POLYCYCLIC AROMATIC HYDROCARBONS AND TOXIC TRACE METALS IN SEAWATER AND SEDIMENTS FROM THE WEST COAST OF INDIA AND THEIR BIOACCUMULATION IN SOME SELECTED SPECIES OF FISHES

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CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried by Mrs. Latha Unnikrishnan, under my supervision and guidance in the Central Institute of Fisheries Technology, Cochin in partial fulfillment of the requirements for the degree of Doctor of Philosophy and that no part of this work thereof has been submitted for any other degree.

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Chapter 1

INTRODUCTION

GENERAL INTRODUCTION

The large Arabian Sea marine ecosystem includes the western Arabian Sea, bordering Somalia, Yemen and Oman, the Central Arabian Sea bordering Iran and the eastern Arabian Sea bordering India and Pakistan. Each sub region has its own special features in terms of current patterns, physical characteristics, physico chemical qualities, dominant fish species, biodiversity, etc. Depending on monsoon winds, local topography, the width and depth of the continental shelf, and drainage of coastal areas, there are three coastal ecosystems, each charecterised by its own productivity and species distribution (Dwidedi and Choubey, 1998). The continental shelf is widest off the north west coast of India. This region often gets tropical cyclonic storms. The Arabian Sea is a monsoonal area. For half of the year from July to December the winds in this region are from the south-west, inducing a great deal of evaporation from the warm waters of Arabian sea, and heavy rain fall along the coast of India. The wind blow towards India and cause upwelling of low oxygen waters. There is a concentration of fish in near shore areas at this time. In the other half of the year, the winds blow in the opposite direction, and not as strongly as during the southwest monsoon. There is clear difference in salinity in water masses of different origin. Off India's south-west coast, mud banks help to increase productivity whereas the north-west coast is influenced by wind-induced upwelling.

1.1 Fishery resources in Arabian Sea

With a long coastline extending along the mainland and the rich areas surrounding the Andaman and Nicobar Islands and Lakshadweep Archipelago and a fairly wide continental shelf and slope, India has rich and varied marine fishery resources. It consists of different species of fishes, crustaceans and molluscs. The total length of India's coastline is about 8129 km. The west coast of India has a sea front of about 2400 km in length extending from Cape Comerine in the south to Rann of Kutch in the north. The fishing grounds on the continental shelf of west coast up to 200 m depths may be roughly estimated to be 168350 km^2 (Balakrishnan, 1985). The seas that surround the Indian coasts are parts of the Indian Ocean, which has an area of 74917000 km^2 (Balakrishnan, 1985) lying between longitudes 20° E and 115° E. The continental shelf of India is more prominent on the west coast than on the east coast. A large scale turbulence involving upwelling and wind phenomenon occur on the south-west coast of India which contributes to the Arabian sea, becoming one of the most productive fishing grounds in the world. About 3/4th of the Indian sea food production is from the west coast. India's south-west coast ecosystem is dominated by small pelagic fishes, such as oil sardines (*Sardinella longiceps*), mackerels (*Rastrelliger kanagurta*) and tunas. Most of this catch comes from a narrow 10-15 km coastal belt, and accounts for 23.6% of India's marine fish catch. The dominant fish species off India's central-west coast ecosystem are Scianids (*Pseudoscianata diacanthus*), carangids (*Caranx* spp) and anchovies. The dominant species of

India's north-west coast are prawn, Scianids and Carangidae. Small tuna migrate this area to breed.

Current fishing methods have resulted in the over-exploitation of coastal resources such as sardines, prawns, pomfrets and mackerel. However, most of the fish stock breed in deeper off shore waters so there is an opportunity to rebuild stocks. The over-exploitation is mostly due to the large fishing vessels that fish illegally near the coast. Population pressure in India will continue to put pressure on coastal resources. India has over 1 billion people to feed. Seventy percent of them eat fish. India would require 13 million tones of fish to meet the minimum requirements. Yet the present production is 3.9 million t. So there is an urgent need for a long-term plan for conservation and management of the marine ecosystem. Indian coasts have a large variety of sensitive eco-systems. Sand dunes, coral reefs, mangroves, and sea grass beds and wetlands are some that deserve special mention. These ecosystems are the spawning grounds and nurseries for a number of commercially important fishes, gastropods and crustaceans. A critical feature of these ecosystems is the variety of bioactive molecules that they host. Recent monitoring of organisms from the tidal and inter-tidal zone has revealed large numbers of molecules with obvious implication for human health and some have industrial applications also. This could be the most commercially important aspect of the Coastal Zone. Molecules that show bioactivity from one ecosystem may not show the same activity, or level of activity, when mined from a different locale or different season. This feature alone should be reason enough for the protection of all such ecosystems, and not only representative isolated units in protected areas / parks.

1.2 Marine Pollution

Exploitation of marine environment for food, minerals, chemicals, ~~etc,~~ recreational activities and waste disposal led to constant pollution of the aquatic environment by anthropogenic inputs. The word pollution generally refers to virtually any substance or energy released into the environment by human activities, which are detrimental to mankind or ecosystem. Increased industrialisation for economic development necessitates a greater awareness of the dangers of consequent environmental pollution. Presence of organic and inorganic pollutants in surface waters continues to be one of the most important environmental issues of the world. Global economy is expected to reach 13 trillion dollars by 2050, more than five times what it is today. Improved and increased utilization of the available resources will invariably lead to increased production of wastes. On account of this, more area is required for waste disposal. The enormous volume of sea naturally appears to offer virtually an unlimited capacity for mineralizing organic refuse. Many coastal towns take advantage of this and release their domestic sewage and industrial effluents into the sea. Deposition of harmful industrial byproducts and contaminants into the surface waters poses serious problems to the environment. Despite the size of the ocean and their thorough intermixing, water circulation is mostly slow and dispersion of materials in sea is sometimes a very gradual process. Consequently, where, large amounts of wastes are discharged into shallow waters, pollutant concentration may reach high proportions. In addition to the waste disposal by man, accidental discharge and natural or accidental calamities also add to the pollution dynamics in the marine environment.

Pollutants can be classified into highly persistent pollutants, moderately persistent pollutants and transient pollutants. The different pollutants that enter the marine environment include heavy metals, hydrocarbons, radioactive wastes, petroleum hydrocarbons, agricultural run-off containing harmful pesticides and domestic and industrial effluents.

Any exposure of the food fishes to the toxic contaminants in the environment will lead to their subsequent bioaccumulation in them. This in turn is transferred to human beings through food chains. This situation necessitates regular monitoring of pollution in the marine environment.

1.3 Pollution of the Indian coast

Pollution in India mostly arises from land-based sources - industrial & domestic wastes and agricultural run-off. Shipping and associated shipbuilding, breaking and port activities are becoming increasingly significant. The crop of recently started coastally located industries use seawater as a resource and the coastal domain as a sink of altered seawater [temperature and density]. These pose newer, more direct threats to sensitive eco areas. Estuaries are natural transport centers providing good, often shallow natural harbours and link sea with rivers. The waste from inland, released into adjacent waters eventually reaches the estuary through canals and rivers. The regular change of tides will often carry the waste out into the sea. All these have made the seas the final dumping ground of wastes (Vinith Kumar, 2001).

1.4 Major coastal areas and cities of India are all situated adjacent to estuaries

Gujarat

Industries manufacture bulk chemicals, dyes, pharmaceuticals and phosphorus pesticides and discharge over 200 MLD of effluents, which are acidic, oxygen depleted and sediment laden. The effluents contain heavy metals, phenols, nitrogen and phosphorous. This has affected the water quality of the Narmada, Tapti and Mahi rivers.

Mumbai

The River Kalu, north of Mumbai, flowing through the industrial towns of Ambarnath, Ulhasnagar and Kalyan has a mercury concentration exceeding 100 ppm. Thana creek in Mumbai receives effluents of over 50 MLD, where high mercury levels are present in the water, sediments and living organisms.

Kochi

The Periyar river receives chemical industry effluents and untreated sewage. Incidents of ulceration in shrimp and fish, and frequent fish mortality have affected traditional fishing, with no pollution abatement efforts made.

Goa

Estuarine and coastal waters are "clean", though there is high sediment load from mining activities. The Mandovi-Zuari estuaries receive over 30 MLD of partly treated domestic sewage and 15 MLD of industrial and agricultural effluents.

Calcutta

The Hoogly River carry effluents that have contaminated fish and shell fish with heavy metals such as Ni, Cu, Cd and Zn. The sediments near Haldia have up to 10 µg/g of pesticides. E.coli, shigella, salmonella and other human pathogens contaminate these river waters - indication of severe sewage contamination.

The data, to date, indicate that the Indian coasts have well circulated oxygenated waters, and that hot spots remain contained within reasonable limits.

1.5 The major rivers in India may be classified as:

(1) Himalayan rivers

(ii) Peninsular rivers

(iii) Coastal rivers

(iv) Rivers of the inland drainage basin

The Himalayan Rivers are perennial as they are generally snow-fed and have reasonable flow throughout the year. During the monsoon the Himalayas receive very heavy rainfall and the rivers discharge the maximum quantity of water causing frequent floods. The Peninsular rivers are generally rain-fed and, therefore, fluctuate in volume. A large number of the streams are non-perennial. The streams of the inland drainage basin of western Rajasthan are few and far between. Most of them are of an ephemeral character. They drain towards the individual basins or salt lakes like the Sambhar or are lost in the sands having no outlet to the sea. The Luni is the only river of this category that drains into Rann of Kuchch. The Ganga sub-basin, which is a part of the larger Ganga-Brahmaputra-Meghana basin, is the largest in India receiving waters from an area, which comprises about one-quarter of the total area of the country. The Ganga flows through Uttar Pradesh, Bihar and West Bengal in India and enters Bangladesh thereafter. It has two main headwaters in the Himalayas: the Bhagirathi and the Alaknanda, the former rising from the Gangotri glacier at Gomukh and the latter from a glacier short of the Alkapuri glacier. The Ganga is joined by a number of the Himalayan Rivers including the Yamuna, Ghagra, Gomti Gandak and Kosi. The western-most river of the Ganga system is the Yamuna, which rises from the Yamunotri glacier and joins the Ganga at Allahabad. Among important rivers flowing north from central India into the Yamuna/ Ganga are the Chambal, Betwa and Sone. The Brahmaputra and the Barak flowing from east to west in north-eastern region are international rivers and have immense water resources potential which is still in the initial stages of development.

The Godavari in the southern Peninsula has the second largest river basin covering 10 percent of the area of India. Next to it is the Krishna basin in the region, while the Mahanadi has the third largest basin. The basin of the Narmada in the uplands of Deccan flowing to the Arabian Sea and of the Kaveri in the south falling into the Bay of Bengal are about the same size, though with different character and shape. Two other river systems, which are small but agriculturally important, are those of the Tapti in the north and Pennar in the south. These west coast rivers are of great importance as they contain as much as 11 percent of the country's water resources while draining about 50 percent of the land area.

A compilation of the type and quantum of pollutants into the coastal ecosystem of India are given below:(Elrich de Sa, 2001).

No	Input / pollutant	Quantum- Annual
1.	Sediments	1600 million tonnes
2.	Industrial effluents	50 x 10^6 m^3 tonnes
3.	Sewage - largely untreated	0.41 x 10^9 m^3 tonnes
4.	Garbage and other solids	34 x 10^6 tonnes
5.	Fertilizer - residue	5 x 10^6 tonnes
6.	Synthetic detergents - residue	1,30,000 tonnes
7.	Pesticides - residue	65,000 tonnes
8.	Petroleum hydrocarbons (Tar balls residue)	3,500 tonnes
9.	Mining rejects, dredged spoils & sand extractions	0.2x10^6 tonnes

Of the world's total crude oil and its products that amounts to 20000 metric tonnes annually, a major part is transported by the sea route. Of this, approximately 60% were shipped along the oil tanker routes across the Indian Ocean. Oil spills along the routes will have serious deleterious effects on the water quality of the marine environment and the fishery resources. The

Arabian Sea is a major oil tanker route to south east Asia, and beyond, probably accounting for the tar like residues deposit on the west coast of India. This chronic problem is however a seasonal feature and is largely regulated by the monsoons and associated winds. Metals, being a conservative pollutant, need careful monitoring since they remain indefinitely in the environment without breakdown. As yet our waters are relatively clear. There are urgent needs to be addressed at the regional and local scale. The decline in fisheries, the increasing pollution by heavy metals, PCBs & pesticides are local effects produced by anthropogenic influence. However, not enough data exists to relate these to changes in the earth system. Such effects are however keenly felt and must be dealt with scientifically to ameliorate local fallout.

1.6 Problem proposed for present study

Although pollution from toxic metals and pesticides are well studied, reports on pollution by Polycyclic Aromatic Hydrocarbons (PAHs) which are established potential carcinogens is scanty. This is an aspect, which calls for urgent systematic study and constant monitoring, as incidence of different types of cancer is going up in the country as a result of modern urban life. Oil tankers emptying residual crude to coastal harbour waters, other hydrocarbons from land and other sources all contribute to this pollution. This study proposes to take up a systematic study (~~perhaps the first of its kind~~) along our entire west coast. As the problem is of gigantic magnitude, the aspects and the variables to be studied are also correspondingly large. An attempt is made here to study the salient aspects of the problem within the scope of a large study on the pollution profile of our coastal seas. The water, sediments and selected four species of fishes available round the year, are studied for the purpose.

The study also covers the pollution of our coastal waters with metals like Hg, Pb, Cd, Cu, Zn and Ni. The seasonal changes in the concentration of these metals along the coast with special emphasise on the waters off Cochin was also studied as a part of this study. In the present study an attempt was made to determine the distribution of trace metals in the tissues of four species of edible fishes from the West Coast of India.

1.7 Objectives of the study

1. To collect base line data on the concentration of PAHs in seawater and sediment from the west coast of India
2. To study the concentration of PAHs in four species of fishes from the west coast
3. To study the comparative levels of PAHs in fish and in the aquatic environment of the west coast of India
4. To study the influence of sediment characteristics on the concentration of PAH in sediment
5. To study seasonal changes in PAH concentration in water, sediment and fish
6. To provide a baseline concentration of trace metals in water, sediment and fish from the west coast of India
7. To study the seasonal change in the content of selected trace metals in water, sediment and fish from the west coast of India

Chapter 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Polycyclic Aromatic Hydrocarbons are wide spread contaminants in the marine environment (Blumer, 1976; Suess, 1976; Harvey, 1996). These are compounds consisting of two or more aromatic rings and two adjacent benzene rings that share two carbon atoms. They contain many structural isomers. So far, over 100 PAHs have been detected in the environment. On the basis of their properties and their molecular weight, two classes of PAHs can be distinguished, i.e the two and three ring aromatics from naphthalene to anthracene and the four to six ring aromatics from fluoranthene to indeno (1,2,3-c,d) pyrene. These compounds have- been widely studied (Neff, 1979; Mc Elroy *et al.*, 1989) because of their carcinogenic and mutagenic character. PAHs containing 24 or fewer ring carbons have been chosen as target compounds for environmental monitoring of their biological effects. Most of these groups have mutagenic effects in some bacterial mutation tests, and roughly 60% of the PAHs have been found to be carcinogenic in mammals (Sivasami *et al.,* 1990). Numerous reports indicate that carcinogenic PAHs are immunotoxicants. The main environmental significance of PAHs are their carcinogenic potential as established by several workers (Marvin *et al.*, 1995; Hall and Glower, 1990). In 1965, Doll *et al.* demonstrated that most human cancer might be attributable to environmental carcinogens, especially through the daily diet. The carcinogenic risk to man from these chemicals in the environment can in principle evaluated only from epidemiological studies (Zander, 1968). International Agency for Research on

Cancer publication has been dedicated to analysis of cancer in processes, which involve exposure to PAHs (Anon, 1983; 1987). Since there is no adequate information regarding carcinogenicity of individual PAHs to human beings from epidemiological studies, data from animal bioassays were extrapolated to estimate human cancer risks (Anon, 1993a). A quantitative cancer potency factor has thus far been developed for benzo (a) pyrene only (Anon, 1992). It is 7.3 per mg/kg/ day and is based on animal studies.

Hydrocarbons in aquatic environments originate from several sources, which has been grouped into the following categories (Anon, 1982; Clark, 1997). 1). Petroleum inputs 2) Hydrocarbons especially PAHs arise from the incomplete combustion of organic matter in flames, engines and industrial processes (Hites *et al.,* 1977; Wakeham *et al.*, 1980). 3) Natural sources of PAHs include forest fires, natural petroleum seeps 4) Post depositional transformation of biogenic precursors (Young and Cerniglia, 1995) and 5) Biosynthesis of hydrocarbons by marine or terrigenous organisms. Biological sources include land plants, animals, bacteria, macro-algae and micro-algae.

Once formed, PAH are known to enter the near-shore marine environment through the spillage of petroleum, industrial discharges atmospheric fall outs, shipping activities, storm water drains and urban run-off (Neff, 1979; Gevao *et al.,* 1998). Because of their low water solubility, and their hydrophobic nature, PAH in the aquatic environment rapidly become associated with inorganic and organic suspended particles (Gearing *et al.,* 1980; Gearing *et al.*, 1976; Chiou *et al.,*

1998) and subsequent deposition in sediments. The favourable partition coefficients and greater persistence of sedimentary PAH compared to PAH in solution, means that in general, sediments contain a PAH concentration which is a factor of 1000 or more times higher than in overlying water column (Witt, 1995; Law and Biscaya, 1994). Therefore sediments are important in monitoring of PAH inputs into aquatic environment. The importance of sediments as reservoirs for PAH is well documented (Naes *et al.*, 1995; Prahl *et al.*, 1984; Maher and Aislabie, 1992). Once deposited in sediments PAHs are subjected to low photochemical or biological oxidation. Thus PAH level is more or less persistent in sediments (Lizia Guzella *and* Paolis, 1994).

PAHs have also been found in phytoplankton, plant leaves, river sediments, suspended solids and worms (Lake *et al.*, 1979). This aquatic biota serves as a food source for all aquatic animals (Andelman and Suess, 1970). A variable proportion of sediment associated PAH is available for uptake by demersal fish. A major factor determining this is the amount of organic carbon present (De Witt *et al.*, 1992; Livingston *et al.*, 1993). The life style and feeding habits of the organism are also important factors in the bioaccumulation of these compounds. As PAH solubility decreases with increasing molecular weight, bioaccumulation of PAH from sediments by marine organisms is generally greater for the lower molecular weight and more water-soluble compounds than for the higher molecular weight compounds.

Some invertebrates are able to degrade and excrete PAH and PAHs are very efficiently metabolized by phase 1 enzymes of cytochrome P450 in fish liver and excreted in bile. Thus although they are exposed to sedimentary PAH via several routes, they do not generally accumulate these compounds at very high levels. Only very high concentrations can be lethal to fish. According to Roberts *et al.* (1989) LC50 values for PAH recorded are 81-3220 μg/g dry weights. But many carcinogenic and mutagenic intermediates such as diol epoxides are produced by cytochrome P-450 while metabolizing the PAH (Vander Oost *et al.*, 1994). The environmental concern of PAH is due to their potential to form carcinogenic and mutagenic derivatives such as diols and epoxides (Woodhead *et al.*, 1999).

Concentration of total PAH as low as 1-3 μg/g dry weight are able to induce cytochrome P-450 enzyme in Winter flounder and Spout (Payne *et al.*, 1988). Carcinogenesis and liver neoplasia have been reported in brown bullhead catfish at total PAH concentrations as low as 0.1 μg/g and as high as 41 μg/g (Baumann *et al.*, 1995). Relatively high concentrations of PAH can be lethal to invertebrates also.

Studies on PAHs in Indian waters are limited. Mithlesh *et al.* (2004) studied petroleum Hydrocarbons (PHC) in the marine environment of Bassein Mumbai. He reported that concentration of PHCs in the water off Mumbai varied widely from 2.9- 39.2 μg/l. Chouksey (2002) studied the migration and fate of selected contaminants from anthropogenic discharges in coastal marine environment. Fondekar and Alagarsami (1984) reported the petroleum hydrocarbon contamination along the oil tanker routes in the Arabian Sea. The distribution of petroleum Hydrocarbons in the Goa coastal waters was reported by Fondekar *et al.* (1980). Petroleum Hydrocarbons in the sediments from Bombay harbour, Dharamtar creek and Amba River was done by Ingole *et al.* (1989). Petroleum hydrocarbon residues in the marine environment of Bassein – Mumbai were reported by Mithlesh *et al.* (2004). Ingole *et al.* (1995) studied the concentration of Petroleum hydrocarbons in inter tidal eco system along the Bombay coast. In his study, the concentration of PHC in the intertidal sediment and water samples collected at Madh, Worli and Colaba were in the ranges 2.9-10.3 μg/l. Petroleum Hydrocarbon concentration in some regions of the northern Indian Ocean was investigated by Sengupta *et al.* (1980). A survey of the petroleum hydrocarbon concentrations in the shelf regions and adjacent waters of the west coast of India was made by Sengupta *et al.* (1978)

Fondekar et al. (1980) reported the effects of oil spill in the Goa coast. The environmental impact of Bombay High oil spill was also estimated by Fondekar *et al.* (1980). State of oil pollution in the Northern Arabian Sea after the 1991 Gulf oil spill was investigated by Sengupta *et al.* (1993). Topgi *et al.* (1982) studied the dissolved petroleum Hydrocarbons along the oil tanker routes in the Southern Bay of Bengal.

Studies on Petroleum Hydrocarbon concentration in selected species of fishes and prawn from north west coast of India was carried out by Mehta *et al.*

(1994). According to his study the concentration of PHCs in 6 fish and prawn species sampled at 10 transects from Veraval and Rętnagiri ranged from 0.2- 10 2µg/g wet weight. Variation of PAHs in sediments in sediments of shrimp farms in Cochin are was studied by Ashraf *et al*, (2003). Indra Jasmine *et al*. (2003) studied Polyaromatic hydrocarbons in seafood. Accumulation and release of Petroleum hydrocarbons by *Mytilopsis sallei* from harbour waters of Visakhapatnam was done by Raghuprakash (2003). Investigations on the stress effects of petroleum hydrocarbons on *Metapenaeus dobsoni* were done by Miriam (2002). Jehosheba (2004) studied the biochemical effects of petroleum hydrocarbons on the tropical teleost *Oreochromis mossambicus*.

The occurrence of inorganic contaminants mainly, metals in surface seawater continue to be one of the most important environmental issues of our time. Though metals have exerted a profound influence on the course of biological evolution, their modern day industrial usage, mainly during the course of last fifty years has led to their bioaccumulation in the environments (Moore and James, 1992). Many of these metals find their way into the living systems through air, water and food and tend to accumulate in the body Some of them even in minor concentrations threaten to affect the metal dependent enzyme catalyzed reactions in the body. At least 11 metals are known to be essential for living organisms and these are Fe, Cu, Zn, Co, Mn, Cr, Mo, V, Se, Ni and Sn. Essential metals always function in combination with organic molecules and most commonly with proteins either tightly bound in metalloproteins or more loosely bound in metal protein complexes (Williams, 1981; Brouwer *et al.,* 1986). There is little evidence that marine organisms ever suffer from metal deficiencies and presumably the optimum concentrations are those that occur naturally.

Trace metals reach the marine environment via river run-off carrying discharged heavy metals from industries, mining activities, shipping, dredging activities and through other anthropogenic inputs (Manhan, 1994). From surrounding seawater, food and through the imbibed seawater these get accumulated in marine organisms directly (Depledge and Rainbow, 1990). They are accumulated to body concentrations, orders of magnitudes higher than the concentrations in an equivalent weight of seawater (Eisler *et al.*, 1981; Rainbow *et al.*, 1990). In aquatic environments metals have been termed as conservative pollutants because once added to the environment they remain there for considerably long time if not forever. These metals cannot be broken down to harmless substances by bacterial action. They are leached into the aquatic system as a result of weathering of rocks and volcanic eruptions and as a result of human activities. These processes and activities change the natural concentrations of metals in the seawater resulting in 10 or even 100 fold increase near the point of effluent discharge. The discharge of toxic heavy metal industrial wastes into the sea through rivers and streams result in accumulation of pollutants in the marine environment especially fishes and bio availability of the trace metal is the key factor determining tissue metal concentration (Goldberg *et al.,* 1976). In marine organisms trace metal uptake occurs directly from the surrounding seawater across the permeable body surface from food and imbibed seawater entering the gut (Depledge *et al.*, 1990). The fishes form an important target for biomagnification of the metals as they are at the top of the food pyramid and act as a possible transfer media to human beings. Elevated levels of toxic metals like Cd, Hg, Pb, As, etc; have been observed in various fishery products, particularly molluscan shellfish, from various parts of the world (Lakshmanan, 1989; Kurihara *et al.*, 1993).

Fishes coming towards the top of the food pyramid, there is maximum possible biomagnification in them, which will get transferred to human beings. Even though a good amount of data on the distribution of trace metals in the Indian seas are available (Braganca and Sanzgiri, 1980; Sanzgiri and Braganca, 1981; Rajendran *et al.*, 1985; Satyanarayana *et al.*, 1985), not much work has been done on the concentration of these metals in fishes from the Indian coast, for evaluating the levels of contamination from the natural environments (Matakar, *et al.,* 1981; Singbal, *et al.,* 1982; Kureishy, *et al.,* 1983). The most important works on trace metal concentrations are of Windom and Smith (1972), Abdulla *et al.* (1972), Chestner and Stoner (1974), Chestner and Stoner (1975), Bender and Gagner (1976). Danielsson (1980) studied trace metals from surface waters from different depths of Indian Ocean. Matakar (1981) studied the concentration of trace metals in Bombay harbour bay. Studies on trace metals in seawater from Sourashtra coast has been done by Kesava Rao and Indusekhar (1986). In the Arabian Sea, the most important works are those of Sengupta *et* *al.* (1978), Sanzgiri and Moraces (1979) and Braganca and Sanzgiri (1980), Kureishy *et al.* (1983). Studies in Cochin coast has been done by Mohapatra (1994), Meenakumari and Nair (1984), Nair and Rao (1980), Nair *et al.* (1990), Meenakumari (1989), Radhakrishnan (1993). Asha Jyothy and Vijayalakshmi (1999) studied concentration of metals in fishes from Thane and Bassein creeks of Bombay, India. Maheswary *et al.* (1997) reported heavy metals in fishes from coastal waters of Cochin.

Study of the physico chemical parameters of the seawater is essential to get background information about the pollution in the marine environment. Earlier works on the physico chemical properties of sea water in the Arabian sea have been done by Gupta and Pylee (1964), Qasim and Sankaranarayanan (1969) Meenakumari (1989), Zingde (1985) and Harvey (1926). Distribution of dissolved oxygen in the Western Bay of Bengal was reported by Naqvi *et al.*(1979). Studies on the nutrient fraction and stoichiometric relationship in the Arabian Sea were done by Sen Gupta *et al.* (1978). The noteworthy contributions pertaining to Cochin backwaters are those of Wallershaus (1972) on the hydrography, Ramamritham and Jayaraman (1963) on the hydrohraphical conditions around Willingdon Island, Haridas *et al.* (1973) on salinity, temperature, oxygen and zooplankton biomass of the backwater, Balakrishnan and Shynamma (1976) on the diurnal variation of physico chemical parameters during the south west monsoon, Joseph (1974) on nutrient distribution of harbour.

The preceding review of literature on PAHs and trace metals in the marine environment indicates that there is a paucity of data on the west coast of India in the west coast. In view of the great concern in the water quality of the Arabian Sea and the safety of the food fishes, the current investigations are aimed to fill the lacunae in information on trace metal levels and PAHs levels in water, sediment and fishes from the west coast of India.

Chapter 3

MATERIALS AND METHODS

3.1 Area of Investigation

Samples were collected from west coast of India during the cruises namely 181,191, 197, and 204 of the Fisheries Oceanographic Research Vessel "Sagar Sampada" under the Department of Ocean Development, Govt. of India during the period from February 2000 to October 2003. The operational area covered lat.8° -21° N and long.69° -75° E. Since the numbers of cruises of Sagar Sampada were limited in the west coast, samples were taken from the off Cochin area from shallower waters, for two years to get enough data for the study so that a comparison can be made between the two. Monthly samples of the water, sediments and fishes were collected between 2000-2002 from off Cochin area from MFB "Matsyakumari" of Central Institute of Fisheries Technology (CIFT), Cochin. The sampling was done at a depth of 20-25m. The operational area covered latitude 9° 54' 28"N to 10° 02' 58"N and Longitude 76° 05' 58"E to 76° 12' 47"E. The details of the study area are given in Table 3.1-3.7and in figures 3.1-3.5.

Cruise No.181

The fishing was conducted along the coastal waters off Gujarat from latitude 20° 18' to 21° 31'N and longitude 69° 10'- 70° 33' E. at a depth range of 50-100m during February 2000 in the premonsoon season (Table 3.1 & Fig. 3.1).

Cruise No. 184

Area covered during the cruise was from latitude 8° 30'-22° 00'N and longitude 69° 20'- 76° 29'E, from off Quilon to off Gujarat during May 2000 in the monsoon season (Table 3.2, 3.3 & Fig. 3.2). The entire area of study was divided into 4 zones and samples were collected from each zone.

Cruise No. 191

Cruise was conducted between latitude 8°47'-11°47'N and longitude 74° 27'- 76° 27'E from off Quilon to off Manglore during the period from 8th August 2001 to 24th August 2001 in the monsoon season (Table 3.4 & Fig. 3.3).

Cruise No.197

Area covered during the cruise was from latitude 9° 08' – 11° 46'N longitude 74° 25'- 76° 00' E from off Cochin to off Manglore during the period from 4th January 2002 to 24th January 2002 in the pre monsoon season (Table 3.5 & Fig. 3.4).

Cruise No.204

The cruise covered the area between latitude 8°14'N and longitude 73° to 74° E during 20th October 2002 to 6th November 2002 in the post monsoon season (Table 3.6 & Fig. 3.5).

3.2 Stations in the Off Cochin area

Vembanad lake is a perennial and extensive backwater system stretching 75 km to the south and 40 km to the north of Cochin harbour with innumerable canals branching out from the lake forming an extensive complex of inland water ways. The port of Cochin is located near the confluence of the Vembanad Lake with the Arabian Sea. Vembanad Lake maintains connection with Arabian Sea through a narrow opening, 365 m broad between the mainland of Cochin and the

Vypeen Island. Several rivers originating from the Western Ghats empty into the Vembanad Lake, the major ones being the Periyar and Muvattupuzha river. There are many factories such as a major fertilizer plant producing K- P- N fertilisers an oil refinery, a rare earth element processing factory and Zn smelters on the banks of the Periyar river and a News Print factory on the Muvattupuzha riverbank. Cochin is located at latitudes 9°58' N and 76° 14' E and is an all weather major port on the southwest coast of India. The maximum rainfall in this region occurs in May, June, July and August and average rainfall is 3000 mm (Soman, 1997).

Seven stations were selected for the present study in the area. The details of the stations are given in Table 3.7. Sampling was done monthly in different seasons for two years (2000-2002).

3.3 Methods of sampling and analysis

From FORV Sagar Sampada water samples (both surface and bottom) were collected using Niskin bottles attached to Conductivity Temperature Depth (CTD) instrument. Temperature and salinity of the water samples were measured using the CTD. pH of the water samples measured using precision pH meter (Jackson, 1973). Salinity of water samples collected from off Cochin area was analysed by the method of Strickland and Parson (1972). Dissolved Oxygen was measured by the Winkler method (Grasshoff, 1983).

Sediment samples were collected from different stations using Smith & Mac Intyre grab for the analysis of trace metals and Polycyclic Aromatic

Hydrocarbons (PAH). The samples were kept in freezer maintained at -15° C in polythene bags until analysis. The sediment organic carbon was determined using the method of Walkly and Black (Allison, 1965). Sediment characteristics were determined using the Robinson combined sieve and pipette method, (Day, 1965). Analysis of nitrite-N, Phosphorus and total nitrogen was carried out by standard methods (Grasshoff, 1983).

3.4 Analysis of PAH in sediment

For PAH analysis, sediment samples (50 gm) were dried under vacuum at room temperature and extracted using dichloromethane. The organic layer was separated and was passed through anhydrous sodium sulphate. The process was repeated three times for the complete extraction of the PAHs. The extract was concentrated to a small volume using rotary evaporator and cleaned up using alumina: silica columns using 1:1 dichloromethane: hexane eluent. The samples were evaporated to dryness and redissolved in a fixed volume of high purity acetonitrile. The quantification of PAH was carried out using in HPLC (Hew lett Packard model 1090) fitted with Emerck PAH column. The maximum elution time was 30 minutes. The samples were analysed for sixteen PAH constituents viz. naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluorene, pyrene, benzo (a) anthracene, chrysene, benzo (k) fluoranthene, benzo (a) pyrene, dibenzo (a, h) anthracene, benzo (g, h, I) perylene and indeno (1,2,3-cd) pyrene. A mixture of 16 PAHs from Sigma chemicals, USA, was used as standard. Solvents like acetonitrile, n-hexane and dichloromethane used for extraction were of HPLC grade. A solvent system of 100% acetonitrile pumped at the rate of 1ml per minute was used (Lizia Guzella and Paolis, 1994).

3.5 Analysis of PAH in water

Water samples (1 litre) were extracted using dichloromethane in a separating funnel. The organic layer was collected and concentrated to a small volume and cleaned up using alumina: silica columns using Dichloromethane: hexane (1:1) mixture. The purified samples were redissolved in high purity acetonitrile and fed into the column of HPLC.

3.6 Analysis of PAH in Fish

Four species of edible fishes namely, *Saurida tumbil*, *Nemipterus japonicus*, *Epinephelus diacanthus* and Sciaenidae (sp) were selected for the study. These species are found abundantly in all seasons through out the west coast of India. *Nemipterus japonicus* is found abundantly in coastal waters, mainly on mud or sand bottoms usually in schools. They feed mainly on crustaceans, mollusks (mainly cephalopods) polychaetes and echinoderm. Sciaenid are small to moderately large fishes, primarily costal water species. Majority of them are found over muddy bottoms. In India about 60% of the total marine fish landings corresponds to sciaenids. Most of them feed on small crustaceans, fishes and benthic organisms. *Saurida tumbil* is a highly abundant species along the west coast of India. They are bottom dwelling and fish eaters. The flesh is said to be of good quality and flavour though bony. *Epinephelus*