

Total metal analysis in the sediment has been used frequently in the initial phase of environmental impact assessment to locate areas of possible concern. But the determination of total metal concentrations does not necessarily reflect bioavailability due to the assumption that all the species of a particular metal have equal impact on the ecosystem (Coetzee, 1993; Thompson et al., 1984). Therefore, the investigations on chemical partitioning of metals by sequential extraction with selective extracting agents give information on the fundamental reactions that govern the behaviour of metals in sediment. Hence, it is recognised that heavy metal speciation studies in sediments are also essential and dominant for estimating the mobility and bioavailability of the metals in the ecosystem (Gleyzes et al., 2002). The speciation of metals in sediments is thus a critical factor in assessing the potential environmental impacts. Because the toxic heavy metals that are released into the coastal environment may significantly affect the ecology, and in extreme cases, may lead to the destruction of the whole ecosystem. Therefore, to assess mobility and toxicity of heavy metals, the proposed index such as Risk Assessment Code (RAC) based on the geochemical fractionation was used as the common tool. This assessment is important due to the fractions introduced by anthropogenic activities are typified by the adsorptive, exchangeable, and bound to carbonate fractions that could equilibrate with the aqueous phase and thus become more rapidly bioavailable (Liu et al., 2008) and make it convenient in evaluating the potential risk associated with the human population.

4.2 Total metal concentration

4.2.1 Results and Discussion

The distribution pattern of heavy metals was in the following sequential order: Fe> Mn> Zn> Pb> Ni> Cu> Co> Cd (Table 4.1 and Fig. 4.1). The present study indicated the concentration range for each metal was: 1.05 to 2.29 mg/kg for Cd, 28.59 to 73.90 mg/kg for Pb, 15.60 to 55.41 mg/kg for Cu, 22.20 to 99.26 mg/kg for Zn, 11.88 to 137.89 mg/kg for Mn, 17.28 to 30.46 mg/kg for Co, 19.67 to 70.23 mg/kg for Ni and 715.31 to 1145.85 mg/kg for Fe. Comparison of spatial variation of heavy metals with respective average shale values (Taylor and McLennan, 1985) indicated that Zn, Mn, and Fe were below this level. Whereas Pb, Cd, Co, Ni and Cu concentrations were exceeded the respective shale average, representing the influence of land-based inputs and may be an indication of anthropogenic sources of these elements. Besides, Zn concentration at Vypin zone exceeded the average shale value and this is due to the Zn manufacturing units which can act as point sources of contamination (Shibu et al., 1995; Deepulal et al., 2012). In the Munambam zone, station M1, situated at the adjacent areas of the fishing harbour is the most polluted site in terms of Pb, Co, Zn, Ni and Fe likely due to high mechanical fishing, shipping and other harbour activities in the area. Earlier, Lepland et al. (2010) published similar trend value noting that the harbours are frequently polluted by metals discharged from ship repair or painting.

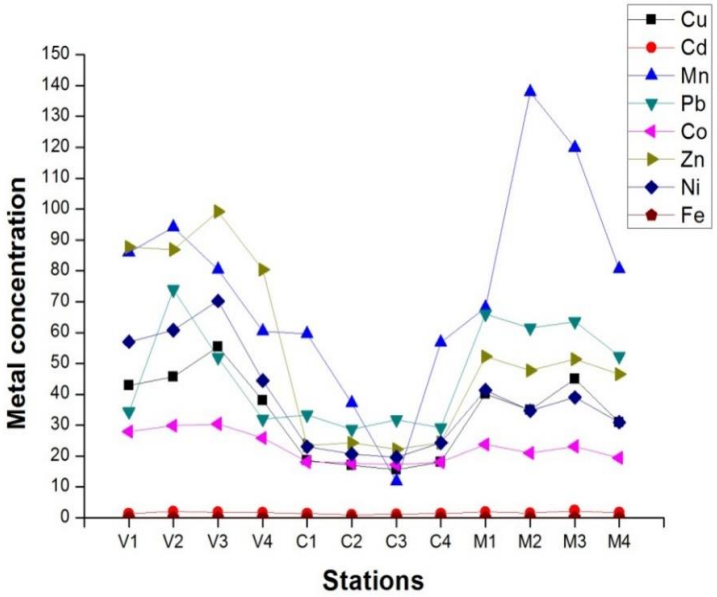

Fig. 4.1: Concentration of total heavy metals (mg/kg; Fe (%)) in the sediment

Heavy metals like Cu, Co, Zn and Ni showed high concentration in the Vypin zone. This aspect is worthy to be noted and it is of great environmental concern. These elevated levels might be due to the enormous amount of wastes released into this area through a number of adjacent tributaries. The industrial assortment at the bank of Cochin backwater includes fertilizers, pesticides, chemical and allied industries, radioactive mineral processing, petroleum refining, metal plating and fish-processing units. According to Salas et al. (2017), processing of phosphorous minerals from nearby factory plant contributed metallic contaminants like Cd, Cu, Hg, Ni, Pb, and Zn. Additionally, the Vypin area also affected by the release of waste oil, paints, metal and paint scrapings from the Cochin port, Cochin Shipyard and from domestic

sewage drains. The discharge of industrial effluents along with the restricted flow due to indiscriminate sand mining in the upper reaches of the river Periyar and dredging operations in shallow regions of ship channel in the estuary have resulted in the accumulation of contaminants enriched with heavy metals (Balachandran et al., 2005; Deepulal et al., 2012; George et al., 2012). Aside from these data, the ANOVA results revealed that all the estimated heavy metals exhibit a strong spatial variation (p«0.01) except Cd and Fe. The wide spatial variation in the metal distribution in the sediments supports the view that anthropogenic activity contributes significantly to the contamination of marine sediments due to both industrial and urban activities of the analysed research area.

Table 4.1: Concentration of the total heavy metals (mg/kg) in the surface sediments

Metal	Minimum	Maximum	Average	ANOVA (P value)
Cu	15.60	55.41	33.21	<0.01
Cd	1.05	2.29	1.57	0.04
Mn	11.88	137.89	72.45	0.01
Pb	28.59	73.90	45.53	0.01
Co	17.28	30.46	22.78	<0.01
Zn	22.20	99.26	54.20	<0.01
Ni	19.67	70.23	39.03	<0.01
Fe	715.31	1145.85	982.69	0.23

To gain a comprehensive understanding of the degree of contamination in the study zones, a comparison of the heavy metal concentrations with other regions were performed (Table 4.2). The mean contents of Pb and Ni in the study area were higher than their corresponding average values reported earlier in the Cochin estuary (Salas et al., 2017), Punnakayal estuary (Magesh et al., 2013), Kallar Estuary (Magesh et al., 2013), Ennore estuary (Raj and Jayaprakash, 2007) and Tamiraparani estuary (Magesh et al., 2013), while the contents of Cd and Fe were lower as compared to these study sites. The Cu concentration in this study was higher than that of the Cochin estuary (Salas et al., 2017), Punnakayal estuary (Magesh et al., 2013) and Kallar estuary (Magesh et al., 2013). The mean concentration of Co in the study area was higher than that of all other representative estuaries in India (Raj and Jayaprakash, 2007; Kumar and Edward, 2009; Magesh et al., 2013; Shah et al., 2013; Reddy et al., 2016; Salas et al., 2017). With regard to Zn and Mn, their contents in the study zones were lower than those in all other representative coastal regions in India (Raj and Jayaprakash, 2007; Kumar and Edward, 2009; Magesh et al., 2013; Shah et al., 2013; Reddy et al., 2016; Salas et al., 2017). In addition, with regard to other elements, the average content of Fe was comparable to those in Nellore coast (Reddy et al., 2016). As compared to the data published for other regions, the metal contamination data for the study area indicated that the sediment is not severely contaminated with Fe, Mn, Zn and Cd.

Table 4.2: Heavy metal content (mg/kg; Fe (%)) in riverine/estuarine sediments from different part of India.

Study Area	Fe	Mn	Cu	Pb	Zn	Ni	Co	Cd	References
Tapti estuary	6.79	NA	148.32	56.69	143.60	80.92	18.34	0.83	Shah et al., 2013
Tamiraparani estuary	1.64	668.50	40.19	26.16	198.60	23.96	3.62	11.13	Magesh et al., 2011
Nellore coast	0.10	1384.00	197.82	159.60	234.70	71.63	4.99	NA	Reddy etal., 2016
Ennore estuary	2.72	373.00	506.20	32.36	126.80	38.61	8.10	6.58	Raj and Jayaprakash, 2007
Kallar estuary	3.40	356.25	27.43	29.11	320.40	33.78	1.38	3.61	Magesh et al., 2013
Punnakayal estuary	2.83	277.63	30.98	28.13	231.00	21.20	3.65	10.40	Magesh et al., 2013
Cochin estuary	3.10	273.78	26.74	21.91	386.10	31.12	13.75	5.07	Salas et al., 2017
Manakudy estuary	0.40	253.81	42.30	163.46	66.40	24.45	4.82	2.89	Kumar and Edward, 2009
Central Kerala	0.10	72.45	33.21	45.53	54.20	39.03	22.78	1.57	In this study

NA- Data not available

4.2.1.1 Anthropogenic Influence Assessment

Assessment of pollution status and potential ecological risk posed by each heavy metal were carried out by applying the geological accumulation index method (Igeo), the enrichment factor (EF), pollution load index (PLI), Contamination factor (CF) and Sediment Quality Guidelines. The calculation of different geochemical indices were detailed in Chapter 2.

4.2.1.1.1 Enrichment Factor

In order to get a better understanding of the heavy metal sources in the present work, enrichment factor (EF) approach was considered (Zhang and Liu, 2002; Han et al., 2006; Han and Lu, 2017). The EF was calculated for a better assessment of anthropogenic origin for each metal (Table 4.3 and Fig. 4.2). Based on the EF values, the sediments of the study area were significantly contaminated in the order Cd> Co>Pb >Ni > Cu> Zn> Mn. The EF values of all the surface sediments ranged from 374.42 to 777.25 for Cd, 60.34 to 136.49 for Co, 50.88 to 127.12 for Pb, 36.81 to 139.46 for Ni, 23.68 to 83.84 for Cu, 11.01 to 60.41 for Zn and 0.75 to 7.69 for Mn. The Cd, Pb, Ni, Co and Cu displayed enrichment above thirty (>30) signifying very severe enrichment of these metals. According to Zhang and Liu (2002), the higher the EF values, the more severe the anthropogenic contribution, for this reason the result indicates an anthropogenic source of these metals. Likewise, Cd exhibited very high EF values in all the study zone, clearly indicating the influence of human activities. Whereas, the mean EF for Ni were below 5 (<5), showing moderate enrichment for this metal. According to Zhang et al.

(2007), EF>1.5 displays that a significant portion of heavy metals is executed from external sources. In the three zones, all the studied metals recorded an EF >1.5 outlining anthropogenic input of these metals and the findings of Deepulal et al. (2012) also strongly support this conclusion.

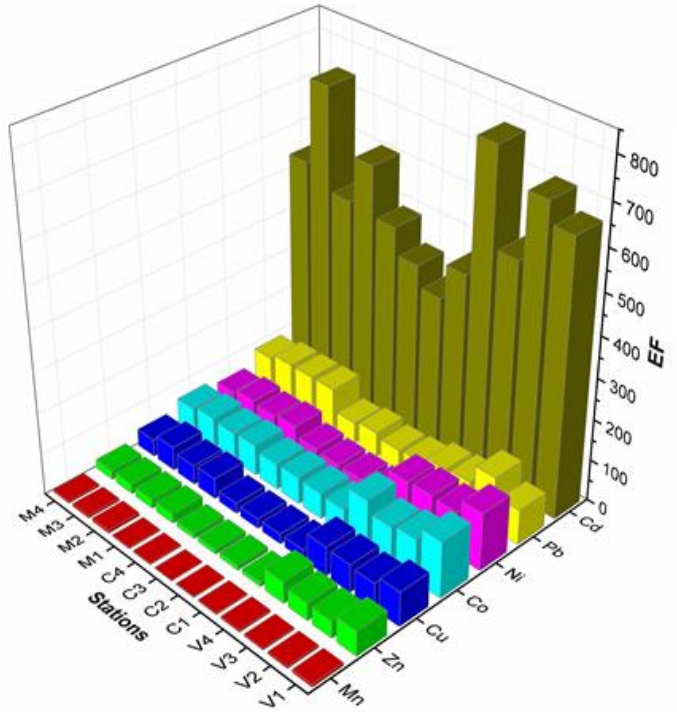

Fig. 4.2: Enrichment factor of heavy metals in the sediments.

Table 4.3: Enrichment factor of metals analysed in the surface sediment of Central Kerala

Enrichment Factor							
Stations	**Cd**	**Pb**	**Ni**	**Co**	**Zn**	**Mn**	**Cu**
V1	661.41	84.31	139.46	136.49	60.41	7.02	83.84
V2	710.33	127.12	104.61	102.75	42.11	5.39	62.95
V3	550.90	79.37	107.26	93.05	42.70	4.09	67.70
V4	777.25	74.70	103.83	120.80	52.84	4.70	71.00
C1	460.05	55.39	38.33	60.34	11.01	3.30	24.80
C2	374.42	50.88	36.81	62.97	12.20	2.21	24.15
C3	423.56	60.31	37.32	65.59	11.87	0.75	23.68
C4	485.71	51.31	42.81	63.58	12.11	3.34	25.42
M1	603.73	103.85	65.12	74.63	23.15	3.57	50.41
M2	493.56	103.01	58.02	70.33	22.53	7.69	46.70
M3	737.68	102.45	62.85	74.61	23.33	6.43	57.87
M4	535.90	88.22	52.22	65.49	22.10	4.53	41.78

4.2.1.1.2 Geo-accumulation index

Igeo values estimated for sediments in the study region are depicted in Fig. 4.3 and Table 4.4. The average Igeo values of the studied metals were -3.80 for Mn, -118 for Zn, -0.29 for Cu, 0.25 for Ni, 0.55 for Pb, 0.57 for Co and 3.37 for Cd respectively. The average Igeo for Cd (3.37) indicated that the sediments were strongly polluted to very strongly polluted with this metal. The estimated Igeo values revealed that Pb, Co and Ni exhibited unpolluted to moderately polluted condition. Whereas, metals like Cu, Mn and Zn can be considered as unpolluted in the study site. The result inferred that the Igeo of Cd was higher compared with those of other metals and suggested its pollution sources are mainly as human activities.

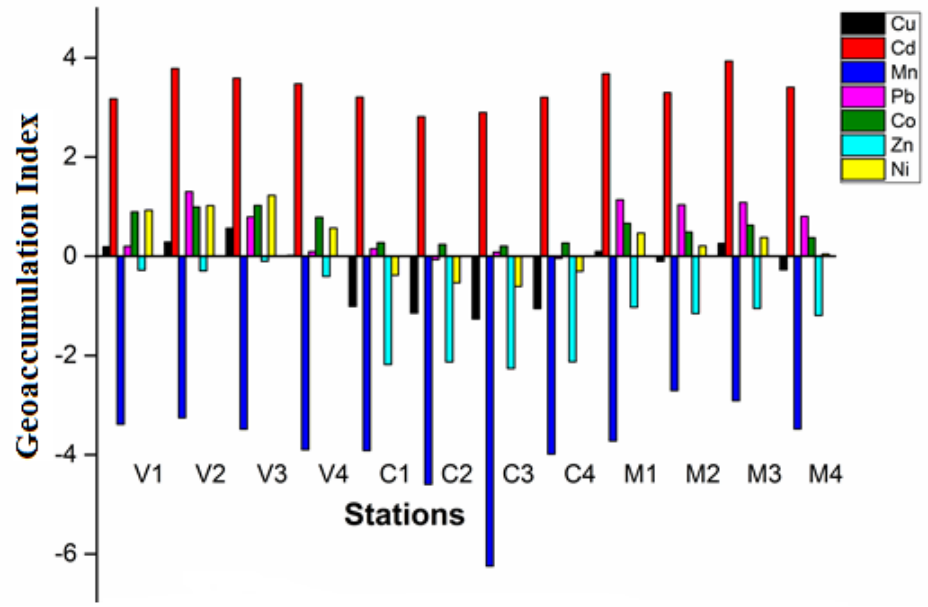

Fig. 4.3: Geo-accumulation index estimated for the heavy metals in the sediments

Table 4.4: Geo-accumulation index of metals analysed in the surface sediment of Central Kerala

Geo-accumulation Index							
Stations	**Cu**	**Cd**	**Mn**	**Pb**	**Co**	**Zn**	**Ni**
V1	0.19	3.17	-3.39	0.20	0.90	-0.28	0.93
V2	0.29	3.78	-3.26	1.30	0.99	-0.29	1.02
V3	0.56	3.59	-3.49	0.79	1.02	-0.10	1.23
V4	0.02	3.47	-3.90	0.09	0.79	-0.41	0.57
C1	-1.01	3.20	-3.92	0.15	0.27	-2.18	-0.38
C2	-1.14	2.81	-4.60	-0.07	0.24	-2.13	-0.54
C3	-1.27	2.90	-6.24	0.08	0.20	-2.26	-0.61
C4	-1.06	3.20	-3.99	-0.04	0.27	-2.13	-0.30
M1	0.10	3.68	-3.72	1.14	0.66	-1.03	0.47
M2	-0.10	3.30	-2.71	1.04	0.49	-1.16	0.21
M3	0.26	3.93	-2.91	1.08	0.63	-1.05	0.38
M4	-0.28	3.41	-3.48	0.80	0.37	-1.19	0.05

4.2.1.1.3 Contamination Factor

In order to understand the heavy metal enrichment in the sediments of the Central Kerala, CF has been estimated for all the metals studied (Fig. 4.4 and Table 4.5). The CF values were 10.52 to 22.90 for Cd, 1.43 to 3.70 for Pb, 1.00 to 3.05 for Co, 0.98 to 3.51 for Ni, 0.62 to 2.22 for Cu, 0.31 to 1.40 for Zn and 0.02 to 0.23 for Mn (Table 4.5). Degree of contamination results verified that heavy metal pollution occurred in the study area, and they varied between low levels to a very high level. Mn and Zn showed low contamination; Cu, Pb, Co and Ni exhibited moderate contamination and Cd recorded very high contamination. This result suggested that Mn and Zn originated, to a large extent from the natural input via weathering of rocks, whereas, sources of Cd, Pb, Cu, Co and Ni pollution was attributed to land runoff and anthropogenic activities.

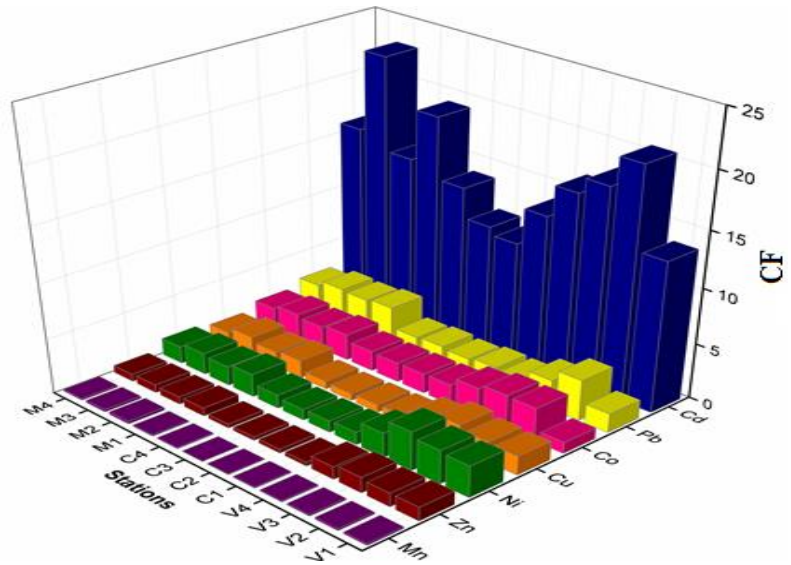

Fig. 4.4: Contamination factor of heavy metals in the sediment

Table 4.5: Contamination factor of metals analysed in the surface sediment of Central Kerala

Contamination Factor							
Stations	**Cu**	**Cd**	**Mn**	**Pb**	**Co**	**Zn**	**Ni**
V1	1.71	13.52	0.14	1.72	1.00	1.23	2.85
V2	1.83	20.65	0.16	3.70	2.99	1.22	3.04
V3	2.22	18.04	0.13	2.60	3.05	1.40	3.51
V4	1.52	16.65	0.10	1.60	2.59	1.13	2.22
C1	0.75	13.83	0.10	1.67	1.81	0.33	1.15
C2	0.68	10.52	0.06	1.43	1.77	0.34	1.03
C3	0.62	11.16	0.02	1.59	1.73	0.31	0.98
C4	0.72	13.79	0.09	1.46	1.80	0.34	1.22
M1	1.60	19.20	0.11	3.30	2.37	0.74	2.07
M2	1.40	14.75	0.23	3.08	2.10	0.67	1.73
M3	1.80	22.90	0.20	3.18	2.32	0.72	1.95
M4	1.24	15.90	0.13	2.62	1.94	0.66	1.55

4.2.1.1.4 Pollution Load Index

The transects were partitioned into polluted (PLI>1) and unpolluted (PLI<1) due to heavy metals on the basis of PLI formulated at each sampled transect. The pollution load index (PLI) calculated for each station are represented in Fig.4.5. At Chettuva, the overall PLI values were 7.60, 1.16, 0.61 and 1.64 at C1, C2, C3 and C4 respectively. However, PLI values at Vypin zone persisted 31.41, 109.18, 100.96 and 36.07 at V1, V2, V3 and V4 whereas at Munambam, the PLI values recorded 8.85, 9.34, 11.59 and 5.56 at M1, M2, M3 and M4 respectively. The values of PLI indicated that the integrated pollution status of combined metal groups is pretty serious and especially the Vypin zone is severely impacted by contamination. The overall value of PLI>1 at these zones could be due to the flow of effluents from several sewage systems and land runoff pouring their water mass enriched with inorganic metallic compounds into this study zones and indicating severe deterioration of study sites by metals.

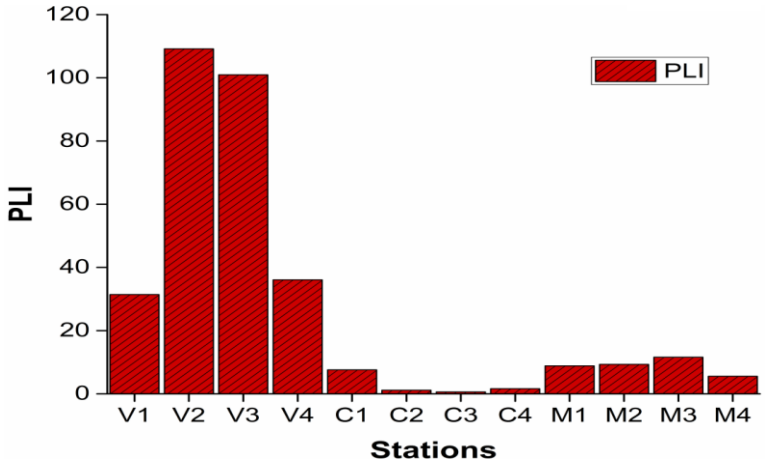

Fig. 4.5: Pollution Load Index of heavy metals in the sediment

4.2.1.1.5 Evaluation of pollution using SQG's

Numerous sediment quality guidelines have been developed to address environmental concerns. The guidelines values namely, threshold effects level (TEL) and probable effects level (PEL) are used to determine whether a specific metal detected in sediment poses a threat to aquatic ecosystems. Three ranges of chemical concentrations are defined by TEL and PEL as represented as 1) concentrations of chemicals in sediments below TEL that are not associated with adverse biological effects; 2) concentrations between TEL and PEL that may occasionally be associated with adverse biological effects; and 3) concentrations above PEL that are expected to be frequently associated with adverse biological effects (MacDonald et al., 2003). In terms of PEL and TEL comparisons (Table 4.6), Ni concentration reported for Vypin zone exceeded the PEL values inferring a possible Ni contamination in the area. The average concentration of Cu, Cd and Pb is above the corresponding TEL value, but substantially lower than the PEL value. The above results lead to the

conclusion that the Cu, Cd and Pb levels may occasionally be associated with adverse biological effects. The Zn average values are below the TEL value and based on the used criteria, Zn was not associated with adverse biological effects. In addition to this, the ERL (effects range-low) and ERM (effects range-mean) concentrations presented in the Sediment Quality Guide also have been adopted as an informal tool to evaluate the sediments chemical data in relation to possible adverse effects on aquatic biota. The values of Cu and Pb in all the zones except Chettuva; Cd in three zones; Ni in all the stations except C2 and C3 were well above the ERL, implying that adverse effects on aquatic biota might frequently occur. The values of Cd were found to be greater than the threshold level in all the zones and there would be of significant impact to the aquatic system.

Table 4.6: Sediment Quality Guideline values for heavy metals (MacDonald et al., 2000; Bakan and Ozkoc, 2007)

Metals	NOAA Guidelines		Canadian Guidelines	
	ERL	ERM	TEL	PEL
Cd	1.2	9.6	0.7	4.2
Cu	34	270	18.7	108
Pb	46.7	218	30.2	112
Ni	20.9	51.6	-15.9	-43
Zn	150	410	124	271

4.2.1.2 Heavy metals source analysis

4.2.1.2.1 Relationship of metals with sediment properties

Cu, Cd and Pb displayed a strong positive correlation with Mn and the association of Mn with these metals suggests that Mn-oxide may be the major phase of these metals in the environment. Cu revealed a positive correlation with TOC; Co exhibited a positive correlation with TC, TOC and TN. The positive correlation revealed that organic matter plays a greater role

in the binding capacity of metals and ligands. For evaluating the efficiency of ecological indicators to reflect different stress situations, the heavy metals were correlated with Shannon-Weaver index, H'. With regard to this correlation, an insignificant negative relationship was observed between Cu, Cd, Mn, Pb, Co, Zn and Ni with H', noted that when these metals in the sediment increased, Shannon diversity decreased. These results provided strong evidence of pollution-induced stress in the study area. From Table 4.7, it can be seen that the relation between them is not significant, indicating that the relationship is complicated and heavy metals are not the only factor controlling the diversity. In addition, possible correlation among different metals were examined and highly significant correlation ($p<0.01$) was demonstrated between Zn with Co ($r=0.977$); Zn with Cu ($r=0.906$); Cd with Cu ($r=0.764$); Pb with Cd ($r=0.805$); Co with Cd ($r=0.600$); Pb with Mn ($r=0.697$); Pb with Cu ($r=0.661$) in sediment. The positive inter-element relationship between these metals suggests a similar mechanism of transport and accumulation within the sediments or sharing of the same chemical environment during transport (Hu et al., 2013). Notably, the correlation and geochemical associations of heavy metals reveal a significant source of contamination reflecting a common origin of similar nature existing from the industrial effluents (Turner, 2000). Hakanson and Jansson, (1983), suggested that even metals with quite different chemical properties might appear with similar distribution in nature due to the fact that they are linked to carrier particles with similar properties. The absence of strong correlation among other metals revealed that the concentrations of these metals are not controlled by a single factor, but a combination of geochemical support phases and their mixed associations (Jain et al., 2005).

Table 4.7: Pearson correlation of parameters with heavy metals in the surface sediment

	Sand	Silt	Clay	TC	TOC	TN	TS	Cu	Cd	Mn	Pb	Co	Zn	Ni	Fe	H'
Sand	1															
Silt	-0.835	1														
Clay	-0.985	0.726**	1													
TC	-0.654*	0.619*	0.621*	1												
TOC	-0.642*	0.564	0.623*	0.921**	1											
TN	-0.622*	0.614*	0.582*	0.989**	.921**	1										
TS	-0.617*	0.615*	0.576	0.986**	0.933**	0.978**	1									
Cu	-0.431	0.542	0.367	0.563	0.658*	0.545	0.553	1								
Cd	-0.455	0.328	0.249	0.309	0.499	0.316	0.654*	0.764**	1							
Mn	-0.346	0.393	0.308	0.414	0.415	0.351	0.417	0.637*	0.607*	1						
Pb	-0.300	0.504	-0.815	0.388	0.534	0.350	0.461	0.661*	0.805**	0.697*	1					
Co	-0.282	0.596*	0.380	0.594*	0.703*	0.601*	0.290	0.918**	0.600*	0.420	0.474	1				
Zn	-0.229	0.243	0.209	0.290	0.434	0.279	0.257	0.906**	0.522	0.422	0.397	0.977**	1			
Ni	-0.367	0.382	0.338	0.364	0.540	0.355	0.343	0.929**	0.575	0.446	0.483	0.987**	0.972**	1		
Fe	-0.532	0.553	0.489	0.512	0.556	0.479	.586(*)	0.117	0.396	0.252	0.561	-0.106	-0.203	-0.030	1	
H'	-0.380	-0.450	-0.510	-0.110	-0.460	-0.470	-0.250	-0.130	-0.400	-0.380	-0.080	-0.006	-0.020	-0.340	0.060	1

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

4.2.1.2.2 Cluster Analysis

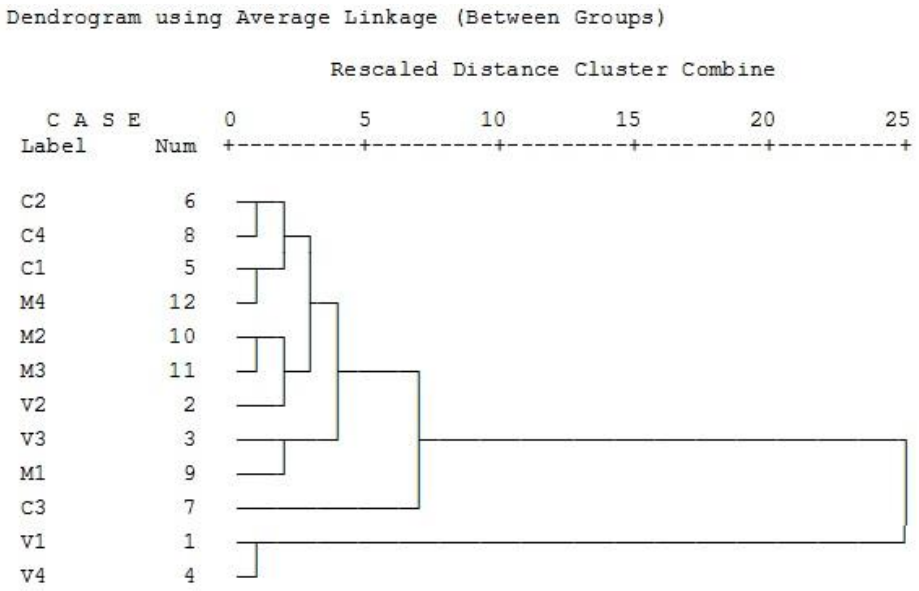

Fig. 4.6: Dendrogram showing the cluster of sampling stations based on analysed variables.

Hierarchical cluster analysis (HCA) is one of the most commonly used methods of multivariate statistical analysis and classifies samples and indicators according to their characteristics. Cluster analysis was performed to identify the similarity between the sampling stations and also to determine the specific areas of contamination. Fig 4.6 represents the result rendered from this analysis for the sediment quality with respect to the sampling stations. From the 12 sampling stations, three associations were evident (i.e.,) cluster 1 (station C1, C2, C4 and M4), cluster 2 (station M1, M2, M3,V2,V3) and cluster 3 (station V1, V4, C3) which revealed that, these three regions have different characteristics in terms of heavy metal enrichment from different sources (natural and anthropogenic). Cluster 1 can be depicted as moderately polluted group, cluster 2 is

denoting the highest pollution level among the sites observed while cluster 3 is the least polluted groups as per the overall data matrix obtained from the study. This technique is useful in affording a reliable classification of heavy metals in the study area and also provides a design for future spatial sampling strategy without losing any significance of the outcome.

4.2.1.2.3 Factor Analysis

To better elucidate the sources of the metal distribution in sediment, Principal Component Analysis (PCA) was performed (Table 4.8). Three principal components were extracted from the available dataset, explained a total variance of approximately 86.49%. Factor 1 is statistically dominant and accounted to 53.93% having strong positive loading (0.8-0.9) on Cu, Cd, clay and silt, moderate loading on (0.5 to 0.7) Ni, Co, Zn, Pb, Mn, TN, TS and TOC and negative loading for sand. The close association of clay particles with organic matter and heavy metals demonstrating the granulometric dependence and also the flocculation and sedimentation of organic matter. The grouping of TOC, TN and TS pointed towards the diagenetic processes taking place in the surface sediments. Diagenesis, a redox process, largely mediated by microorganisms and the suitable indicators of this process is TOC, TN and TS. The second principal component (PC2), accounts for 24.80% of the total variance and is dominated by Cu, Co, Zn and Ni. This factor reflected the contribution from anthropogenic input and it can be identified as the pollution factor. Moreover, the loading of Zn along with TOC in factor 1 with high significance implied the terrigenous contribution in addition to industrial

sources. Factor 3 accounts for 7.77% of the total variance and exhibited positive loading on Pb and Fe showing the association of Pb with ferric hydroxide and this binding affects the bioavailability of Pb and are less available to organisms.

Table 4.8: Results of PCA carried out on the analysed parameters in the study

Variables	Component		
	1	2	3
Sand	-0.700	0.459	0.462
Silt	0.847		
Clay	0.804		
TOC	0.706	-0.641	
TN	0.544	-0.656	
TS	0.796		
Cu	0.849	0.508	
Cd	0.858		
Mn	0.728		
Pb	0.762		0.586
Co	0.685	0.676	
Zn	0.680	0.700	
Ni	0.756	0.618	
Fe		-0.649	0.491
% Variance	53.93	24.80	7.77

Note: Only Factor loadings > ±0.45 are shown

4.3 Distribution of Heavy Metals in the Geochemical Fractions

4.3.1 Results and Discussion

A summary of geochemical fractionation results and its mobility pattern is described (Fig. 4.7, Table 4.9). The overall concentration of Cd in the various fractions followed the order: CA> EXC> OM> FMO>

RES. An average of 73% of the total Cd in the sediment is present in the EXC, CA and FMO fraction, with the CA fraction accounting for the highest amount of 30%, while the exchangeable fraction accounted for 26%. Cadmium seems to be homogeneously distributed in all the zones but there observed alternation of Cd level in different fractions. In the exchangeable, carbonate and reducible fraction, Cd is found associated in the Vypin, Chettuva and Munambam zones with an average of 96%, 70% and 53% respectively. The chemical speciation data showed that Cd is in an unstable form in the study sites and bounded to exchangeable, carbonate and reducible fraction and it can be concluded that Cd exchanged easily.

The affinity order of Pb in different fractions is EXC> CA> RES> FMO> OM. In most of the stations, Pb was associated with the mobile phases in particular with the exchangeable phase. Lead is distributed principally between the 28% in the exchangeable fraction, 21% in the carbonate bound fraction, 16% in the Fe/Mn oxide fraction and 15% in the organic/sulphide fraction and on average, about 20% of Pb is found in the residual phase. It is noteworthy that the association of Pb in OM fraction does not hike with the increasing organic matter level in the sediments. Because the binding property is influenced by numerous factors, such as total metal load, competition between heavy metals and sediment organic matter concentration (Chakraborty and Chakrabarti, 2008; Chakraborty et al., 2012).

On the average, Cu concentration of all the fractions was in the order: CA> OM> FMO> EXC> RES. The high content of Cu in the bioavailable forms at Vypin zone (on average of 71%) could be attributed to the use of antifouling paint on ships in addition to other industrial and domestic activities around the zone. This finding strongly supports the conclusion drawn by Deepulal et al. (2012). Fractionation profile of Cu showed that a major portion is bound to exchangeable and carbonate fraction at Vypin and Chettuva zone respectively, indicating contributions from the nongeogenic source. While at Munambam zone, maximum portion is bound to organic matter probably due to its more pronounced tendency for complexation with organic matter at this site. Furthermore, Cu was also found to be mainly bound to the organic fraction in the samples collected from the station near the Munambam harbour (M1; 26%). This may be due to the high input of organic waste material from the fish market spot. The significant association of Cu with organic matter bound fraction in this study may be an indication of the high organic content of the sediment. Since some literature on this aspect have already known that there exists a high formation constant of organic Cu complexes (Nierop et al., 2002). In addition to this, there is high retention capability of organic matter, especially humic substances, which are the component of natural organic matter and chemically active in complexing metals (Fytianos and Lourantou, 2004; Jain et al., 2008) for Cu. Likewise, the proportion of metals such as Cd, Mn, Co, Zn and Ni at M3 and Mn, Pb, Co and Ni at V3 recorded higher proportion in the oxidisable phase implying that these metals may be carried by organic matter in this station. It is observed that higher TOC content (5.15% and 4.60%; Table

3.4) in the sediments at M3 and V3 stations suggested that organic matter is the primary factor impacting the behaviour of heavy metals in these stations. However, at V2 and C3, relatively low amounts of metals were bound to organic matter fraction and this was due to the low organic carbon content (C3:0.16%; V2:0.18%, Table 3.4) at these sites.

The overall concentration of Zn in the various fractions followed the order. FMO> EXC> CA> OM> RES. The fractionation pattern showed that Zn mostly occurs in the first three fractions of the sediments averaging 68%. These three fractions are the most labile segment and constitute the most dangerous phases for the environment. The sequential extraction results of the current study suggested that Fe-Mn oxides may be the main carriers of Zn and the higher concentration of Zn associated with this fraction are adsorbed to the Fe-Mn colloids (Jenne, 1968; Okuku et al, 2010). This illustrated the ability of Fe-Mn oxides for scavenging heavy metals from solution through processes such as adsorption and co-precipitation (Lim and Kiu, 1995).

The fractionation profile of Mn followed the pattern: CA> FMO> OM> RES> EXC. More than 50% of the manganese was associated with the first three fractions i.e. exchangeable, bound to carbonate and reducible fractions and may pose risk to aquatic life. The highest percentage of Mn (a mean of 30%) was existing in the labile carbonate bound phase. This is probably because of the known close association of Mn with carbonates as endorsed by other research reports (Kiratli and Ergin, 1996; Morillo et al., 2004). Mn retained on sediment surface by relatively weak electrostatic interactions which may be released by ion

exchange processes and dissociation of Mn-carbonate phase (Tessier et al., 1979). In addition, a substantial Mn percentage was obtained in the reducible phase (28%) and the relatively high concentration of manganese in reducible fraction suggests that the metal exists in the reduced form (Tessier et al., 1979). Moreover, in this reduced phase, Mn exists as oxides and may be released if the sediment is subjected to more reducing conditions (Panda et al., 1995). Comparing the zones, the Munambam zone has slightly higher contents of Mn in their oxidisable fractions (27%), whereas in the Vypin and Chettuva zones, Mn is more strongly associated with the potentially more mobile phases such as carbonate and reducible fractions.

The affinity order of Ni in the various fraction decreased in the following order RES> OM> FMO> CA> EXC. The fractionation pattern of Ni indicates that Ni is associated to organic and residual compounds in all the sites with the exception of V1 and V2, representing their principally non-anthropogenic origin and their less availability to the aquatic fauna. Nickel is mainly related to the residual fraction, varying from 29% in the Vypin zone to 50% in the Chettuva zone. The overall concentration of Fe in the various fraction followed the order: RES> FMO> OM> CA> EXC. Fe is present mainly in the residual phase, representing the average of 51% of the total concentration characterizing their stable compounds in sediments. The metal associated with this fraction cannot be remobilized under normal conditions encountered in the nature. The concentrations were very low in exchangeable phase (3% as mean value), and this level is toxicologically insignificant for causing any adverse effect on biota. These results were in good agreement with

data reported by several previous studies carried out worldwide in the marine coastal waters (Usero et al., 1998; Martin et al., 1998; Takarina et al., 2004). The largest amount of Fe is available in the residual fraction, probably because Fe originates from the geochemical background rather than the anthropogenic source and Fe in the residual phase suggested that most of the iron retained within the crystal lattice as iron peroxides (goethite, limonite, magnetite, etc.). The results of this work, therefore, inferred that sediment-bound Fe and Ni along Central Kerala are mostly of natural geochemical origin.

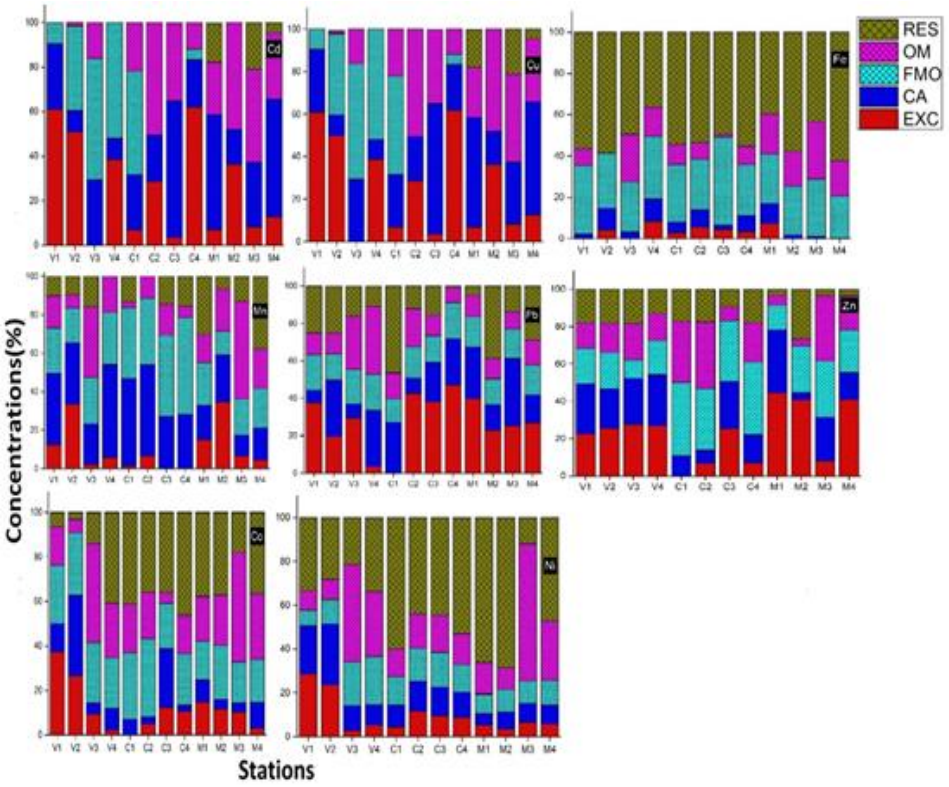

Fig. 4.7: Fractionation profile of heavy metals in the surface sediments

Table 4.9: Fractionation profile of surface sediments

Metals	Zone	Exchangeable	Carbonate	Fe-Mn oxides	Organic	Residual
Cu	Vypin	4.53-18.24 (12.64)	0.94-25.01 (9.93)	0.68-16.12 (9.01)	4.68-14.91 (9.59)	ND-6.08 (3.55)
	Chettuva	ND-2.49 (1.71)	1.45-7.54 (5.42)	0.50-4.50 (2.92)	ND-9.50 (3.14)	2.17-3.93 (3.90)
	Munambam	0.69-11.03 (5.63)	0.42-13.69 (7.18)	4.68-17.87 (9.89)	8.02-16.25 (11.12)	2.1-7.50 (5.65)
	Average	6.45	7.51	7.27	7.95	4.37
Cd	Vypin	ND -1.03 (0.80)	0.15-0.51 (0.31)	0.12-0.94 (0.66)	ND -0.28 (0.16)	ND
	Chettuva	0.04-0.78 (0.30)	0.22-0.69 (0.38)	ND -0.06 (0.34)	0.15-0.53 (0.34)	ND
	Munambam	0.12-0.53 (0.25)	0.23-0.84 (0.64)	ND	0.40-0.87 (0.61)	ND -0.44 (0.27)
	Average	0.45	0.44	0.33	0.37	0.09
Mn	Vypin	1.75-30.92(11.50)	16.98-30.21 (26)	15.32-19.45 (17.66)	6.28-29.75 (15.05)	ND -12.78 (9.98)
	Chettuva	ND -2.36 (1.58)	3.06-23.78 (14.98)	4.85-28.43 (16.30)	1.59-4.06 (2.68)	ND -8.81 (5.80)
	Munambam	3.38-46.08 (16.68)	11.91-32.81 (17.42)	14.95-20.57 (16.90)	9.94-55.92 (27.81)	8.39-20.39 (17.95)
	Average	9.92	19.47	16.95	15.18	11.24
Pb	Vypin	1.14-14.99 (10.93)	2.39-22.16 (9.59)	6.49-10.51 (8.31)	4.05-14.65 (9.75)	3.64-18.53 (9.73)
	Chettuva	ND -13.76 (12.65)	2.4-8.96 (6.36)	4.19-5.71 (4.81)	2.29-5.8 (4.06)	0.31-15.34(6.00)
	Munambam	13.92-26.17 (17.53)	7.8-23.03 (14.39)	8.4-10.65 (9.43)	5.7-7.54 (6.72)	3.29-23.72 (12.68)
	Average	13.70	10.11	7.52	6.84	9.47
Co	Vypin	0.53-10.89 (5.60)	1.67-10.82 (4.68)	5.75-8.90 (7.66)	1.82-14.55 (6.91)	0.95-10.31 (4.44)
	Chettuva	ND -2.14 (1.62)	0.52-4.60 (1.75)	3.54-6.1 (4.85)	0.88-4.08 (2.92)	6.21-8.41 (7.14)
	Munambam	0.59-3.80 (2.30)	0.94-2.62 (1.71)	3.77-5.33 (4.41)	4.9-10.86 (6.69)	3.99-9.81 (7.27)
	Average	3.17	2.71	5.64	5.51	6.28
Zn	Vypin	19.8-26.99 (22.29)	17.36-24.57(21.81)	9.57-16.94 (14.23)	11.61-18.21 (13.85)	10.61-15.73 (14.85)
	Chettuva	ND -5.61 (3.03)	1.73-5.67(3.4)	7.27-9.50(8.52)	1.63-8.83 (5.80)	2.15-4.42 (3.74)
	Munambam	4.21-23.23 (16.33)	1.84-17.83(9.63)	6.84-11.82 (11.20)	1.82-17.95 (7.68)	1.72-12.55 (4.48)
	Average	13.89	11.62	11.32	9.11	7.69
Ni	Vypin	1.90-14.35 (7.68)	4.03-16.96 (9.61)	3.13-14.01 (8.33)	3.75-31.27 (13.31)	14.37-17.16 (15.25)
	Chettuva	1.00-2.50 (1.87)	2.31-2.94 (2.66)	3.03-3.21 (3.09)	2.83-3.44 (3.26)	8.74-13.85 (11.23)
	Munambam	1.24-2.55 (1.94)	2.23-3.41 (2.73)	3.5-4.08 (3.71)	3.42-24.63 (10.59)	4.81-27.75 (17.74)
	Average	3.83	5.00	5.04	9.05	14.74
Fe	Vypin	0.46-60.19 (25.36)	15.61-100.65 (58.65)	221.47-290.34 (251.33)	0.51-278.22 (109.59)	266.09-562.01 (455.78)
	Chettuva	30.12-50.72 (38.44)	19.63-79.95 (57.75)	228.69-390.76 (243.95)	11.27-102.57 (69.01)	456.11-566.45 (517.63)
	Munambam	0.08-80.60 (20.44)	3.46-108.86 (34.91)	203.86-282.73 (249.57)	169.34-283.29 (210.23)	438.14-624.04 (526.22)
	Average	28.08	50.44	263.18	129.61	499.88

Notes: metal concentration has been defined as mg-metal/kg-dry sediment; ND: not detected

4.3.1.1 Availability of heavy metals

The analytical data have been treated summing the results from the first, second and third geochemical phases to get the bioavailable phase (BAP) and from the fourth and fifth to obtain the non-bioavailable phase (NBAP) as summarised in Table 4.10. These metals are classified as bioavailable and non-bioavailable groups on the basis of their relative mobility and toxicity to the aquatic environment. Metals associated with BAP, mostly originates from anthropogenic sources, they are easily adsorbed on surface sediments through weak bonds (Forstner, 1989; Horowitz, 1985) and are readily available to aquatic biota (Pempkowiase et al., 1999). The concentration in the bioavailable fraction is a serious environmental concern (Sundaray et al., 2011). While metals associated with NBAP are available only in short term for bio uptake under certain condition, due to strong bond which exists between these metals and organic matter or crystal lattice of silicates in sediments. The experimental data shows that the mobility of heavy metals decreases in the following order: Cd (73%)> Mn (68%)> Pb (65%)> Cu (63%)> Zn (56%)> Co (47%)> Fe (36%)> Ni (35%). The present study has demonstrated that Fe and Ni were the least bioavailable metals in the sediments across all locations and therefore is in a non-available form. A high percentage of metals such as Cu (71%), Cd (96%), Mn (71%) and Zn (68%) recorded in BAP at Vypin zone is due to greater mobility, as the metal originating from industrial effluents. The maximum Co concentration has been found in NBAP at all sites with the exception of V1, V2 and C3 where its highest portion is in BAP and elevated concentrations of Co could be due to the fishing and boating activities (Nemati et al., 2011).

Results of this study detected that Cd, Pb, Zn, Cu and Mn are more mobile rather than other metals which would make them more dangerous to the area.

Table 4.10: Bioavailable and non-bioavailable fractions (%) of heavy metals in the sediment

Locations	Fractions	Cu	Cd	Mn	Pb	Co	Zn	Ni	Fe
V1	BAP	69	100	73	63	76	67	58	35
	NBAP	31	0	27	37	24	33	42	65
V2	BAP	81	98	84	64	91	65	63	41
	NBAP	19	2	16	36	9	35	37	59
V3	BAP	74	84	47	55	42	71	34	27
	NBAP	26	16	53	45	58	29	66	73
V4	BAP	60	100	81	53	35	67	36	50
	NBAP	40	0	19	47	65	33	64	50
C1	BAP	77	78	84	40	37	28	28	36
	NBAP	23	22	16	60	63	72	72	64
C2	BAP	66	50	89	68	43	32	40	38
	NBAP	34	50	11	32	57	68	60	62
C3	BAP	85	65	70	74	59	60	38	49
	NBAP	15	35	30	26	41	40	62	51
C4	BAP	11	88	79	91	37	40	33	36
	NBAP	89	12	21	9	63	60	67	64
M1	BAP	55	59	55	84	42	82	19	41
	NBAP	45	41	45	16	58	18	81	59
M2	BAP	71	52	71	51	40	71	21	25
	NBAP	29	48	29	49	60	29	79	75
M3	BAP	47	37	36	77	33	35	25	29
	NBAP	53	63	64	23	67	65	75	71
M4	BAP	59	66	42	58	34	59	26	21
	NBAP	41	34	58	42	66	41	74	79
BAP: Bioavailable phase; NBAP: Non bioavailable phase									

Spatial distribution patterns of Zn, Cu, Co and Ni in the various fractions also indicated a higher potential for mobilization of these metals in the sediments of the Vypin zone which is a part of Cochin estuary. The higher levels of metal in non-residual fractions implied the anthropogenic inputs to surface sediments from the recent industrial development and urbanization happened in the surrounding areas. With envisaged increased development, dredging activities and disposal of dredged material produced changes in oxic and anoxic conditions, which may, in turn, affect metal mobility. It was evident from the data that the metals in the sediments were bound to various chemical fractions with different strengths; therefore, the value could establish a clear indication of sediment reactivity, which would assess the risk of heavy metals in the aquatic environment (Sundaray et al., 2011). To assess the risks, a risk assessment code (RAC) was utilized (Singh et al., 2005; Sundaray et al., 2011).

4.3.1.2 Assessment of potential risk of heavy metals

Risk Assessment Code (RAC) was used to assess the environmental risk of heavy metals in the sediments (Jain, 2004). The mobility and toxicity of heavy metals are evaluated using the results of RAC. This index was suggested by Perin et al. (1985) and is defined as follows:

$$\text{RAC}= \frac{F1+F2}{Ct} * 100$$

where F1 and F2 are concentrations of metal in exchangeable and carbonate fractions respectively, Ct is the total concentration of metal or sum of the metal contents in five fractions. The risk level of RAC is classified in Table 4.11.

Table 4.11: Classification of Risk Assessment Code

Risk Assessment Code (RAC)		
Category	**RAC Value**	**Risk level**
1	<1	No risk
2	1-10	Low risk
3	11-30	Medium risk
4	31-50	High risk
5	>50	Very high risk

The statistical results of RAC for investigated heavy metals are presented in Fig.4.8 in the form of a box plot. The total percentage at 1 to 10 %, 11 to 30 %, 31 to 50% and over 50% indicates low risk, moderate risk, high risk and very high risk, respectively and it also shows that metal can easily enter into the food chain (Perin et al., 1985). The RAC values of all the metals revealed that metals had low risk to very high risk. Thus, according to the Risk Assessment Code (RAC), the order of potential ecological risk generally followed the pattern: Cd> Pb> Cu ≈ Mn> Zn> Co> Ni> Fe. The results revealed that Fe pose low risk whereas Ni and Co exhibit moderate risk (22% and 23% respectively). However, concentrations of Cu (41%), Mn (40%), Pb (48%) and Zn (43%) had at high risk whereas Cd (56%) comes under the very high risk category implicating anthropogenic derived activities which could pose serious problems to the ecosystem and can easily enter into the food chain.

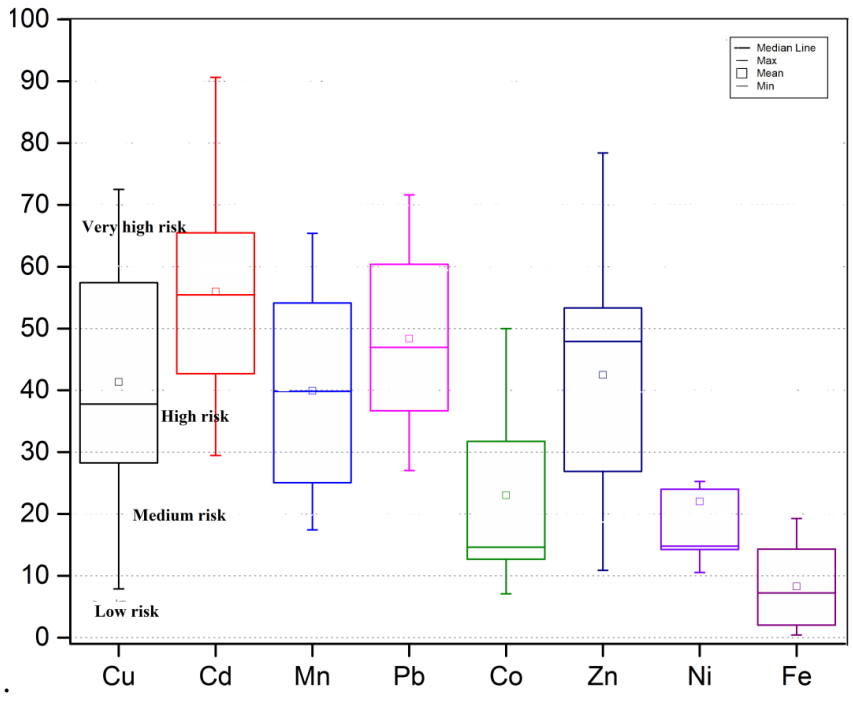

Fig. 4.8: Box-and-whisker plots for the risk assessment of heavy metals

4.4 Conclusions

Heavy metal contamination along the prominent fishing zones of Central Kerala is investigated by analysing the total concentrations and fractionation in sediments at twelve monitoring locations. The contamination was characterized by using multivariate statistical analysis, including correlation analysis, PCA, and cluster analysis. The principal component analysis suggested that Fe, Ni, Co, Zn, Pb and Mn can be attributed to natural sources and its association with clay particle inferring a granulometric dependence. Moreover, separate loading of Mn, Cu, Cd, Pb, Co and Zn implies its anthropogenic input in addition to its natural

source. The enrichment factor, revealed moderate enrichment for Mn; very severe enrichment for Cd, Pb, Ni, Co and Cu signifying anthropogenic contribution. However, the levels of the investigated Ni in the Vypin zone exceeded the PEL, whereas Cd in three zones, Cu and Pb in all the zones except Chettuva were well above the ERL, implying that adverse effects on aquatic biota might frequently occur. The heavy metal fractionation studies presented in this chapter add a new dimension in the assessment of sediment quality studies for the determination of the ecotoxic potential of metal ions. The speciation analysis established that none of the analysed metals, except Fe possesses safer levels in the study zone because appreciable levels of them are found to be associated with mobile geochemical forms. The results of the sequential extraction revealed that each heavy metal has various risk percentages in the five phases. Whereas the mobility rate of the metals is different from one another, the highest mobility rate is related to cadmium, lead, copper, manganese and zinc (as based on RAC) and as such, these metals indicated a high level of risk and suggest that certain pockets of Central Kerala are polluted by heavy metals due to human influences. This study revealed that enhanced concentrations of heavy metals in Vypin and Munambam were due to strong anthropogenic influences from urban areas, as well as due to industrial establishments. These may have a severe impact on marine livings and other organisms in the river. Therefore, it is necessary to give more attention to the accumulation of heavy metals in organisms as far as the seafood industry and public health are concerned. More precise environmental protection measures should be taken to control the discharge of heavy metals from anthropogenic sources. Although pesticides, which

are anthropogenic organic pollutants, also enhance the contamination of the aquatic environment. The characteristics of organochlorine insecticides (OCIs), such as high lipophilicity, their bioaccumulation, long-range transport, have increased the chances of contaminating the air, water and soil, even after many years of application. Therefore, it is important for this study to continue monitoring OCI residues in sediments, and surface water, hence the research objective also encountered this aspect and the salient features of the distribution of OCIs are detailed in Chapter 5.

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Chapter 5

CONCENTRATION OF OCI RESIDUES IN THE SURFACE WATER AND SEDIMENTS

Contents

5.1 Introduction

Organochlorine insecticides (OCIs) are persistent hydrophobic broad-spectrum toxicants and they may accumulate and magnify in the ecosystem because of their resistance to biotic and abiotic degradation. These are characterised by less polarity, low aqueous solubility and high lipophilicity. Therefore, the residues and metabolites of many OCIs are very stable and remains in the environment for decades (El-Mekkawi et al., 2009). Once entering OCIs into water bodies, these toxic chemicals will partition between the phases of sediments, water and biota. They have a potential for bioaccumulation and leading to biomagnification in the food chain and pose the problem of chronic toxicity to animals and humans via air, water and food intake (Afful et al., 2010; Enbaia et al., 2014; Kafilzadeh, 2015). In order to understand the possible emission sources of OCIs, it is necessary to elucidate the distribution, behaviour

and fate of these contaminants in various environmental compartments. Water is one of the primary ways in which pesticides are transported from an application site to other far locations in the environment. As sediments may receive OCI discharges, they are trapped and accumulates the contaminants received, and behave as an ultimate sink for years (Wu et al., 2008). Hence sediments are used for evaluating the health of aquatic ecosystem for decades back (Sujatha and Chacko, 1992; Sujatha et al., 1994(a); Babu, 2001; Cesar et al., 2007; Philips et al., 2010; Akhil and Sujatha, 2015). An assessment of these compounds in the sediments and water column therefore indicated the extent of aquatic contamination as well as their accumulation characteristics in the marine ecosystems and thereby formulated it as an important approach to assess the fate of these organic contaminants (Sujatha et al., 1994 (b); Pandit et al., 2001). Furthermore, the analysis of sediments eliminates the problem of erratic fluctuations, which are often observed in water. Therefore, sediment would act as an important tool for providing valuable information about their toxicity to organisms in the marine environment. There is a conspicuous lack of information on the residual concentration of insecticides in the study sites. Hence the present investigation seeks to collect enough data on the extent of contamination levels of organochlorine insecticides in water and sediments of Central Kerala, India. Methods for the determination of OCIs were described in chapter 2.

5.2 Results and Discussion

5.2.1 Contamination status of OCIs in sediment and water samples

The total organochlorine residue detected in the water sample was 1001.58 ng/L and the level of OCIs was found to be in the order ∑HCH> heptachlor> aldrin> ∑DDT> heptachlor epoxide> dieldrin> endrin. Whereas in the sediment samples, total concentration was 408.68 ng/g and were in the order ∑HCH > aldrin > heptachlor > ∑DDT > heptachlor epoxide > dieldrin > endrin. HCH isomers, aldrin and heptachlor were the most commonly detected organochlorine insecticides. The total organochlorine insecticides (OCIs) concentration in sediments varied from 5.87 to 75.28 ng/g with an average value of 37.15 ng/g. Furthermore, the overall concentrations of OCIs in sediments from Chettuva zone (17.34 to 75.28 ng/g, mean 43.20 ng/g) were higher than those from Munambam zone (5.87 to 57.26 ng/g, average 34.55 ng/g) and Vypin zone (23.44 to 45.39 ng/g, average 32.56 ng/g). However, in the water sample, total OCIs varied from 2.78 to 189.76 ng/L with an average value of 91.05 ng/L. This analysis has indicated that the agricultural activities in and around the region in combination with the atmospheric bulks represent the source of insecticide residues in the investigated samples. While comparing with the existing data (Table 5.3), it is also revealed that the present data on total OCI concentrations in sediments were lower than those concentrations reported in polluted riverine and coastal sediments from the Cochin estuary (Akhil, 2014) and Yamuna river (Pandey et al., 2011). In contrast, the residual concentration of OCIs detected in the current study was considerably high as compared with those measured in the sediment from Hugli estuary (Bhattacharya et al., 2003; Guzzella et al., 2005).

Table 5.1: OCIs in the sediment and water samples collected from Central Kerala

	Water(ng/L)											Sediment(ng/g)										
OCIs	**C1W**	**C2W**	**C3W**	**C4W**	**M1W**	**M2W**	**M3W**	**M4W**	**V1W**	**V2W**	**V3W**	**C1S**	**C2S**	**C3S**	**C4S**	**M1S**	**M2S**	**M3S**	**M4S**	**V1S**	**V2S**	**V3S**
α-HCH	10.27	13.47	17.09	13.72	41.76	9.84	11.72	15.01	10.58	22.63	0.06	8.10	6.68	5.04	5.17	2.95	6.37	0.02	9.35	8.24	5.38	4.72
β-HCH	8.50	14.65	16.20	11.05	29.10	46.54	15.43	26.81	37.52	22.78	0.19	12.37	6.12	4.27	4.37	ND	5.61	0.09	4.48	4.20	3.99	4.15
γ-HCH	7.84	5.37	10.85	10.19	20.54	46.44	ND	ND	5.63	38.79	0.04	22.64	10.20	ND	5.10	12.91	7.26	5.75	3.42	11.44	3.91	3.76
Heptachlor	12.54	31.85	11.53	29.10	29.10	20.78	8.54	18.73	26.75	21.45	0.16	6.84	2.47	5.32	6.52	ND	8.59	0.01	9.17	6.94	6.44	ND
Heptepox	5.14	ND	5.17	6.02	ND	6.85	ND	ND	ND	5.40	0.05	2.70	4.21	2.71	2.80	0.21	2.96	ND	2.41	2.39	ND	2.52
Aldrin	17.55	23.72	19.28	17.47	ND	18.98	ND	22.21	23.16	20.25	0.10	10.13	8.89	ND	9.77	8.68	ND	ND	8.19	8.69	9.13	8.29
Dieldrin	9.21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.99	4.20	ND	ND	ND	ND	ND	4.17	ND	ND	ND
Endrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.80	ND	ND	ND	ND	4.07	ND	ND	ND	ND	ND	ND
p,p'-DDE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07	ND	ND	ND	ND	ND	ND	ND	3.10	ND	ND	ND
o,p'-DDD	7.37	ND	ND	ND	ND	7.37	ND	ND	ND	ND	0.74	ND	ND	ND	3.67	3.49	ND	ND	ND	3.49	ND	ND
p,p'-DDD	ND	ND	ND	ND	ND	15.71	ND	ND	ND	ND	0.16	7.51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
o,p'-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.30	ND	ND	ND	ND	15.73	ND	ND	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	ND	ND	17.25	ND	ND	ND	ND	0.11	ND	ND	ND	ND	9.22	ND	ND	ND	ND	ND	ND

ND - Not Detected

As shown in Table 5.1, ΣHCH concentrations in the sediment samples ranged from 5.87 to 43.12 ng/g dry weight, with an average of 18.01 ng/g. This residual level was higher than those reported by the work carried out by Bhattacharya et al. (2003) and Guzella et al. (2005) in the Hugli estuary. Moreover, the highest detected concentration of organochlorine residue in water was ∑HCH. It ranged from 0.30 to 102.82 ng/L (mean 49.15 ng/L) with the frequency of detection 100%. When comparing the concentrations of HCH residues in the water sample (Table 5.2), it was observed that the residual levels reported in this study was lower than those recorded at the Cauvery river and Ganga river (Agnihotri et al., 1994; Abida et al., 2009), but were higher than those reported in the Gomti river and Tamiraparani river (Malik et al., 2009; Kumaraswamy et al., 2012). These remarkable variations may be due to the different application history and sources of OCIs in the study sites, and other environmental factors such as climatic conditions, the season of the year as well as the physicochemical characteristics of the water and the underlying sediments (Hellar-Kihampa, 2011).

As shown in Table 5.1, the predominant HCH isomer in the sediment sample was γ -HCH in Vypin zone, representing for 38.38% of the total HCHs, followed by α -HCH and β -HCH, accounting for 36.83% and 24.78% of the total HCHs, respectively. The compositional profile of HCH isomers in Munambam was similar to that of Vypin, and it accounts for 50.40%, 32.11% and 17.49% for γ-HCH, α-HCH, and β-HCH, respectively. But at Chettuva, γ-HCH was dominant isomer displaying 42.13% followed by β-HCH and α-HCH, exhibiting 30.12% and 27.75% of the total HCHs, respectively. These results suggested that

contamination of HCHs in the study area may be of point source pollution from the neighbourhood agricultural areas surrounding the sampling site where the chances of using these HCH compounds. HCH has two formulations, i.e., technical grade and lindane. The ratio of α-/γ-HCH is relatively stable, with values of 4–7 for technical HCH mixtures and values near zero for lindane (> 99% γ-HCH), The ratio of α- to γ-isomer (α / γ ratios) in the sediment samples range from 0 to 2.73 and this resulted value are well below those in the technical mixture, that implies the use of lindane in this region. Furthermore, the values of α/γ-HCH ratio in the water samples ranged from 0.56 to 1.68 with an average value of 1.19 and thus it could also be assumed that the presence of HCHs in this study area may be received as a recent input of lindane. The technical mixtures of HCH was produced and used in India until it was banned in 1997, and high concentration of lindane was observed in the sampling stations which may be due to the use in the agricultural area to control vector-borne diseases and previous research work of Srimurali et al. (2015) also found similar inference.

As hinted earlier, DDT and its primary breakdown product are highly lipophilic, persistent with a long half-life of 6 to 10 years and bioaccumulate in human body (Wolff et al., 2000; Longnecker, 2005; Jayaraj et al., 2016). Although the use of DDT has long been banned (UNEP, 2003), analysis of the OCIs in sediments revealed the presence of DDT at M1 and the degradation products of DDT (DDE at M4 and DDD at M1, V1, C4 and C1). At the same time, the analysis of the same in water revealed the presence of DDT at M2 and V3; DDE at V3 and DDD at C1, M2 and also at V3. Presence of p,p'-DDE in the sediment of station

M4 (3.10 ng/g) indicated a significant risk to human health due to their persistent character in the environment, more accumulative nature in the marine biota and its xenoestrogenic effects (Svobodova et al., 2003; Won et al., 2009). Although, the relative concentration of the parent DDT compound and its metabolites, DDD and DDE, are very useful in providing information on possible pollution sources. In this investigation, the concentrations of parent compound (o,p'-DDT) were always higher than their metabolite o,p'-DDD. As estimated, it contributed 55.3% of the total DDT from the sediment samples collected from M1 station. These results revealed that the site, M1 may be considered dramatically affected by organic pollution, indicating that these contaminants may be from the harbour related activities, such as fish feed residues (Yu et al., 2011) or urban activities which are trapped in the sediments nearby. The most possible DDT source in the fishing harbours is also by the impact of antifouling paints mainly used in boat maintenance in harbour areas (Lin et al., 2009; Xin et al., 2011).

To determine the source of DDT, o,p'-DDT/p,p'-DDT can also be used, since technical DDT and dicofol has different proportions of o,p'-DDT and p,p'-DDT. Thus, for technical grade DDT, the ratio o,p'-DDT/p,p'-DDT ranged from 0.2 to 0.3, whereas it ranged from 1.3 to 9.3 or higher for dicofol (Qiu et al., 2005). In the present study, ratio o,p'-DDT/p,p'-DDT was 4.5 at M1, suggested that the application of dicofol-type DDT may have occurred in the same station. Although the high concentration of biological metabolites p,p'-DDD at C1, o,p'-DDD at C4 and p,p'-DDE at M4 as the derived metabolites of the parent DDTs indicated that DDT contamination was mainly from the aged and

weathered agricultural soils as similar research work reported earlier (Zhou et al., 2006; Eqani et al., 2011). Besides, the ratio of the content o,p'-DDT/o,p'- DDD also provides a useful index to assess whether DDT at a given site is fresh or aged. A value below 0.33 generally indicates an aged input (de Mora et al., 2004). This o,p'-DDT/o,p'- DDD ratio obtained from M1 is 4.51 and this high value in the sediment sample suggests the recent usage of this insecticide in the surrounding agricultural areas or for boat maintenance.

The residue of heptachlor was detected in all water samples, constituted 21.02% of total organochlorine residues. Level of heptachlor was ranged between 0.16 and 31.85 ng/L (average 19.14 ng/L), whereas heptachlor epoxide was found at a low level with the frequency of detection was only 13.51%, it varied between ND and 6.85 ng/L (average 2.60 ng/L). However, the average concentration of heptachlor occasionally within the WHO guideline value (100 ng/L). Moreover, the heptachlor in the sediment samples fluctuated between ND to 9.17 ng/g (average 4.75 ng/g) and heptachlor epoxide was extended between ND to 4.21 ng/g (average 2.07 ng/g). The concentration of heptachlor did not show a significant spatial variation in the studied sites. A previous study in the Cochin estuary reported values similar to the present study, where heptachlor concentration having a mean value of 6.46 ng/g (Akhil, 2014). The concentration of heptachlor detected in the present study revealed the continuing use for the insect control and also in the seed and wood preservation. Their presence can also be related to surface runoff from agriculture cropland and also through urban areas, municipal and

industrial effluents as well as by atmospheric deposition (Zhou et al., 2006).

Generally, aldrin, the probable carcinogen is mainly used as an insecticide for the control of termites (Mukherjee and Gopal, 2002). Aldrin was detected in most of the water samples and accounts for 81.8% and dieldrin concentrate only 9.1% of the total samples. However, no dieldrin residues were detected in water samples collected from Munambam and Vypin zone (Table 5.1). Total aldrin residues (aldrin + dieldrin) in the study (15.63 ng/L) were less than those found by Malik et al. (2009) from the water of Gomti river and also by Kumarasamy et al. (2012) from the water of Tamiraparani river. Measured concentrations were all below the WHO guidelines for drinking-water quality, which recommends a maximum of 30 ng/L for the compounds (WHO, 2006). Although in the sediment samples, the residue level of aldrin ranged from ND to 10.13 ng/g. Aldrin transformed to more toxic persistent dieldrin at C1, C2 and M4 (4.99, 4.20 and 4.17 ng/g respectively) through microbial oxidation (WHO, 2003). According to Akhil and Sujatha (2014), the persistent nature of heptachlor and aldrin in the regions indicated its direct input to the aquatic environment from nearby point sources, followed by rapid burial and preservation under anoxic sediments.

In India, the use of dieldrin was restricted in May 1990 and was completely banned in July 2003 and endrin was banned in May 1990 (Pandey et al., 2011). In the present study, low amount of endrin and dieldrin were detected as 0.37 ng/g; 1.21 ng/g and 0.07ng/L; 0.84 ng/L in the sediment and water samples respectively. The relatively higher

concentrations of these two compounds in the sediment samples was attributed to the fact that both endrin and dieldrin are not very soluble in water and therefore more likely to adsorb in sediments (Konstantinou et al., 2006; Akan et al., 2014). The spatial distribution of OCIs was site-specific and the maximum concentration of OCIs were found in the water sample (189.76 ng/L) collected from M2, near the prawn farm. In the case of sediment samples, higher concentration (75.28 ng/g) was observed at C1 near poultry-yard. The most striking point of observation is that almost all the pesticides were detected in the sediment sample from M1 (total of 57.26 ng/g), being contaminated with harbour discharge as well as domestic sewage around Munambam harbour. The total OCIs detected in the Vypin zone were 237.73 ng/L from water and 97.68 ng/g from sediment samples respectively and these residues may be arising from accidental or intentional discharges of pesticides and pesticide waste from manufacturing plants situated near the vicinity (Dsikowitzky et al., 2014). Aside from these data, analysis of variance (One-way ANOVA) records no significant spatial variation and this outcome illustrates that meteorological input, such as precipitation, has influenced not only on the occurrence of pesticide compounds in surface waters and sediments but also on the concentration levels of pollutants as well.

Table 5.2: Organochlorine residues in water (ng/L) samples from different part of India

	α-HCH	β-HCH	γ-HCH	Heptachlor	Heptaepoxide	Aldrin	Dieldrin	Endrin	p,p'-DDE	o,p'-DDD	p,p'-DDD	o,p'-DDT	p,p'-DDT	Reference
Central Kerala	0.06-41.76 (15.10)	0.19-46.54 (20.80)	ND-46.44 (13.24)	0.16 - 31.85 (19.13)	ND- 6.85 (2.60)	ND-23.72 (14.79)	ND-9.21 (0.84)	ND-0.80 (0.07)	ND-0.07 (0.01)	ND-7.37 (1.41)	ND-15.71 (1.44)	ND-0.30 (0.03)	ND-17.25	In this study
Gomti river	BDL-44.58 (6.64)	BDL-301.4 (24.21)	BDL-63.38 (8.25)	BDL-29.64 (5.14)	BDL-5.36 (0.16)	BDL-77.93 (13.20)	BDL-22.45 (5.72)	BDL-4.25 (0.17)	BDL-10.20 (0.83)	NA	BDL-15.57 (0.65)	BDL-68.87 (4.07)	BDL-133.76 (0.42)	Malik et al., 2009
Cauvery river	BDL-8.70	BDL-560 (288.3)	BDL-930 (520)	NA	NA	NA	NA	NA	BDL-960 (311.66)	NA	BDL-530 (303.33)	NA	BDL-640 (490)	Abida et al., 2009
Tamiraparani river	<0.01	<0.05	<0.06-0.78	<0.06-0.33 (0.31)	NA	<0.02	<0.03-2.28	<0.02	<0.01-0.02	<0.02-0.05	<0.01-0.2	NA	<0.01	Kumarasamy et al., 2012
Ghaggar river	NA	21.13-241.6 (115.60)	BDL-48.72 (4.06)	NA	NA	NA	NA	NA	27.33-367.5 (105.03)	BDL-5.21 (1.46)	9.29-104.12 (48.53)	BDL-7.19 (3.01)	165.62-675.41 (426.18)	Koushik et al., 2010
Ganga river	13-312 (107.4)	BDL-181 (60.7)	BDL-535 (76.4)	BDL-412 (36.7)	NA	BDL-99 (25)	BDL-49 (12.7)	NA	BDL-532 (48)	NA	NA	BDL-346 (72.7)	BDL-431 (128.7)	Agnihotri et al., 1994

BDL- below detectable limit; NA- Not available ;ND-Notdetected

Table 5.3: Organochlorine residues in Sediment (ng/g) samples from different part of India

	α-HCH	β-HCH	γ-HCH	Heptachlor	Heptaepoxide	Aldrin	Dieldrin	Endrin	p,p'-DDE	o,p'-DDD	p,p'-DDD	o,p'-DDT	p,p'-DDT	Reference
Central Kerala	0.02-9.35 (6.17)	ND-12.37 (4.51)	ND-22.64 (8.23)	ND-9.17 (4.75)	ND-4.21 (2.07)	ND-10.13 (6.52)	ND-4.99 (1.21)	ND-4.07 (0.37)	ND-3.10 (0.28	ND-3.67 (0.97)	ND-7.51 (0.68)	ND-15.73 (1.43	ND-9.22 (0.84)	In this study
Hugli estuary	BDL-0.003	BDL-0.25 (0.13)	BDL-0.16 (0.01)	NA	NA	NA	NA	NA	BDL-0.008 (0.003)	NA	NA	BDL-0.03 (0.003)	BDL-0.11 (0.044)	Battacharya et al., 2003
Hugli estuary	0.05-0.26 (0.14)	<0.05-0.06 (0.06)	0.06-0.15 (0.1)	NA	NA	NA	<0.05	<0.05	<0.01-0.40 (0.17)	<0.01-0.08 (0.04)	<0.01-0.35 (0.14)	0.04-0.14 (0.1)	0.12-1.29 (0.53)	Guzella et al., 2005
Cochin estuary	NA	NA	NA	BDL-63.6 (6.46)	BDL-69.4 (4.06)	BDL-33.2 (2.43)	BDL-142.4 (10.58)	BDL-103.1 (3.49)	BDL-21.5 (2.47)	BDL-42.7 (4.31)	BDL-.(6.94)	NA	BDL-162.5(9.56)	Akhil, 2014
Yamuna river	0.24-25.49 (10.08)	0.31-30.27 (9.43)	0.37-27.85 (11.04)	0.79-25.23 (8.96)	2.09-78.18 (24.73)	1.05-29.1 (11.66)	5.49-81.8 (21.81)	12.14-67.63 (26.82)	NA	NA	NA	NA	NA	Pandey et al., 2011
East and west coast of India	BDL-721.88	BDL-251.7	BDL-55.6	NA	NA	BDL-3.00	NA	NA	NA	NA	NA	NA	NA	Pandit et al., 2001
Gomti river	BDL-32.06 (3.14)	BDL-14.88 (1.73)	BDL-30.39 (2.52)	BDL-40.44 (3.85)	BDL-7.18 (0.21)	BDL-10.89 (0.98)	BDL-1.65 (0.19)	BDL-11.96 (0.54)	BDL-14.03 (0.99)	NA	BDL-95.73 (6.24)	BDL-345.66 (23.94)	BDL-206.12 (18.67)	Malik et al., 2009

BDL- below detectable limit; NA- Not available ;ND-Notdetected

Overall, the contamination levels of OCIs in the sediments were higher than those in the water. The lindane was detected in a higher concentration of 90.48 ng/g in sediment, whereas 145.68 ng/L level was occurring in the water sample. This result suggested that lindane is more prevalent and persistent in the sediments than in water. Similarly, aldrin, heptachlor, heptachlor epoxide and dieldrin in the sediment samples were also having the highest value of 71.77, 52.30, 22.91 and 13.35 ng/g compared to surface water (162.72,210.53, 28.62, 9.21 ng/L respectively). The organic matter contents in sediment (Gupta et al., 2016) and low solubility of OCI in water could have been the main factors of the recorded organic contamination in sediment. From the observation, it is asserted that the fine texture of the sediments, their anoxic properties and the presence of both the autochthonous and allochthonous forms of organic matter are all directly related to the enrichment by means of trace organic compounds (Sarkar et al., 2007).

5.2.2 Potential source analysis of OCIs

5.2.2.1 Correlation of OCIs with characteristics of sediments and water

The results of the correlation analysis of OCIs with sediment properties are listed in Table 5.4. In this study, significant correlations were observed between DDT with HCH and endrin ($p<0.05$, $r =0.689$; $r =0.704$). In addition, no significant correlations exist between OCIs with clay, silt, sand and TOC contents except the negative correlation of dieldrin with silt ($P<0.05$, $r=-0.634$) and positive correlation of heptachlor with sand ($P<0.05$; $r=0.603$). In the aquatic environment, the total organic carbon (TOC) in sediments was an important factor that control the

distribution of OCI compounds. Basically, high OCIs concentration are accompanied by the greater TOC content and OCIs adsorbed on to the particulate matter due to their high affinity for organic matter (Mai et al., 2002). However, this correlation was not achieved in this work and this inconsistency was also reported in other previous studies (Mai et al., 2002; Tan et al., 2009). Besides, the adsorption of OCIs in the sediment is also largely related to the particle size of sediments. Though only a low significant correlation was found between all OCIs and the proportion of fine sediments suggest that high concentrations of contaminants at specific sites are more related to the anthropogenic influence than grain size.

The relationship between the physicochemical properties (pH, temperature, salinity, DO and nutrients) and the OCIs concentration in the surface waters of the Central Kerala was also described using a Pearson product–moment correlation coefficient (r) (Table 5.5). Silicate achieved significant moderate correlation with HCH ($r = 0.625$, $p<0.05$) and heptachlor epoxide ($r = 0.645$, $p<0.05$). Similarly, a significant correlation was observed between phosphate with heptachlor epoxide ($r = 0.917$, $p<0.01$) and nitrite with endrin ($r = 0.668$, $p<0.05$). The strong positive correlation between these parameters may indicate that an increase in the concentration of silicate, phosphate and nitrite could have enhanced the adsorption of the insecticides. In addition, a negative significant correlation exists between ammonia with dieldrin ($r = 0.741$, $p<0.01$); pH with endrin ($r = 0.622$, $p<0.05$) and DO with endrin ($r = 0.603$, $p<0.05$) showing an increase in ammonia, pH and DO resulted in the decreased concentration of respective OCIs and vice versa.

Table 5.4: Pearson correlations of OCIs with general sedimentary parameters

	Sand	Silt	Clay	TOC	HCH	Heptachlor	Aldrin	Heptepox	Dieldrin	Endrin	DDT
Sand	1										
Silt	-.773**	1									
Clay	-.855**	.838**	1								
TOC	-.805**	.831**	.759**	1							
HCH	0.41	-0.261	-0.454	-0.337	1						
Heptachlor	.603*	-0.363	-0.343	-0.481	0.597	1					
Aldrin	-0.074	-0.33	-0.064	-0.15	0.319	0.033	1				
Heptepox	0.082	-0.468	-0.341	-0.302	0.272	0.115	0.367	1			
Dieldrin	0.455	-.634*	-0.579	-0.242	0.492	0.16	0.466	0.426	1		
Endrin	0.024	0.461	0.032	0.314	0.388	0.295	-0.418	-0.487	-0.193	1	
DDT	0.115	0.189	-0.045	0.185	.689(*)	0.547	0.055	-0.155	0.167	.704*	1

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

Table 5.5: Pearson correlation analysis of OCIs in the surface water sample with physicochemical parameters

	HCH	Heptachlor	Aldrin	Heptepox	Dieldrin	Endrin	DDT	Ammonia	Silicate	Phosphate	Nitrite	Nitrate	pH	TEMP	Salinity	DO
HCH	1															
Heptachlor	0.585	1														
Aldrin	0.449	0.468	1													
Heptepox	0.315	0.434	0.5	1												
Dieldrin	-0.069	0.073	0.162	0.317	1											
Endrin	-.921**	-0.482	-0.5	-0.27	-0.1	1										
DDT	0.043	0.101	0.142	0.473	0.41	0.069	1									
Ammonia	0.056	-0.163	-0.22	-0.25	-0.741**	-0.021	-0.25	1								
Silicate	0.625*	0.453	0.44	0.645*	0.271	-0.589	0.046	-0.292	1							
Phosphate	0.181	0.323	0.434	0.917**	0.352	-0.256	0.388	-0.268	0.47	1						
Nitrite	-0.536	-0.106	-0.15	-0.21	-0.05	0.668*	-0.075	-0.512	-0.27	-0.206	1					
Nitrate	-0.142	-0.256	-0.56	-0.32	-0.121	0.324	-0.001	-0.361	-0.12	-0.314	0.638*	1				
pH	0.484	0.074	0.151	0.137	0.113	-.622*	0.03	0.452	0.381	0.088	-.946**	-0.627*	1			
TEMP	0.057	-0.126	-0.13	-0.52	-0.57	0.091	-0.495	0.048	-0.01	-.604*	0.49	0.565	-0.37	1		
Salinity	-0.091	-0.353	-0.55	-0.44	-0.494	0.366	0.006	0.133	-0.28	-0.546	0.419	0.794**	-0.42	0.58	1	
DO	0.59	0.227	0.287	-0.05	0.108	-.603*	0.264	0.221	0.186	-0.191	-.634*	-0.373	.670*	-0.06	-0.2	1

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

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

5.2.2.2 Factor Analysis

Principal Component Analysis (PCA) was carried out to identify the possible sources of OCIs, and the component matrixes were rotated using the Varimax method. Four principal components with eigenvalues above one was extracted, accounting for 88.96% of the total variance (Table 5.6). PC1 was correlated well with silt, TOC, clay, o,p'-DDD, o,p'-DDT, p,p'-DDT, heptachlor epoxide and endrin, as o,p'-DDT was the major impurity in dicofol and they could be degraded into o,p'-DDD; thus, PC1 might reflect the source of dicofol (Brown et al.,1986). PC2 was highly associated with clay, β-HCH, heptachlor and aldrin indicating that all of these OCI isomers might originate from a similar kind of sources. PC3 had significant loading for aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, endrin, o,p'-DDT and p,p'-DDT, which illustrated that they might have another common source. The fact that DDT and its metabolites had the highest loading on PC1 and PC3, suggested that PC1 and PC3 were mainly related to adsorption of OCIs to TOC, as DDT and its metabolites had relatively lower water solubility and vapour pressure than HCH isomers (Zhang et al., 2017). PC4 had a strong correlation with β-HCH, γ-HCH and negative correlation with α-HCH. It was similar to the composition of fresh lindane and therefore, PC4 was mainly related to lindane sources.

Table 5.6: Result of Principal Component Analysis

	Component			
	1	2	3	4
% of variance	36.09	23.9	18.09	10.88
Cumulative % of variance	36.09	59.99	78.08	88.96
correlation coefficient				
Sand	-0.612	-0.689		
Silt	0.887			
Clay	0.481	0.752		
TOC	0.678		0.662	
α -HCH	-0.579		0.478	-0.491
β-HCH	-0.644	0.545		0.418
γ-HCH	-0.439	0.468		0.670
Heptachlor		0.735		
Aldrin		0.581	0.613	
Heptepox	0.705		0.607	
p,p' -DDE			0.532	-0.666
Dieldrin	-0.540		0.702	
Endrin	0.760		0.521	
o,p' -DDD	0.598			
p,p' -DDD	-0.461		0.404	0.759
o,p' -DDT	0.760		0.521	
p,p' -DDT	0.760		0.521	

Note: Only Factor loadings > ±0.40 are shown

5.2.2.3 Cluster Analysis

Hierarchical cluster analysis identified sampling sites into three regions based on spatial similarities in texture and TOC parameters and OCI residual concentrations. Region 1 consisted of sites C4, V3 and M3,

OCIs concentration were measured in considerably low concentration in this region and the sediments at this site were also dominated by clay and TOC. Region 2 comprised four sites (C1, C2, C3, M1) which were characterized by relatively higher amounts of OCIs at this site were also were dominated by sand. M1 station was located near Munambam harbour, receives harbour discharge as well as domestic sewage around the station. Sites C1, C2 and C3 are from Chettuva zone receive agricultural runoff from adjoining cropland areas of banana, tapioca and grains and also from domestic wastes. Heptachlor was detected in considerably higher concentration at sites C1, C3 and M1, indicating their usage in large quantities in the rice- growing areas. Region 3 consisted of four sites (V1, V2, M2, M4), OCIs detected were in the average range. Stations V1 and V2 are in the Vypin zone and the OCIs contamination in the Vypin zone appears to be primarily due to the effluents from nearby industries and may it from adjoining agricultural fields, whereas the presence of OCIs at M2 and M4 may from agricultural or domestic sources.

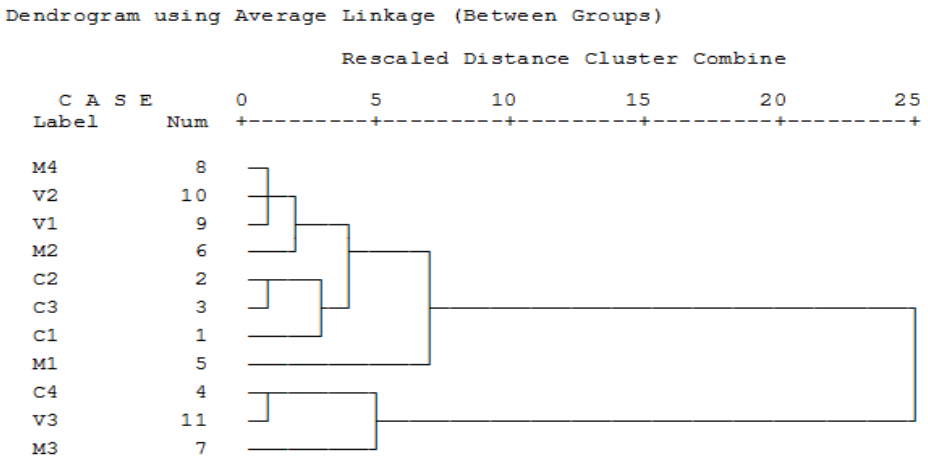

Fig. 5.2: Hierarchical dendrogram of sampling sites

5.2.3 Potential ecological risk of OCIs

OCI concentrations in the evaluated sediment samples were compared with sediment quality guidelines to find out the potential risk to aquatic life in the studied sites (Table. 5.7). Potential environmental risk of OCIs residues was evaluated by two widely used sediment quality guidelines (SQG). They are Threshold Effect Level (TEL) and Probable Effect Level (PEL) of the Canadian Sediment Quality Guidelines (CCME, 1999), as well as the Effect Range-Low (ERL) and Effect Range-Median (ERM) of the US National Oceanic and Atmospheric Administration (NOAA) guidelines (Long et al., 1995). The ERL and ERM separated concentrations of a particular chemical into three ranges to represent adverse biological effects: "rarely", "occasionally" and "frequently be observed", respectively (Long et al., 1995). Likewise, Canadian Council of Ministers of the Environment (CCME) adopted TEL and PEL values to separated concentrations of a specified chemical into three ranges: "no effect range", "possible effect range" and "probable effect range" (CCME, 2002). The concentration of γ-HCH detected in the samples ranged from ND to 22.64 ng/g, which exceeded ISQGs, TEL (0.94 ng/g) and PEL values (1.38 ng/g), indicating severe contamination for lindane. Concentrations of dieldrin (ND to 4.99 ng/g) and heptachlor epoxide (ND to 4.21 ng/g) were also found to be well above their ERL and TEL values. Concentrations of ∑DDTs ranged from ND to 28.4 ng/g (average of 4.2 ng/g), which were far greater than the ERL (1.58 ng/g) value, showing an intermediate ranking of sediment toxicity within the neighbouring benthic community by the exposure of these OCIs. Concentration levels of p,p' -DDE was higher than ERL values at the M4 site. Meanwhile, the

concentration of endrin at M1 was greater than ERL and TEL values. The level of dieldrin exceeded the ERL value in Chettuva and Munambam zone, whereas lindane was greater than PEL and TEL in all the zones. Heptachlor epoxide was above the PEL values at Chettuva zone and greater than TEL at Munambam and Vypin zone. These results suggested that the highest possibility of ecotoxicological risk to the sediments dwelling species which are associated with exposure to ∑DDTs, lindane, heptachlor epoxide, endrin and dieldrin in this area. Although, the potential adverse biological effects, combined with the five OCI isomers (ie, γ-HCH, dieldrin, endrin, heptachlor epoxide and ∑DDT) were also evaluated by sediment quality guideline quotient (SQGQ) (Tian et al., 2013). At first, the PEL quotient (PELQ) for each contaminant, based on the published guideline values for sediments (namely the probable effects level: PEL), was calculated according to the formula (Long et al., 1998):

$$PELQi = \frac{C_i}{PEL}$$

where PEL is the guideline value for the contaminant i and Ci is the measured concentration of the same contaminant. Then the SQGQ were calculated for each site as:

$$SQGQ = \frac{\sum_{i=1}^{n} PELQi}{n}$$

where n is the total number of analysed contaminants whose sediment quality guidelines are available. According to the potential adverse biological effects of sediment contamination, three ranges of SQGQ were applied (Costa et al., 2011): no effects (SQGQ< 0.1),

moderate effects (0.1≤SQGQ<1), as well as high adverse biological effects (SQGQ≥1). As per the above equation, SQGQs for C1, C2, M1, M2 and V1 were above 1, indicating that OCIs in the sediments would cause high adverse biological effects. While SQGQs for C3, C4, M3, M4, V2 and V3 in surface sediments were grouped into 0.1≤ SQGQ<1, which suggested moderate biological effects in the sediments of these sites. Thus, monitoring and controlling OCIs are crucial and should be paid much more attention to these sampling sites. This highlights that sediments sampled from Central Kerala pose a serious threat to aquatic life, and urgent restoration and management are warranted to safeguard the aquatic system.

Table 5.7: Sediment Quality Guideline values of selected OCIs in the surface sediments

Compounds	SQGs, ng/g d.w			
	ERL	ERM	PEL	TEL
∑DDTs	1.58	46.1	51.7	NA
o,p' and p,p' -DDD	NA	NA	8.51	NA
o,p'-and p,p'-DDE	NA	NA	6.75	NA
o, p'-and p,p'-DDT	NA	NA	4.77	NA
p,p'-DDD	2	20	7.81	1.22
p,p'-DDE	2.2	27	3.74	2.07
p,p'-DDT	1	7	4.77	1.19
Endrin	0.02	45	62.4	2.67
Dieldrin	0.02	8	6.67	2.85
Lindane	NA	NA	1.38	0.94
Heptepox	NA	NA	2.74	0.6

NA- Not available

In order to assess the ecotoxicological risk of OCIs in water samples, all data were compared with Environmental Quality Standards (EQS) for priority substances in inland surface waters and coastal waters (US EPA, 2010). According to the environmental quality standard for surface water set by the European Union (2000/60/EC), the concentration of DDTs should not exceed 25 ng/L. In accordance with the China seawater quality guideline (GB3097-1997), the DDT concentration which is less than 50 ng/L is generally considered to pose no hazard to the ecosystem. In the present study, it was observed that the average concentration of DDTs in water from Central Kerala was 4.46 ng/L, which was lower than the above-mentioned environment quality standards, showing the ecological risk for DDT from the water phase is low. The present inferred data showed that the mean concentrations of HCH (49.15 ng/L), which was found to exceed the environmental quality standard of 20 ng/L of HCHs for surface water set by European Union (2000/60/EC). Similarly, ∑aldrin, dieldrin and endrin (15.70 ng/L) was higher than the EQS value of 10 ng/L, showing high ecological risk. While considering the individual concentration levels of aldrin (14.79 ng/L) was also found to be exceed the respective criteria levels of 0.13 ng/L, whereas dieldrin (0.84 ng/l) and endrin (0.07 ng/L) recorded in the present study were lower than criteria standards 65.1 ng/L, 61 ng/L for dieldrin and endrin as set by US EPA for protection of freshwater aquatic organisms. Thus, it appears that the average concentration of HCH and ∑aldrin, dieldrin and endrin residues in water from Central Kerala are more concern to the benthic organisms creating an adverse ecological risk to the marine ecosystem.

5.3 Conclusions

The composition of organochlorine insecticides varied significantly with different sampling sites. A high concentration of o,p'-DDT in sediments at M1 reflected the use of dicofol. Ratios between OCI isomers (α/γ HCH and o,p'-DDT/p,p'-DDT) and principal component analysis were used to verify the results of source apportionment of DDTs and HCHs. All the results indicated that the HCH pollution was from lindane sources, and DDT pollution input was due only from dicofol sources. Although, the concentration levels of p,p'-DDE, o,p'-DDD, p,p'-DDD, p,p'-DDT, o,p'-DDT, endrin and dieldrin at Chettuva and Munambam zone were higher than the sites at Vypin zone. Thus, the sediments at sites Chettuva and Munambam could potentially cause acute biological impairment. The results also highlight that OCIs contamination should be considered as an important environmental issue due to their excessive use in agriculture, domestic and industrial sector. Based on the sediment quality guidelines, lindane, ∑DDTs, heptachlor epoxide, endrin and dieldrin would be more concerned for the ecotoxicological risk. Based on the SQGQ, sediments in the C1, C2, M1, M2 and V1 exhibited a higher combined ecotoxicological risk than those of sediments in the other stations. Based on known toxicological data, the average concentration of HCH, ∑aldrin, dieldrin and endrin in the water samples were at concentrations high enough to exhibit a potential biological risk. The results indicated the existence of certain potential health hazards for the organisms and biomonitoring assessment is needed concurrently to evaluate the accumulation of insecticide in the food web. Thus, for providing a better insight into the health hazards of OCIs and heavy metals, the bioaccumulation process in the bivalves were discussed in Chapter 6.

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Chapter 6

STATUS OF ANTHROPOCENES IN THE BIVALVES

Contents

6.1 Introduction

Anthropogenic pollutants such as organochlorine insecticides (OCIs) and heavy metals contributes a great extend for causing pollution in the marine environment. Extensive use of such group of compounds has been restricted and banned years back because of their ability to bioaccumulate in the organisms. One common notable property of organochlorine compounds is their high solubility in the fatty component of the living matter (Al-Aamri, 2013). The contamination of marine ecosystems with these pollutants, both pesticides and metals, induce stress in marine life, especially in sedentary organisms such as bivalve molluscs (Sindermann, 2005). Marine organisms accumulate chemical pollutants in their tissues when the uptake processes from the environment are more active than the metabolic process or removal of these compounds from the body. The physicochemical properties of the contaminant, its distributions in the aquatic system and the feeding behavior, as well as the

metabolism of the marine organism are all factors which also affects the accumulation of these contaminants in the tissue of benthic organisms. Furthermore, these toxic contaminants bioaccumulated through different mechanisms: bioconcentration by the direct uptake from water, or biomagnification through the consumption of contaminated food. Humans, as a final link in the food chain, are mostly affected, and consequently the public has become increasingly concerned regarding the potential risk associated with the human health from the consumption of the aquatic live stock as a food source (Mwevura, 2002 (a); Thompson et al., 2017). For the protection of human beings and other nontarget organisms from the adverse effects of chemical exposure, it is important to understand the dynamic process of bioaccumulation and it has become a critical issue and have to focus on the regulation of chemicals. Therefore, the establishment of biomonitoring the chemical contaminant in the given ecosystem has become an important priority (Abd-Allah and Bream, 2001).

Marine molluscs such as oysters and clams are known to be the sentinel bio-indicator for monitoring the contaminants in the marine environment (Krishnakumar et al., 2018). The wide use of shellfish as biomonitor reflects not only due to their high ability to bioaccumulate organic and inorganic pollutants but also their extensive distribution in the aquatic realm. Besides, the bioaccumulation of heavy metals and OCIs into the biota is not only influenced by the chemical characteristics of the compounds and their surrounding environmental conditions, but also depends on the characteristics of the species like sex, age, body size, lipid content and health status of an organism (Schäfer et al., 2015;

Rajeshkumar and Li, 2018). In the present study, the types of bivalves collected included an edible oyster *C. madrasensis* from all the three zones, the clam species M. *casta* (MAC, VAC, CAC) from M1, V1 and C1 and V. *cyprinoides* (C1C, C2C, C3C, C4C) from Chettuva zone and from Vembanad Lake (S1C, S2C, S3C). Both clams and oyster are commercially important bivalves, because they maintain a part of fishery food resource in Kerala for both indigenous and export use. The major advantages for selecting these as biological indicators are due to the direct measurement of the organism to record the biological availability of pollutants than from water or sediments (Philipps, 1978; Parmar et al., 2016).

Shellfish represent an important protein source for the human diet. Regardless of these nutritional properties, the consumption of shellfish, particularly raw or undercooked, is not risk-free (Serratore, 2017). Ecological magnification in organisms of the food chain appears to be the most harmful environmental effect resulting from the indiscriminate usage of OCIs (Nair and Sujatha, 2012). Metals and pesticides accumulate in the tissues of bivalve mollusc at concentrations that can cause carcinogenic and mutagenic effects in the consumers. Furthermore, the threshold level contaminant concentrations in the bivalve tissues was more accurately reflect the magnitude of environmental contamination (Phillips, 1980, 1990). It could also provide reliable information on the potential impact of seafood consumption on public health (Fang, 2004; Otchere, 2005; Salem et al., 2014). Thus, understanding the bioaccumulation mechanisms in this aquatic organism is critical for predicting their potential toxicity to human health. No systematic study has been carried

out in Central Kerala for identifying the presence of pesticides in aquatic organisms like molluscs. Hence, to provide data on the residual levels of organic and inorganic contaminants, the concentrations of heavy metals and OCIs were determined, which have not been reported so far in Central Kerala.

6.2 Results and Discussion

6.2.1 Accumulation of OCIs in the bivalves

The results showed that the 13 OCIs were detected in the bivalves (Table 6.1) and among analysed OCIs in the shellfish, residual levels of ∑HCHs were predominant followed by ∑DDT, heptachlor, aldrin, heptachlor epoxide, dieldrin and endrin. The average concentrations of the OCIs detected were 44.23 ng/g, 110.39 ng/g and 165.67 ng/g in the M. *casta*, V. *cyprinoides* and C. *madrasensis* species respectively. No clear pattern was observed in the general spatial distribution of OCI levels in organisms due to the great intra-spatial and interspecies variability. The high OCI concentration was determined in the oyster C. *madrasensis* with an average of 163.87, 195.48 and 112.52 ng/g in the Vypin, Chettuva and Munambam zone respectively. In addition, the bivalves collected from Vypin zone have higher OCI content with an average of 161.90 ng/g. Furthermore, the highest concentration was estimated for C. *madrasensis* at V1 site (385.59 ng/g), the location adjacent to busy shipping channel. The lowest concentration was observed for M. *casta* (57.24 ng/g) collected from the same station. Contamination by organochlorine insecticides, especially by DDTs (200.13 ng/g), was present in this area due to the discharge of

effluents mixed with pesticides released by nearby industries and from agricultural runoff (Dsikowitzky et al., 2014).

The ∑HCH content accounted for 45.59% of total OCI concentration. Compared to other OCI compounds, HCHs revealed relatively a homogenous distribution pattern resulting from long-range transport. HCH compounds have high vapour pressure and they volatilize easily from the contamination sources and are redistributed through the atmosphere (Jiang et al., 2000). Because of this characteristic, HCH compounds were the most frequently detected OCIs in the study area. The ∑HCH content accounted for 43.00, 50.87 and 64.17% of total OCI concentration in the *C. madrasensis, V. cyprinoides* and in the *M. casta* species respectively. The maximum concentration of HCH measured in *C. madrasensis* was 151.37 ng/g at C3 site, which was far above the US EPA Screening Criteria reported as 70 ng/g ww (US EPA, 1995; Maret and Dutton, 1999). Furthermore, comparing ΣHCH levels in shellfish from around the world, it is notable that the concentrations estimated in the present study were in the lower end in relation to data from Indian coastal water (Monirith et al., 2003). Relating ΣHCH levels presented herein with the previous studies (Ramesh et al., 1990; Kan-Atireklap et al., 1998; Tanabe et al., 2000; Sankar et al., 2006; Pikkarainen, 2007), which indicated higher levels were recorded in the present study (Table 6.2). These findings suggested that in the recent years, the use of such compounds was frequent near the study area.

The combined DDT content was the second highest, accounting for 14.99% of the total OCI content in the bivalves. The ∑DDT content recorded 16.96, 9.38 and 6.10% of total OCI content in the *C. madrasensis*, *V. cyprinoides* and in the *M. casta* species respectively. The metabolites of DDTs in the seafood were primarily o,p'-DDT, p,p'-DDT, o,p'-DDD, p,p'-DDD and p,p'-DDE. The concentrations of o,p'-DDT was highest among the five DDTs isomers, which represents 31.34% of the total DDTs content and other metabolites having 25.42, 20.99, 13.24 and 9.00% of p,p'-DDD, p,p'-DDT, p,p'-DDE and o,p'-DDD respectively. A significant level of ∑DDTs contaminations in the present study was inferred as compared with the previous reports of OCIs in aquatic biota from the different regions of India (Ramesh et al., 1990; Kan-Atireklap et al., 1998; Tanabe et al., 2000; Sankar et al., 2006). These results revealed that DDT is still being used in the regions of Kerala, although the governments have banned the use of DDTs in 1983. The heptachlor concentration in bivalves collected from the studied environment was in the range of ND to 89.95 ng/g with an average concentration of 20.01 ng/g (Table 6.1). The heptachlor concentration was highest in the *C. madrasensis* having an average value of 26.60 ng/g and the lowest value was found in the *M. casta* with an average of 6.08 ng/g (Fig. 6.1). The levels of endrin were not detected from all the samples except for V. *cyprinoides* sampled from C1 (9.90 ng/g) and for *C. madrasensis* collected from V1 (18.42 ng/g).

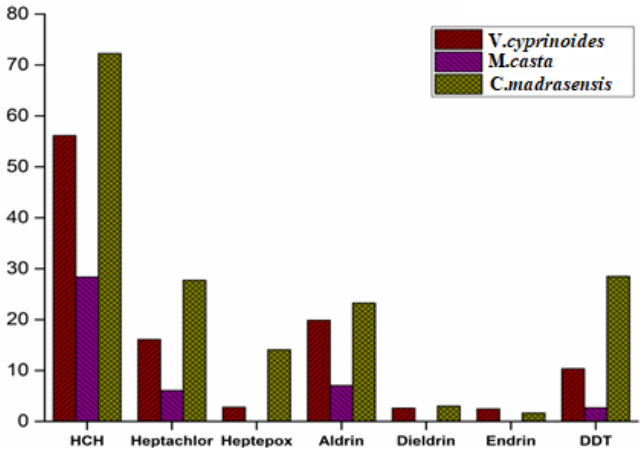

Fig. 6.1: Concentration of OCIs in the three bivalves collected from Central Kerala

The contributions of individual OCI compounds varied widely between marine species (Fig.6.1). From the results, it is clear that, all these marine bivalves had strong accumulation ability for HCHs, in addition, the *C. madrasensis* species exhibited strong accumulation ability also for DDTs, heptachlor, heptachlor epoxide and aldrin. Further, One-way ANOVA was used to set up the database of OCIs in bivalves and a value of $P<0.05$ was considered to indicate a significant difference in the level of OCIs in these species. The results indicated that ∑HCH, heptachlor, heptachlor epoxide and aldrin levels have significant difference ($P=0.04$, df=2; $P=0.002$, df=2; $P=0.02$, df=2; $P=0.04$, df=2 respectively) among *C. madrasensis*, *V. cyprinoides* and *M. casta;* whereas the other pollutant levels were not significantly varied between these species. These results suggested that the bioaccumulation of ∑HCH, heptachlor, heptachlor epoxide and aldrin in bivalves were highly species-specific, probably due to different ecological characteristics for different aquatic species such as feeding habits and habitats and earlier reports by Yang et al. (2006) also supported this view.

Table 6.1: OCIs concentration in the bivalves (ng/g)

Bivalve	Sample code	α-HCH	β-HCH	γ-HCH	Heptachlor	Hepta epoxide	Aldrin	Dieldrin	Endrin	o,p'-DDD	p,p'-DDD	o,p'-DDT	p,p'-DDT	p,p'-DDE
M. *casta*	VAC	36.29	12.81	8.14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MAC	10.91	ND	8.27	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	CAC	ND	ND	8.73	18.24	ND	21.21	ND	ND	ND	ND	ND	ND	8.09
C. *madrasensis*	C1M	35.15	9.08	40.65	44.04	5.13	25.79	ND	ND	ND	ND	ND	ND	ND
	C2M	7.19	17.80	16.02	16.87	15.72	36.39	12.85	ND	ND	ND	ND	ND	ND
	C3M	75.57	41.61	34.18	41.11	22.43	64.39	ND	ND	24.68	ND	ND	ND	ND
	C4M	19.48	18.40	47.46	89.95	19.99	ND	ND	ND	ND	ND	ND	ND	ND
	M1M	41.35	12.21	ND	14.17	5.33	17.63	ND	ND	ND	16.02	ND	ND	ND
	M2M	27.01	9.50	16.03	10.61	5.82	ND	ND	ND	ND	ND	ND	ND	7.67
	M3M	21.02	23.58	14.52	14.98	8.33	29.54	ND	ND	ND	ND	46.38	ND	10.77
	M4M	42.83	10.00	13.18	22.16	ND	27.86	ND	ND	ND	ND	ND	ND	7.80
	V1M	23.59	21.17	ND	12.06	35.41	54.24	20.57	18.42	ND	56.57	67.37	76.19	ND
	V2M	ND	19.01	42.78	ND	29.11	ND	ND	ND	ND	ND	ND	ND	ND
	V3M	12.34	20.74	61.35	11.54	7.89	ND	ND	ND	ND	ND	ND	ND	ND
V. *cyprinoides*	C1C	18.96	43.97	15.32	33.47	6.86	26.40	ND	9.90	ND	19.67	ND	ND	ND
	C2C	48.62	9.90	5.48	11.63	ND	19.50	10.51	ND	8.01	ND	ND	ND	8.26
	C3C	18.25	14.20	13.63	12.21	4.35	19.72	ND	ND	ND	ND	ND	ND	ND
	C4C	22.98	7.04	6.28	7.09	ND	13.85	ND	ND	ND	ND	ND	ND	5.48

ND-Not detected

Table 6.2: Organochlorine insecticide concentrations (ng/g wet weight) in bivalves from various geographical locations of India

Shell Fish	Compounds		Sample collection year	Reference
	∑DDT	∑HCH		
Green mussel (*Perna viridis*)	0.93-40 (10.96)	1.5-12 (5.82)	1994-1997	Tanabe et al., 2000
Green mussel (*Perna viridis*)	2.8-39 (17.72)	4.3-16 (7.49)	1988-1989	Ramesh et al., 1990
Green mussel (*Perna viridis*)	0.93-40 (10.9)	1.5-12 (5.83)	1994-1995	Kan-Atireklap et al., 1998
Shell fishes	BDL-0.16 (0.08)	BDL-26.8 (6.71)	2003	Sankar et al., 2006
Green mussel (*Perna viridis*)	29-3000 (373.88)	19-430 (119)	1994-2001	Monirith et al., 2003
Bivalve	ND-200.13 (20.17)	19.18-151.37 (64.46)	2014	In the present study

ND: Not detected; BDL: Below detectable limit

Numerous factors, both external and internal, affect the ability of seafood to accumulates OCIs. The presence of lipid content (LC) in the tissue of organism has been shown to be an important factor which determines the hydrophobic contaminant accumulation as they act as the primary storage site for these compounds (Muncaster et al., 1990; Kidd et al., 1998; Waszak and Dabrowsak, 2009). According to their observation, the relative size of the lipid pool affects the movement of these organic compounds into the biota tissues, implying that higher LC causes faster uptake and slower elimination. This gives rise to minimum accumulation of contaminants in the tissue having low LC as compared to high accumulation in those tissues with high LCs (Hanson et al., 1979; Oyo-Ita et al., 2014). As similar trend was observed in the present research work.

In the present study, the lipid content was 6.30 to 10.87% in the *V. cyprinoides*, 7.78 to 9.34% in the *M. casta* and 10.88 to 19.81% in the *C. madrasensis* species respectively (Table 6.3). The average lipid content of the bivalves was 12.26% on a wet weight basis and was significantly different across the species ($P=1.26x10^{-8}$, df=2) but insignificant spatial variation was reported ($P>0.05$).

Table 6.3: General characteristics of bivalves collected from Central Kerala

Zone	Bivalve	Sample code	Lipid (%)	Shell length(cm)
Chettuva	V. *cyprinoides*	C1C	9.88	4.00
		C2C	10.87	3.50
		C3C	7.67	3.20
		C4C	6.30	2.50
	C. *madrasensis*	C1M	19.81	5.90
		C2M	15.58	6.10
		C3M	13.33	4.30
		C4M	14.29	6.50
	M. *casta*	CAC	7.78	2.70
Munambam	C. *madrasensis*	M1M	11.33	13.30
		M2M	10.88	3.70
		M3M	17.38	3.10
		M4M	11.96	4.90
	M. *casta*	MAC	8.20	2.50
Vypin	C. *madrasensis*	V1M	17.33	8.50
		V2M	13.58	10.50
		V3M	15.13	6.40
	M. casta	VAC	9.34	2.30

Besides, the effect of LC on OCIs accumulation in sediment-dwelling organisms along with the effect of body size (shell length) was also examined. One-way analysis of variance (ANOVA) of OCIs concentrations in the bivalves was performed in order to test the significant relationship between LC and shell length of organisms with the OCI levels. The calculated F values showed 32.88 (df =1; $P=1.91X10^{-6}$) for LC and 36.80 (df= 1; $P=7.06X10^{-7}$) for shell length. Significant correlations were also observed between OCIs (HCH and heptachlor epoxide) concentrations and bivalve lipid contents ($r =0.497$. $P<0.05$; $r=0.679$. $p<0.05$) respectively (Table 6.4). In addition, significant correlation was also observed between shell length with HCH and heptachlor epoxide ($r= 0.502$, $P<0.05$; $r=0.682$, $P<0.01$). Hence, it was clear that lipid content and shell length are played a key role in the bioaccumulation of HCH and heptachlor epoxides (Table 6.4). Besides, the variations of other OCI concentrations (heptachlor, aldrin, dieldrin, endrin and DDT) were not ascribed to the lipid content of the bivalves investigated, which was supported by the insignificant correlation between OCI concentrations and lipid contents ($r=0.349$ for heptachlor; $r=0.143$ for aldrin; $r=0.302$ for dieldrin; $r =0.161$ for endrin; $r=0.125$ for DDT). Furthermore, the same OCIs were not significantly correlated with shell length ($r=0.346$ for heptachlor; $r=0.135$ for aldrin; $r=0.302$ for dieldrin; $r=0.16$ for endrin; $r=0.126$ for DDT). The variation of OCI concentrations other than HCH and heptachlor epoxides might be, therefore dependent on the presence of localized sources of contaminant rather than on the physiological variation of bivalves.

Table 6.4: Pearson correlation of OCIs in the bivalves with lipid and shell length

	HCH	Heptachlor	Heptaepoxide	Aldrin	Dieldrin	Endrin	DDT	Lipid	Shell length
HCH	1								
Heptachlor	0.341	1							
Hepta epoxide	0.494*	0.303	1						
Aldrin	0.025	0.535*	0.071	1					
Dieldrin	-0.052	0.085	0.222	0.404	1				
Endrin	0.046	0.144	0.344	0.323	0.623**	1			
DDT	0.032	0.301	0.127	0.603*	0.355	0.561*	1		
Lipid	0.497*	0.349	0.679*	0.143	0.302	0.161	0.125	1	
Shell length	0.502*	0.346	0.682**	0.135	0.302	0.163	0.126	1**	1

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

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

6.2.1.1 Relationship between water and sediment loadings with tissue concentrations

6.2.1.1.1 Bio-concentration factor (BCF) of organochlorines

Bioconcentration of compounds in biota is the process by which chemicals are enriched in the organisms relative to the water in which they reside. This parameter describes the ability of a chemical to concentrate on the aquatic organisms. As already mentioned, bioaccumulated hydrophobic organic pollutants are associated with the lipid content of biota. The freely dissolved chemical concentration in water is only able to pass through biological membranes and is readily bioavailable for the uptake by the organism. Thus, the BCF is usually calculated from the measured total water concentration and is independent of the organic matter in the water column (Arnot and Gobas, 2006). BCF was calculated (Zhou et al., 2008) according to the following equation

$$BCF=\frac{[Bivalve]/lipid}{[Water]}$$

By convention, a BCF value lower than 250 is considered as having a low potential for bioconcentration. Hence, BCF was calculated in the different sampling sites (Table 6.5) and its average value extended from 3.13 to 714.32 in *C. madrasensis* and 0.76 to 10.51 in the *M. casta* species. Likewise, the mean BCF in the *V. cyprinoides* collected from Chettuva zone was 56.44. For ∑HCH, heptachlor and heptachlor epoxide, the residual levels exist in the tissues of *C. madrasensis* at V3 was relatively higher (2112.27, 462.78 and 996.53 respectively) than 250, the bioconcentration cut off point, which exhibited its high abilities for bioaccumulation relative to other selected molluscs. Although, the BCF

deduced in the present study for the *C. madrasensis* were higher than those for the *M. casta* and *V. cyprinoides*. This could be attributed to the fact that *C. madrasensis* being larger in size which might have higher lipid content and thereby, prone to bioaccumulate more organochlorines.

Table 6.5: Bio Concentration Factor of organochlorines in the bivalves

	Zone	Bivalve	Sample code	∑HCH	∑DDT	Heptachlor	Heptepox	Aldrin
BCF	Chettuva	V.*cyprinoides*	C1C	29.77	27.02	27.02	13.53	15.23
			C2C	17.58		3.36		7.56
			C3C	13.61		13.81	10.99	13.34
			C4C	16.48		3.87		12.59
		C.*madrasensis*	C1M	32.30		35.55	10.11	14.88
			C2M	11.27		4.87		14.11
			C3M	44.73		46.50	56.65	43.57
			C4M	38.75		49.07	52.68	
		M.*casta*	CAC	4.22	14.10	18.69		15.54
	Munambam	C.*madrasensis*	M1M	5.17		4.30		
			M2M	4.70	1.75	4.69	7.81	
			M3M	12.53		10.09		
			M4M	13.19		9.89		10.49
		M.*casta*	MAC	7.64				
	Vypin	C.*madrasensis*	V1M	4.81		2.60		13.51
			V2M	5.40			39.72	
			V3M	2112.27		462.78	996.53	
		M.*casta*	VAC	3.82				

6.2.1.1.2 Biota Sediment Accumulation Factor (BSAF)

The Biota sediment accumulation factor (BSAF) is a screening tool also used to detect and predict the bioaccumulation potential of lipophilic compounds. It is a valuable tool index adopted in the present study and

this attempt provided a bioavailability profile of OCIs in the aquatic biota and BSAF was calculated according to the equation (Ankley et al., 1992):

$$BSAF=\frac{C_b / f_1}{C_s / f_\infty}$$

Where Cb is the biota contaminant concentration, f_1 is biota lipid concentration, Cs is the sediment contaminant concentration and f_∞ is the organic carbon fraction of the sediment. OCI concentration in bivalves is calculated as ng/g of bivalve; lipid content of bivalve is calculated as g lipid/g of bivalve; OCI concentration in sediment is calculated as ng/g of sediment; organic carbon in sediment is calculated as g TOC/g of sediment.

BSAF of *C. madrasensis* was much higher than those in *M. casta* in all the zones (Table 6.6). The calculated BSAF values for ∑HCHs were in the range 0.01 to 3.83, with the highest value found in *C. madrasensis* at the C4 site, even though the associated sediment exhibited lower ∑HCH concentration. On the other hand, the lowest BSAF value was observed in *M. casta* from the C1 site, with higher associated sedimentary ∑HCH concentration. Similarly, the BSAF values for heptachlor ranged from 0.02 to 389.58, with the highest value observed in *C. madrasensis* at the M3. The lowest BSAF value for heptachlor was found in *C. madrasensis* at V1. Bioaccumulation is implied when a BSAF is significantly greater than one. A theoretical value of 1.7 has been estimated based on the partitioning of non-ionic organic compounds between tissue lipids and sediment carbon (ASTM, 1997). A value of less than 1.7 indicates less partitioning of the organic compound into lipid profile than the predicted value, whereas a value greater than 1.7 reflected more uptake of the compound than can be explained by the partitioning theory alone

(Brunson et al.,1998; US EPA, 1998; Ozkoc et al., 2007). In the present investigation, the BSAF for ∑HCH was 3.83, 2.99 and 2.27 in the *C. madrasensis* collected from C4, M3 and V3 sites respectively; for heptachlor, it was 9.07 and 389.58 in the *C. madrasensis* collected from C4, M3 and in the same species, BSAF of heptachlor epoxide was 4.69 and 29.02 from C4 and M1. These values were significantly greater than the theoretical benchmark value of 1.7. This higher BSAF value may be due to the exposure to contaminants in the overlying water, or due to the spatial differences in sediment contamination. The BSAFs calculated except than this, were well below the cut-off point (1.7), pointed out the fact that ∑DDT, aldrin, dieldrin and endrin were in safe limit.

Table 6.6: Biota Sediment Accumulation Factor of organochlorine insecticides in bivalves

	Zone	Bivalve	Sample code	∑HCH	∑DDT	Heptachlor	Heptepox	Aldrin	Dieldrin
BSAF	Chettuva	V.*cyprinoides*	C1C	0.05	0.07	0.13	0.07	0.07	
			C2C	0.14		0.23		0.11	0.12
			C3C	0.12		0.06	0.04		
			C4C	1.63	0.98	0.71		0.93	
		C.*madrasensis*	C1M	0.05		0.17	0.05	0.07	
			C2M	0.09		0.34	0.19	0.2	0.15
			C3M	0.39		0.19	0.2		
			C4M	3.83		9.07	4.69		
		M.*casta*	CAC	0.01	0.04	0.09		0.07	
	Munambam	C.*madrasensis*	M1M	0.53	0.18		29.02	0.65	
			M2M	0.14		0.06	0.1		
			M3M	2.99		389.58			
			M4M	0.71	0.47	0.45		0.63	
		M.*casta*	MAC	0.78					
	Vypin	C.*madrasensis*	V1M	0.02	0.75	0.02	0.19	0.08	
			V2M	0.06					
			V3M	2.27			0.95		
		M.*casta*	VAC	0.20					

6.2.2 Partitioning of metals in bivalves

Knowing the characteristics of sediments is not enough to predict clearly the bioavailability of metals. Benthic species such as bivalves have been utilized as biomonitor to find out the metal availability in marine environments. The concentrations, expressed on a dry weight basis of metals (Ni, Fe, Zn, Mn, Cd, Cu, Pb and Co) in three species of bivalves are shown in Table 6.7. The pattern of heavy metal content occurrence in the soft tissue part of the three bivalve samples is in the following order: Fe> Zn> Cu> Pb> Mn> Co> Ni> Cd for C. *madrasensis*; Fe> Mn> Zn> Pb> Ni> Cu> Co> Cd for M. *casta* and Fe> Pb> Zn> Mn> Ni> Co> Cu> Cd for V. *cyprinoides* (Table 6.7; Fig. 6.2). Fe is a vital element for nearly all living organisms due to its role in various metabolic processes (Rout and Sahoo, 2015). A high content of Fe was observed in all the species. The range of heavy metals in C. *madrasensis* were 39.34 to 439.48 mg/kg (average: 122.41mg/kg) for Cu, 3.05 to 12.54 mg/kg (average: 7.91 mg/kg) for Cd, 34.33 to 91.15 mg/kg (average: 61.84 mg/kg) for Mn, 33.68 to 174.52 mg/kg (average: 88.02 mg/kg) for Pb, 25.28 to 136.21 mg/kg (average: 58.47 mg/kg) for Co, 23.08 to 422.04 mg/kg (average: 140.10 mg/kg) for Zn, 26.53 to 128.97 mg/kg (average 58.30 mg/kg) for Ni and 566.36 to 1291.94 mg/kg (average: 854.61 mg/kg) for Fe. The range and average concentrations in V. *cyprinoides* were 14.28 to 16.58 mg/kg (average: 15.74 mg/kg) for Cu, 1.86 to 3.04 mg/kg (average: 2.27 mg/kg) for Cd, 25.83 to 28.70 mg/kg (average: 26.85 mg/kg) for Mn, 34.90 to 41.10 mg/kg (average: 36.81 mg/kg) for Pb, 23.79 to 25.89 mg/kg (average: 24.79 mg/kg) for Co, 30.29 to 34.68 mg/kg (average: 32.90 mg/kg) for Zn, 23.41 to 26.25 mg/kg (average: 25.01 mg/kg) for Ni and 584.10 to 724.31 mg/kg

(average: 624.42 mg/kg) for Fe. Furthermore, the concentration in the M. *casta* were 19.15 to 40.66 mg/kg (average: 33.46 mg/kg) for Cu, 2.47 to 21.56 mg/kg (average: 9.26 mg/kg) for Cd, 38.02 to 123.56 mg/kg (average: 89.97 mg/kg) for Mn, 32.93 to 81.08 mg/kg (average: 54.57 mg/kg) for Pb, 22.43 to 46.15 mg/kg (average: 30.99 mg/kg) for Co, 34.46 to 116.87 mg/kg (average: 66.76 mg/kg) for Zn, 25.76 to 69.07 mg/kg (average: 52.00 mg/kg) for Ni and 546.67 to 700.90 mg/kg (average: 627.74 mg/kg) for Fe (Table 6.7; Fig. 6.2).

As concentrations displayed slight variations with regard to the three kinds of species and the concentrations of Cu, Zn and Pb were significantly higher ($p<0.05$, ANOVA) in C. *madrasensis* than M. *casta* and V. *cyprinoides*. Whereas, Cd and Mn level in M. *casta* was significantly higher than that in C. *madrasensis* and V. *cyprinoides*. The pollution levels in the marine organisms presented a characteristic feature according to the species selected, due to the heavy metal sources and the different capacities of the organism for metal accumulation (Villar et al. 1999; Jeng et al., 2000). The highest average concentrations of metals in the soft tissue of C. *madrasensis* were recorded at V2, followed by M2, M3 and C3 with a content of 280.79, 240.73, 230.47 and 183.95 mg/kg respectively. The maximum concentrations of Pb (174.52 and 147.71 mg/kg) were recorded in C. *madrasensis* at V2 and M2 followed by M3 and C1 (119.51 and 114.29 mg/kg). The enrichment of Cd was much higher in the M. *casta* than the other two kinds of species such as C. *madrasensis* and V. *cyprinoides* having an average value of 9.26, 7.91 and 2.26 mg/kg respectively. The high concentrations of Cd in the bivalves may be from land-based inputs of fertilizers and pesticides from agricultural activities

and anticorrosive paints applied on ships. These contributes to a great extend in Cd enrichment (Cheriyan et al., 2015; Ragi et al., 2017). The accumulation of Mn has also followed the same pattern and the average concentration in the M. *casta*, C. *madrasensis* and V. *cyprinoides* were 89.97, 61.84 and 26.85 mg/kg respectively.

Bivalve exhibited a wide range of body Cu concentrations with significant differences between species ($P<0.05$; $P=0.03$, $df=2$). Average higher concentrations of Cu were found in C. *madrasensis* (122.41 mg/kg), in which significant increased levels were observed in the species collected from V2 (439.48 mg/kg) and M2 (367.82 mg/kg). Whereas lower content was recorded in the V. *cyprinoides* species (15.74 mg/kg) collected from Chettuva zone. The concentration of Zn varied among the different bivalves ($P<0.05$; $P=6.9x10^{-5}$, $df=2$) with an average of 140.10, 66.76, 32.90 mg/kg in C. *madrasensis*, M. *casta* and V. *cyprinoides* respectively. Mostly, the enrichment of metals (Cu, Pb, Co, Zn, Ni) displayed the difference between the three kinds of bivalves: C. *madrasensis*> M. *casta*> V. *cyprinoides*. These results showed that pollutants easily enriched in the oyster, while the minimum enrichment viewed in black clam collected from Chettuva zone. Accumulation processes and heavy metal concentration variation in mollusc tissues are influenced by a host of biotic and abiotic factors (Phillips and Rainbow, 1994). Besides, the differences may have been also caused by variations in natural processes as well as anthropogenic activities around the sites (Mensah et al., 2007; Kacar et al., 2016). Environmental factors, such as the salinity, pH, dissolved oxygen, water temperature (Elder and Collins, 1991; Dhanakumar, 2012) and the marine sediment characteristics such as

organic carbon, grain size could have significantly control over the presence of heavy metal concentrations in these bivalve molluscs. Biological parameters such as food acquisition capability (Saavedra et al., 2004), sex (Sokolowski et al., 2004) and related factors such as size/weight (Phillips, 1976 (a,b); Szefer and Szefer, 1990), tissue composition modification and the reproductive cycle (Boyden and Phillips, 1981; Brown and Depledge, 1998) also play an important role in the accumulation processes of heavy metals in molluscs. With regards to the sampling sites, elevated level of heavy metals was detected in the soft tissues of the bivalves collected from Munambam and Vypin than Chettuva zone which could be due to exposure of environmental contamination related to anthropogenic activities originating from various sources like discharge of industrial, agricultural, domestic and port activities with subsequent bioavailability of metals to bivalves (George et al., 2013).

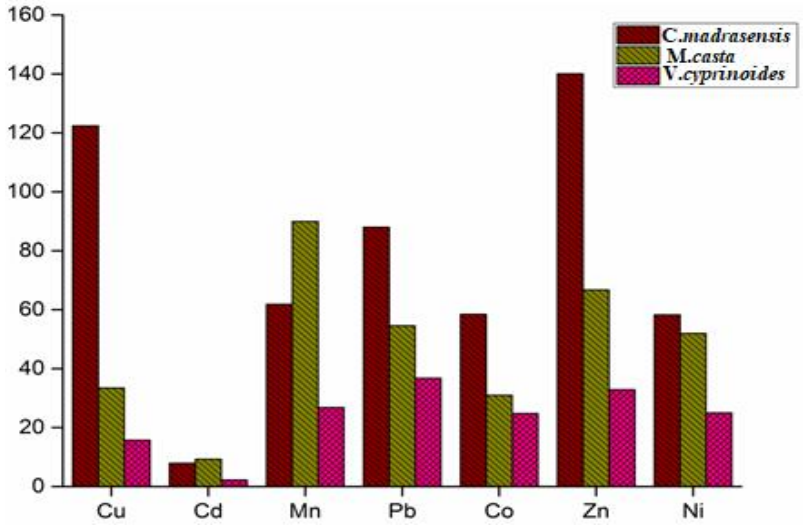

Fig. 6.2: Heavy metal concentration in the bivalves

Table 6.7: Heavy metal concentration in the bivalves (mg/kg)

Bivalves	Sample code	Cu	Cd	Mn	Pb	Co	Zn	Ni	Fe
C. *madrasensis*	C1M	65.45	12.54	59.99	114.29	90.48	111.68	82.04	787.90
	C2M	60.94	6.92	39.16	48.49	30.51	23.08	29.99	708.32
	C3M	93.90	5.64	34.33	68.15	43.62	56.10	38.26	1131.61
	C4M	43.47	3.74	47.67	33.68	25.28	55.04	26.53	761.62
	M1M	39.34	7.54	48.12	69.57	29.89	132.60	35.26	742.01
	M2M	367.82	10.02	72.71	147.71	86.76	163.34	77.23	1000.24
	M3M	62.59	10.72	88.46	119.51	65.12	146.46	58.98	1291.94
	M4M	73.10	10.11	59.37	87.24	39.45	138.89	42.60	755.57
	V1M	42.13	6.46	78.22	38.50	41.29	186.48	55.59	811.48
	V2M	439.48	10.28	91.15	174.52	136.21	422.04	128.97	843.68
	V3M	58.29	3.05	61.07	66.58	54.57	105.39	65.87	566.36
M. *casta*	VAC	40.66	3.75	108.33	49.69	46.15	116.87	61.17	700.90
	MAC	40.56	21.56	123.56	81.08	22.43	48.94	69.07	635.64
	CAC	19.15	2.47	38.02	32.93	24.39	34.46	25.76	546.67
V. *cyprinoides*	C1C	14.28	1.86	25.94	35.17	25.89	32.88	23.41	584.10
	C2C	15.59	3.04	25.83	34.90	24.43	33.76	24.55	598.47
	C3C	16.58	2.17	26.91	36.07	25.05	34.68	26.25	590.78
	C4C	16.50	1.99	28.70	41.10	23.79	30.29	25.82	724.31

6.2.2.1 Bioaccumulation of metals in tissues

The Biota-Sediment Accumulation Factor (BASF) was calculated in order to evaluate the rate of heavy metal accumulation in the bivalve tissues (Lau et al., 1998; Szefer et al., 1999). BSAF calculated by using the equation,

$$BSAF = \frac{Metal\ concentration\ in\ Organism}{Metal\ concentration\ in\ bulk\ sediment}$$

The behaviour of heavy metals in an environment is critically dependent on their chemical form, which influences the mobility, bioavailability and thereafter leads to deleterious effect to the organisms (Passos et al., 2010). Although, metal speciation provides detailed information on concerning the origin, mode of occurrence, biological and physico-chemical availabilities and mobilisation of heavy metals. However, there are gaps for the information related to bioavailability and bioaccumulation where the organism accumulates more than the bioavailable forms. The records on both the sediment geochemistry and biology of the specific organism will provide an explanation for the mechanisms that control metal bioaccumulation (Griscom and Fisher, 2004).

Therefore, further, an attempt was made to compute BSAF using equation (modified)

$$\text{BSAF}=\frac{\text{Metal concentration in Organism}}{\text{Metal concentration in sum of bioavailable phases}}$$

Metal enrichment in the tissues of the bivalves were ranked in the following order: Cd> Cu> Zn> Co> Pb> Ni> Mn> Fe; Cd> Co> Ni> Zn> Mn> Pb> Cu> Fe and Cd> Zn> Co> Pb> Ni> Mn> Cu> Fe in the C. *madrasensis*, M. *casta* and V. *cyprinoides* respectively. However, higher values were obtained for Cd in all the bivalve species and therefore their bioaccumulation rate was higher than the bioavailability (Table 6.8a). It was also noted that although Fe concentration in the sediments was higher but its BSAFs were lower and this is probably due to the faster deposition of Fe than all other metals. Further, BSAF (modified) values were computed using the sum of bioavailable phases (BAP) (Table 6.8b) in the

place of bulk metal concentration; also indicated that Cd as most preferred elements by the organisms. Except for Ni and Fe, all other metals were highly bioaccumulated in C. *madrasensis* bodies. High BSAF for Cu were found in all the species, with the exception of V. *cyprinoides* collected from C1, C2 and C4. Moreover, the highest BSAF values were recorded in C. *madrasensis* collected from station M2 (10.54). According to Szefer et al. (1999), bioaccumulation is expected to occur in organisms if the BSAF value is >1. In the present research study, for the three bivalve species, BSAF was higher than one for Cd, Mn, Pb, Co, Zn and Ni. Besides, BSAF of Cu in the C. *madrasensis* and M. *casta* was also greater than one, inferring obvious bioaccumulation of these elements in the studied species. Regardless of the grain size of the sediments that do not favour the retention of contaminants and organic matter; metals in sediments were bioavailable and accumulated in bivalves. The low OM concentrations in sediments may help the bioaccumulation of metals (Atkinson et al., 2007). Sediments with lower organic matter content might lead to an increase in the ingestion rate of organisms to satisfy their nutrient requirement and hence to record an increase in the bioaccumulation of metals in organisms (Kalman et al., 2012). As demonstrated, the bioavailability of almost all the analysed metals was comparatively low at C4, M3 and V3; TOC content was high (TOC 4.14%, 5.15%, 4.60% respectively).

Table 6.8a: The Bio sediment Accumulation Factors (BSAF) for each metal considering bulk metal concentration.

	Bivalve	Sample code	Cu	Cd	Mn	Pb	Co	Zn	Ni	Fe
BSAF	C.*madrasensis*	C1M	3.51	9.07	1.01	3.43	4.99	4.75	3.56	0.75
		C2M	3.59	6.58	1.05	1.70	1.72	0.95	1.45	0.72
		C3M	6.02	5.05	2.89	2.14	2.52	2.53	1.95	1.23
		C4M	2.41	2.72	0.84	1.16	1.40	2.26	1.09	0.77
		M1M	0.98	3.93	0.71	1.05	1.26	2.54	0.85	0.67
		M2M	10.54	6.80	0.53	2.40	4.13	3.42	2.23	0.96
		M3M	1.39	4.68	0.74	1.88	2.81	2.85	1.51	1.19
		M4M	2.36	6.36	0.74	1.67	2.03	2.98	1.37	0.73
		V1M	0.98	4.78	0.91	1.12	1.48	2.13	0.98	1.13
		V2M	9.61	4.98	0.97	2.36	4.56	4.86	2.12	0.83
		V3M	1.05	1.69	0.76	1.28	1.79	1.06	0.94	0.49
	M. *casta*	VAC	0.95	2.78	1.26	1.44	1.65	1.33	1.07	0.98
		MAC	1.01	11.23	1.81	1.23	0.95	0.94	1.67	0.57
		CAC	1.03	1.78	0.64	0.99	1.34	1.47	1.12	0.52
	V. *cyprinoides*	C1C	0.77	1.34	0.44	1.06	1.43	1.40	1.02	0.56
		C2C	0.92	2.89	0.69	1.22	1.38	1.39	1.19	0.61
		C3C	1.06	1.94	2.27	1.13	1.45	1.56	1.33	0.64
		C4C	0.91	1.44	0.51	1.41	1.32	1.24	1.06	0.73

Table 6.8b: The Bio sediment Accumulation Factors (BSAF) for each metal considering bulk metal concentration and sum of bioavailable phases.

Heavy metals	C. *madrasensis*		M. *casta*		V. *cyprinoides*	
	BSAF	BSAF (bioavailable)	BSAF	BSAF (bio available)	BSAF	BSAF (bioavailable)
Cu	3.86	7.15	1	1.56	0.92	3.08
Cd	5.15	8.45	5.26	8.97	1.9	3.1
Mn	1.01	1.66	1.24	2	0.98	1.36
Pb	1.84	3.14	1.22	2.07	1.21	1.89
Co	2.61	5.79	1.31	2.56	1.39	3.26
Zn	2.76	4.28	1.25	1.98	1.4	2.4
Ni	1.64	5.37	1.29	5.02	1.15	3.32
Fe	0.86	2.66	0.69	1.88	0.63	1.64

Although, the different biomonitoring species in the same habitat may show slightly different metal contamination pattern. According to Rainbow (1995), even closely related species may be feeding on different food sources with consequently different inputs of metals for accumulation. Hence it is, thus, not surprising that the three bivalve species do not have similar metal burdens even when present together at the same site. In the present study, the bivalves C. *madrasensis* and M. *casta* live in the same habitat, relatively large differences could be recorded in the BSAF for these organisms, especially for Cu, Cd, Zn and Pb. Variation in the heavy metal bioaccumulation for the bivalves living in the same environment may be due to their differences in the bioaccumulation pattern, metabolic processes and their physiology as earlier reported in similar type of research work (Goodyear and McNeil, 1999). Finally, the outcome could be concluded that C. *madrasensis* have a greater capacity for metal bioaccumulation than M. *casta* and V. *cyprinoides*.

The heavy metal and OCI concentration in the V. *cyprinoides* from Chettuva zone were compared with the contamination accumulation data in the same bivalve species (S1C, S2C and S3C) sampled from Vembanad Lake during the monsoon season in the mid-June (Table 6.9).

Table 6.9: Heavy metal and OCI concentration in the V. *cyprinoides* collected from Vembanad Lake

Bivalve	Organochlorine Insecticides(ng/g)													
	Sample code	**α-HCH**	**β-HCH**	**γ-HCH**	**Heptachlor**	**Hepta epoxide**	**Aldrin**	**Dieldrin**	**Endrin**	**o,p'-DDD**	**p,p'-DDD**	**o,p'-DDT**	**p,p'-DDT**	**p,p'-DDE**
V. *cyprinoides*	SIC	ND	3.72	ND	ND	ND	ND	ND	ND	ND	ND	ND	28.50	ND
	S2C	0.85	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	S3C	ND	ND	ND	5.28	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Heavy metals (mg/kg)													
	Sample code	**Cu**		**Cd**		**Ni**		**Pb**		**Zn**		**Mn**	**Co**	**Fe**
	S1C	16.00		0.10		12.00		6.80		83.70		ND	ND	6537.66
	S2C	13.00		2.80		22.30		4.50		267.80		ND	ND	16759.69
	S3C	12.50		1.70		15.60		ND		130.40		ND	ND	11636.12
ND- Not detected														

The average concentration of Cu, Cd, Pb and Ni showing higher levels in the V. *cyprinoides* collected from Chettuva zone (15.74, 2.26, 36.81 and 25.01 mg/kg respectively). Whereas Zn concentration was reported at higher levels in the bivalve sampled from Vembanad lake (160.63 mg/kg) due to these stations from Vembanad Lake are located near to the Zn manufacturing industry and the source is also from antifouling paints applied on ship. In addition, the OCIs was detected in V. *cyprinoides* is in the very low level and α-HCH, β-HCH, p,p'-DDT and heptachlor (0.85, 3.72, 28.50 and 5.28 ng/g respectively) was the only detected OCIs from Vembanad lake. Whereas, the OCI levels from Chettuva zone was comparatively high especially ∑HCH, heptachlor and aldrin (56.15, 16.10 and 19.87 ng/g respectively), which must be due to the pre-monsoonal accumulation of pesticides (Sujatha et al., 1993) in this zone. Overall, the result indicated that the accumulation of insecticides for the bivalve was time and season dependent.

6.3 Public health concern of heavy metals and OCIs

Seafood is an essential source of protein, fatty acids, vitamins and minerals. Despite the numerous beneficial effects on human health from its regular intake, various other contaminants could pose a risk on consumers (Jović and Stanković, 2014). The condition index (CI) is a useful marker of the health status of bivalve. Moreover, it has been observed that under stressful environmental conditions, CI could be diminished (Widdows et al., 2002; Blanck, 2002; Buzzi and Marcovecchio, 2018.) and it was proved by the derived negative correlation among some heavy metals and OCIs with the CI (Table 6.10). Bivalves with higher

levels of Cu, Co, Zn, Fe and heptachlor epoxide posed decline in the condition index of bivalves than those with lower values of these contaminants (Pearson coefficient r = − 0.636, − 0.561 and − 0.541, -0.633 and -0.653 respectively; Table 6.10). Although the influence of other factors is not discarded, the results of this research attempt suggested that the amount of these contaminants in soft tissue affected the health status of bivalves.

Table 6.10: Pearson correlation of heavy metals and OCIs with CI

Condition Index														
Cu	Cd	Mn	Pb	Co	Zn	Ni	Fe	HCH	heptachlor	heptepox	Aldrin	Dieldrin	Endrin	DDT
-.636(**)	-0.292	-0.369	-0.417	-.561(*)	-.541(*)	-0.392	-.633(**)	-0.174	-0.202	-.653**	0.043	0.079	0.051	-0.056

6.3.1 Hazard assessment of heavy metals

To prevent health risks, National and International organizations have established maximum acceptable levels of heavy metals in seafood (Table 6.11). The standards are mainly expressed on a wet weight basis and so according to recommendations in the literature, metal concentrations in the bivalve tissue in dry weight can be converted into wet weight by dividing them by factors ranging from 4 to 6 (George et al., 2010; George et al., 2013; Anandkumar et al., 2017; Anandkumar et al., 2018; Anandkumar et al., 2019). In this study, a factor 4.76 (i.e. 79% moisture) was adopted. The wet weight Cd levels in the C. *madrasensis* and M. *casta* samples were higher than the permissible levels recommended

by the Food and Agricultural Organization (FAO, 1983), World Health Organization (WHO, 1989), Europian Communities (EC, 2001), Food Safety and Standards Authority of India (FSSAI, 2015), and Malaysian food regulation (MFR, 1985). The average concentrations of Pb in the three bivalve species (18.49, 7.73 and 11.46 ww in the C. *madrasensis*, V. *cyprinoides* and M. *casta* respectively) exceeded the threshold values recommended by all the above-mentioned international guidelines (Table 6.11), indicating a potential risk to both the organisms and consumers. *C. madrasensis* exceeded the maximum permitted limit for Cu recommended and reported by Ministry of public health (MPHT, 1986) but it is lower than all other international limits. The present study followed a set of standards laid out by the Food and Agricultural Organization (FAO, 1983) in conjunction with the World Health Organization (WHO, 1982) called the CODEX Alimentarius which determines the levels of heavy metals safe for human consumption, (CODEX, 2014). The Codex Alimentarius sets the limit of Pb in bivalve molluscs as 1 mg/kg which means that the average concentration of Pb was noted in this study is in elevated levels (14.93 ww). The Codex Alimentarius places the maximum allowed limit of Cd in bivalve molluscs as 2 mg/kg. In this study, the average concentration of Cd in M. *casta* were more than CODEX Alimentarius limit (CODEX, 2014). Moreover, Zn concentration in all the three bivalve species were lower than the guideline issued by above mentioned organizations. Therefore, among the investigated heavy metals, Pb and Cd concentrations found in this study seem to be high as compared to the threshold limits and may pose risk to human health.

Table 6.11: Permissible limits (Maximum Permissible limits (MPL) of OCIs and heavy metals by various organizations

Organization	Metals ww (mg/kg)				OCIs (mg/kg)							
	Pb	Cd	Zn	Cu	Lindane	βHCH	heptachlor	Endrin	Aldrin	Dieldrin	4,4'-DDT	∑DDT
European Communities, 2001	1.5	1										
Food and Agriculture Organization (FAO,1983)	0.5	0.5	40	30	0.3	0.3	0.3	0.3	0.3		0.3	
World Health Organization (WHO 1989)	2	1	100	30								
Ministry of public health(MPHT 1986)	1	2	100	20								
Malaysian Food regulation (MFR 1985)	2	1	100	30								
CODEX 2014	1	2										
Food Safety and Standards Authority of India (FSSAI 2015)	0.3	0.3										
US Food and Drug Administration (FDA 2001)		4			300	300	300	300	300		5000	
USEPA, 1989					0.1-2							
NAS/NAE, 1972							100	100		100		1000

6.3.2 Hazard Assessment of OCIs

Organochlorines have posed a serious potential health hazard and therefore maximum residual limit has been recommended for human consumption by various agencies and are listed in Table 6.11. The γ-HCH concentrations measured in the bivalves were well below those of the reported legal limits for human health set in Canada (100 ng/g) or in Germany (2,000 ng/g) for fish and fisheries products (Haines et al., 1994). In addition, the limits of HCHs and DDTs for marine molluscs of Marine Organism Pollution Assessment Standard (MOPAS) recommended by the Technical Group of Guangdong Coastal Zone Resource Comprehensive Survey are 2 and 0.12 mg/kg (ww). FAO recommends a level of 300 ng/g as the maximum acceptable limit for DDT; while the Canadian limit is 500 ng/g (Mwevura et al., 2002 (b)). The FDA (2001), limits are 5000 mg/kg for p,p'-DDT and 300 mg/kg for aldrin, dieldrin, heptachlor, heptachlor epoxide in the edible aquatic organism. National Academy of Sciences and National Academy of Engineering (NAS/NAE, 1972) recommends a limit of 1000 mg/kg for total DDTs and 100 mg/kg for dieldrin, endrin and heptachlor. In the present study, the residual levels of DDT in the C. *madrasensis* collected from V1 exceeded the recommended levels of Technical Group of Guangdong Coastal Zone Resource Comprehensive Survey. Compared to the maximum admissible concentration for DDT (50 ng/g ww) established by the European Union (Binelli and Provini. 2003), C. *madrasensis* collected from M3 (57.15 ng/g) and from V1 (200.13 ng/g) which were also considered overloaded but the DDT concentrations are rather low compared with the maximum acceptable limit established by other above said International agencies.

Furthermore, compared with the maximum admissible concentration (10 ng/g ww) suggested by the European Union (Binelli and Provini, 2003), the residual levels of γ-HCH in all the samples, except V. *cyprinoides* from C2 and C4, M. *casta* from all the zones and C. *madrasensis* from M1 and V1 have exceeded this level. From a public health point of view, the data presented herein indicate that the observed levels of the lindane and DDT pose a risk for humans consuming shellfish from this area. However, the levels of heptachlor, heptachlor epoxide, aldrin, dieldrin and endrin concentrations were far below the above said tolerance levels and the present residual levels are not going to cause any serious health hazards. In addition, it cannot be excluded from the bioaccumulation of these OCIs to higher organisms which may leads to serious health hazards.

6.4 Conclusions

In conclusion, the results of the current study provide a valuable information on the status of heavy metals and OCI in the bivalves residing in the coastal belt. The overall analysis showed that the levels of Cd, Cu and Zn in Central Kerala have relatively existed a serious issue. In this study, for the three bivalve species, BSAF was higher than one for Cd, Mn, Pb, Co, Zn and Ni. Besides, BSAF of Cu in the C. *madrasensis* and M. *casta* was also greater than one, showing a clear bioaccumulation status of these elements in the studied species. BSAF found for C. *madrasensis* were significantly higher than M. *casta and* V. *cyprinoides* indicating its high efficiency in accumulating metals. Organochlorine insecticides (OCIs) residues in the bivalve tissues revealed a widespread contamination along the major fishing zones of Central Kerala. Among

the OCIs determined in the shellfish in the present examination, residual levels of ∑HCHs were predominant, followed by ∑DDTs and other OCIs and the highest levels of ∑HCH and ∑DDT were due to their resistance to microbial degradation. ∑DDTs contamination in the present study revealed elevated levels. The results confirmed that DDT is still being used in and around the study zone, which also require enough concern due to the toxicological effects. The BSAF for ∑HCH, heptachlor, heptachlor epoxide in the C. *madrasensis* collected from V3, M3 and C4 stations were significantly greater than the theoretical benchmark of 1.7 suggesting that these zones are under dangerous condition. Comparison with the oyster C. *madrasensis,* exhibited a marked difference in the contaminant levels of M. *casta* and V. *cyprinoides* suggesting a difference in exposure levels between species. The three species of bivalve studied, the bioaccumulation of OCIs was higher in C. *madrasensis* tissues and it was related to the lipid content of bivalve. As such, the consumption of these bivalve may pose biomagnification to human consumers, thereby constituting a public health risk.

From the health point on seafood safety, the concentrations of Cd and Pb in the bivalve species were exceeded the threshold values recommended by National and/or International seafood safety standard, indicating a potential risk to both the organisms and thereby leads to toxicity and a dangerous health situation creates when ingested. Although, the analyzed OCI concentrations in bivalves reveals that the lindane and DDT were found enough to be considered as potential human health hazard. In this context, the health risk assessment was declared that the heavy metals especially Cd, Pb and OCIs particularly HCH and DDT load

in examined bivalves especially C. *madrasensis* must increase the possible toxicological complications. People who continuously consume these bivalves contaminated with metals and OCIs as found in the present study are under the occurrence of target health risk in the long run.

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Chapter 7
SUMMARY

This is the first study that has established a comprehensive evaluation of the residual level and the fate of heavy metals and organochlorine insecticides in the triad system, using sediment, water and bivalves from the major fishing zones of Central Kerala, Southwest coast of India. The impacts of pollutants in the study area are much more serious owing to their toxicological and chemical properties as well as due to the limited knowledge among users for the safe practice. Therefore, there is an urgent need to conduct an assessment of the adverse effects of these pollutants in the aquatic ecosystems. Hence, in this study, the bioaccumulation levels of heavy metals and OCIs in edible bivalves and also their ecotoxicological risk were assessed to have a better understanding of the exposure hazard through consumption.

The levels of Pb and Cd in this study was generally high in the analysed compartments. Elevated concentration of heavy metals especially in the Munambam and Vypin zone outlined the strong anthropogenic influences from domestic areas, shipping activities as well as effluent discharged due to certain industrial establishments. The Munambam fishing harbour is one of the most polluted areas due to the adverse effect of effluent discharges attributable to the fishing activities.

In some instances, Cu, Cd and Pb pollutant levels were above sediment quality guidelines, suggesting the existence of environmentally damaging ecotoxicological effects. Metals are weakly bound to the sediment moieties, are readily available in the aquatic system and causes a greater environmental risk leading to a chaos to the ecosystem. The metal fractionation data presented here opens a new dimension in quality monitoring and the assessment of the ecotoxicological potential of metal ions. From speciation analysis, Cd, Pb, Zn, Cu and Mn were showed higher percentage with exchangeable, carbonate bound and Fe-Mn oxide bound, all are considered to be weakly bound fractions for becoming more bioavailable to the environmental niche. Whereas, the mobility rate of the metals was different from one another, the highest mobility rate was related to cadmium (as based on RAC) and reveal a very high level of risk. The BSAF's evaluated the efficiency of metal uptake by the organisms and coupled to this, speciation studies predict the bioavailability of metals which could serve as a meaningful measure to examine the link between bioaccumulation and toxicological endpoints in order to understand the risks posed by the environment.

The presence of persistent organochlorine insecticides and their degradation products at varying concentrations were detected in the study area. The OCIs compounds; (α-HCH, β-HCH, γ-HCH, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide) were assessed and found to be present in the different segments. DDTs, HCHs and heptachlor compounds were the most predominant residues attributing to their current use in vector control, illegal usage of these compounds for high yield of crops, contamination

from past usage and spills from obsolete pesticides. Generally, the concentrations of the OCIs in the bivalve tissues were significantly higher as compared to those in the sediments and water compartment. Ecotoxicology impact of sediments on aquatic species using sediment guidelines suggested that the level of ∑DDTs, lindane, heptachlor epoxide, endrin and dieldrin may pose a threat to the aquatic species. Although, based on known toxicological data, the average concentration of HCH, ∑aldrin, dieldrin and endrin in the water samples are at elevated levels, which are enough to exhibit a potential biological chronic risk in the coming years. Among OCIs determined in the shellfish, residual levels of ∑HCHs were predominant followed by ∑DDT, heptachlor, aldrin, heptachlor epoxide, dieldrin and endrin. Analysis of the triad system indicated that HCH was shown in the priority list of OCI residues and the source of HCHs was potentially received fresh lindane. This outcome may be due to the use in the agricultural area for the control of vector borne diseases. The sources of DDTs in the M1 station near the fishing harbour was due to the release from fishing boats with DDT-containing antifouling paint and the recent usage of this insecticide in the surrounding agricultural areas. The levels of DDE and DDD from the surface sediments showed ecological concern on Central Kerala because DDT is still being used in the form of different commercial name in these areas.

The maximum OCI concentration was measured in the oyster C. *madrasensis;* whereas the minimum concentration was always measured in M. *casta.* In most cases, the concentrations of the OCIs such as ∑HCH, heptachlor, heptachlor epoxide and aldrin levels were found to be a significant ($P< 0.05$) difference among C. *madrasensis*, V. *cyprinoides*

and M. *casta*, whereas the other pollutant levels are not significantly varied between these species. BCF and BSAF values showed that C. *madrasensis* had accumulated more OCIs than M. *casta* and V. *cyprinoides*. This could be attributed to the fact that C. *madrasensis* being larger in size which might have higher lipid content and therefore, prone to bioaccumulate more organochlorine residues. Altogether, the level of heavy metals especially Cd, Pb, Cu, Zn and OCIs particularly HCH and DDT load in the examined bivalves were above the recommended maximum permissible limits, and thus identified as a potential ecological concern owing to the shellfish consumption in Central Kerala. The negative correlation of Condition index with the Cu, Co, Zn, Fe and heptachlor epoxide is also given an evidence that the amount of these contaminants occurring on biota possess a wide concern on the health status of bivalves. All other metals and OCIs were below the tolerance limit, but the potential health risk associated with bivalve consumption cannot be neglected for those who often adapt to consume more seafood products as dietary components.

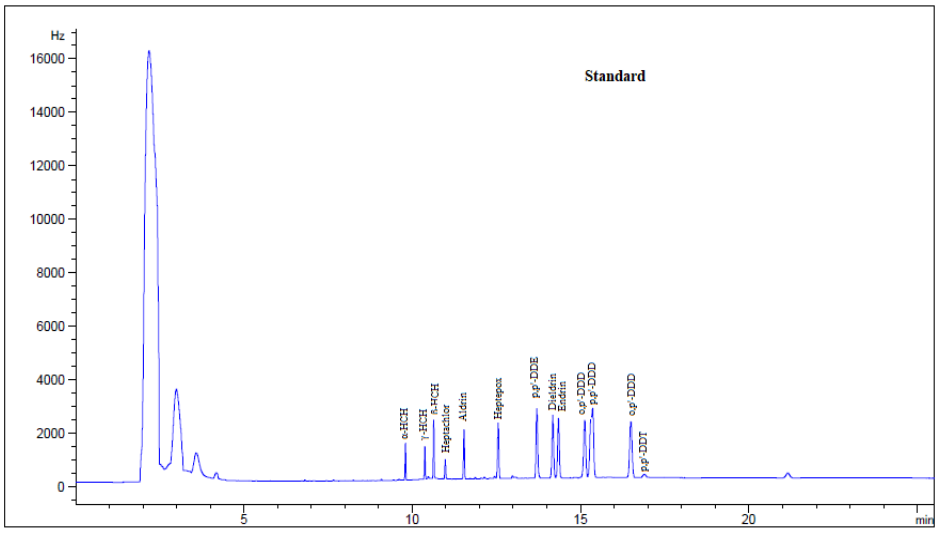
Therefore, it is worthwhile to continue the routine ecotoxicological risk assessment of persistent inorganic and organic pollutants in the environment and specific to biota for the purpose of protecting public health. Further work should be initiated and necessary to determine the accumulation capacity of metals and OCIs in different organs of marine organisms such as muscle, mantle and adductor. Since the response of pollutant concentrations in specific organs of marine shellfishes to environmental exposures have not been examined in the study area. In estimating the risks of the pollutants to human, actual average body

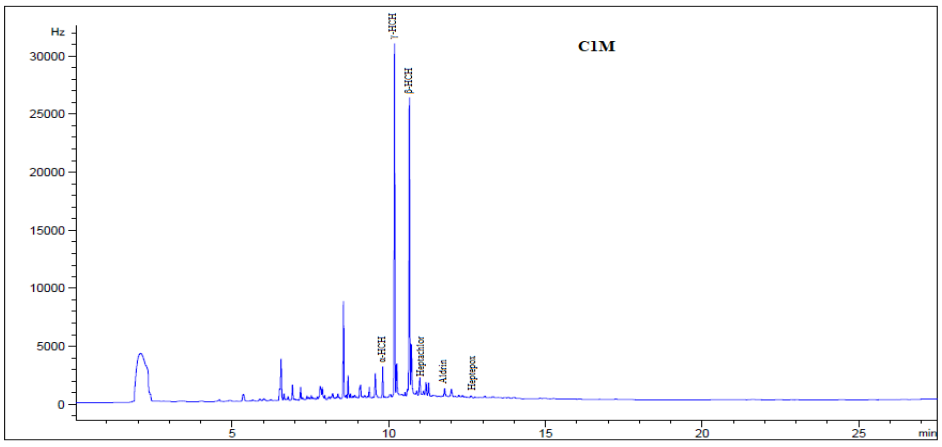
weights of children and adult should be determined and used for computation. Public health authorities should take initiative to conduct a proper orientation programme for farmers about the positive and negative aspects of harmful residues to ensure the proper handling of pesticides. Because the misuse of pesticides is alarming due to the less awareness about the environmental consequences. It is recommended that a social study should be carried out to find the level of public awareness on the dangers of aquatic pollution for the users of shellfishes. In addition, to avoid the health risk, an awareness programme should arrange by instructing consumers to understand the potential and indirect risk of consuming edible seafood cultivated in the polluted areas.

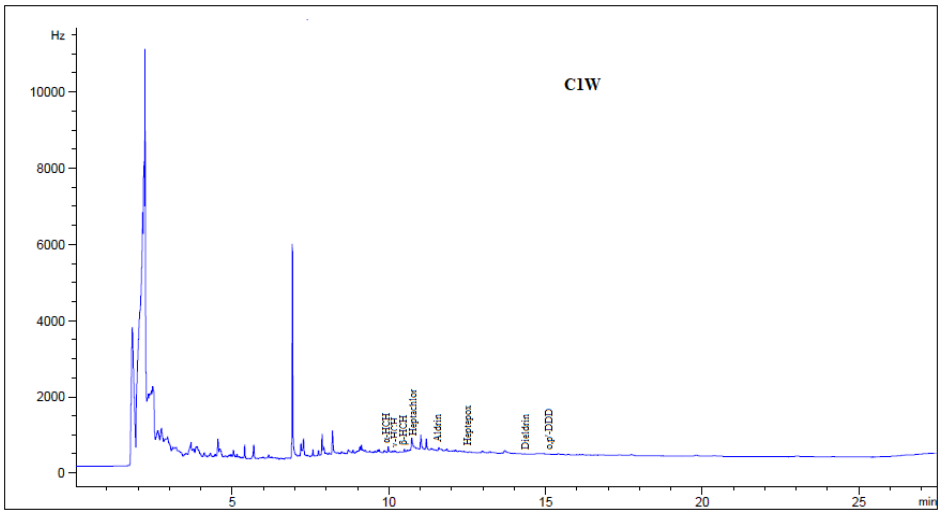
ANNEXURES

Annexure 1

CHROMATOGRAM OF OCIS DETECTED FROM PROMINENT FISHING ZONES OF CENTRAL KERALA



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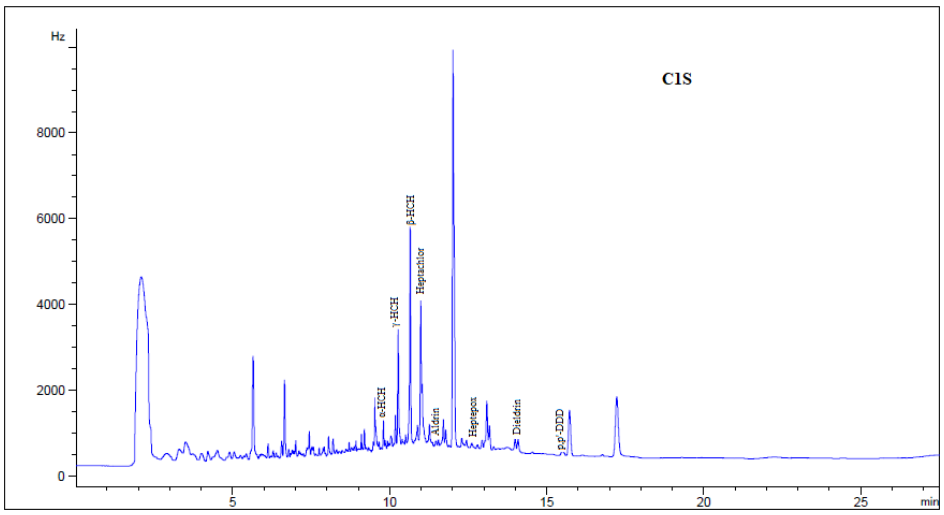
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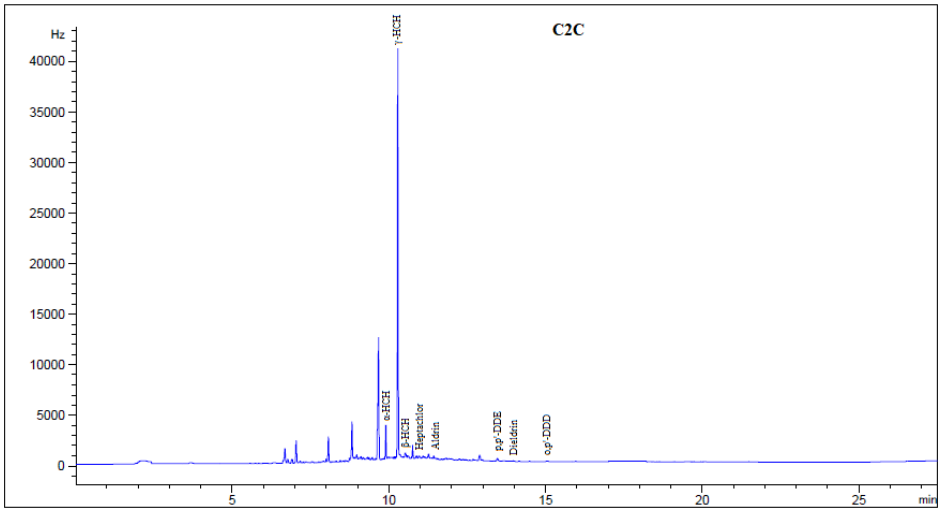
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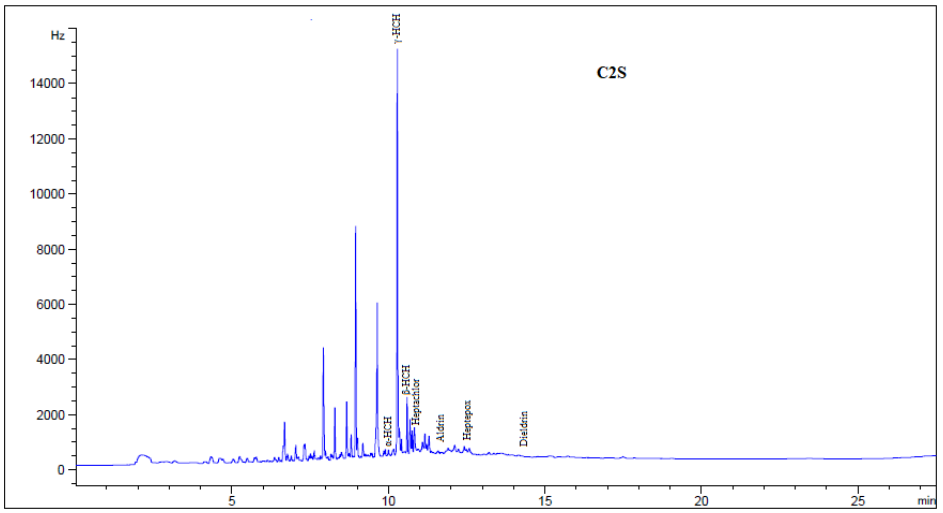
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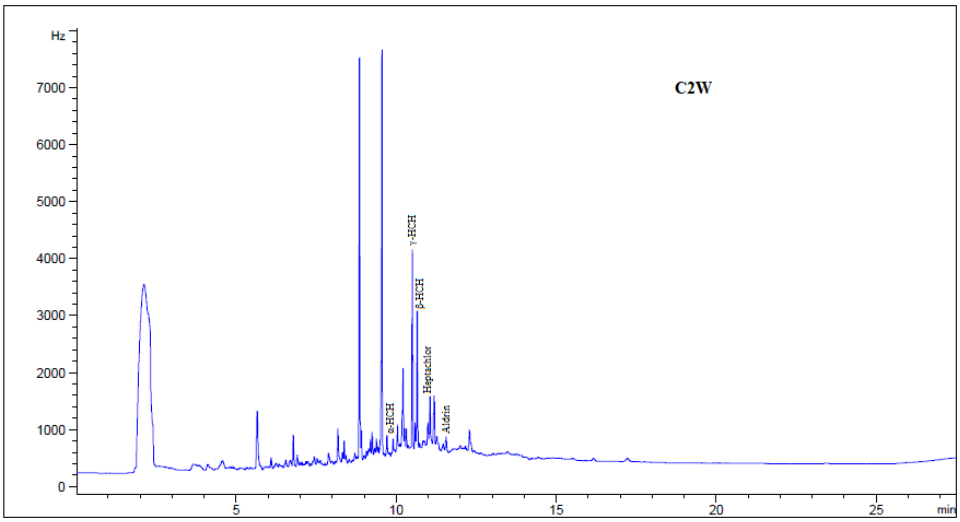
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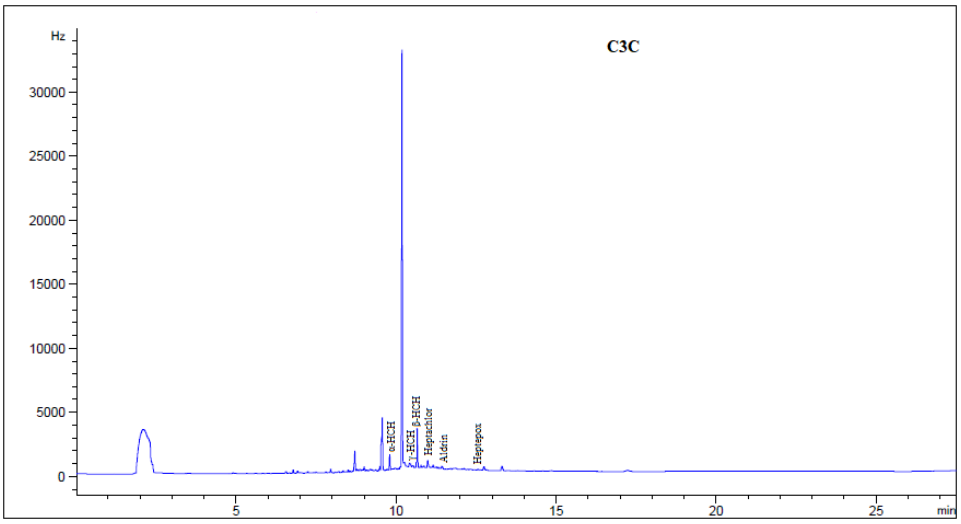
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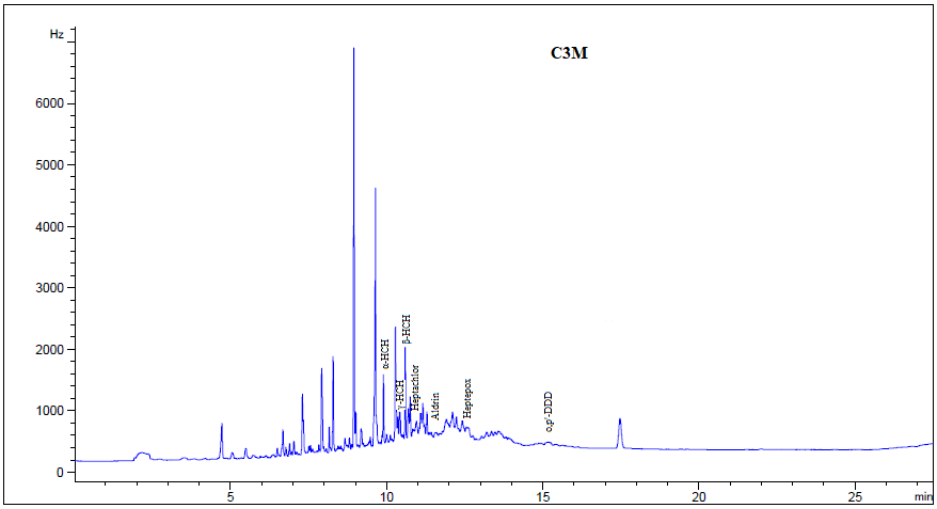
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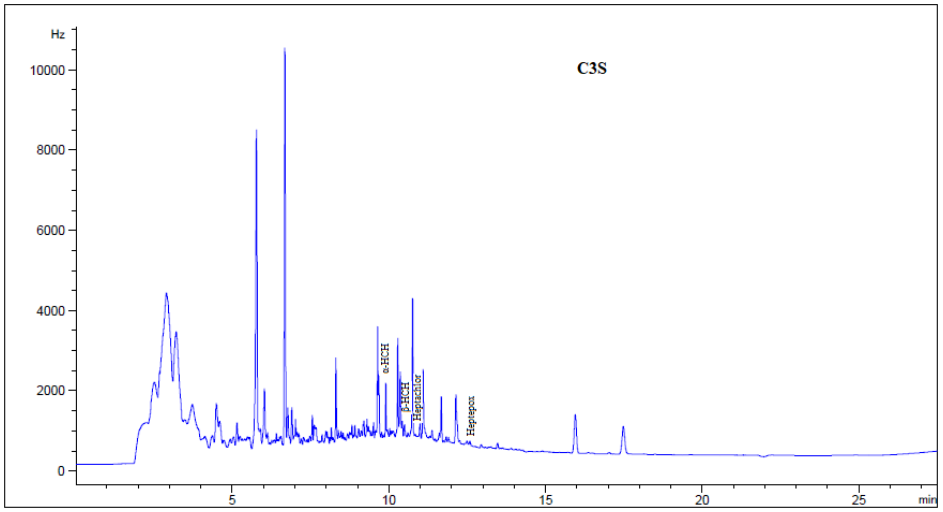
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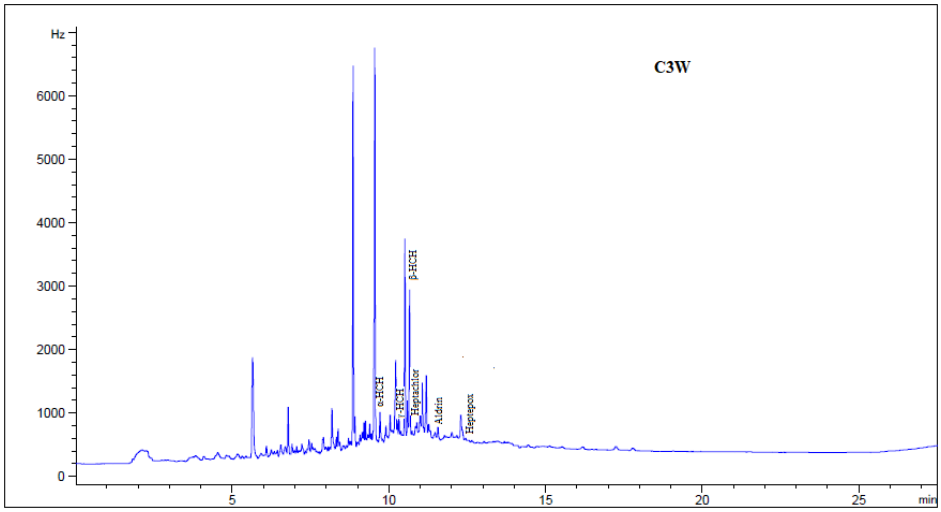
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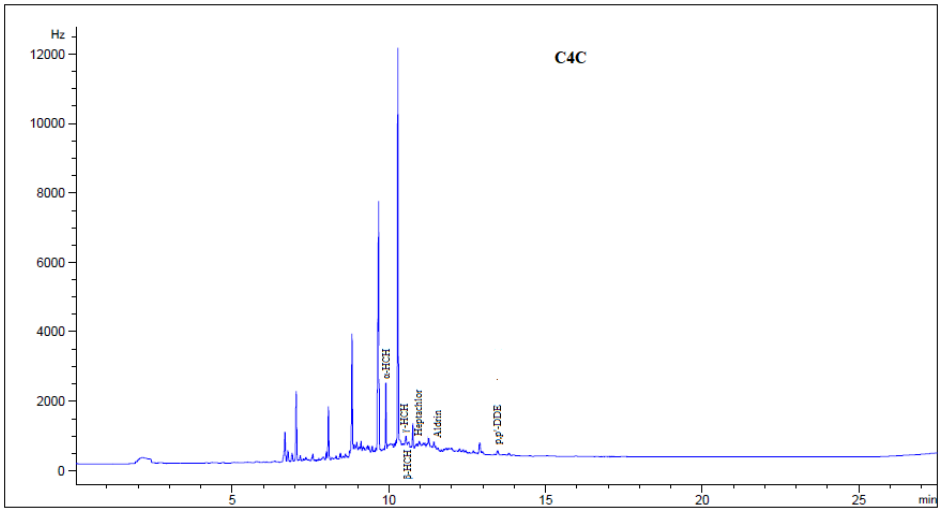
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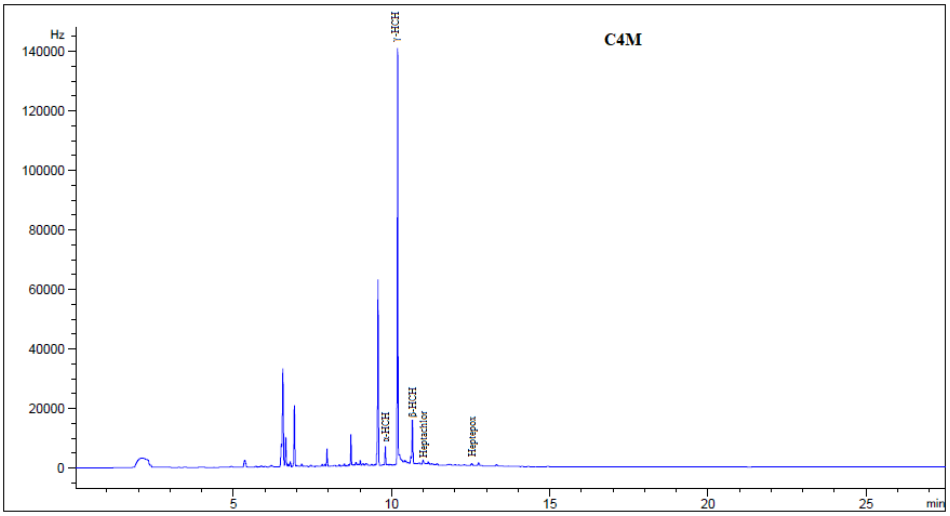
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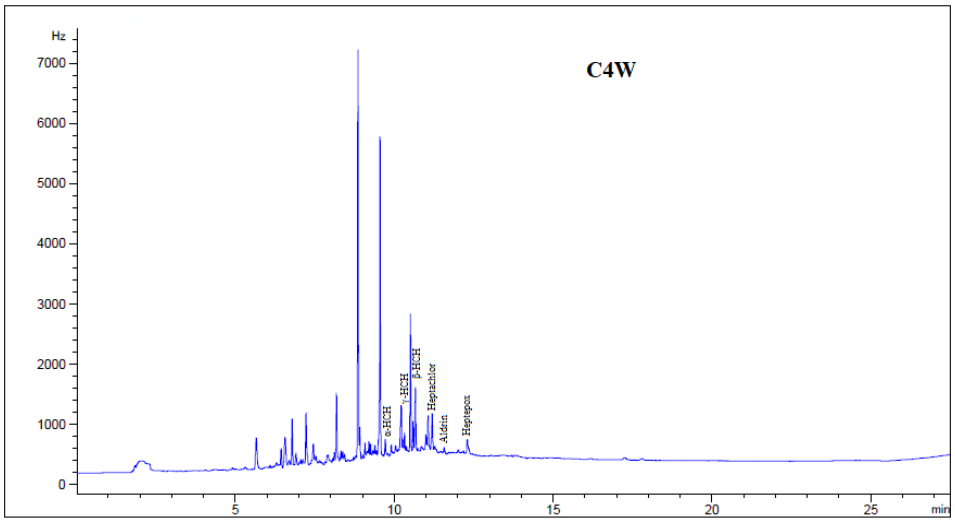
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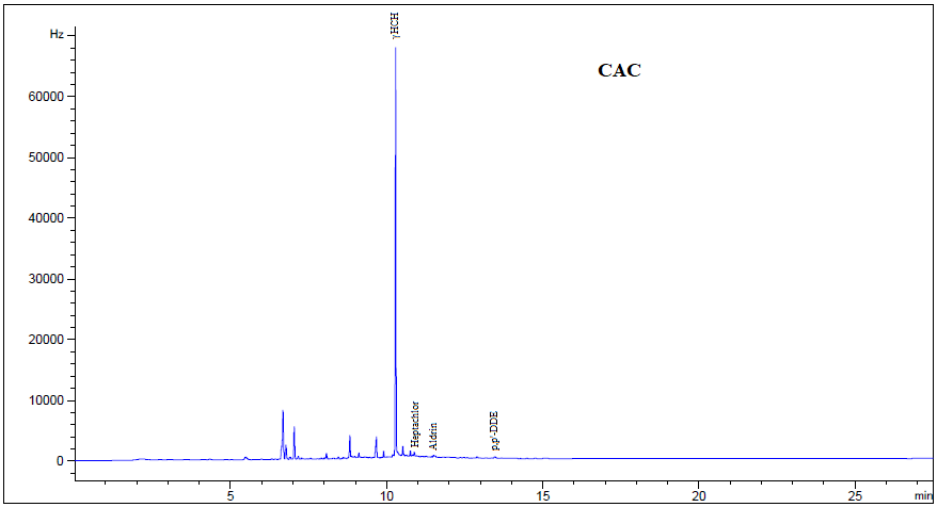
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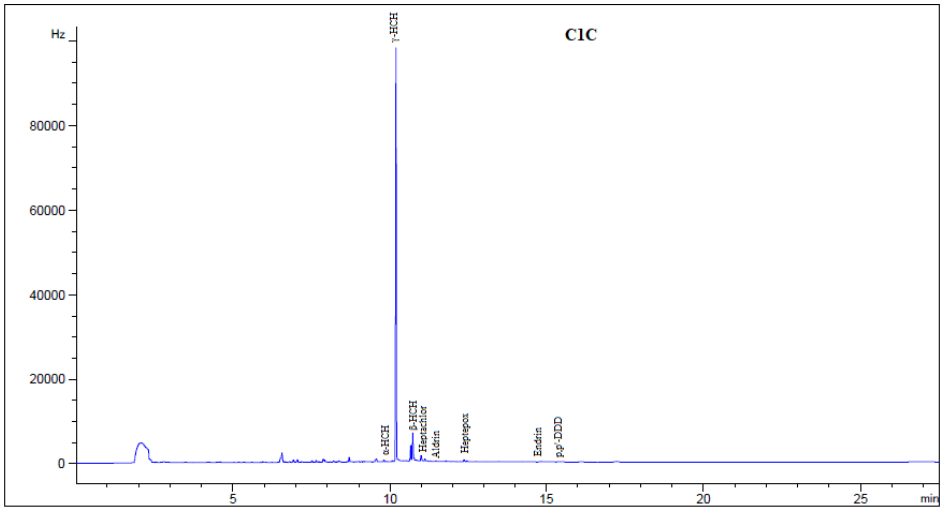
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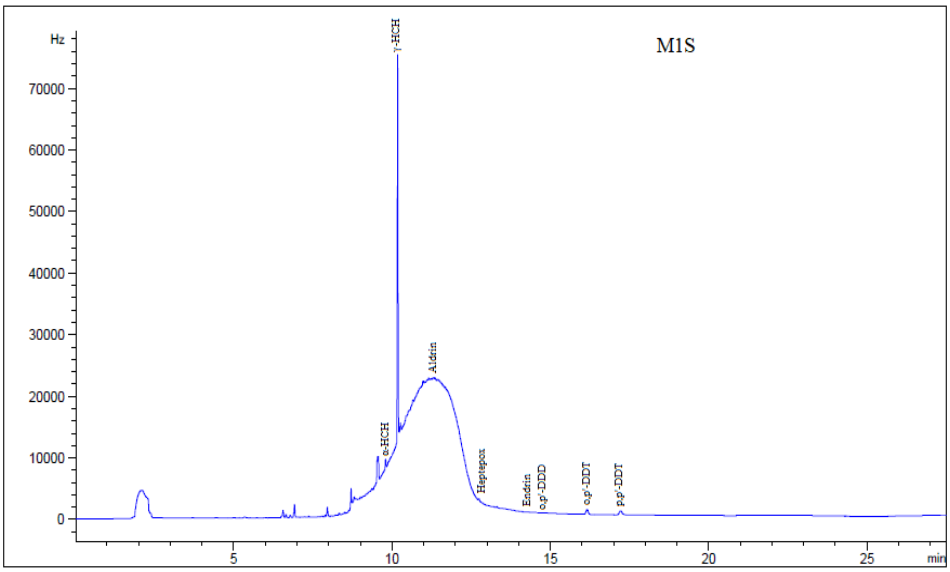
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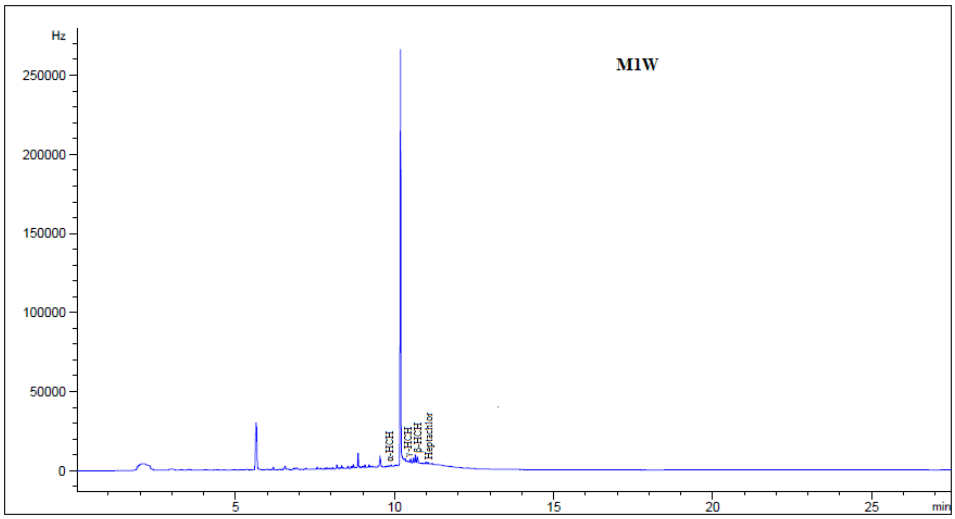
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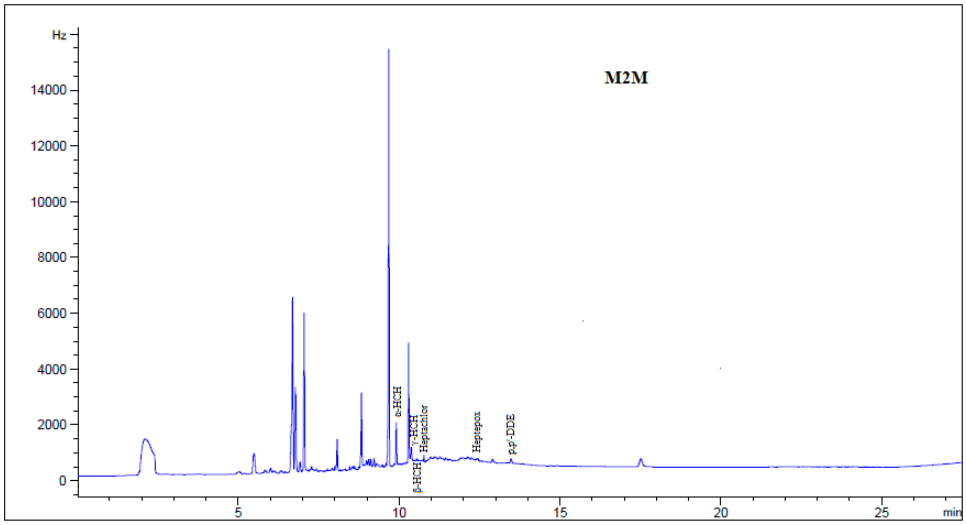
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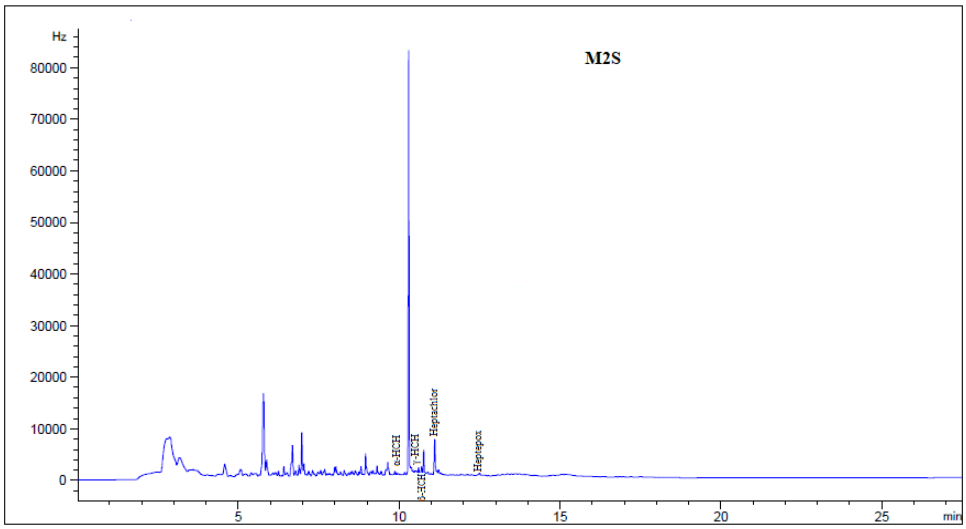
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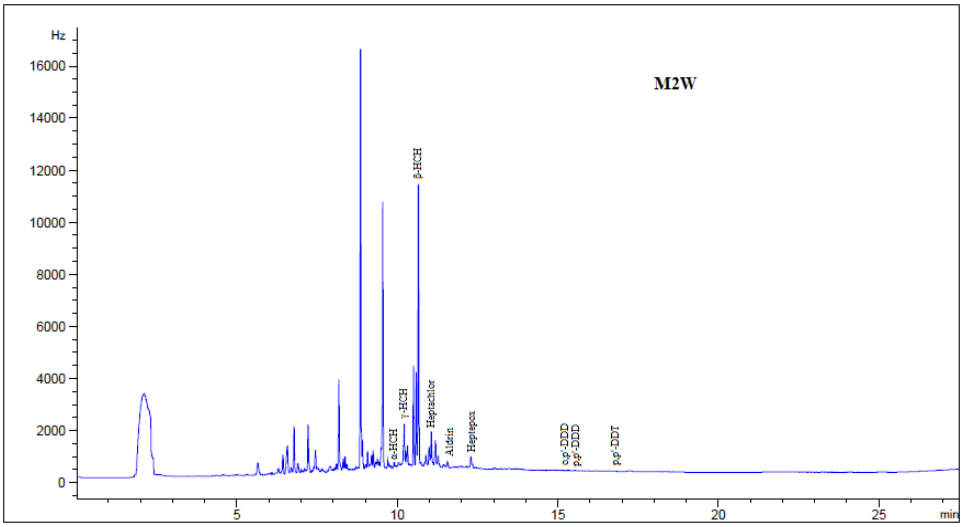
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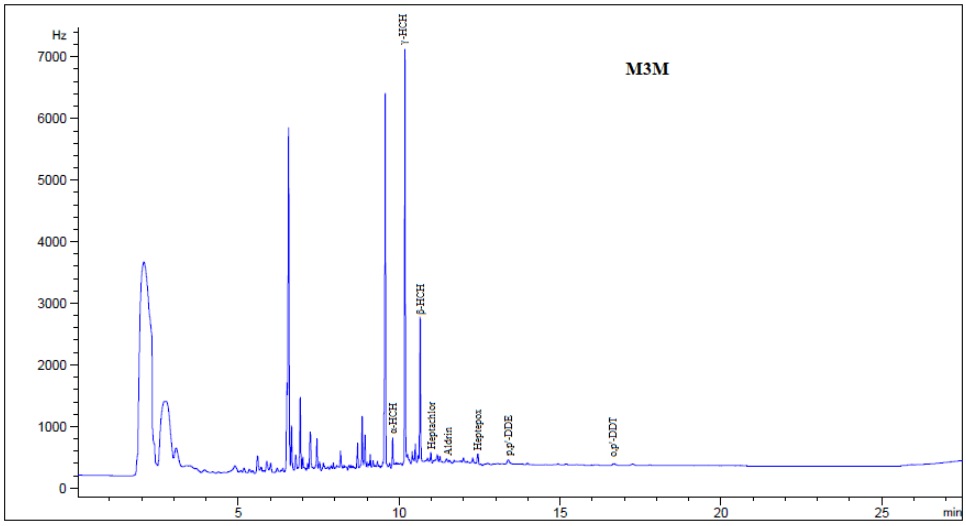
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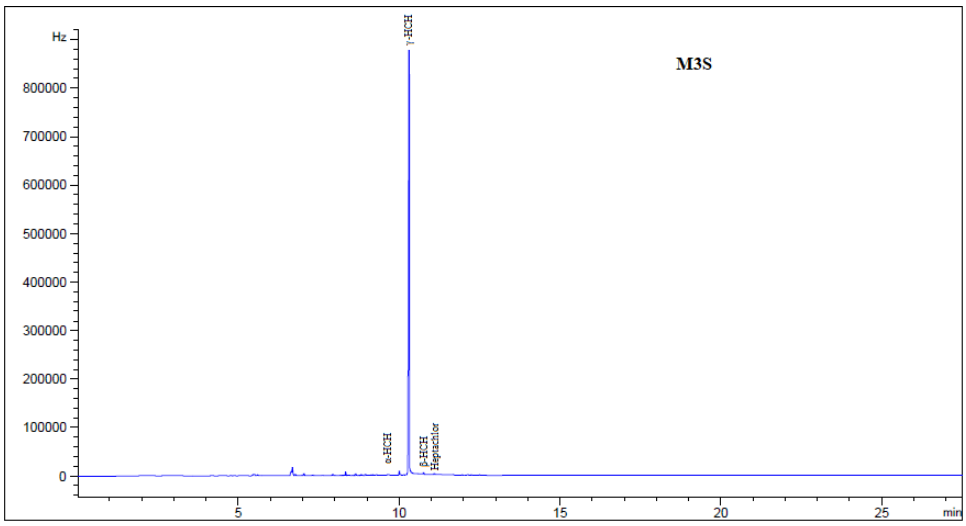
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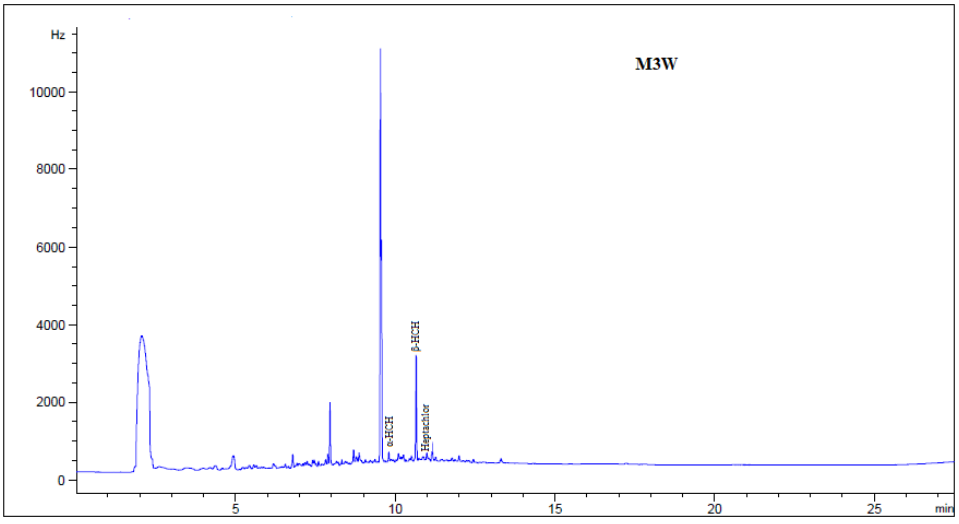
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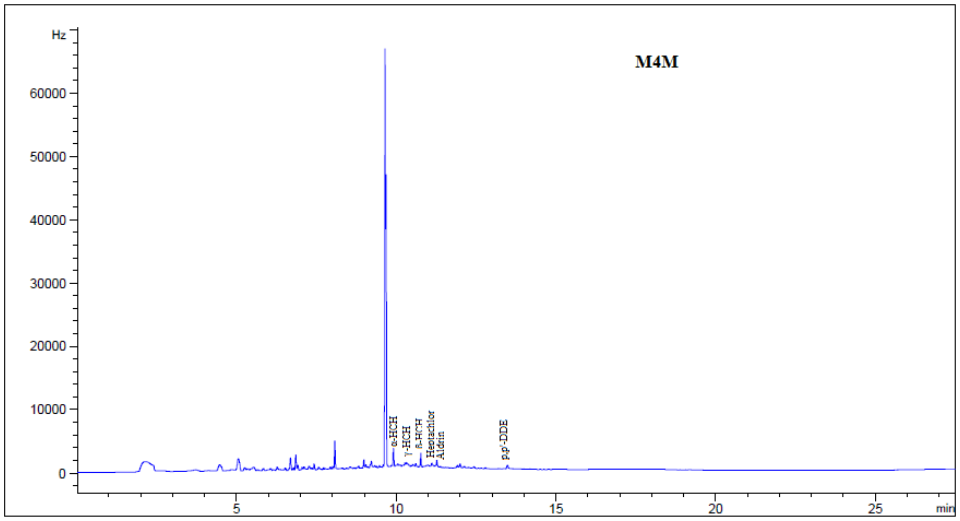
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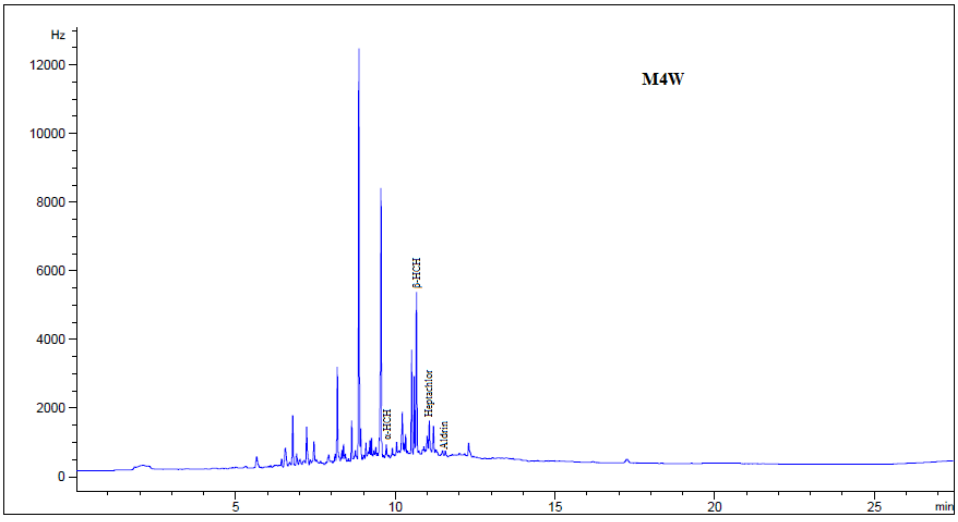
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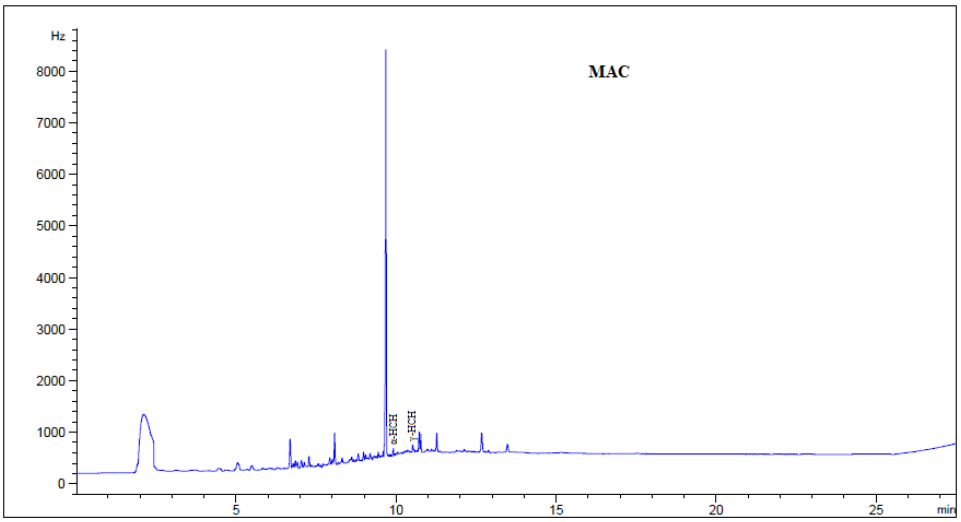
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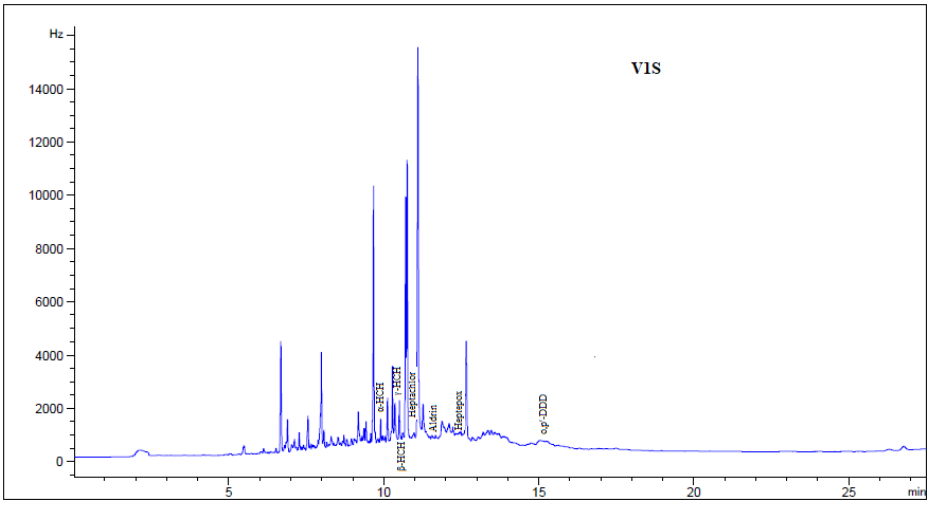
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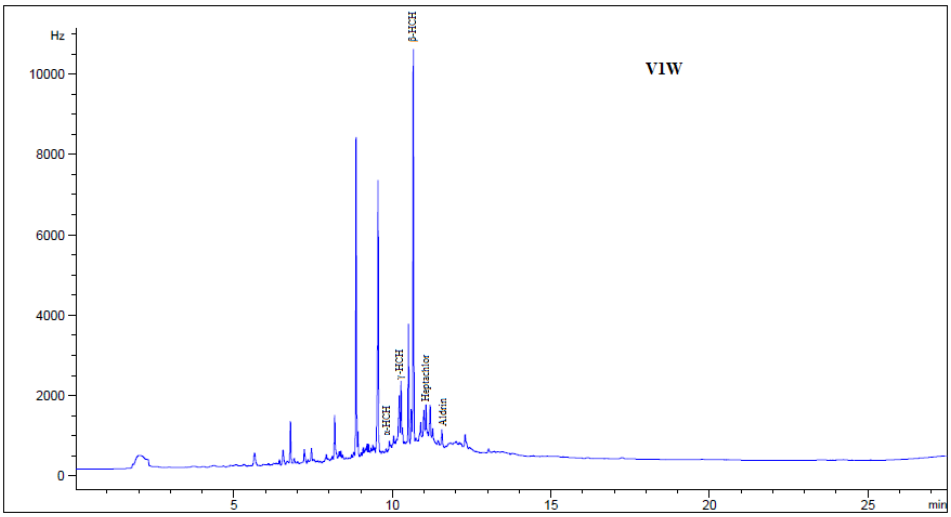
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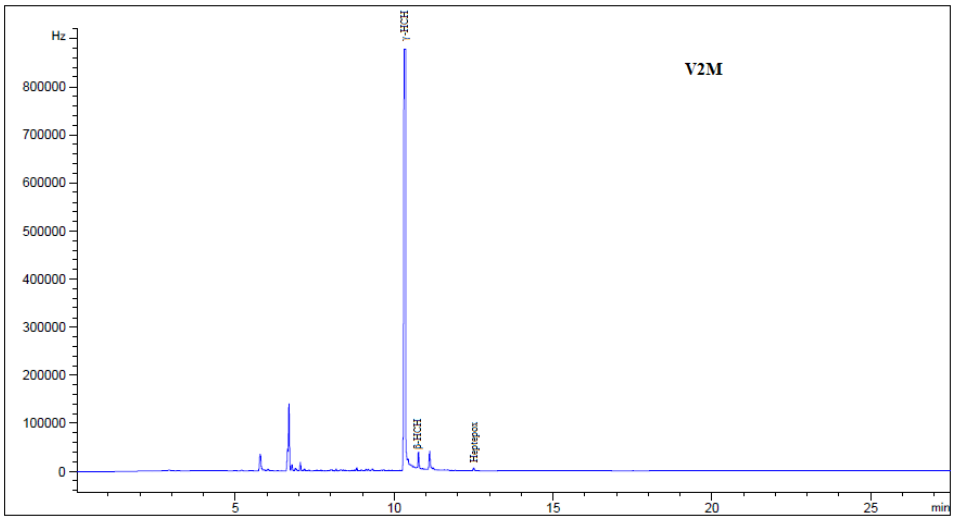
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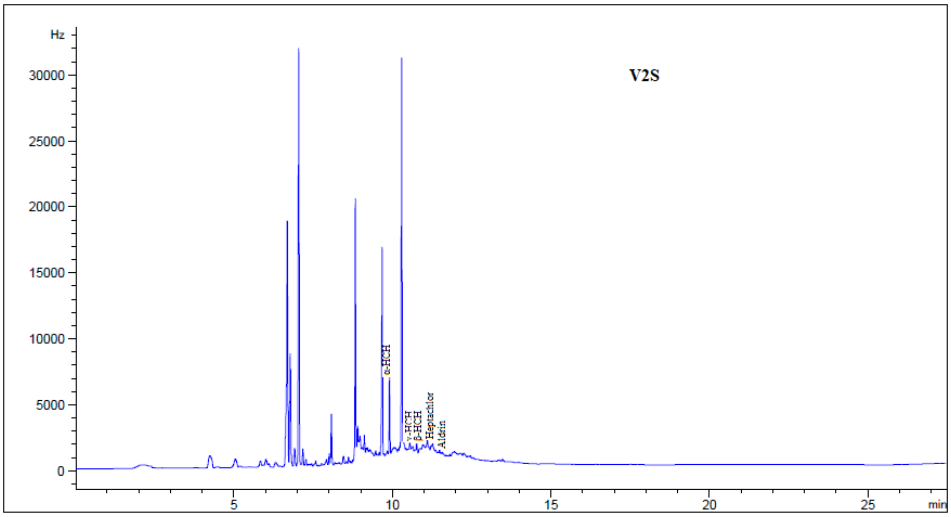
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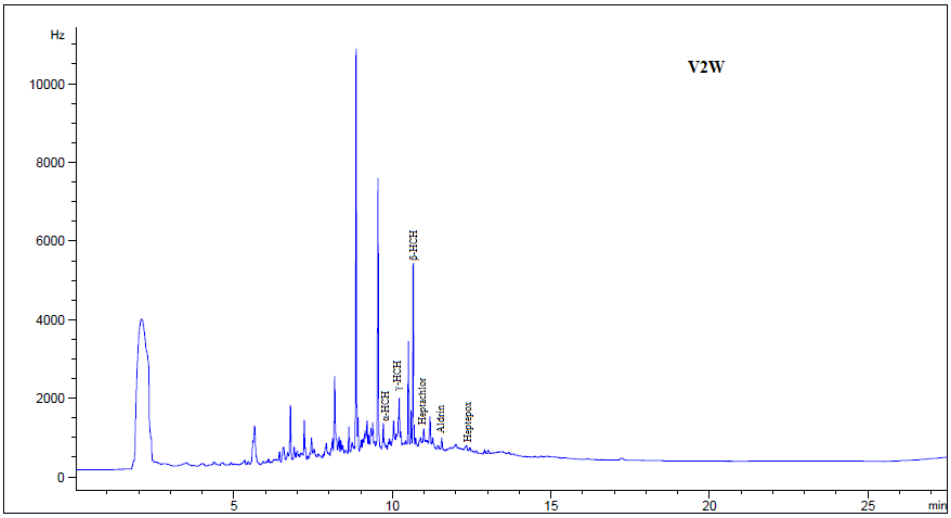
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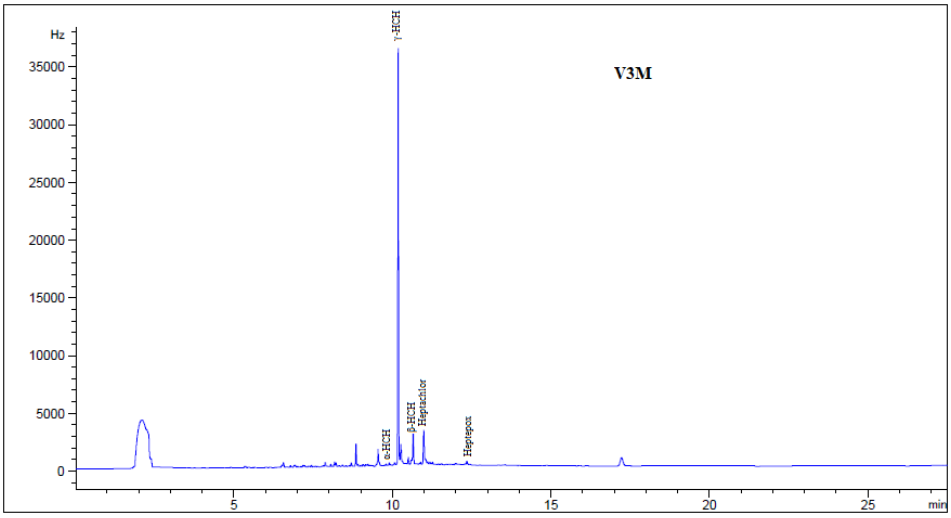
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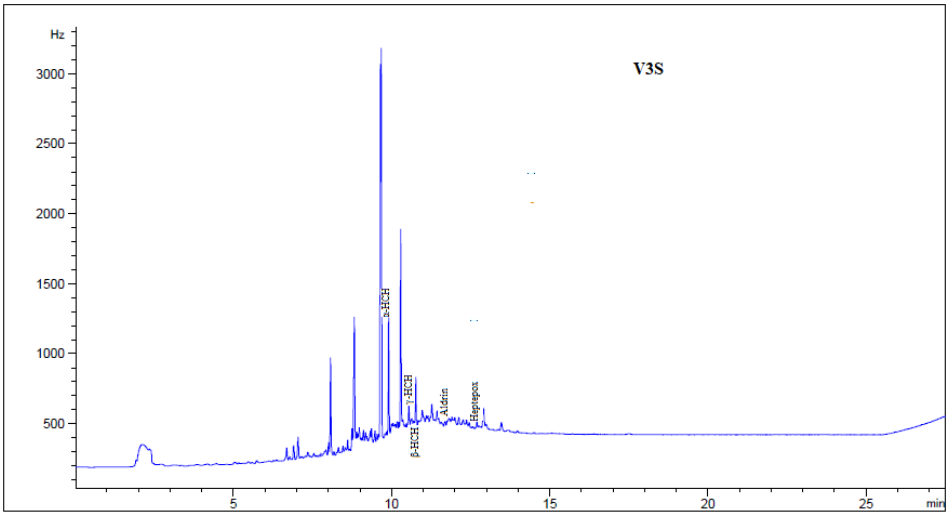
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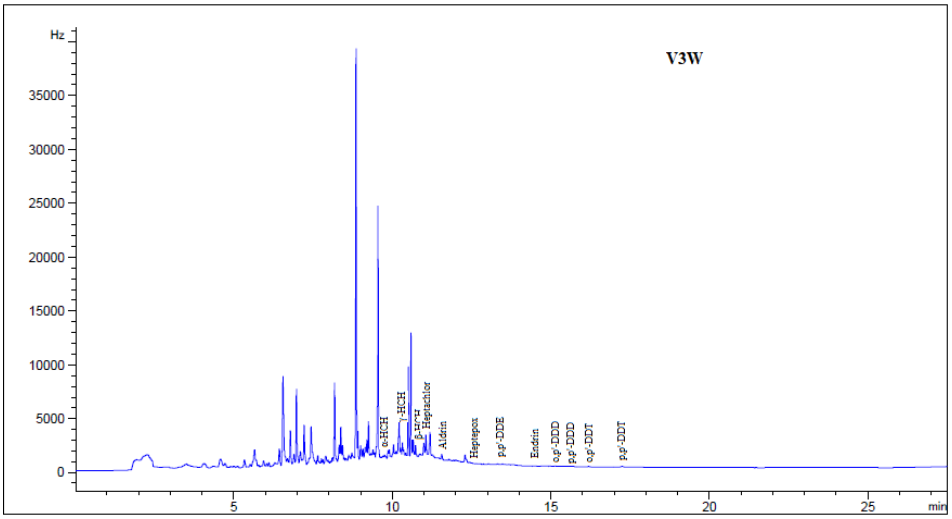
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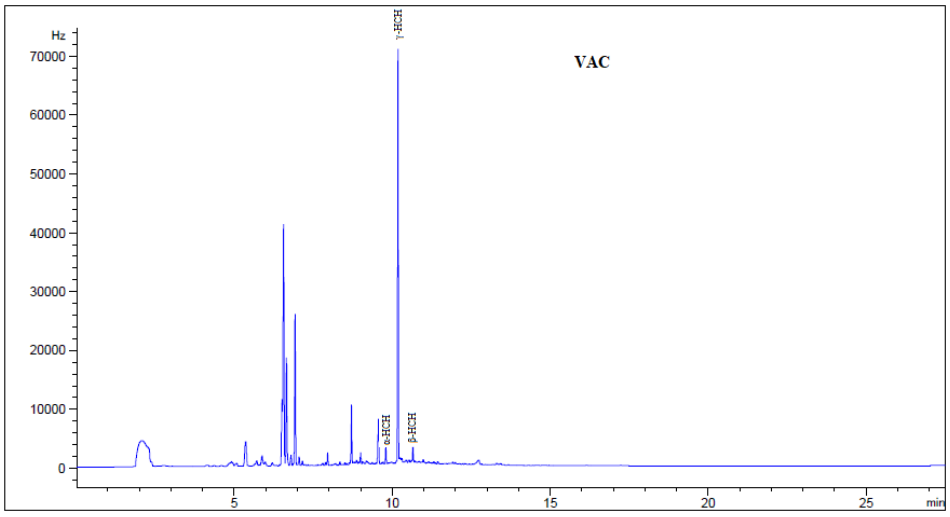
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Annexure 2

List of Abbreviations

OCIs	-	Organochlorine insecticides
POPs	-	Persistent Organic Pollutant
DDD	-	Dichloro-diphenyl-dichloro-ethane
DDE	-	Dichloro-diphenyl-dichloro-ethylene
DDT	-	Dichloro-diphenyl-trichloro-ethane
HCH	-	Hexachlorocyclohexane
Heptepoxide	-	Heptachlor epoxide
L	-	Litre
mL	-	milliliter
%	-	Percent
Chl-*a*	-	chlorophyll-*a*
Chl-*b*	-	chlorophyll-*b*
Chl-*c*	-	chlorophyll-*c*
Pheao	-	pheaopigments
CHO	-	Carbohydrate
PRT	-	Protein
LPD	-	Lipid
ANOVA	-	Analysis of variance
AAS	-	Atomic Absorption Spectophotometry
M. *casta*	-	*Meretrix casta*
V. *cyprinoides*	-	*Villorita cyprinoides*
C.*madrasensis*	-	*Crassostrea madrasensis*
TC	-	Total Carbon
TN	-	Total nitrogen
TS	-	Total Sulphur
TOC	-	Total Organic Carbon
OM	-	Organic matter

CI	-	Condition Index
ww	-	wet weight
h	-	Hour
H_2O_2	-	Hydrogen peroxide
mg/kg	-	milligrams per kilogram
mg/L	-	milligrams per liter
µM/L	-	micromoles per liter
µg/L	-	micrograms per liter
ng/g	-	nanograms per gram
ng/L	-	nanograms per liter
NA	-	data not available
ND	-	not detected
EXC	-	exchangeable
CA	-	Bound to carbonate
FMO	-	Bound to Fe-Mn oxide
OM	-	Bound to organic matter
RES	-	residual
DDW	-	Double distilled water
DO	-	Dissolved oxygen
BAP	-	Bioavailable phase
NBAP	-	non- bioavailable phase
RAC	-	risk assessment code
LC	-	Lipid content
BCF	-	Bioconcentration factor
BSAF	-	Biota sediment accumulation factor
EQS	-	Environmental quality standards

Annexure 3

List of Publications

Publications

1. **Shibini Mol P.A,** Sujatha C.H, Arsha Krishnan, Deepak K, Sruthy Mol PP, Priyanka BR and Dhanya (2019). Spatial distribution of organic geochemical record in the core sediments along the prominent zones of Central Kerala, India. Environmental Forensics. 20(1):1-14. DOI:10.1080/15275922.2019.1566294.

2. **Shibini Mol P.A,** Ranjitha Raveendran, Sujatha C.H (2015). Elucidation of contaminant induced toxic responses in the biota of Vembanad Lake, Kerala. Human Ecological and Risk Assessment. 21(6):1–17.

3. **Shibini Mol P.A,** Dayala V.T, Sujatha C.H (2013). Spatial relationship among phytoplankton abundance and physico chemical parameters around the coastal waters of Kerala. International Journal of Current Research. 5(10):3094-3099.

4. **Shibini Mol P.A,** Sujatha C.H. Distribution and Geochemical Speciation of Sediment Bound Heavy Metals in the Specific Zones of Central Kerala, India. Environmental Nanotechnology, Monitoring and Management. (With Editor).

Conference Abstracts

1. **Shibini Mol P A**, Sujatha C H. Relationship between Phytoplankton Abundance and Environmental variables in the major fishing zones of Central Kerala, South West coast of India. National seminar on Aquatic Chemistry "AQUASEM 19" organized by Department of Chemical Oceanography, Inter University Centre for Development of Marine Biotechnology & Society of Aquatic Chemists, Cochin University of Science and Technology.

2. **Shibini Mol P A**, Sujatha C H. Spatial variation and source identification of heavy metals in the surface sediments of Central Kerala, South west coast of India. Multi-disciplinary International Conference on Green economy and environment sustainability, Al-Ameen College in collaboration with College Development Council, 2018,

3. **Shibini Mol P A**, Sujatha C H. Heavy metal accumulation in the bivalves along the specific fishing zones of Central kerala, India. fifth national conference of Ocean Society of India (OSICON-17), NCESS, Trivandrum, 2017,

4. **Shibini Mol PA**, Ranjitha Raveendran, Sujatha C H. Bioaccumulation of Organochlorine Insecticides in the Bivalve (Villorita Cyprinoids Var Cochinensis) of Vembanad Lake, Kerala. Abstract of papers, National Conference of Ocean Society of India on Role of Oceans in Earth System (OSICON' 13), Pune, India, 2013, 325-328.

5. **Shibini Mol P A**, Vani Venu, Sujatha C H. Distribution and enrichment evaluation of heavy metals in Kerala coast, West Coast of India. National seminar on Green chemistry and Environmental Conservation, Morning Star Home Science College, Ankamali, 2013,58.

6. **Shibini Mol P A**, Dayala V T, Sujatha C H. Spatial assemblage of phytoplankton and its relation to physicochemical parameters in the shore regions of Kerala Coast. National symposium on Coastal Oceanographic Studies: Modeling and Observations (COSMOS), NPOL, Cochin, 2013, 214-220.