POLYCYCLIC AROMATIC HYDROCARBON CONTAMINATION IN PALLIKARANAI WETLAND, CHENNAI, TAMIL NADU, INDIA; FISH AS AN INDICATOR

Thesis submitted to Bharathiar University, Coimbatore for the award of Degree of

DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL SCIENCE

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Dr. S. MURALIDHARAN

Senior Principal Scientist

DIVISION OF ECOTOXICOLOGY

SÁLIM ALI CENTRE FOR ORNITHOLOGY AND NATURAL HISTORY

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डॉ. एस मुरलीधरन / Dr. S Muralidharan
वरिष्ठ प्रधान वैज्ञानिक / Senior Principal Scientist
सालिम अली पक्षिविज्ञान एवं प्रकृति विज्ञान केन्द्र
Sálim Ali Centre for Ornithology and Natural History
आनैकट्टी, कोयम्बटूर – 641 108
Anaikatty (Post), Coimbatore - 641 108

Countersigned

Head of the Department
डॉ. एस मुरलीधरन / Dr. S Muralidharan
वरिष्ठ प्रधान वैज्ञानिक / Senior Principal Scientist
सालिम अली पक्षिविज्ञान एवं प्रकृति विज्ञान केन्द्र
Sálim Ali Centre for Ornithology and Natural History
आनैकट्टी, कोयम्बटूर – 641 108
Anaikatty (Post), Coimbatore - 641 108

Director
डॉ. के शंकर / Dr. K. Sankar
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Sálim Ali Centre for Ornithology and Natural History
आनैकट्टी, कोयम्बटूर – 641 108
Anaikatty (Post), Coimbatore - 641 108

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Research Supervisor
डॉ. एस मुरलीधरन / Dr. S Muralidharan
वरिष्ठ प्रधान वैज्ञानिक / Senior Principal Scientist
सालिम अली पक्षिविज्ञान एवं प्रकृति विज्ञान केन्द्र
Sálim Ali Centre for Ornithology and Natural History
आनैकट्टी, कोयम्बटूर – 641 108
Anaikatty (Post), Coimbatore - 641 108

Countersigned

Director (Research Institute)
डॉ. के शंकर / Dr. K. Sankar
निदेशक / Director
सालिम अली पक्षिविज्ञान एवं प्रकृति विज्ञान केन्द्र
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आनैकट्टी, कोयम्बटूर – 641 108
Anaikatty (Post), Coimbatore - 641 108

Signature of the Researcher

R&D Director (BU) / Head of the Department

(Seal)

डॉ. एस मुरलीधरन / Dr. S Muralidharan
वरिष्ठ प्रधान वैज्ञानिक / Senior Principal Scientist
सालिम अली पक्षिविज्ञान एवं प्रकृति विज्ञान केन्द्र
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Chapter 1

CHAPTER 1

INTRODUCTION

"Only within the moment of time represented by the present century has one species - human - acquired significant power to alter the nature of the world"

- Rachel Carson

1.1. Wetland Pollution

Wetlands are amongst the most productive ecosystems of the world with immense socio-economic, ecological and bio-aesthetic significance. From time immemorial, wetlands have played a crucial role in development of culture and civilization (Matthews., 1993). However, wetlands have not received their due credit. Consequently, wetlands have diminished in quality and sizes at alarming rates globally. Wetlands in India and elsewhere are increasingly facing several anthropogenic pressures. Apparently, wetlands in India are considered as wastelands, to be filled up, occupied and diverted for various human needs, disregarding the ecological services that these ecosystems provide to humans (Gopal and Sah., 1995). Burgeoning developmental projects, improper use of watershed, large scale changes in land use in addition to the ever growing human population have resulted in the substantial decline of wetlands (Vijayan., *et al.,* 2004). Real estate activities are becoming a major threat to wetlands in and around cities in India. With respect to species richness and species diversity, wetland ecosystems stand higher than most other ecosystems (Saurabh., 2013). Compared to tropical rain forests and coral reefs, wetlands are remarkable in their biological productivity (Vijayan *et al.,* 2004).

Wetlands are diverse and unique in structure, characteristics and functions, probably much more than other ecosystems. Wetlands are dynamic and complex and are under the influence of an array of biotic and abiotic factors (Saurabh., 2013). Nevertheless, the single most important factor that determines the nature of a wetland is its hydrologic regime (Millennium Ecosystem Assessment, 2005).

The hydrologic regime determines, modifies or changes the chemical and physical properties of a wetland. Changes in the physico-chemical settings in turn have direct impact on biotic and abiotic systems of the wetlands (Vijayan *et al.,* 2004). Even for

minor changes in the hydrologic regime of wetlands, biota may respond at times markedly, in terms of species composition, richness, trophic relations and ecosystem productivity (Saurabh., 2013). Wetlands provide a variety of ecological goods and services. However, recognition of their importance and roles defied time, until perhaps late sixties or early seventies of the last century (Vijayan *et al.,* 2004). In recent years, research has shed light on the ecological and environmental intricacies of wetlands; their values are being recognized, and wetland protection is considered imperative in many parts of the world (Vileisis., 1999).

Ecosystem services are functions that contribute to human welfare and help sustain the biosphere (Costanza *et al.* 2014). The value of a wetland is an estimate of the importance or worth of one or more of its functions to society (Richardson., 1994). Ecosystem services offered by wetlands include floodwater storage and control, recharge of aquifers, treatment of waste water and pollution abatement, general water quality improvement, habitats for fish, wildlife and several other animals and plant species, and biological productivity (Millennium Ecosystem Assessment., 2005). In addition, wetlands are of high aesthetic and heritage value and provide opportunities for recreation, research, and education (Saurabh., 2013).

Wetlands also contribute to the stability of global levels of available nitrogen, atmospheric sulphur, carbon dioxide, and methane (Millennium Ecosystem Assessment, 2005). Although they are known methane emitters, they are also the most productive ecosystems helping in scavenging carbon dioxide (Ayers *et al.,* 1994). These beneficial services, considered valuable to societies worldwide, are the result of inherent and unique natural characteristics of wetlands. However, the processes by which environment produces resources, ecosystem goods and services, often are taken for granted (Tiner *et al.,* 2002). Wetlands function like natural tubs or sponge, storing water and releasing it gradually (Pervez *et al.,* 2005). The water storage capacity is noteworthy in reducing chances of flooding (Acreman *et al.,* 2013). The trade-off between environmental protection and development is most acute in dynamic and complex ecosystems such as wetlands.

Wetlands worldwide face a range of anthropogenic threats (Thornton *et al.,* 2003). The chief indirect drivers of change are human population growth around wetlands coupled with growing economic and commercial activities (Saurabh., 2013). Major direct threats for inland wetlands are infrastructure development, land reclamation and over-harvesting. Major indirect ones are aquaculture, agriculture, reduced water flow, depletion of ground and surface water supplies, introduction of invasive alien species, and organic and inorganic pollutants (Sahgal., 2003). With increasing human use of freshwater, ecological balance of water has considerably reduced. In India, momentous losses of wetlands have resulted from conversion to industrial, agricultural and various other developments (Saurabh., 2013). Unsustainable levels of grazing and fishing activities have also resulted in degradation of wetlands (Kanaujiya *et al.,* 2014). Only a few ecologically sensitive regions are protected by various acts such as forest conservation and wildlife protection acts, while rest of such areas especially wetlands have continued to be easy targets to anthropogenic pressures and damages (Sahgal *et al., 2003*).

Development of human activity has always been closely associated with different water resources but, unfortunately anthropogenic activities have often resulted in chemical contamination of these resources. Anthropogenic activities like vehicular and ship transport, industrial plants, refuse incineration, mining, burning of biomass (forest fires, agricultural purposes), domestic cooking, household heating, spillage of oil/petroleum products, urban runoff (Gnandi *et al.,* 2011), and agricultural activities (N'guessan *et al.,* 2009) represent the most abundant sources of trace metals and organic pollutants in the environment (Bandowe *et al.,* 2014).

In an urban landscape like Chennai, the area and infrastructure required for safe disposal of household, corporation, industrial and agricultural waste is enormous. Unfortunately, water bodies in and around the city are being treated as dump yards. The rapidly developing Asian countries may face threatening environmental contamination that however remains largely unassessed (Zhang *et al.,* 2006). Environments in the rapidly developing Asian countries are getting subjected to increasing pollution and environmental degradation, therefore monitoring such pollution is inevitable. Although, there are many studies on wetland contamination in North American and European countries, such studies are far and few in India.

The state of Tamil Nadu is blessed with a number of water bodies and wetlands. Several of these are facing serious threats or are increasingly disappearing due to multifarious pressures (Samant., 2000). This is especially true for those wetlands located close to growing urban centres or bustling metropolises.

Pallikaranai wetland located in the outskirts of Chennai is one such wetland that is very likely to disappear in the near future, if active appropriate interventions are not made now. Alarmed by the rapid expansion of the Metropolis, realizing the environmental and ecological significance of the area and recognizing the need to protect, the Tamil Nadu state government, declared a part of the Pallikaranai marsh (317.00 ha) as a reserve forest (under section 4 of Tamil Nadu Forest Act, 1882) (Gazette notification GO. Ms. No. 52, dated 09/04/2007).

During the north-east monsoon, when there is influx of sea water from Bay of Bengal into this fresh water body, the salinity of the water rapidly increases. There is considerable increase in the water level and during this time some species of sea fish may also find their way into Pallikaranai wetland.

The Chennai Corporation and many NGOs in the city are making efforts to minimize impacts of contaminants on the water and the organisms by adopting various strategies, but their efforts appear to turn futile. Callous dumping of municipal waste along the water body, discharge of sewage, construction activities (buildings, railway stations, roads) have shrunk the Pallikaranai wetland to a great deal. The marshland has been under severe pressure through rampant constructions, ranging from flyovers to educational institutions, IT parks, restaurants, shopping malls, hospitals and other commercial establishments, which has affected the free flow of water. In 2007, as an effort to protect the remaining wetland from shrinking further, the area was notified as a reserve forest. The Pallikaranai salt marsh is a transitional intertidal area between land and brackish water in the thickly populated area of South Chennai metropolis, Tamil Nadu State, India (Jayaprakash *et al.,* 2010). The rapid development and urbanization of Chennai City in the last couple of decades have added stress on the local aquatic environment (Srinivasan *et al.,* 2013). Pallikaranai is a the largest marsh land in the region and is linked to the Bay of Bengal through several networks of backwaters and the Buckingham Canal (Jayaprakash *et al.,* 2010).

Presently, Pallikaranai faces severe threats from improper waste disposal, land conversion / reclamation and other various anthropogenic pressures. The high population of migratory as well as wetland birds in this area emphasizes the urgent need of conservation of this urban wetland. Moreover, according to local bird-watchers, the number of fish-eating birds has increased considerably. This appears to be a result of increasing fish abundance in the wetlands. The fishes are collected by people from nearby human settlements for their consumption. As fishes are being bioaccumulators, there exists a very high possibility of transfer of residues of harmful chemicals to humans. During biotransformation of xenobiotics, metabolites are formed and sometimes these metabolites may induce DNA strand breakage or formation of adducts, and even modify the chromosome structure (clastogenicity) and sequence of base pairs of DNA (mutagenicity) and heading to incomplete repair of DNA adducts (Steinnert, 2003).

There is little or no research carried out to understand the importance given for potential impacts of leachate from dump yard on the ground water and also the marshland . Polycyclic Aromatic Hydrocarbons have become a predominant group of contaminants in Pallikaranai. It becomes all the more important to study the effects of contaminants, particularly PAH in this region after the 2015 floods as changes in floral and fauna patterns have been observed by locals and regular visitors. With the common man being amnesiac about the causes of this particular nature's fury, it is the responsibility of researchers to monitor the impact regularly. The effects of PAHs on wetland ecosystems in the country are less studied and there is no information available in this wetland as well.

1.2. Polycyclic Aromatic Hydrocarbons

They are ubiquitous in nature and occur in the air, water, soil, plant, and animal components of the global environment (Albers, 2006). PAH may be formed in three ways: by high temperature pyrolysis of organic materials, by low to moderate temperature digenesis of sedimentary organic material to form fossil fuels and by direct biosynthesis by microorganisms and plants (Blumer 1976, Neff 1985). PAHs are produced by incomplete combustion and are formed when complex organic substances are exposed to high temperature or pressure. At ambient temperatures, they are solid

(Shafy and Mansour., 2016). PAHs are non-polar and this non-polar nature increases with increase in molecular weight. They are lipophilic in nature (Douben, 2003). PAHs are a complex group of chemicals with similar chemical structure, but a variety of chemical and physical attributes and are usually produced and occur in the environment as mixtures (Logan., 2007). They are widely distributed in the environment and human exposure to them is unavoidable (Muntean *et al.,* 2013). As a group, PAHs are chemically complex and contain many individual compounds. PAHs, which are defined by having two or more fused benzene (aromatic) rings, include more than 10,000 possible individual compounds (Gupte *et al.,* 2016). For the purposes of toxicity assessment, the U.S. Environmental Protection Agency (USEPA 2003) identified 34 PAHs that are generally abundant (Wise *et al.,* 2015). PAH compounds vary in physical (e.g., vapor pressure, solubility) and chemical (e.g., resistance to oxidation and reduction) characteristics and molecular weight (USEPA 2003).

The different size and structure of various PAH compounds also influence the type and magnitude of toxicological effects such carcinogenicity, mutagenicity, and genotoxicity. The presence of other chemicals and exposure to light can also affect the toxicity of individual PAHs or PAH mixtures (Logan 2007). PAH mixtures can be divided into three or four classes based on environmental source (Neff 1979; USEPA 2003): pyrogenic (formed by combustion of organic matter such as fossil fuels in short time periods, high temperature, and typically low pressure); petrogenic, such as petroleum (formed in the earth by geological process at low temperature, long time periods, and possibly high pressure); diagenic (formed in place in sediments from biogenic compounds, possibly from anaerobic processes) (Gao *et al.*, 2018); and biogenic (formed directly in sediments by animals, plants, fungi, and bacteria) (Logan 2007). A number of them, such as benzo[*a*]pyrene, are carcinogenic and mutagenic, and they are widely believed to make a substantial contribution to the overall burden of cancer in humans (Krajian and Odeh., 2013).

Their presence in the environment is reflected in their presence at detectable levels in many types of uncooked food. In addition, cooking processes can generate PAHs in food (Mahugija and Njale., 2018). PAHs can also be formed during the curing and processing of raw food prior to cooking (Phillips., 1999). Several studies have been carried out to

determine the levels of exposure to PAHs from representative human diets, and the proportion of the overall burden of environmental exposure to PAHs that is attributable to the diet (Magee and Connolly., 2014). In most cases, it is concluded that diet is the major source of human exposure to PAHs (Polycyclic aromatic hydrocarbons) (Phillips 1999).

The contamination by PAHs has been linked to a range of sub-lethal effects which includes reduced reproductive outputs, immune-suppression, liver and kidney damage, inflammation and general health impairment in birds (Leighton, 1993; Troisi *et al.,* 2007). Due to their mutagenic, carcinogenic and toxic properties, they are considered as a potential threat to wildlife (Eisler, 2000; Albers and Loughlin, 2003; Malcolm and Shore, 2003; Abdel-Shafy and Mansour, 2016). They are also capable of bioaccumulating in invertebrates (Meador, 2003; Achazi *et al.,* 2003).

Epidemiological data are inadequate to evaluate the possible interaction between PAHs and particles in the induction of lung cancer in humans. Finally, exposure to PAHs is ubiquitous, and many non-occupational sources of exposure exist, mainly tobacco smoke and diet; the comparison of occupationally exposed populations and 'unexposed' reference populations therefore is biased to a great extent (Caruso *et al.,* 2015).

General characteristics common to this class of compounds: -

1. High melting and boiling point
2. Low vapour pressure
3. Low water solubility which tend to decrease with increasing molecular mass

(WHO., 2003; Douben, 2003).

PAHs are formed by a variety of natural activities such as forest fires, volcanoes, coal and petroleum formation, and anthropogenic activities such as burning of coal, petroleum products, wood, waste, etc. (Murphy and Morrison., 2014). Sewage sludge, fly ash and creosote preserved wood are predominant sources of PAH in the hydrosphere. Chlorophyllous plants, fungi and bacteria also synthesize PAHs (McElroy *et al.,* 1989; Eisler, 2000; Brazkova and Krastanov.,2013). Although, the PAHs are produced by natural and anthropogenic activity, the major sources affecting the presence and distribution of PAHs in the environment are anthropogenic (Hsieh *et al.,* 2019).

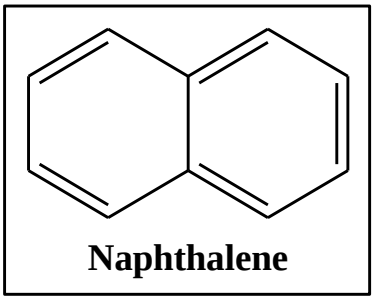
The compounds containing four or more aromatic rings are known as high molecular weight PAHs, whereas those having less than three rings are named as low molecular weight PAHs (Pozdnyakova., 2012). While low molecular weight PAHs volatilise and readily dissolve in water, high molecular weight PAHs, with four to seven rings, have low vapour pressure and are nearly insoluble in water. Due to these characteristics of volatility and water-solubility, low-molecular weight PAHs are abundant in the environment (Choi *et al.,* 2010). Heavy PAHs are more toxic in comparison, due their to stable nature and ability to remain in bound forms (Albers, 2006). These omnipresent environmental contaminants are wide spread in the air, bonded to particulate matter and readily bioavailable (Baird, 2007; Duan *et al.,* 2007). PAHs in the environment can be degraded through photo-oxidation, photolysis or chemical oxidation (Neff, 1985; Baek *et al.*, 1991).

The toxicity of PAHs to aquatic organisms is affected by metabolism and photo-oxidation. They are generally more toxic in the presence of ultraviolet light. PAHs have moderate to high acute toxicity to aquatic life and birds. PAHs in soil are unlikely to exert toxic effects on terrestrial invertebrates, except when the soil is highly contaminated. Adverse effects on these organisms include tumors, reproduction, development, and immunity. Mammals can absorb PAHs by various routes e.g. inhalation, dermal contact, and ingestion (Abdel-Shafy and Mansour, 2016).

Studies have demonstrated the carcinogenic, mutagenic and teratogenic behaviour of many of the PAHs (Eisler, 2000; Douben, 2003; Abdel-Shafy and Mansour, 2016). Based on this, PAHs are considered to becompounds of concern by many environmental organizations, and their concentration in water is strictly regulated to protect human health, Based on the toxicity, some of the PAHs are included in the "priority pollutants" list by the U.S. Environmental Protection Agency (EPA). Among those, 16 have been listed to be checked in waste water (US EPA method 610), drinking water (US EPA method 525 and 550) and ground water and wastes (US EPA method 8100 and 8310) (Longbottom, 1982; Eichelberger, 1988; Lerda, 2010). The listed 16 PAHs have been measured and monitored in different environmental components in the ecosystem throughout the globe (Eisler, 2000; Douben, 2003; Lerda, 2010)). The physio chemical parameters of the listed PAHs are explained as below;

1.2.1. Naphthalene

Naphthalene is a white, volatile, solid polycyclic hydrocarbon with aromatic odour. It is insoluble in water and freely soluble in ethanol, benzene and toluene. Naphthalene is obtained from either coal tar or petroleum distillation and is primarily used to manufacture phthalic anhydride and also used in moth repellents (ATSDR, 1990). Exposure to naphthalene is associated with haemolytic anaemia, damage to the liver and neurological system, cataract and retinal haemorrhage. Naphthalene is considered to be a human carcinogen and may be associated with an increased risk of developing laryngeal and colorectal cancer. Examination of the available toxicity data indicates that naphthalene is of high to moderate acute toxicity to saltwater life, with the majority of effect concentrations ranging from 0.4 to 5 mg l^{-1}. No major taxonomic group appears to be significantly more sensitive to naphthalene than any other, although acute effects have been observed for molluscs, crustaceans and fish at concentrations below 1 mg l^{-1} (ATSDR, 1990, https://pubchem.ncbi.nlm.nih.gov.compound/ naphthalene).



Molecular Formula	$C_{10}H_8$
Molecular Weight	128.17
CAS No	91-20-3
Melting Point	80.2ºC
Boiling Point	218ºC

Figure 1.1: Aromatic structure of Naphthalene

1.2.2. Acenaphthylene

Acenaphthylene is a colourless crystalline solid. It is insoluble in water, soluble in ethanol, ether, benzene and slightly soluble in chloroform. It is used in synthesis of dyes, insecticides, fungicides and in the manufacturing of plastics (Huchcraft *et al.,* 2010). It is produced industrially by catalytic gas-phase dehydrogenation of acenaphthene (https://pubchem.ncbi.nlm.nih.gov/ compound/acenaphthylene).

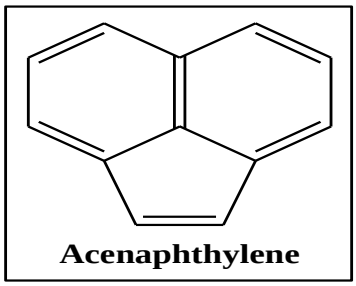
 Acenaphthylene	Molecular Formula	$C_{12}H_8$
	Molecular Weight	152.20
	CAS No	208-96-8
	Melting Point	92.5 ºC
	Boiling Point	280 ºC

Figure 1.2: Aromatic structure of Acenaphthylene

1.2.3. Acenaphthene

Acenaphthene appears as white needles. It is insoluble in water, soluble in ethanol and methanol, freely soluble in chloroform, benzene and toluene. Acenaphthene may irritate skin and mucous membranes. It emits acrid smoke and irritating fumes when heated. It is derived from coal tar and used to make dyes, pharmaceuticals, insecticides, fungicides and plastics (https://pubchem.ncbi.nlm.nih.gov/compound/acenaphthene).

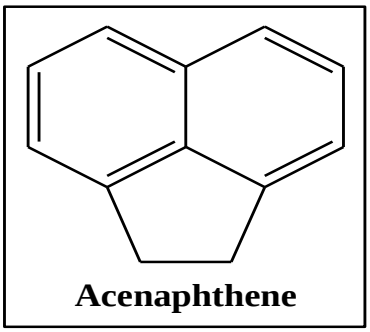
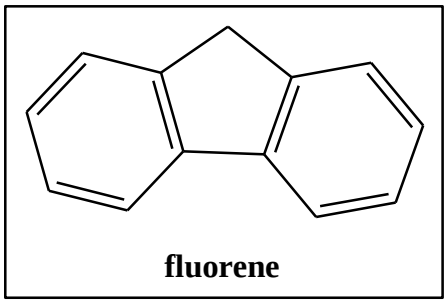
 Acenaphthene	Molecular Formula	$C_{12}H_{10}$
	Molecular Weight	154.21
	CAS No	83-32-9
	Melting Point	93.4 ºC
	Boiling Point	279 ºC

Figure 1.3: Aromatic structure of Acenaphthene

1.2.4. Fluorene

Fluorene appears as white leaflets consisting of three aromatic hydrocarbons. It is insoluble in water, soluble in hot alcohol and freely soluble in acetic acid. It sublimes easily under vacuum and appears fluorescent when impure. This compound is slightly flammable and sensitive to temperature and it produces toxic fumes at higher temperature. Polyfluorene polymers and fluorene derivatives are synthesised using fluorene (https://pubchem.ncbi.nlm.nih.gov/compound/6853#section=Top).

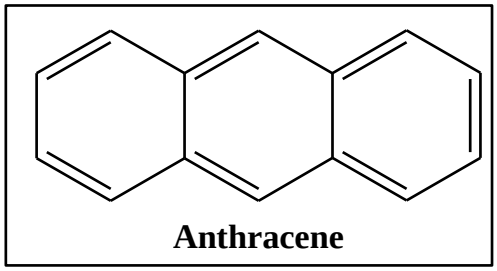


Molecular Formula	$C_{13}H_{10}$
Molecular Weight	166.22
CAS No	86-73-7
Melting Point	115 ºC
Boiling Point	295 ºC

Figure 1.4: Aromatic structure of fluorene

1.2.5. Anthracene

Anthracene, also called paranaphthalene or green oil, is a white to yellow solid with weak aromatic odour. It consists of three benzene rings. It is insoluble in water and sparingly soluble in ethanol, methanol and chloroform. It is derived from coal-tar. It is primarily used as an intermediate in the production of pesticides, dyes, smoke screens, scintillation counter crystals and in organic semiconductor research. Prolonged exposure of anthracene causes a variety of topical and systemic adverse reactions. (https://pubchem. ncbi.nlm.nih.gov/ compound/anthracene).



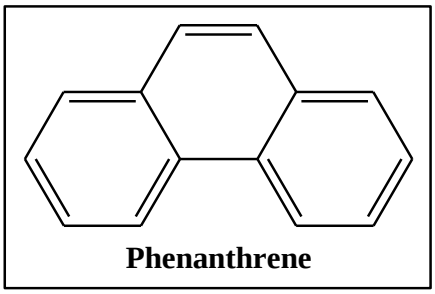
Molecular Formula	$C_{14}H_{10}$
Molecular Weight	178.23
CAS No	120-12-7
Melting Point	215 ºC
Boiling Point	340 ºC

Figure 1.5: Aromatic structure of anthracene

1.2.6. Phenanthrene

It occurs as a colourless monoclinic crystal with faint aromatic odour and consists of three aromatic fused rings. It is insoluble in water and soluble in organic solvents such as alcohol, toluene, benzene and acetic acid. The solutions of phenanthrene exhibit a blue fluorescence. Symptoms following exposure to this compound may include skin sensitization, dermatitis, bronchitis, cough, dyspnoea, respiratory neoplasm, kidney neoplasm, skin irritation,

and respiratory tract irritation. When heated till decomposition, it emits acrid smoke and fumes (https://pubchem.ncbi.nlm.nih.gov/compound/phenanthrene).



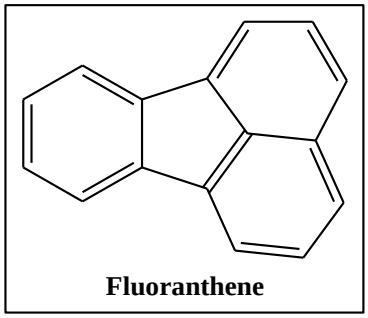
Phenanthrene

Molecular Formula	$C_{14}H_{10}$
Molecular Weight	178.23
CAS No	85-01-8
Melting Point	99.2 ºC
Boiling Point	340 ºC

Figure 1.6: Aromatic structure of phenanthrene

1.2.7. Fluoranthene

It is a light-yellow fine crystal consisting of four fused rings. It is almost insoluble in water (IARC, 1983); but is soluble in alcohol, ether, benzene and acetic acid (Lide, 1991). The major hazards encountered in the use and handling of fluoranthene stem from its toxic properties. It is toxic by all routes of exposure (i.e., inhalation, ingestion, dermal contact). Exposure to this pale-yellow crystalline substance may occur from its presence in coal tar products. It is used to coat pipes and storage tanks, in roofing materials and asphalt. Effects from exposure (based on an analogy to phenol) may include contact burns to the skin and eyes, nausea, tachycardia, cardiac arrhythmias, liver injury, pulmonary oedema, and respiratory arrest. In addition, NIOSH recommends that fluoranthene be regulated as an occupational carcinogen (https://pubchem.ncbi.nlm.nih.gov/ compound/fluoranthene).



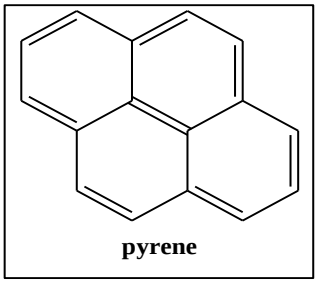
Fluoranthene

Molecular Formula	$C_{16}H_{10}$
Molecular Weight	202.26
CAS No	206-44-0
Melting Point	108 ºC
Boiling Point	384 ºC

Figure 1.7: Aromatic structure of fluoranthene

1.2.8. Pyrene

Pyrene is a parent class of polycyclic aromatic hydrocarbon containing four fused rings. It is a colourless solid and the solution has a slight blue fluorescence. Pyrene is insoluble in water and soluble in ethanol, benzene and toluene. It is derived from coal tar pitch and used to manufacture dyes, plastics, pesticides and is also used in biochemical research (https://pubchem.ncbi.nlm.nih.gov/compound/pyrene).

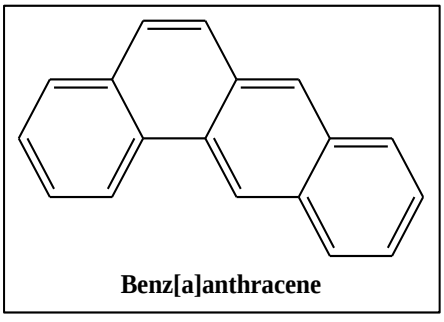


Molecular Formula	$C_{16}H_{10}$
Molecular Weight	202.26
CAS No	129-00-0
Melting Point	151 ºC
Boiling Point	404 ºC

Figure 1.8: Aromatic structure of pyrene

1.2.9. Benz[a]anthracene

Benz[a]anthracene is a polycyclic aromatic hydrocarbon consisting of four fused benzene rings. It appears as colourless leaflets or plates or coarse gold powder with a greenish-yellow fluorescence. It is insoluble in water and soluble in some organic solvents namely ether, ethanol, acetone and benzene. Benz[a]anthracene arises from a range of exhausts namely, gasoline, diesel, cigarette smoke tobacco, coal combustion, wood and soot smoke etc. It is also found in coal tar and coal tar pitch, charcoal broiled foods, amino acids, fatty acids and carbohydrate pyrolysis products, creosote, asphalt and a few mineral oils. Benzo[a]anthracene is reasonably considered to be a human carcinogen (https://pubchem. ncbi.nlm.nih.gov/compound/Benz_a_anthracene).

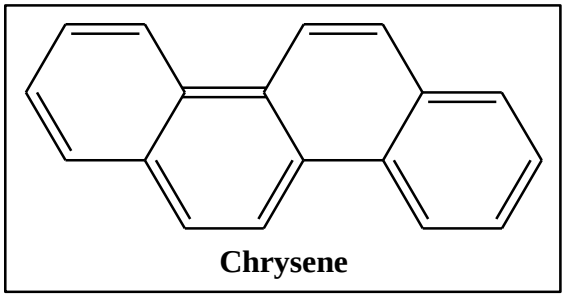


Molecular Formula	$C_{18}H_{12}$
Molecular Weight	228.29
CAS No	55-56-3
Melting Point	167 ºC
Boiling Point	435 ºC

Figure 1.9: Aromatic structure of Benz[a]anthracene

1.2.10. Chrysene

It is a four ring PAH; pure form of chrysene appears in white colour but commonly seen in pale yellow due to some impurities and it is allied to naphthalene and anthracene. It is insoluble in water and soluble in benzene. It is found in coal tar and creosote. It is mostly used in chemical laboratories for research purposes (DHHS 1995; https://pubchem.ncbi.nlm. nih.gov/compound/chrysene).

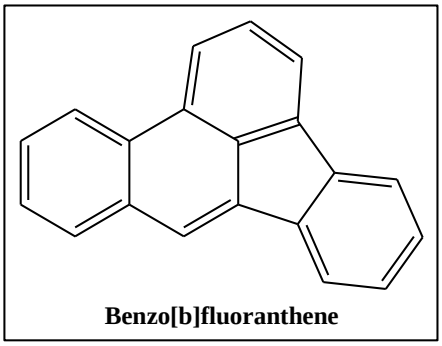


Molecular Formula	$C_{18}H_{12}$
Molecular Weight	228.29
CAS No	218-01-9
Melting Point	258 ºC
Boiling Point	448 ºC

Figure 1.10: Aromatic structure of chrysene

1.2.11. Benzo[b]fluoranthene

Benzo[b]fluoranthene is a colourless needle shaped solid made up of four benzene rings and one five-membered-ring. It is insoluble in water and slightly soluble in benzene and acetone. Benzo[b]fluoranthene is found in combustive fuel exhaust, smoke from tobacco products, coal tar, soot, amino acids and fatty acid pyrolysis products. Benzo[b]fluoranthene is considered to be a human carcinogen (DHHS, 1995; https://pubchem.ncbi.nlm.nih.gov/compound/Benzo_b_fluoranthene).

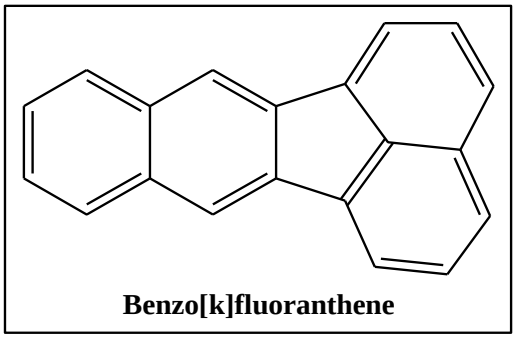


Molecular Formula	$C_{20}H_{12}$
Molecular Weight	252.32
CAS No	205-99-2
Melting Point	168 ºC
Boiling Point	481 ºC

Figure 1.11: Aromatic structure of benzo[b]fluoranthene

1.2.12. Benzo[k]fluoranthene

Benzo[k]fluoranthene is a pale-yellow needle or crystalline solid consisting of five fused rings. It is insoluble in water and soluble in benzene, ethanol and acetic acid. Sources of Benzo[k]fluoranthene include smoke from cigarettes, gasoline, oil and coal. It is also found in lubricating oils, used motor oils and crude oils. This substance is used only for research purposes and is reasonably considered to be a human carcinogen. (DHHS, 1995; https://pubchem.ncbi.nlm.nih.gov/compound/Benzo_k_fluoranthene).



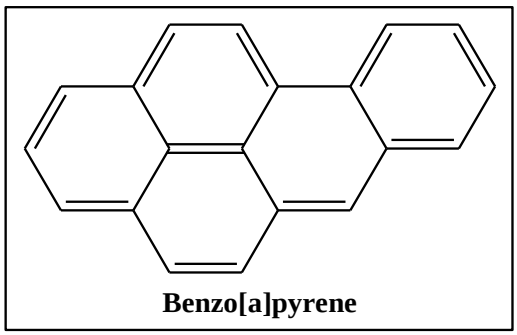
Benzo[k]fluoranthene

Molecular Formula	$C_{20}H_{12}$
Molecular Weight	252.32
CAS No	207-08-9
Melting Point	217 ºC
Boiling Point	480 ºC

Figure 1.12: Aromatic structure of benzo[k]fluoranthene

1.2.13. Benzo[a]pyrene

Benzo[a]pyrene is a colourless crystalline aromatic hydrocarbon consisting of five fused benzene rings and formed during the incomplete combustion of organic matter. It is insoluble in water, slightly soluble in alcohol and soluble in benzene, toluene, xylene and ether. Benzo[a]pyrene occurs in charcoal broiled foods, amino acids, fatty acids and metabolites of carbohydrates on pyrolysis. It is also found in vehicle exhaust (particularly gasoline and diesel), tobacco smoke, coal and tar pitch, creosote oil, petroleum asphalt and shale oils. Benzo[a]pyrene is considered to be a potent mutagen and human carcinogen. 60% of benzo[a]pyrene gets oxidised by light. Suitability for human consumption of food materials and water is studied by assuming PAH arbitrary values as equivalents of Benzo[a]pyrene because toxicity data is available only for Benzo[a]pyrene (Douben, 2003; Albers, 2006; IARC, 2010; Abdel-Shafy and Mansour, 2016; https://pubchem.ncbi.nlm. nih.gov/compound/benzo_a_pyrene).

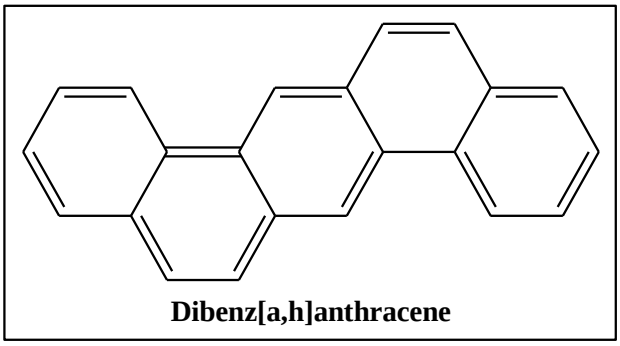


Molecular Formula	$C_{20}H_{12}$
Molecular Weight	252.32
CAS No	50-32-8
Melting Point	177 ºC
Boiling Point	495 ºC

Figure 1.13: Aromatic structure of Benzo[a]pyrene

1.2.14. Dibenz[a,h]anthracene

Dibenzo [a, h] anthracene is a white to pale yellow solid. It is insoluble in water, slightly soluble in alcohol and soluble in acetone, benzene and acetic acid. It is a carcinogenic aromatic hydrocarbon consisting of five fused benzene rings produced by the incomplete combustion of organic matter. Dibenzo [a, h] anthracene is formed on combustion of gasoline, tobacco, tar and soot. It is abundant in smoked and barbequed foods. It is used only for research purposes to induce tumorogenesis. Dibenzo [a, h] anthracene is a known mutagen and is reasonably considered to be a human carcinogen. (DHHS, 1995; https://pubchem.ncbi.nlm.nih.gov/compound/Dibenz_a_h_ anthracene)



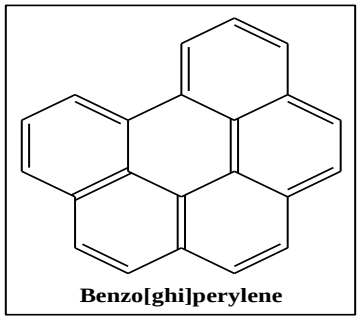
Molecular Formula	$C_{22}H_{14}$
Molecular Weight	278.35
CAS No	53-70-3
Melting Point	270 ºC
Boiling Point	524 ºC

Figure 1.14: Aromatic structure of dibenz[a,h]anthracene

1.2.15. Benzo[ghi]perylene

Benzo[ghi]perylene is a colourless to white crystalline solid. It is insoluble in water and soluble in acetone, benzene and 1,4 dioxane. Inhalation of this material may be harmful. Contact may cause burns and irritate eyes. It may produce irritating, corrosive

and/or toxic gases on combustion. There is no commercial production of this compound and it is used only for research purposes (https://pubchem.ncbi.nlm.nih.gov/compound/ Benzo_ghi_ perylene).

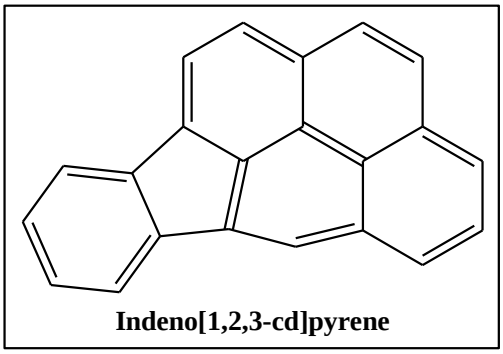


Molecular Formula	$C_{22}H_{12}$
Molecular Weight	276.34
CAS No	255-883-8
Melting Point	278 ºC
Boiling Point	550 ºC

Figure 1.15: Aromatic structure of benzo[ghi]perylene

1.2.16. Indeno[1,2,3-cd] pyrene

Indeno [1,2,3-cd] pyrene are yellow needles or plates consisting of six fused rings and formed by the incomplete combustion of organic matter. It is insoluble in water and soluble in organic solvents. Indeno[1,2,3-cd] pyrene is predominantly found in gasoline exhaust, diesel vehicle emissions, cigarette smoke, certain foods, soot, coal tar, coal tar pitch, and petroleum asphalt. This substance is also used only for research purposes. Indeno[1,2,3-cd] pyrene is reasonably considered to be a human carcinogen (DHHS, 1995; https://pubchem.ncbi.nlm.nih.gov/compound/Indeno_1_2_3-cd_pyrene).



Molecular Formula	$C_{22}H_{12}$
Molecular Weight	276.34
CAS No	193-39-5
Melting Point	164 ºC
Boiling Point	536 ºC

Figure 1.16: Aromatic structure of indeno[1,2,3-cd]pyrene

1.3. BIOLOGICAL AND NON-BIOLOGICAL COMPONENTS INCLUDED IN THE STUDY

Towards understanding the magnitude of PAH contamination in Pallikaranai wetland, two indicator components, namely sediment and fishes have been chosen.

1.3.1. Fish

Fish are a diverse group that can have complicated life cycles and behaviour and can be exposed to PAH-contaminated sediments and water by a variety of routes, including respiration; ingestion of food, sediment, and detritus; and dermal absorption (Huang and Batterman., 2014). Individual PAHs may elicit a variety of effects in different fish species, and different PAHs may elicit different effects in any single species of fish (Sereshk and Bakhtiari., 2014). The variation in both fish and PAHs is reflected in the wide range of adverse effects observed in fish exposed to PAHs (Santana *et al.,* 2018). Some of the effects observed include mortality in all life stages, decrease in growth, cardiac dysfunction, a variety of deformities, lesions and tumours on the skin and in the liver, cataract, compromised immunity, estrogenic activity, bioaccumulation, bioconcentration, trophic transfer, and biochemical changes, some of which can be used as biomarkers (Logan 2007).

Fish can serve as natural indicators of PAH contamination in surface waters. Because of commercial and recreational importance, fish are often the most visible members of aquatic communities. Damage to the skin, barbels, fins, and eyes from exposure to PAH-contaminated sediments and water can be observed with the naked eye. It is no wonder that such damages have resulted in some of the earliest investigations (e.g., Murchelano and Wolke 1985) of the effects of PAHs on fish and continue to attract attention.

Through their life cycle, fish may be exposed to PAHs in sediments as well as in the water column (Belfroid *et al.,* 1996). Some species excavate nests and many have demersal eggs, which put the eggs in contact with the sediments. Larvae may live close to the bottom and be exposed to sediment contamination (Eldridge *et al.,* 1978). Eggs and larvae of some species also inhabit the surface microlayer where elevated PAH concentrations can occur (Wurl and Obbard 2004). Some adult fish are benthic, such as

flatfish and catfish, and come into direct contact with sediments, and many eat benthic invertebrates that accumulate PAHs (Oliva *et al.,* 2010). All fish have gills comprised of thin membranes to transfer oxygen from water. Fish actively pass large quantities of water, and thus the gill tissues can be exposed to PAHs present in the water (Logan 2007). Potential effects on fish are also of interest, because these animals occupy key positions in aquatic and terrestrial food webs (Shurin *et al.,* 2006). They are fed upon by many species including other fish, humans and other mammals, piscivorous birds, and various scavengers. Therefore, changes in fish populations can translate into other populations dependent on them for food. Fleeger *et al.,* (2003) recently reviewed types of indirect effects of contaminants on fish and identified trophic cascade and competitive release of PAH contamination.

1.3.2. Sediment

Sediment is an essential, integral and dynamic part of wetlands. In natural and agricultural basins, sediment is derived from weathering and erosion of organic material and soils in upstream areas and from the erosion of river banks and other in-stream sources (Brils., 2002). As surface-water flow rates decline in lowland areas, transported sediment settles along the river bed and banks by sedimentation (Maljević and Balać 2007). This also occurs on floodplains during flooding, and in reservoirs and lakes. Often the natural sedimentation areas are severely restricted, e.g. because of embankments and the loss of flooding areas as a result of these embankments (Förstner *et al.,* 2004). At the end of most rivers, the majority of the remaining sediment is deposited within the estuary and in coastal zone (Sednet., 2002-2004). Natural river hydrodynamics maintain a dynamic equilibrium, regulating small variations in water-flow and sedimentation by re-suspension and resettlement (Brils., 2002). In estuaries, sediment transport occurs both downstream and upstream, mixing fluvial and marine sediment as a result of tidal currents (Allen *et al.,* 1980). Sediments are widely used as geo-marker for monitoring and identifying the possible sources since sediment can act as sink for the pollutants (Chuan and Yunus., 2019).

Sediment forms a variety of habitats. Many aquatic species live in the sediment. Microbial processes cause regeneration of nutrients and important functioning of nutrient

cycles for the whole water body (Sednet., 2002-2004). Sediment dynamics and gradients (wet-dry and fresh-salt) form favourable conditions for a large biodiversity, from the origin of the river to the coastal zone (Brils., 2002). A healthy river needs sediment as a source of life. Sediment is also a resource for human needs. For millennia, mankind has utilised sediment in river systems as fertile farmland and as a source of construction material.

Sediment acts as a potential sink for many hazardous chemicals. Since the industrial revolution, human-made chemicals have been emitted to surface waters (Bekić., 2013). Due to their properties, many of these chemicals stick to sediment. Hence in areas with a long record of sedimentation, sediment cores reflect the history of the pollution in a given river basin (Sednet., 2002-2004). Where water quality is improving, the legacy of the past may still be present in sediments hidden at the bottom of rivers, behind dams, in lakes, estuaries, seas and on the floodplains of many river basins (Förstner and Salomons., 2010). These sediments may become a secondary source of pollution when they are eroded (e.g. due to flooding and channel bank erosion) and transported further downstream (Brils., 2008). Along the course of the river to the sea, transportation, dilution and redistribution of sediment associated contaminants occur (Bekić., 2013). Many relatively small inputs, all complying with emission regulations, accumulate to reach higher levels by the time sediment reaches the river delta (Lebreton *et al.,* 2017). In Pallikaranai marsh, uncontaminated marine sediments are mixed with contaminated fluvial sediments.

The nature of human-made, often impervious, land surfaces and heavily engineered waterways results in hydrological and sedimentological systems in urbanised basins which contrast significantly to those within more natural (i.e. pristine, forested, agricultural) aquatic systems (Taylor and Owens., 2009). In addition, the abundance of contamination sources in urban systems results in chemical pressures often manifested as high pollution concentrations or loadings, which in turn have detrimental impacts on human and ecosystem health (Brils., 2002). These lead to management and sustainability issues in more natural environments (De Vries *et al.,* 2003).

Contaminants can be degraded or get bound to sediment, thus decreasing their bioavailability (Reid *et al.,* 2000). At a certain level, contaminants in sediment will start to impact the ecological or chemical water quality status (Apitz *et al.,* 2006). In the end, effects may occur such as the decreased abundance of sediment dwelling (benthic) species or a decreased reproduction or ill-health of animals consuming contaminated benthic species (Minns *et al.,* 1993). Contaminated sediments remain potential sources of adverse effects on water resources through the release of contaminants to surface waters and groundwater (Junakova and Balintova., 2012).

With rapid urbanization, uncontrolled vehicle emissions, rampant oil spills and emissions from construction work, Polycyclic Aromatic Hydrocarbons have become a predominant group of contaminants of a marsh. Pallikaranai, an urban wetland in Chennai is no exception. Turning a marsh that is a vital habitat for numerous species of animals into a wasteland, and continue contaminating it, may have tremendous consequences on the communities living around the marshland. There is little to no importance given for measurement of potential impacts of leachate generation from dump yard on ground water in Pallikaranai. It becomes imperative to study the effects of contaminants in this region, particularly after the 2015 Chennai floods, as the flora and fauna patterns seem to have changed. With the common man being amnesiac about the implications of this particular nature's fury, it is the responsibility of environmental researchers to raise concern, if need be, time and again. In this context, to answer these questions, a study was conducted in Pallikaranai with the following **objectives**: -

1. To check the usefulness of fish as an indicator to understand the magnitude of PAH contamination in Pallikaranai wetland, Chennai by estimating the PAH residues in sediment and select species of fishes in Pallikaranai wetland.
2. Assess suitability of fishes for human consumption.

Chapter 2

CHAPTER 2

REVIEW OF LITERETURE

2.1. International context

PAHs being hydrophobic in nature have a greater tendency to accumulate in the fat tissues of organism and sink in the sediments as they do not remain in the water column. They are generally low in the water column. The hydrocarbons that are most strongly ad/ab-sorbed onto sediment organic matter will be most resistant to loss or alteration by the other processes. Conversely, the more volatile/soluble hydrocarbons will be more susceptible to change by volatilization/reaction/leaching/biodegradation. The result will be "weathering" of the hydrocarbon mixture discharged into the sediment, with an accompanying change in its composition and a preferential transport of certain fractions to other environmental compartments (Ross Sadler and Des Connell., 2003). The PAHs present in the water column are available to fishes via different uptake routes, such as diet and/or direct transport across external membranes (Richardson *et al.,* 2004). PAH are the most toxic among hydrocarbon families (Catoggio, 1991). PAH reveal their toxicity following biotransformation to toxic metabolites (Varanasi & Stein 1991, Stein *et al.,* 1992) through metabolic activation in the organism (Cavalieri & Rogan., 1985). After absorption and distribution, these compounds are readily metabolized, chiefly by the cytochrome P-450 enzymes (Oost *et al.,* 2003). Factors influencing bioconcentration of PAHs in fish include food chain hierarchy, efficiency of assimilation, degree and nature of substance of biotransformation, rate of depuration, and growth rate of fish (Dórea., 2008). Xu *et al.,* (2011) studied the residue levels of PAHs in different tissues of four common edible freshwater fishes namely, grass carp, snakehead fish, crucian carp and silver carp, collected from lake Small Bai-Yang-Dian in northern China and found the levels to be relatively high. Binelli and Provini (2004) observed that the total PAH levels in Carp, Allice, Bleak, Chub, Tench, Perch and Pike collected from Lake Iseo were high. PAHs are all-pervasive contaminants and they are persistent organic pollutants of the aquatic environment. They are also known for their carcinogenic and teratogenic properties (Ravindra *et al.,* 2001). Their hydrophobic character and low biodegradability

cause accumulation in organisms much higher than their concentrations in the environment (Spies *et al.,* 1988; Zuloaga *et al.,* 2009).

PAHs may pose toxicity in fish and birds (Payne *et al.,* 2003) , by interfering with their cellular membrane functions and the associated enzyme systems (Neff, 1985), and metabolites of PAHs may bind to proteins and DNA which causes biochemical disruptions and cell damage in animals (Varanasi *et al.,* 1989; Eisler, 2000).

The carcinogenicity of PAHs can be increased by exposure to ultraviolet radiation, and 11 out of the 16 PAHs listed by the US Environmental Protection Agency as priority pollutants were photo-mutagenic (Yan *et al.,* 2004). It was suggested that levels of PAHs commonly found in many aquatic environments are an important risk factor for various health aspects of fish (Payne *et al.,* 2003), such as adverse histopathologic and immunological responses in Tilapia species (Holladay *et al.,* 1998; Hart *et al.,* 2002;).

For non-metabolized PAHs, small fish usually have a higher oxygen uptake than large ones, and the rate of organic compound loss increases much more than that of uptake. This will lead to less bioaccumulation of organic compounds in small fish (Randall *et al.,* 1998). For the more water-soluble PAHs, most uptake is thought to take place through the gills, especially for PAHs (Baussant *et al.* 2001). Bioconcentration of PAHs from water into fish tissue depends on the balance between uptake and elimination rates (Baussant *et al.,* 2001; Jonsson *et al.,* 2004). Most fish species (except agnaths) have enzymatic systems that metabolize and detoxify PAHs (Hahn *et al.,* 1994), which are eliminated at different rates for different PAHs. The influence of metabolism can result in a pattern of PAH accumulation that differs among fish species. Metabolism also accounts for observed differences between PAHs in fish. Once in an organism, PAHs tend to partition to the most lipid-rich tissues, such as the liver and bile of fish (Meador *et al.,* 1995). Elimination of PAHs from fish depends on both passive diffusion and active biotransformation in the liver followed by excretion with the bile. Passive diffusion across the gills and through the skin is more important for low molecular weight PAHs, and biotransformation and excretion are more important for high molecular weight PAHs (Logan, 2007). Fish can be exposed to PAHs present in their food. Bivalves

(eastern oyster, *Crassostrea virginica*, and blue mussels, *Mytilus edulis*) can accumulate high levels of PAHs from contaminated sediments, and winter flounder *Pseudopleuronectes americanus* fed contaminated blue mussels will in turn be adversely affected (Gardner *et al.* 1991). Laboratory studies have also shown a trophic exposure pathway for benzo(a)pyrene from sediments to deposit-feeding polychaete worms *Armandia brevis* to flatfish (English sole, *Peuronectes vetulus*) (Rice *et al.,* 2000). PAHs may elicit a variety of effects in different fish species, and different PAHs may elicit different effects in any single fish species; effects also depend on the life stage during which the exposure occurs (Aas *et al.,* 2001). When chronically exposed to PAHs as embryos, fish may exhibit mortality, decreases in growth, deformities, and oedema (Gundersen *et al.,* 1996; Barron *et al.,* 2004). Fish embryos exposed to petrogenic PAH mixtures (Incardona *et al.,* 2004). productive failure. PAHs damage DNA through metabolic products that covalently bond to DNA to form carcinogen-DNA adducts (Perlow and Broyde 2001), which may be the initial step in carcinogenesis (Reichert *et al.,* 1998; Baan *et al.,* 1994; Rose *et al.,* 2000).

Cytochrome P450 1A (CYP1A) enzymes are active in PAH metabolism and excretion (Stegeman and Lech, 1991), and PAH metabolites generated by this system have been implicated in chronic toxicity to larval fish (Hawkins *et al.,* 2002). Responses of the hepatic aryl hydrocarbon hydroxylase system can also be used as biomarkers (Collier and Varanasi, 1991). Aryl hydrocarbon receptor (AhR) agonism is a model for chronic toxicity of PAHs to embryonic fish (Nacci *et al.,* 2002; Barron *et al.,* 2004). Fish are particularly vulnerable and visible receptors for PAH contamination. Their aquatic habitats are naturally close to human habitats and potential sources of contamination, and PAHs tend to move with or in the same direction as surface and groundwater to aquatic habitats. Because of their vulnerability to exposure, the visibility of some adverse effects such as tumours and lesions, their centrality in aquatic food webs, and both their recreational and commercial importance, fish and the effects of PAHs on them have been the object of continued investigation.

Fish have several mechanisms that can cope with exposure to polycyclic aromatic hydrocarbons (PAHs). One system involved in defence against PAHs is the induction of drug-metabolizing enzymes (Tuvikene, 1995). Fish can metabolize PAHs mainly by oxidation, reduction, hydrolysis and conjugation reactions catalysed by various enzymes,

for example, cytochrome P450 monooxygenases, glutathione S-transferase (GST), and uridine 5 diphosphate-glucuronosyltransferase (UDP-GT), which are mainly localized in the liver but are also found in extrahepatic tissues. PAHs exert their toxicity following biotransformation to toxic metabolites, which can be bound covalently to cellular macromolecules such as proteins, DNA and RNA, which causes cell damage, mutagenesis, teratogenesis, and carcinogenesis (Tuvikene, 1995)

McDonald *et al.,* (1992) assessed the PAH exposure in fish from Arthur Harbor, Antarctica and sites remote from human activities by measuring the concentrations of Polycyclic Aromatic Hydrocarbon (PAH) metabolites in bile and PAH in tissues and stomach contents. The confirmation of PAH metabolites in the bile of fish from Arthur Harbor is direct evidence that the presence of humans has resulted in Antarctic fish being exposed to petroleum derived PAH.

Oost *et al.,* (1994) reported PAH contamination levels in eel samples collected from six sites in Amsterdam. A significant increase in PAH-DNA adduct levels was observed in the liver of eel from all polluted sites.

Fernandez *et al.,* (2007) designed a study to assess levels of exposure to Polycyclic Aromatic Hydrocarbons in farmed sea bass *Dicentrarchus labrax* from five aquacultures located in Southern Europe.

Liang *et al.,* (2007) reported Polycyclic aromatic hydrocarbons levels in fish collected from eight shrimp shallow ponds from July 2003 to January 2004. The results showed that significantly higher PAHs were accumulated in fish viscera (505–854ng/g) than in muscle (184–194ng/g). They also observed that viscera appeared to be a promising tissue for indicating PAH levels in Tilapia species, because it contained much higher concentrations than the muscle.

Bandowe *et al.,* (2014) reported the concentrations of 28 PAHs, 15 oxygenated PAHs (OPAHs) in muscle and gut + gill tissues of demersal fishes (*Drapane africana, Cynoglossus senegalensis* and *Pomadasys peroteti*) from three locations along the coast of the Gulf of Guinea (Ghana). The concentrations of 28PAHs in muscle tissues averaged 192 ng g−1 dw (range: 71–481 ng g−1 dw) and were not statistically different between locations. The concentrations of∑28 PAHs were higher in guts + gills than in muscles.

The PAH composition pattern was dominated by low molecular weight compounds (naphthalene, alkyl-naphthalenes and phenanthrene).

The scientific committee on food in 2002 assessed the toxicity of 33 PAHs and concluded that 15 of them are potentially genotoxic and carcinogenic to human (Ruiz and Wenz, 2009). Does the fish by itself get genetically disturbed during the process of bioaccumulation is yet another question that needs to be answered in future.

2.2. National Context

Studies in India have reported that a considerable quantum of petroleum hydrocarbons enters the aquatic environment, gets distributed in water, concentrates in sediment and gets transferred to biota (Chouksey *et al.*, 2004), and hence sediments, being repositories of hydrocarbons, act as a major tool to monitor contaminants in aquatic environments (Chernova *et al.,* 1999). Long-term PAH stress may lead to adverse effects such as DNA lesions (covalent bond DNA adducts) and later irreversible PAH induced tumours. (Although, for most people, fish and seafood represent only a small part of their dietary intake, the contribution of this food group to the daily intake of PAHs in some individuals may be comparatively important (Domingo., 2007). Ray *et al.,* (2008) documented the sum of 12 PAHs ranging from 2.39 to 7.53 µg/g in the soil around the International Airport in Delhi due to jet turbine exhaust in the peripheral soil. Muralidharan *et al.*, (2012) recorded that the PAHs levels in water and sediments samples collected along the harbour line, Mumbai, India, appear to be several folds greater than existing sediment quality criteria suggested by US EPA. The PAH composition pattern in water and sediments suggested the dominance of high molecular weight compounds and indicative of pyrolytic and petrogenic sources.

A study by Singh *et al.*, (2008) on PAH levels in Indian children (sample size chosen from Lucknow) revealed significantly elevated levels in blood samples of urban than rural population (125.55ng/g in urban and 23.96 ng/g in rural regions). Studies need to be conducted to evaluate if consumption of PAH contaminated food like fish are amongst the reasons for such high toxic levels.

Although, information is available on the ecological parameters, little information is available on contaminants, particularly PAHs. Jayanthi and Padmavathi., (2014),

reported higher than permissible levels of lead, cadmium, chromium, manganese and nickel in Pallikaranai region. The soil of the Pallikaranai marshland consists mostly of clay (Vencatesan, 2014), which is, on an atomic level, slightly negatively charged. Since metal-ions are positively charged, the ions tend to attach themselves to a soil if they get a chance. Also, organic matter, which is also present within the marshland, has a negative charge and has the same effect on trace elements as a clay-soil. Oxides can be both positively and negatively charged, depending on the pH. With a higher pH, the oxides are more likely to attract positively charged trace elements. This means that if heavy metals enter a fresh water source, they are rapidly removed from the water and attaches themselves to the sediments, causing the concentration of heavy metals in the surface water to decrease (Parsons, 2013). These heavy metal concentrations have already increased by almost sixty percent for most elements compared to all other ecosystems in the world, and is more heavily contaminated with the elements namely, cadmium (Cd), mercury (Hg), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) compared to other regions in the southeast coast of India. The high concentrations of these metals in the northern part of the marshland is said to be due to dumping of waste, while enrichment of metal concentrations in central Pallikaranai is mainly due to the circulation from upstream. Researchers have also found that the concentrations are higher in the northern part of the marshland than in the southern part (Jayaprakash *et al.,* 2010).

A study by students from SIPCOT, Chennai in 2005 concluded that an air sample from Pallikaranai wetland region had 69 volatile organic chemicals and 20 sulphur containing compounds inclusive of carcinogens such as benzene, 1,3 butadiene and chloromethane at levels greater than permissible limits prescribed by US EPA (Jayaprakash *et al.,* 2010). Distribution of PAH in fish tissue and sediment samples in Indian wetlands are still not well documented and Pallikaranai is no exception.

Chapter 3

CHAPTER 3

METHODOLOGY

3.1. Study Area

Pallikaranai wetland (12° 56′ 15.72″ N, 80° 12′ 55.08″ E) is a fresh water marsh adjacent to the Bay of Bengal situated in south Chennai with a geographical area of 80 km^2 (Figure1). The state government of Tamil Nadu declared 317 hectares (780 acres) of the Pallikaranai wetland as a reserve forest (Malleshappa and Jayanthi, 2013). Palikaranai has a rich biodiversity and is a bird watchers' paradise. It provides suitable habitat for many organisms particularly birds, fishes, amphibians.

A project on 'Inland Wetlands of India' conducted by SACON commissioned by the Ministry of Environment, Forests and Climate Change, Government of India, had prioritised Pallikaranai marsh as one of the most significant wetlands of the country (Vijayan *et al.,* 2004). Pallikaranai is among the 11 wetlands in Tamil Nadu chosen under the five-year National Action Plan for Conservation of Migratory Birds and their Habitats along Central Asia Flyway (2018-2023).

Despite several court rulings, burning of garbage continued unabated at the marsh and the adjacent area of Perungudi (News report, TNN 16 July, 2009).Official statistics reveal that, in the absence of source segregation of waste, the dump yard is eating up to 4 hectares (9.9 acres) of marshland every year. The ground water around Pallikaranai marsh has high levels values of TDS, anions and cations which make the water non-potable and its use as dumpsite is mostly responsible reason for accelerating the ground water contamination in the area (Jayaprakash *et al.,* 2010).

The water level at Pallikaranai marsh is usually around 3 meters. During floods the excess water is discharged into Okkiyum Madavu, then the water is channelled into Cooum and is drained finally into the Bay of Bengal sea coast through Muttukkadu - Kovalam creek (Jose *et al.,* 2016).

Pallikaranai marsh supports 349 species of flora and fauna including 133 species of birds, 10 species of mammals, 21 species of reptiles, 10 species of amphibians,

50 species of fishes, nine species of molluscs, five species of crustaceans, and seven species of butterflies and about 114 species of plants including 29 species of grass.

3.1.1. Threats to the Marsh

The Chennai metropolis waste dump yard and Perungudi Sewage Treatment Plant occupy 250 acres of the prime marsh lands of Pallikaranai wetland (Nikhil Raj *et al.,* 2010). Perungudi dump yard receives around 5000 tons of waste per day. Contaminated liquid in the form of leachates generated from liquids present in the refuse, incursion of ground water into the landfill and different sources of moisture entering the uncovered waste by precipitation (Vasanthi *et al.,* 2008) migrates from the dumpsite and form a plume in approximately 1.5km to 1.75km spatially and to a depth of 100m (Mathiazhagan, 2013) polluting the hydrological system of Pallikaranai marsh. Another emerging great threat to the fragile ecosystem is the Alandur Municipal Solid Waste facility on the Eastern side of Tambaram to Velacherry road. Perungudi Sewage Treatment Plant (STP) treats around 54 million litres/ day by activated sludge process (CMW, 2011). Treated effluents from STP are let off into the marsh land, since 2005. Unchecked entry of sewage from apartments and housing colonies are yet another source of pollution in the marsh. The wetland which extended to more than 50sq.km originally (about 30 years back) is reduced to 5.9 sq.km in 2007 and is further reduced to 3.17 sq.km (793 acres) at present (Gazette notification GO.Ms. No. 52, Environment dated 9th April, 2007).

Pallikaranai is acting as a carbon source rather than a sink with an annual carbon dioxide emission of 18.4 gigatonnes per year, and the carbon sinking rate is a negligible 0.0020 gigatonnes per year (News report, The Indian Express, February, 2019)

With rapid urbanization, indiscriminate dumping of waste, uncontrolled vehicle emissions and rampant oil spills, PAH have become a predominant group of contaminants in Pallikaranai. Part of Pallikaranai being a reserve forest, contaminating it, may have tremendous consequences on the ecology of the wetland (Vencatesan, 2014).

Figure 2: Perungudi lake located in Pallikaranai wetland, Chennai, Tamil Nadu

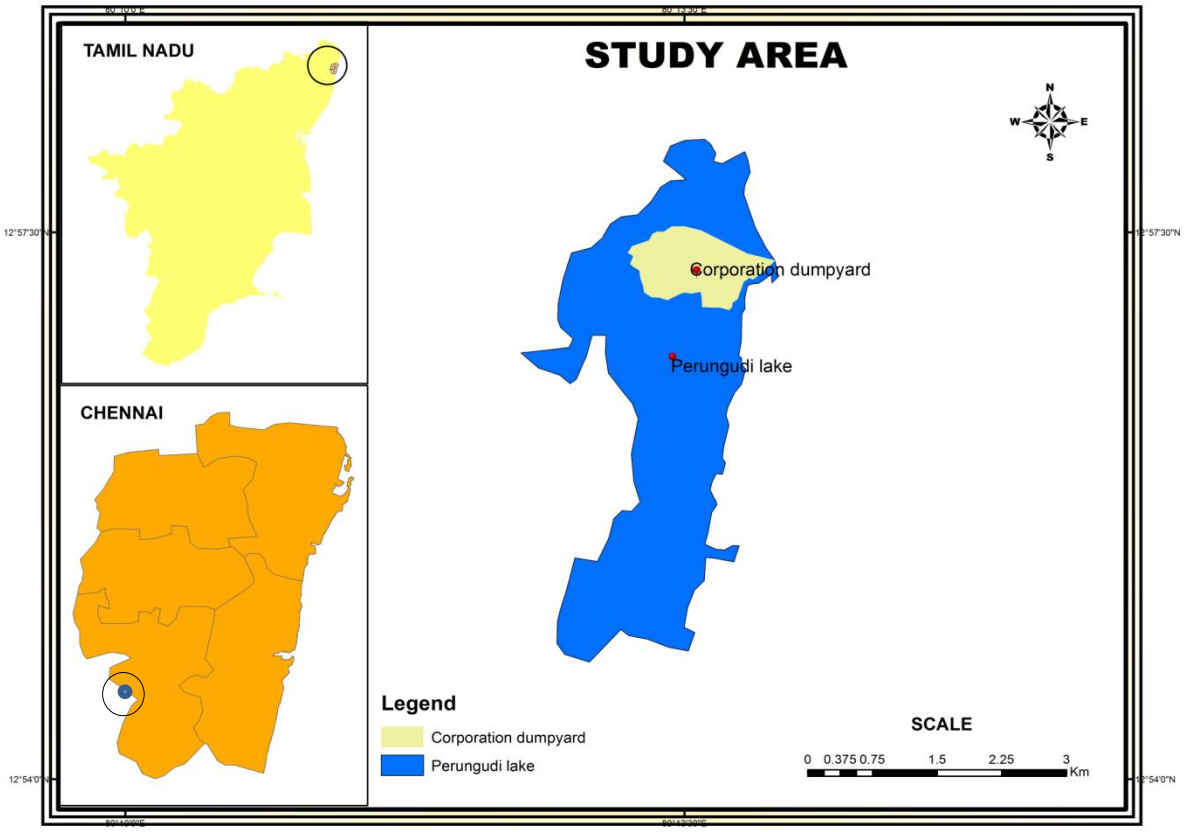


Figure 3: The garbage dump located in Perungudi at Pallikaranai Wetland, Chennai

Figure 4: Other anthropogenic activities in Pallikaranai Wetland, Chennai

Figure 5: Warning signs by the Tamil Nadu Forest Department on display to prevent dumping in Pallikaranai Wetland, Chennai

Figure 6: An aerial view of the shrinking Pallikaranai Wetland, Chennai

Figure 7: Bird diversity in Pallikaranai Wetland, Chennai

3.2. Analytes included in the study

Among the US EPA listed 16 PAHs (Lerda, 2010), only 15 of them, namely Naphthalene, Acenaphthene, Phenanthrene, Anthracene, Fluorene, Fluoranthene, Pyrene, Chrysene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenzo[a,h]anthracene, Benzo[g,h,i]perylene and Indeno[1,2,3-cd]pyrene have been included in the study. Acenaphthylene is also listed in the US EPA's list, but since it does not fluoresce, it cannot be detected with florescence detector (Krahn *et al.,* 1984). Besides, specificity and selectivity are is less in UV detector for Acenaphthylene due to high matrix effect of tissues. Hence, it has not been included in the study.

3.3. Chemicals and reagents

PAH standard (certified reference materials) mixture was purchased from AccuStandard, US which contained the USEPA-listed 16 PAHs in solvent mixture of 50:50 methanol and dichloromethane at 200µg/ml concentration. Chromatography grade acetonitrile and sodium chloride were purchased from Merck, India. Anhydrous magnesium sulphate was purchased from HiMedia. Clean up reagents, namely Primary Secondary Amine (PSA), C18 bulk sorbent and graphitized carbon blocks (GCB) were purchased from Agilent Technologies, USA. Fresh ultrapure water with conductivity less than 0.056 µs/cm (> 18.0 MΩ) was produced from Milli-Q DQ3 (Millipore) water purification in the laboratory.

3.4. Sample preparation

QuEChERS multi residue extraction procedure as described by Ramalhosa *et al.*, (2003) was followed to extract all the 15 PAHs from the tissue samples. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive solid-phase extraction (SPE) method initially developed for extracting multi-residue pesticides from vegetables and fruits (Anastassiades *et al.,* 2003). Because of its efficiency, it is being extended to extract other contaminants from different environmental samples. The advantages of this methodology are speed, ease of execution, ruggedness, minimal solvent requirement and cost effectiveness. The method was tested for recovery in the laboratory and the results are presented in the results and discussion section. This extraction method involves two steps; first is the extraction of analytes from the matrix using organic solvents while the

second step is clean-up of unwanted co-extractives from the matrix to reduce matrix effects and to improve method's robustness.

Two to ten grams (based on availability) of homogenised tissue were weighed and placed in a 50 mL centrifuge tube. 5 mL of water was added, shaken well for 30 minutes to homogenize and left for 15 minutes. Subsequently 10 mL of acetonitrile was added to it and shaken again vigorously for a minute. Four grams of anhydrous magnesium sulphate ($MgSO_4$) and one gram of sodium chloride (NaCl) were added to the centrifuge tube, shaken vigorously for a minute and centrifuged at 2,000 G for 5 minutes to separate. After centrifugation, 4 mL of the organic layer of centrifugate was transferred into a 15 mL centrifuge tube which contained 100 mg of Primary Secondary Amine (PSA), 100 mg of C18 bulk sorbent and 500 mg of $MgSO_4$ and shaken well. If the organic layer was pigmented, required quantity of graphitized carbon was added to remove it. Then, this mixture was centrifuged at 1,000 G for 5 minutes. 1 mL of the centrifugate was transferred into vials for instrumental analyses. Stock standard mix (10 ppm) was prepared by diluting the PAH standard mixture twenty times with acetonitrile. Calibration standards (1.0, 2.5, 5, 10, 25, 50 and 100 ppb) were prepared from stock solution by diluting with required volume of acetonitrile.

3.5. HPLC Analysis

Analyses for PAH residues were performed on an Agilent 1100 High Performance Liquid Chromatograph (HPLC) equipped with programmable Florescence Detector (FLD) and controlled by Chemstation software. Fluorescence detection is employed generally for trace analysis of PAHs for better selectivity and sensitivity. The HPLC/fluorescence technique has been the most suitable tool for PAH quantification (Krahn *et al.,* 1984). Hence, fluorescence detector was used to quantify the PAH. The optimum excitation and emission wavelengths varied with individual PAHs, and hence FLD was programmed accordingly to obtain high sensitivity and low limit of detection for individual PAHs. The chromatographic separation was achieved on an Agilent Zorbax Eclipse PAHs (4.6*150mm, 3.5µm) column by gradient elution with a binary system of water and acetonitrile with 1.5ml per minute flow rate. Chromatographic conditions were as follows;

Column : Agilent Zorbax Eclipse PAHs (4.6*150mm, 3.5µm)

Mobile phase : (A) Water

(B) Acetonitrile

Table 1: Gradient Program for elution of PAH

Time in minutes	% of A (Water)	% of B (Acetonitrile)
0.0	50	50
2.0	50	50
22.0	100	0
30.0	100	0
30.1	50	50
36.0	50	50

Stop time : 30 minutes

Post run : 6 Minutes

Flow rate : 1.5 ml/min

Injection volume : 20µl

Column temperature : 25° c

Table 2: FLD Detector program

Time (Time (minutes)	Excitation wave length (nm)	Emission wave length (nm)	Polycyclic aromatic hydrocarbon detected
0.0	220	330	Naphthalene,
7.0	210	330	Acenaphthene, FluoreneAcenaphthene, Fluorene
9.6	250	363	Phenanthrene
10.7	250	405	Anthracene
12.0	240	470	Fluoranthene
13.2	270	400	Pyrene, Chrysene, Benzo[a]anthracene
18.5	270	415	Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Benzo[g,h,i]perylenei] perylene, Indeno[1,2,3-cd]pyrenecd] pyrene
25.1	250	490	Dibenzo [a,h] anthracene

3.6. Quality assurance/quality control

The instrument was calibrated with 1.0, 2.5, 5, 10, 25, 50 and 100 ng/ml (ppb) of PAH standard mix and linear calibration curves with R^2 values ≥ 0.99 were obtained for all individual PAHs (table 4). PAH residues were identified by comparing their retention time to the peaks from the calibration standards and quantified using calibration curve with help of Chemstation software. Limit of detection (LOD) and limit of quantification (LOQ) were evaluated by the concentration of the analyte (individual PAHs) required to give signal to noise ratio of 3 and 10 respectively. LOQ of individual PAHs ranged from 0.05 ng/g to 1 ng/g. Since, calibration curves were drawn from 1.0 ng/g for all PAHs, values below 1 ng/g have not been included and 1 ng/g was fixed

as (below) detection limit (BDL) for all the compounds. LOD and LOQ for all compounds are given in results and discussion section (table 3.3). Average recoveries of the all compounds from fortified samples were above 86.30%, recoveries at three concentring levels (10 ng/g, 20 ng/g and 30 ng/g, n=3 for each concentration) are also presented in the results and discussion section (table 3.4). Results have not been corrected for per cent recovery. Samples were analysed in batches of 10 plus three quality controls, namely blank, method blank and midrange standard. Estimated values are expressed in ng/g (wet weight basis) or ppb.

3.7. Data analyses

As the data were not normally distributed (Shapiro-Wilk test, $p < 0.05$), non-parametric tests were employed. Individual PAH concentrations in fish tissues are presented as median and range in ng/g (wet weight). But to facilitate comparison, the values are expressed as mean with standard error. Values below detection limit were not considered to calculate mean. Variation in percentage of detection and percentage of composition among the PAH was tested using Chi-square test. Kruskal-Wallis test was used to check variation in concentration among organs, individual PAHs, sexes, seasons (fish and sediment), endemic and introduced species and feeding guilds of fish. Pearson correlation was employed to correlate levels of PAH in tissues with that of fins and gut contents. Spearman correlation was employed for correlating levels of PAH in sediment with that of fishes. Analysis of Covariance (ANCOVA) was employed to analyse the effects of covariates on the levels of PAH in endemic and introduced species of fishes. P values less than 0.05 were considered statistically significant. Statistical tests were performed using statistical software SPSS student version 17 and Graphpad Prism 5. Since the levels of High Molecular Weight PAH in fishes were non-detects in most samples, they have not been included for statistical tests while still being discussed contextually at appropriate junctures.

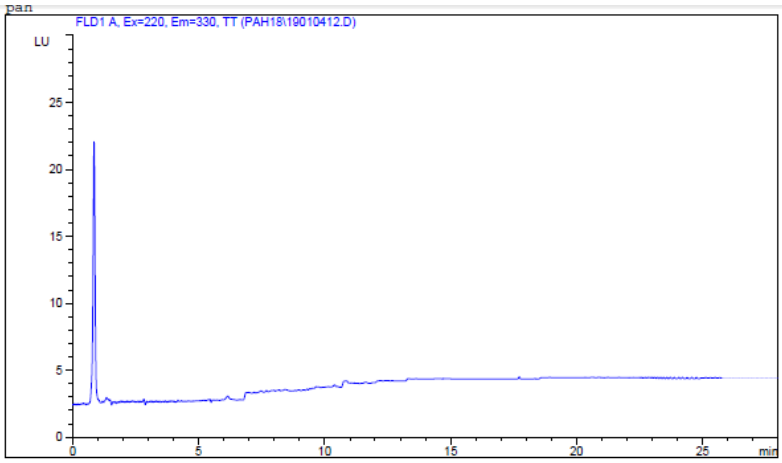
Chapter 4

CHAPTER 4

RESULTS AND DISCUSSION

Methodology adopted for analyses of PAH was tested for robustness before actual analyses of samples. The HPLC methods (EPA 550, 610, 8310 and NIOSH 5506) determine PAHs in the sample extracts using fluorescence (FL) detectors connected in series. As shown in the 10 ppb standard chromatogram (figure 9), peaks of all the 15 individual PAH peaks were baseline separated. The resolution factors of the most closed peaks namely, acenapthene and fluorene, benzo[g,h,i]perylene and indeno[1,2,3-cd] pyrene were greater than 2.04 and 2.21 respectively. None of the target PAHs was detected in the procedural blanks (figure 8). The standard, sample and blank chromatograms are presented (figure10) here under.

Figure 8: Chromatogram of method blank



External Standard Report

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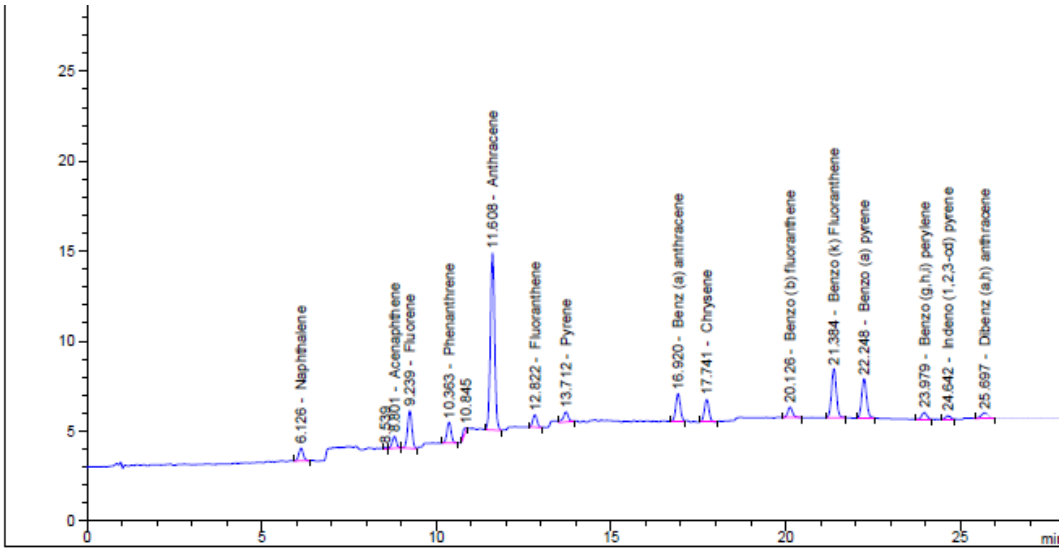
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=220, Em=330, TT

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
6.138		-	-	-			Naphthalene
8.819		-	-	-			Acenaphthene
9.252		-	-	-			Fluorene
10.377		-	-	-			Phenanthrene
11.619		-	-	-			Anthracene
12.833		-	-	-			Fluoranthene
13.727		-	-	-			Pyrene
16.924		-	-	-			Benz (a) anthracene
17.740		-	-	-			Chrysene
20.130		-	-	-			Benzo (b) fluoranthene
21.376		-	-	-			Benzo (k) Fluoranthene
22.240		-	-	-			Benzo (a) pyrene
23.952		-	-	-			Benzo (g,h,i) perylene
24.633		-	-	-			Indeno (1,2,3-cd) pyrene
25.667		-	-	-			Dibenz (a,h) anthracene

Figure 9: Chromatogram of 10 PPB standard mixture

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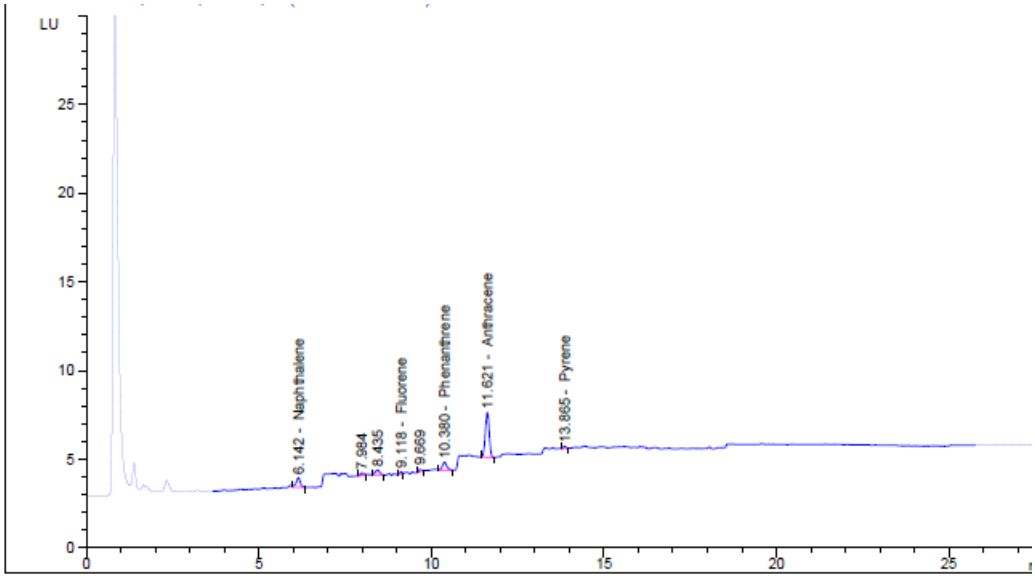
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=220, Em=330, TT
```

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
6.126	BBA		6.33332	1.72282	10.91117		Naphthalene
8.801	VBA		5.67433	1.80582	10.24682		Acenaphthene
9.239	VBA		18.21041	5.47807e-1	9.97579		Fluorene
10.363	PBA		9.65255	1.07107	10.33858		Phenanthrene
11.608	BBA		83.58629	1.23898e-1	10.35616		Anthracene
12.822	PBA		5.82981	1.73150	10.09429		Fluoranthene
13.712	BBA		5.71490	2.31533	13.23186		Pyrene
16.920	BBA		13.51992	7.34984e-1	9.93692		Benz (a) anthracene
17.741	PBA		11.58606	8.65214e-1	10.02443		Chrysene
20.126	PBA		5.45622	1.92453	10.50066		Benzo (b) fluoranthene
21.384	PBA		26.20422	3.90401e-1	10.23015		Benzo (k) Fluoranthene
22.248	PBA		21.46232	4.90616e-1	10.52976		Benzo (a) pyrene
23.979	PBA		4.16212	2.45640	10.22385		Benzo (g,h,i) perylene
24.642	PBA		2.29256	4.34093	9.95184		Indeno (1,2,3-cd) pyrene
25.697	BBA		4.18061	2.37126	9.91332		Dibenz (a,h) anthracene
Totals :					156.46559		

Figure 10: Chromatogram of fish sample



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Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=220, Em=330, TT

RetTime [min]	Type	Area LU *s	Amt/Area	Amount [ppb]	Grp	Name
6.142	BBA	4.62192	1.69790	10.59422		Naphthalene
8.819		-	-	-		Acenaphthene
9.118	PBA	6.71713e-1	0.00000	0.00000		Fluorene
10.380	PBA	3.66818	1.06798	5.28868		Phenanthrene
11.621	PBA	19.88636	1.20789e-1	3.24276		Anthracene
12.833		-	-	-		Fluoranthene
13.865	PBA	7.21557e-1	2.62969	2.56159		Pyrene
16.924		-	-	-		Benz (a) anthracene
17.740		-	-	-		Chrysene
20.130		-	-	-		Benzo (b) fluoranthene
21.376		-	-	-		Benzo (k) Fluoranthene
22.240		-	-	-		Benzo (a) pyrene
23.952		-	-	-		Benzo (g,h,i) perylene
24.633		-	-	-		Indeno (1,2,3-cd) pyrene
25.667		-	-	-		Dibenz (a,h) anthracene

Totals : 21.68725

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In chromatographic analyses, analytes are identified by retention time (RT). Hence, stable RT is important for correct identification of analytes in complex environmental matrices such as tissues. The stable RTs are also very important when using a time-programmable fluorescence detector to set wavelength switching by RTs during an analysis. Precision of peak areas is important for obtaining reliable quantitative data. Precision of retention time and peak area will decide the superiority in qualitative

and the quantitative results of a chromatographic method. To assess the method precision for all PAHs, 20ppb standard was injected consecutively for 10 times. Retention time reproducibility and peak area reproducibility are presented in table 3. As shown in the referred table, Relative Standard Deviation (RSD) of retention times of all compounds were less than 0.2%. Similarly, the RSD of the peak areas of all compounds were less than 1.0 except the last eluting dibenz[a,h]anthracene (1.77%).

Table 3 Retention time and peak area reproducibility of the chromatographic method adopted (20ppb standard, n=10)

Retention time	PAH Compound	Retention time (RSD)	Peak area (RSD)
6.12	Naphthalene	0.179	0.662
8.79	Acenaphthene	0.111	0.911
9.22	Fluorene	0.087	0.680
10.34	Phenanthrene	0.055	0.531
11.57	Anthracene	0.021	0.465
12.78	Fluoranthene	0.008	0.574
13.67	Pyrene	0.015	0.860
16.84	Benz[a]anthracene	0.066	0.718
17.63	Chrysene	0.084	0.712
20.02	Benzo[b]fluoranthene	0.105	0.760
21.22	Benzo[k]fluoranthene	0.112	0.604
22.09	Benzo[a]pyrene	0.110	0.794
23.74	Benzo[g,h,i]perylene	0.139	0.627
24.45	Indeno[1,2,3-cd]pyrene	0.146	0.862
25.41	Dibenz[a,h]anthracene	0.186	1.772

To assess the method linearity, a calibration curve was drawn using a software for each of the PAH for concentration ranging from 1 ppb to 100 ppb (seven point calibration with 1.0, 2.5, 5.0, 10, 25, 50 and 100 ppb). The correlation coefficients of all PAHs were above 0.99, and are presented in table 3.3 demonstrating excellent method linearity for this range.

Table 4: Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ) of all 15 PAHs quantified

Retention time	PAH Compound	R^2 (Linearity range 1 to 100 ppb)	LOD (ng/g)	LOQ (ng/g)
6.12	Naphthalene	0.9980	0.10	0.30
8.79	Acenaphthene	0.9988	0.15	0.50
9.22	Fluorene	0.9983	0.10	0.30
10.34	Phenanthrene	0.9984	0.10	0.30
11.57	Anthracene	0.9963	0.01	0.05
12.78	Fluoranthene	0.9985	0.10	0.30
13.67	Pyrene	0.9986	0.16	0.50
16.84	Benz[a]anthracene	0.9985	0.07	0.21
17.63	Chrysene	0.9985	0.08	0.24
20.02	Benzo[b]fluoranthene	0.9984	0.20	0.60
21.22	Benzo[k]fluoranthene	0.9983	0.03	0.10
22.09	Benzo[a]pyrene	0.9984	0.03	0.10
23.74	Benzo[g,h,i]perylene	0.9983	0.25	0.80
24.45	Indeno[1,2,3-cd]pyrene	0.9981	0.30	1.00
25.41	Dibenz[a,h]anthracene	0.9980	0.30	1.00

The best signal-to-noise ratio for all PAHs was achieved using time programmed fluorescence detector. Limit of detection and limit of quantification of the method were evaluated as concentration of PAH required to give a signal to noise of ratio three and ten respectively. Limit of detection ranged between 0.01 and 0.30 ng/g. Limit of quantification ranged between 0.05 and 1.0 ng/g. LOD and LOQ for all individual PAHs

are presented in table 4. Since, calibration curves were drawn from 1.0 ng/g for all PAHs, values below 1 ng/g have not been included and 1 ng/g was fixed as (below) detection limit (BDL) for all the compounds.

Recovery study was conducted for all 15 PAHs at three different concentration levels, namely 10, 20 and 30 ng/g. Three extractions of spiked samples at three concentration levels were injected in the experiment. Recovery percentage and the RSD for the triplicate are presented in table . Average recovery of all PAHs was above 86.3% and the RSD (table 5) was less than.

Table 5 Percentage recoveries and RSD for 15 individual PAHs quantified in fish tissues

PAH	10 ng/g (n=3)		20 ng/g (n=3)		30 ng/g (n=3)	
	% recovery	RSD	% recovery	RSD	% recovery	RSD
Naphthalene	86.30	3.65	90.97	1.58	91.23	1.44
Acenaphthene	93.80	2.48	92.97	1.86	95.68	0.93
Fluorene	93.87	1.62	91.73	0.63	95.30	0.97
Phenanthrene	96.77	1.43	97.30	1.13	98.26	1.53
Anthracene	97.63	1.28	98.50	0.77	97.77	0.95
Fluoranthene	92.13	1.51	98.12	1.04	96.23	0.99
Pyrene	92.97	2.04	95.00	1.47	93.49	0.87
Benz[a]anthracene	95.90	1.57	96.40	1.38	97.14	1.05
Chrysene	95.57	1.32	97.00	1.37	97.07	0.88
Benzo[b]fluoranthene	93.57	2.31	94.33	1.68	95.39	1.11
Benzo[k]fluoranthene	94.43	1.41	96.68	1.29	97.58	0.99
Benzo[a]pyrene	94.63	1.48	97.68	1.28	97.44	0.98
Benzo[g,h,i]perylene	88.77	2.39	91.50	1.70	89.32	1.38
Indeno[1,2,3-cd]pyrene	90.47	1.90	92.30	2.24	92.42	1.24
Dibenz[a,h]anthracene	90.57	2.97	90.33	2.33	91.38	2.51

4.1. Polycyclic aromatic hydrocarbon (PAH) residues in tissues of select species of fishes collected from Pallikaranai Wetland, Chennai (2017-2018)

Concentrations of toxic contaminants in fish tissue reflect the concentrations in surrounding water and sediment (Bright *et al.,* 1995). Thus fish tissue PAH concentrations could serve as an indicator for assessing health of the ecosystem particularly, water and sediment (US EPA 2000). Fishes near highly contaminated industrial areas are reported to contain high levels of POPs (Fensterheim., 1993). Bioaccumulation of POPs in fishes have been known to cause ill effects in humans (Cabes *et al.*, 1999; Menone *et al.*, 2000; Vigano *et al.*, 2000)

Organs, namely muscle, liver, kidney, gills and reproductive organs of 358 fish comprising 21 species, namely *Anabas tetudineus, Clarias batrachus, Clarias gariepinus, Chana gachua, Chana punctata, Chana striata, Labeo fimbriatus, Heteropneustes fossilis, Oreochromis aureus, Oreochromis mossambicus, Oreochromis niloticus, Pseudotropis maculatis Puntius chola, Puntius dorsalis, Puntius sophore, Puntius sarana, Puntius ticto, Sperata aor, Stolephorus indicus Salmostoma acinaces* and *Trichopodus trichopterus* from Pallikaranai, Chennai, Tamil Nadu, India were analysed for USEPA-listed 15 PAHs. In addition to these organs, fins, scales and gut contents were also analysed. Totally 886 tissues from 358 individuals were analysed. Out of the above mentioned species, 14 of them (*Clarias batrachus, Clarias gariepinus, Anabas testudineus, Labeo fimbriatus, Sperata aor, Oreochromis aureus, Oreochromis mossambicus, Oreochromis niloticus, Puntius chola, Puntius dorsalis, Puntius ticto, salmophasia acinases, Channa striata* and *Trichopodus trichpterus*) which had more than 10 individuals were chosen for assessing species –wise variation of PAH. Invariably all the fishes included in the study had one or more PAHs in their tissues. The late eluting six membered ring structured PAH, Indeno (1,2,3-cd) pyrene was detected only in eight samples while Dibenzo anthracene was detected in five samples of the total of 886 samples analysed. The total PAHs recorded ranged from below detection limits (BDL) to 1890 ±286 ng/g. The individual PAH levels ranged from below detection limit (BDL) to 1396 ±242 ng/g of naphthalene (Table 6.1) in *Oreochromis niloticus*, 64 ±10 ng/g of fluorene (table 6.2) in *Anabas testudineus*, 510 ±57.10 ng/g of phenanthrene (table 6.3) in *Trichopodus trichopterus*, 71± 7.61 ng/g of anthracene (table 6.4) in *Oreochromis mossambicus* and 959 ± 10.78 ng/g of pyrene (table 6.5) in *Oreochromis mossambicus*.

Plate 1: List of Fish Species included in the Study

Anabas testudineus

Clarias batrachus

Clarias gariepinus

Channa gachua

Channa puntatus

Channa striata

Labeo fimbriatus

Heteropneustus fossilis

Oreochromis aureus

Oreochromis moassambicus

Oreochromis niloticus

Pseudotropis maculatus

Puntius chola

Puntius dorsalis

Puntius sophore

Salmostoma acinaces

Trichopodus trichopterus

Systomus sarana

Stolephorus indicus

Sperata aor

High molecular weight PAH ranged between BDL and 158.29±10.46 ng/g in *O.mossambicus*, carcinogenic PAH4 (Sum of chrysene, benzo (a) anthracene, benzo (b) flouranthene and benzo (a) pyrene) ranged between BDL and 76.12± 6.43 ng/g in *Anabas testudineus* and benzo (a) pyrene ranged between BDL and 16.6±0.8 ng/g in *Anabas testudineus* (figure 11). Results are discussed based on individual compounds, sources, accumulation pattern among organs and species as follow;

The results are expressed as in the tables as mean, median and range. Since the data was not normally distributed, median and range have been given while for comparison with literature available elsewhere, mean has been used.

Table 6.1 : Levels of naphthalene (ng/g ww) in fishes collected from Pallikaranai wetland, Chennai between January 2017 and December 2018.

	NAPHTALENE					
SPECIES	**Mean**	**N**	**SD**	**Median**	**Min**	**Max**
CB	39.31	60	45.75	23.95	BDL	317.48
CG	50.92	25	95.64	24.84	BDL	485.74
AT	43.22	40	73.27	26.09	BDL	396.23
LF	28.62	13	30.30	20.89	BDL	106.25
SA	12.40	16	6.60	12.43	BDL	25.46
OA	100.75	77	208.31	22.63	BDL	280.72
OM	58.84	144	83.32	29.98	BDL	546.24
ON	60.61	242	127.67	22.26	BDL	1396.30
PC	27.24	11	14.43	25.01	BDL	56.34
PS	20.21	34	18.27	16.09	BDL	79.28
PT	8.31	10	6.77	8.31	BDL	21.02
SPA	47.69	25	95.71	21.55	BDL	418.97
CP	19.61	26	12.21	17.15	BDL	45.09
TT	92.59	800	243.47	28.81	BDL	1366.94

CB: *Clarius batrachus*, CG: *Clarius gareipinus*, AT: *Anabas Testudineus*, LF: *Labeo fimbriatus*, SA: *Sperata aor*, OA:*Oreochromis aureus*, OM: *Oreochromis mossambicus*, ON: *Oreochromis niloticus*, PC: *Puntius chola*, PS: *Puntius sophore*, PT: *Puntius ticto*, SPA: *Salmophasia acinaces*, CP: *Channa punctata*, TT: *Trichopodus trichopterus*

Table 6.2 : Levels of fluorene (concentration in ng/g ww) in fishes collected from Pallikaranai Wetland, Chennai (2017-2018)

	FLUORENE					
SPECIES	**Mean**	**N**	**SD**	**Median**	**Min**	**Max**
CB	1.81	60	3.12	0.63	BDL	17.27
CG	1.82	25	2.46	1.00	BDL	10.26
AT	3.00	40	10.13	0.47	BDL	64.63
LF	0.41	13	0.67	0.00	BDL	2.27
SA	2.07	16	5.86	0.00	BDL	23.44
OA	1.82	77	4.39	0.00	BDL	26.87
OM	1.98	144	4.25	0.03	BDL	23.50
ON	1.27	242	2.42	0.15	BDL	17.27
PC	1.04	11	1.74	0.06	BDL	5.89
PS	0.64	34	1.67	0.00	BDL	7.18
PT	0.36	10	0.78	0.00	BDL	2.13
SPA	0.41	25	0.67	0.00	BDL	2.44
CP	0.67	26	1.31	0.00	BDL	5.26
TT	1.90	800	3.82	0.38	BDL	19.08

CB: *Clarius batrachus*, CG: *Clarius gareipinus*, AT: *Anabas Testudineus*, LF: *Labeo fimbriatus*, SA: *Sperata aor*, OA:*Oreochromis aureus*, OM: *Oreochromis mossambicus*, ON: *Oreochromis niloticus*, PC: *Puntius chola*, PS: *Puntius sophore*, PT: *Puntius ticto*, SPA: *Salmophasia acinaces*, CP: *Channa punctata*, TT: *Trichopodus trichopterus*

Table 6.3 : Levels of Phenanthrene (concentration in ng/g ww) in fishes collected from Pallikaranai Wetland, Chennai (2017-2018).

	PHENANTHRENE					
SPECIES	**Mean**	**N**	**SD**	**Median**	**Min**	**Max**
CB	7.61	60	6.58	5.46	BDL	25.92
CG	5.62	25	4.76	4.51	BDL	18.95
AT	7.48	40	7.97	5.56	BDL	41.14
LF	9.78	13	12.42	6.65	BDL	43.22
SA	6.72	16	5.50	5.55	BDL	20.65
OA	12.77	77	15.42	5.50	BDL	81.56
OM	11.49	144	20.69	7.14	BDL	156.48
ON	9.02	242	10.01	6.68	BDL	99.26
PC	8.01	11	5.59	7.13	BDL	19.59
PS	8.97	34	13.03	4.64	BDL	68.82
PT	4.44	10	3.81	4.37	BDL	13.41
SPA	9.12	25	10.73	6.32	BDL	42.35
CP	6.43	26	5.56	4.74	BDL	17.23
TT	11.46	800	57.10	2.61	BDL	510.41

CB: *Clarius batrachus*, CG: *Clarius gareipinus*, AT: *Anabas Testudineus*, LF: *Labeo fimbriatus*, SA: *Sperata aor*, OA:*Oreochromis aureus*, OM: *Oreochromis mossambicus*, ON: *Oreochromis niloticus*, PC: *Puntius chola*, PS: *Puntius sophore*, PT: *Puntius ticto*, SPA: *Salmophasia acinaces*, CP: *Channa punctata*, TT: *Trichopodus trichopterus*

Table 6.4 : Levels of Anthracene (concentration in ng/g ww) in fishes collected from Pallikaranai Wetland, Chennai (2017-2018).

	ANTHRACENE					
SPECIES	**Mean**	**N**	**SD**	**Median**	**Min**	**Max**
CB	2.64	60	2.89	1.60	BDL	13.26
CG	1.34	25	2.35	0.62	BDL	11.26
AT	0.83	40	0.68	0.67	BDL	3.27
LF	2.78	13	3.04	3.08	BDL	11.47
SA	2.88	16	3.11	2.46	BDL	10.25
OA	2.39	77	2.91	1.47	BDL	7.76
OM	2.49	144	7.61	0.94	BDL	71.29
ON	2.27	242	4.65	1.00	BDL	56.17
PC	2.83	11	3.95	1.06	BDL	13.61
PS	1.65	34	6.35	0.34	BDL	37.42
PT	1.90	10	2.75	1.60	BDL	9.40
SPA	3.43	25	4.61	2.27	BDL	18.10
CP	2.88	26	2.87	2.40	BDL	10.30
TT	1.48	800	2.11	0.77	BDL	12.91

CB: *Clarius batrachus*, CG: *Clarius gareipinus*, AT: *Anabas Testudineus*, LF: *Labeo fimbriatus*, SA: *Sperata aor*, OA:*Oreochromis aureus*, OM: *Oreochromis mossambicus*, ON: *Oreochromis niloticus*, PC: *Puntius chola*, PS: *Puntius sophore*, PT: *Puntius ticto*, SPA: *Salmophasia acinaces*, CP: *Channa punctata*, TT: *Trichopodus trichopterus*

Table 6.5 : Levels of Pyrene (concentration in ng/g ww) in fishes collected from Pallikaranai Wetland, Chennai (2017-2018)

	PYRENE					
SPECIES	**Mean**	**N**	**SD**	**Median**	**Min**	**Max**
CB	23.81	60	90.27	0.00	BDL	602.11
CG	2.64	25	9.10	0.00	BDL	46.42
AT	27.28	40	60.68	0.00	BDL	269.62
LF	24.01	13	50.61	8.82	BDL	193.03
SA	7.85	16	12.42	0.00	BDL	36.13
OA	26.82	77	40.89	0.00	BDL	80.94
OM	41.43	144	105.08	10.78	BDL	959.11
ON	30.21	242	66.47	4.09	BDL	602.11
PC	26.06	11	41.49	10.74	BDL	139.79
PS	4.89	34	18.39	0.00	BDL	98.20
PT	3.61	10	11.41	0.00	BDL	36.08
SPA	11.04	25	18.15	0.00	BDL	68.41
CP	7.94	26	15.29	0.00	BDL	53.72
TT	7.71	800	26.17	0.00	BDL	156.31

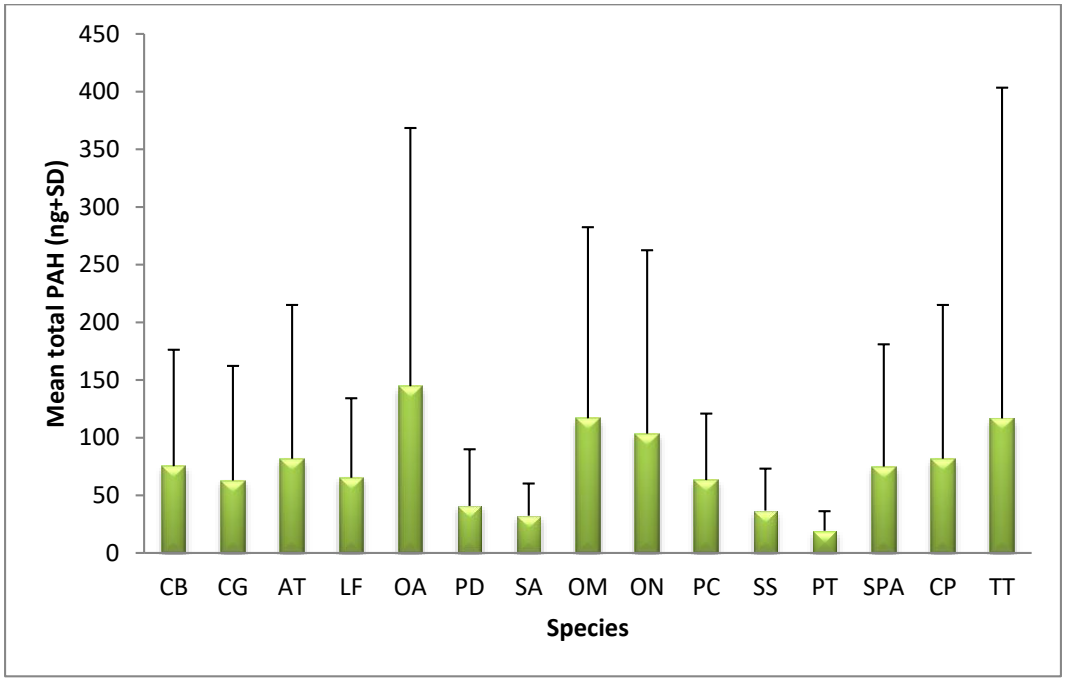
CB: *Clarius batrachus*, CG: *Clarius gareipinus*, AT: *Anabas Testudineus*, LF: *Labeo fimbriatus*, SA: *Sperata aor*, OA:*Oreochromis aureus*, OM: *Oreochromis mossambicus*, ON: *Oreochromis niloticus*, PC: *Puntius chola*, PS: *Puntius sophore*, PT: *Puntius ticto*, SPA: *Salmophasia acinaces*, CP: *Channa punctata*, TT: *Trichopodus trichopterus*

Table 6.6: Levels of mean total PAH (concentration in ng/g ww) in fishes collected from Pallikaranai Wetland, Chennai (2017-2018).

	TOTAL					
SPECIES	**Mean**	**N**	**SD**	**Median**	**Min**	**Max**
CB	75.36	60.00	100.93	42.50	BDL	629.17
CG	62.91	25.00	99.33	32.94	BDL	495.90
AT	81.80	40.00	133.22	47.80	BDL	711.19
LF	65.78	13.00	83.74	33.73	BDL	319.47
SA	32.38	16.00	27.94	23.37	BDL	90.11
OA	144.76	77.00	223.72	43.25	BDL	308.43
OM	116.89	144.00	165.66	60.72	BDL	1171.84
ON	103.62	242.00	158.97	49.65	BDL	1453.07
PC	63.37	11.00	57.58	49.23	BDL	221.59
PS	36.77	34.00	36.35	24.10	BDL	161.90
PT	19.35	10.00	16.95	18.04	BDL	58.16
SPA	74.91	25.00	106.11	40.77	BDL	466.16
CP	38.10	26.00	26.85	34.31	BDL	91.11
TT	116.70	80.00	286.81	37.08	BDL	1890.26

CB: *Clarius batrachus*, CG: *Clarius gareipinus*, AT: *Anabas Testudineus*, LF: *Labeo fimbriatus*, SA: *Sperata aor*, OA:*Oreochromis aureus*, OM: *Oreochromis mossambicus*, ON: *Oreochromis niloticus*, PC: *Puntius chola*, PS: *Puntius sophore*, PT: *Puntius ticto*, SPA: *Salmophasia acinaces*, CP: *Channa punctata*, TT: *Trichopodus trichopterus*

Figure 11: Mean concentration of total PAHs (in ng/g±SD) in the different species of fish from Pallikaranai Wetland, Chennai (2017-2018)



4.2. Accumulation pattern of PAHs among the organs of various species of fishes collected from Pallikaranai Wetland, Chennai (2017-2018)

Variation in total PAHs load among the organs irrespective of species is shown in figure 11. Significant variation was observed in total PAHs load among the organs (Kruskal-Wallis test, n = 886, H = 99.89, $p < 0.05$) studied. The data has been analysed irrespective of species, sex and season taking only organs into consideration. Accumulation pattern of total PAHs residues among the organs were in the order of liver (113.33 ± 23.87 ng/g) > kidney (107.67 ± 28.95 ng/g) > gills (102.18 ± 13.20 ng/g)> reproductive organ (87.52±14.85 ng/g)> muscle (48.43± 5.03 ng/g) (figure 12). Among the organs, liver had the highest concentration of mean total PAH residue, and it has been used in earlier studies in other organisms such as birds to measure PAHs contamination (Troisi *et al.*, 2006; Roscales *et al.*, 2011; Luzardo *et al.*, 2014).

High level of PAHs load in liver and kidney may be due to the fact that they are the primary target organs, and as they are involved in the biotransformation and elimination in the detoxification process (Lee *et al.*, 2017). Liver is also a temporary

storing station for PAH due to its high lipid content (Troisi *et al.*, 2006). Fish have several mechanisms that can cope with exposure to polycyclic aromatic hydrocarbons (PAHs). One system involved in defence against PAHs is the induction of drug-metabolizing enzymes (Tuvikene, 1995). Fish can metabolize PAHs mainly by oxidation, reduction, hydrolysis and conjugation reactions catalysed by various enzymes, for example, cytochrome P450 monooxygenases, glutathione S-transferase (GST), and uridine 5 diphosphate-glucuronosyltransferase (UDP-GT), which are mainly localized in the liver but are also found in extrahepatic tissues. PAHs have been known to have a deleterious effect on the vitellogenesis of fish such as reduction in circulating hormones and plasma vitellogenin, estrogenic and antiestrogenic effects, retardation of oocyte maturation and reduction of reproductive success from both feral populations as well as in laboratory experiments (Nicolas, 1999). While the levels of PAH recorded in the current study do not seem to pose any immediate danger, the levels cannot be considered without concern either.

Figure 12: Mean concentration of total PAHs (in ng/g at 95% confidence interval) in the organs of fishes collected from Pallikaranai Wetland, Chennai (2017-2018)

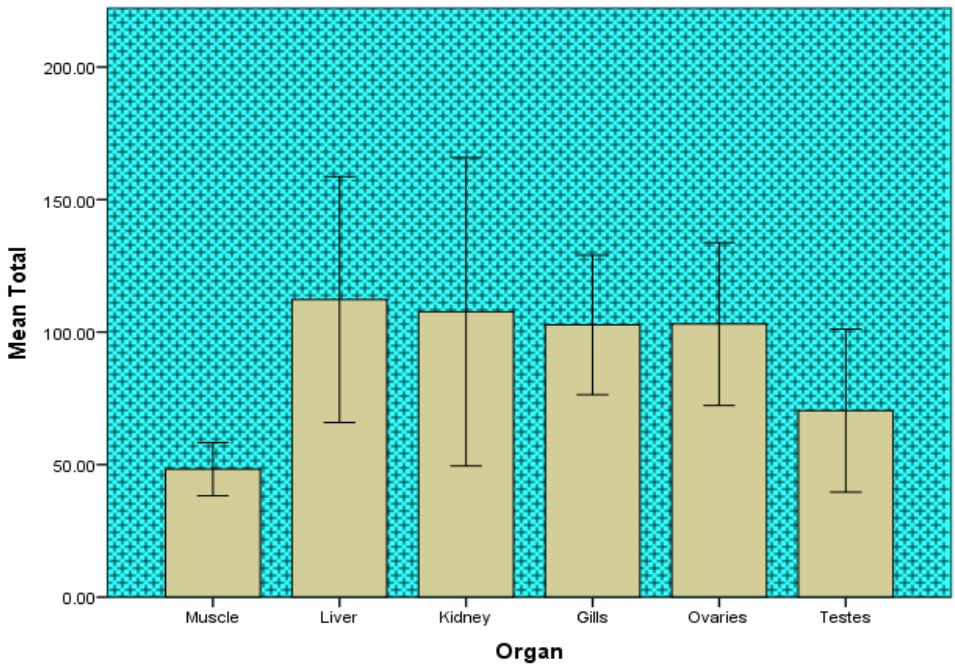
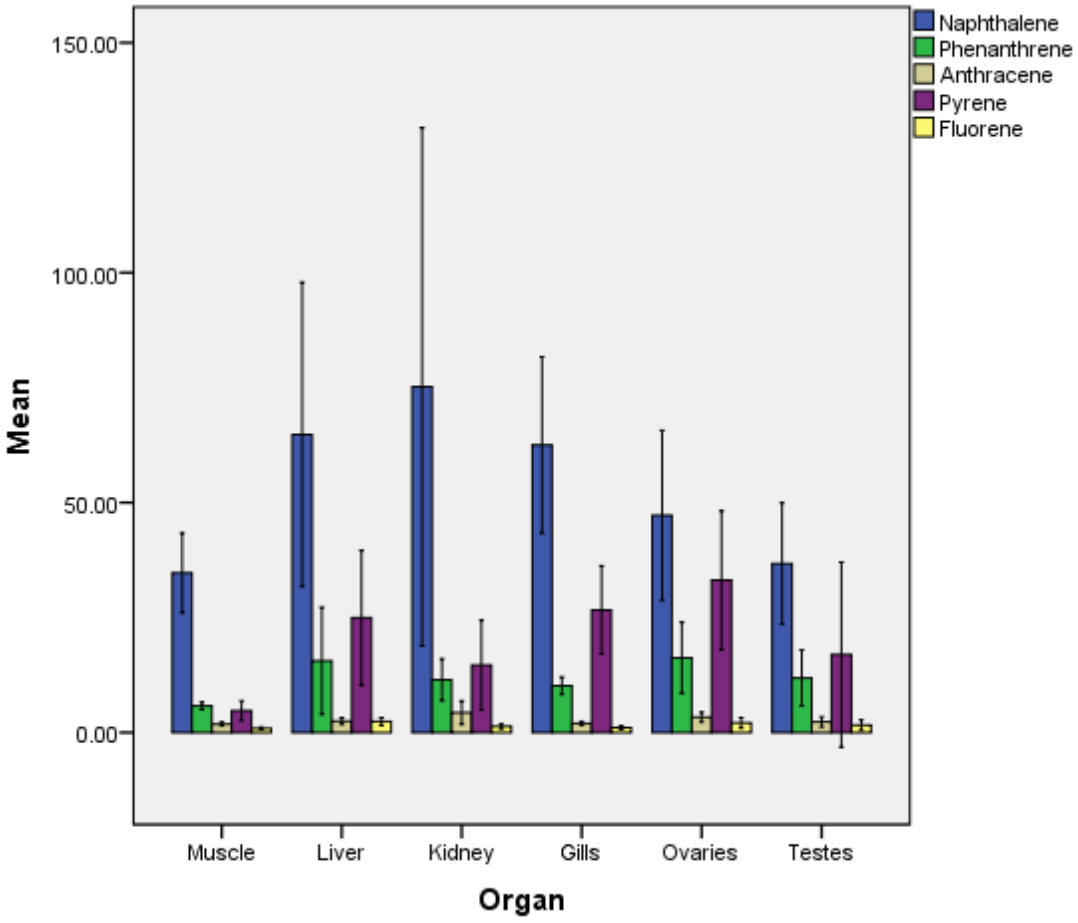


Figure 13: Mean concentration of individual PAHs (in ng/g±SD) in the organs of fishes collected from Pallikaranai Wetland, Chennai (2017-2018)



While naphthalene, fluorene, phenanthrene and pyrene followed a similar pattern to that of mean total PAH (liver > kidney > gills > reproductive organ >muscle) (figure 13), it was slightly different for anthracene with (kidney>liver>reproductive organs> gills>muscle). The variation among organs with respect to individual PAH was significant (n=886, H= 47.35, 51.92, 46.85, 20.69, 130.11 for naphthalene, fluorene, phenanthrene, anthracene and pyrene respectively ; $p<0.05$).

PAH in fish are rapidly metabolized by the liver (Hellou and Payne., 1978). They either become intermediates which bind to liver DNA (forming adducts) or pass to the bile by forming conjugates (Varanasi and Gmur., 1980). Individual PAH in liver samples of the present study are comparable to the levels detected by Pointet and Milliet (2000) in liver tissues of fishes collected from Natural Reserve Camargue (25-84 ng/g of

naphthalene, 5-26 ng/g of fluorene, 32 to 140 ng/g of phenanthrene, 11-76 ng/g of anthracene, BDL- 6 ng/g of pyrene, BDL-28 ng/g of Benzo(a) anthracene, BDL- 15 ng/g of chrysene, BDL to 10 ng/g of benzo flouranthenes and BDL-6 ng/g of benzo (a) pyrene). The levels of total PAH in liver tissue of catfish in the current study is very less when compared to the levels reported by Pointet and Milliet (2000). The levels of mean total PAH in the muscle (mean- 77.5 ng/g, range-BDL- 488.5 ng/g) are very much higher than the levels recorded by Cheung *et al.,* (2007) (Mean- 79.21, range- 15.5-118 ng/g). In order to assess the extent of contamination, criteria reported by Varanasi *et al.,* (1993) was employed. While taking mean total PAH (ng/g±SD) into consideration, the samples were mildly contaminated (table 7). In the all the fish tissue samples analysed, 7.76% of the fishes were not contaminated, 65.57% of the fishes were mildly contaminated. 24.52% of the fishes moderately contaminated while 1.34 % of the samples fell in the highly contaminated range. While the mild and moderately contaminated fishes dominated the wetland, they are still indicative of the threat that looms large on the wetland. While the levels detected in this study are already indicative of the health situ wetland, it may not be long before the wetland becomes deemed unfit to support.

Table 7: Fish tissue contamination criteria (Varanasi *et al.,* 1993)

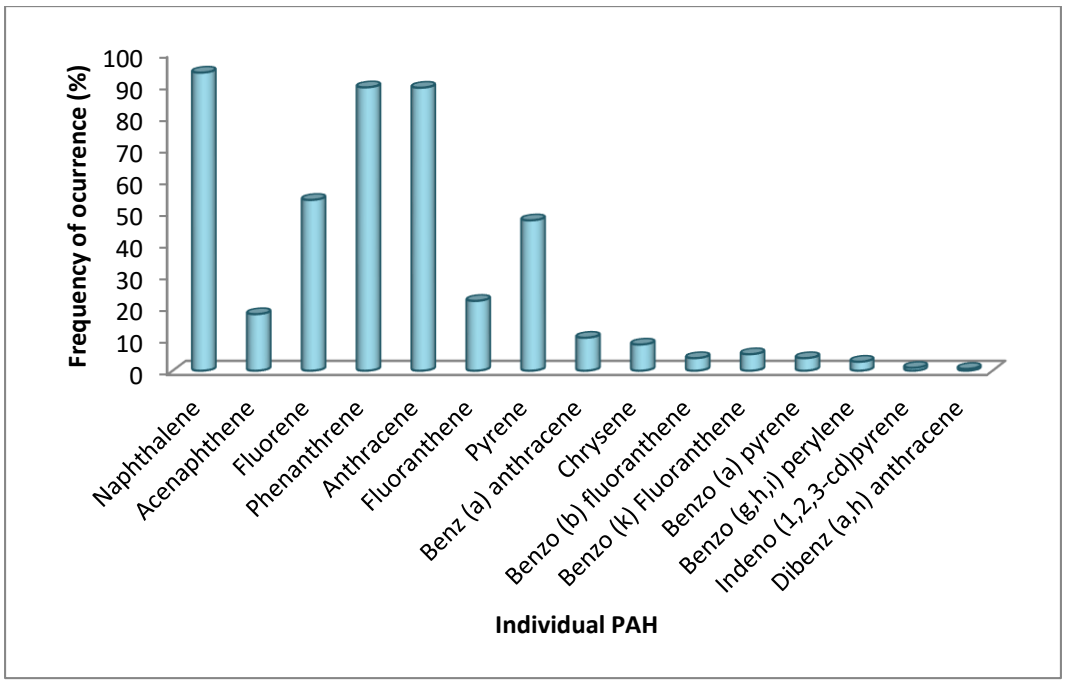
Classification	PAH Concentration(ng/g)
Not contaminated	<10
Mildly Contaminated	10-99
Moderately contaminated	100-1000
Highly contaminated	>1000

4.3. Frequency of occurrence, percentage composition and concentration of individual PAHs in the tissues of fish collected from Pallikaranai Wetland, Chennai (2017-2018)

Presence of PAH in fish organs is useful to reveal recent pollution in the environment by PAH (Speer *et al.,* 1990 and Hellou *et al.,* 2004). Comparison of individual PAHs is made irrespective of species and organs to understand their presence

in the environment. Percentage detection of individual PAHs in the tissues of fishes irrespective of organs and species are presented in figure 14. Six-ring PAHs, namely indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene were detected in none of the samples analysed. Naphthalene was detected in 94% the samples, and it was the most frequently detected PAH in the study followed by phenanthrene (89%) and anthracene (89%), fluorene (54%) and pyrene (44%). While dibenz (a,h) anthracene was detected in only 0.5% of the samples and was the least detected followed by indeno (1,2,3-cd) pyrene was detected in 1% of the samples (figure 14).

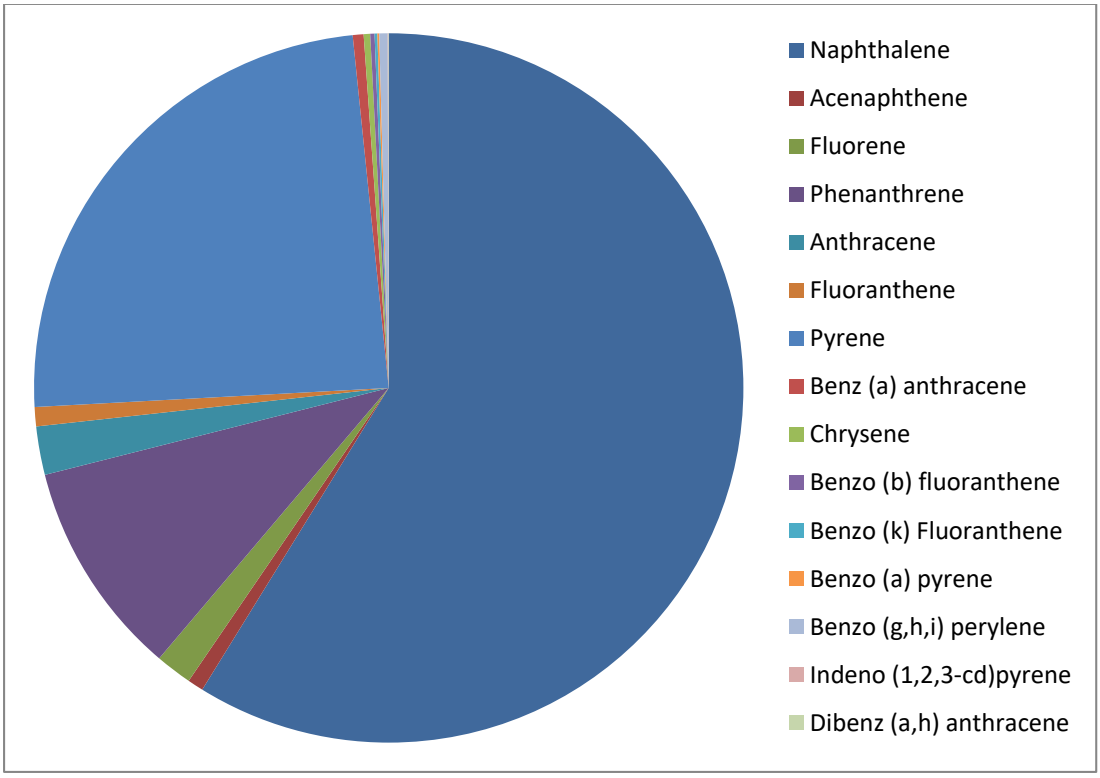
Figure 14: Frequency of occurrence (in percentage) of individual PAH in tissues of fishes collected in Pallikaranai Wetland, Chennai, between January 2017 and December 2018



Contribution of individual PAHs to the total PAH load irrespective of organs and species is shown in figure. Significant differences were observed in percentage composition of individual PAHs to total PAH load. As shown in figure 15, naphthalene (58.81%) contributed the maximum to the total PAH load followed by pyrene (24.23%) and phenanthrene (9.86%). Similarly, dibenz(a,h)anthracene (0.02%) contributed the least to the total PAH load followed by indeno(1,2,3-cd)pyrene (0.03%). The levels of individual PAHs in fishes irrespective of species and organs are shown in figure 14.

The levels varied among the individual PAHs and the variation was significant (Kruskal-Wallis test, n=886, H= 163.10, $p<0.05$) (analyses were carried out using Graphpad Prism 5). In general, the distribution profile of individual PAHs and their relative contribution to the total concentration, that is low molecular weight PAHs being dominant in the tissues of the fishes studied, reflect their abundance in the environment. This may be due to the high volatilization potential and increased solubility of low molecular weight PAHs in water (Albers, 2006). This pattern was similar to that observed in studies by Cheung *et al.*, (2007) in Hong Kong, Da Silva *et al.*, (2007) in Brazil.

Figure 15: Percentage composition of individual PAH in fishes collected from Pallikaranai Wetland, Chennai, between January 2017 and December 2018



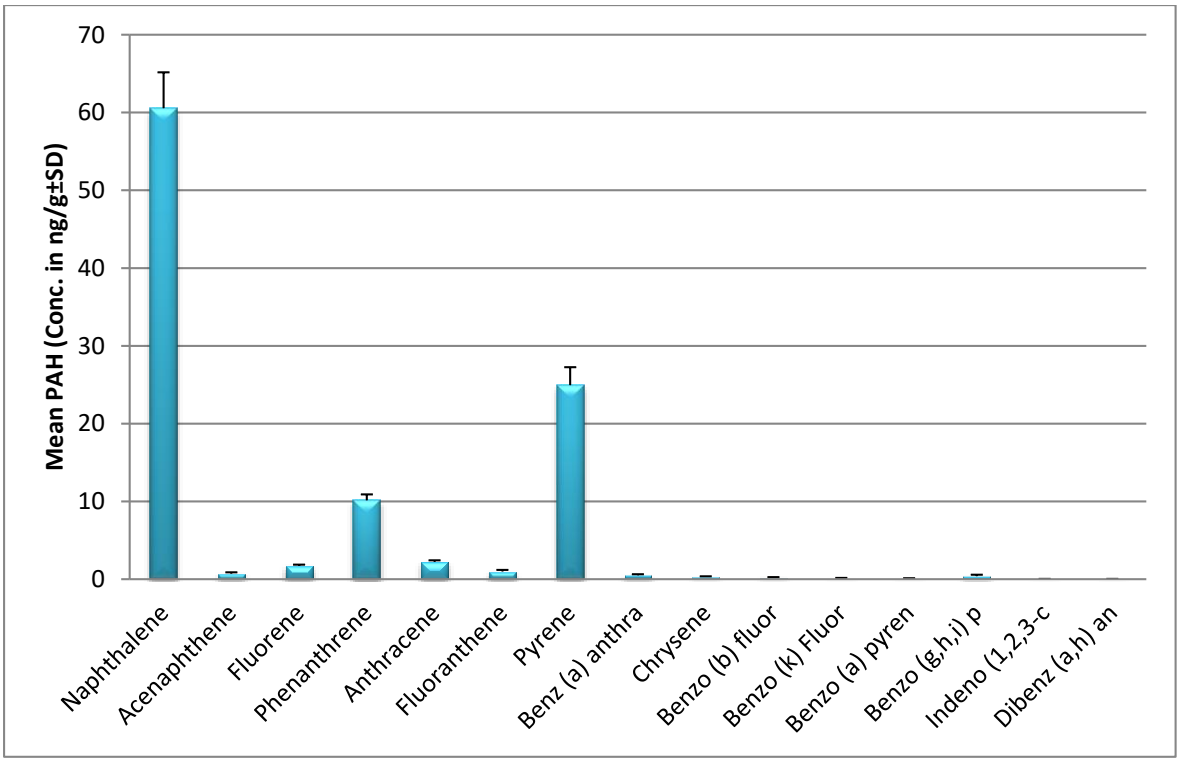
Naphthalene was detected the maximum in most of the fishes possibly due to its high volatilization potential. Due to this nature, it occurs loosely bound to sediment/soil and water and hence, becomes readily available for uptake by organisms including fishes. Common household materials such as moth balls, pesticides, tobacco fumes, burning wood, coal and fuel, thermoset plastics and dyes contain naphthalene and thus contribute heavily to the naphthalene load (ATSDR, 1990; DHSS, 2013) in the atmosphere, and

eventually in biological matrix. The low detection of HMW PAH in fish tissue may either indicate fast rate of depuration metabolically (Black., 1983) or they could point to the absence of these pollutants in the environment (Lacorte *et al*, 2006)

4.4. Ratio of individual PAHs

PAHs were classified based on their ring structure. As 2 ring PAHs include naphthalene, acenaphthene and fluorene; 3 ring PAHs include phenanthrene and anthracene; 4 ring PAHs include fluoranthene, pyrene, benzo[a]anthracene, chrysene, and benzo[b]fluoranthene; 5 ring PAHs include benzo[k]fluoranthene, benz[a] pyrene and dibenz[a,h]anthracene and 6 ring PAHs include benzo[g, h, i] perylene and indeno[1,2,3-cd] pyrene. Although it is difficult to interpret the individual profiles, the distributions in all samples were apparently, dominated by the 2 and 3 rings PAHs. They were found in almost all species. The composition patterns of PAHs by ring size in fishes are given in figure 16. Considering individual PAHs, high levels of benz[a]pyrene were found in all fishes, which on average accounts for 48.8% of the total PAHs.

Figure 16 Mean concentration (in ng/g at 95% CI) of individual PAHs in tissues of fishes of Pallikaranai Wetland, Chennai (2017-2018)



4.5. Sex-wise variation in levels of PAH in fishes collected from Pallikaranai Wetland, Chennai, between 2017 and 2018

Fish reproduction is very sensitive to chemical and physical stressors (Gerking, 1982; Spies *et al.,* 1988; Thomas, 1990a; Johnson *et al.,* 1992). PAHs have been shown to produce endocrine disruption in fish, which has been revealed by disruption of circulating steroid hormones in males and females (Seruto *et al.,* 2005). Ovaries from females exposed to petrogenic fractions have been known to present a significantly reduced number of maturing follicles (Vignet *et al.,* 2016). Studies showed that fish exposure to PAHs results in gonadal structure disruption indicative of maturation defects in females and in males (Peterson *et al.,* 2013, Sun *et al.,* 2011, Sundt *et al.,* 2011 Sun *et al.,* 2015). Most studies post exposure of PAH report a reduction in gonadal and egg maturation (Spies *et al.,* 1988) and a lower reproductive success (Spies and Rice, 1988;). Some authors have reported the maternal transfer of PAH (anthracene) to their offspring (Tilghman Hall and Oris, 1991, Nicolas., 1999). There is evidence, however, that males may also be susceptible to PAH-related reproductive dysfunction (Johnson *et al.,* 2002). PAH exposure has been associated with reduced sperm production and reduced sperm quality (Nagler and Cyr, 1997) With the aim to characterize a possible influence of sex, a comparison of the PAH concentrations was carried out in the fish species. To assess if there was any variation of PAH among sexes, the samples were categorized in three brackets. One being distinctly male with only testis as its reproductive organ, one being distinctly female with only ovary as its reproductive organ and the third being unknown. Individuals whose gender couldn't be identified and individuals who had reproductive organs of both sexes were grouped under unknown category.

While the pattern was Female>Male> Unknown (figure 17) for naphthalene, fluorene and pyrene, phenanthrene, anthracene, total PAH followed the pattern of Female> Unknown> Male (table 8). This is similar to the pattern followed in the study by Almroth *et al.,* (2008). In complete contrast to the present study, female fish exhibited significantly lower mean ΣPAH concentrations than male in all the species in a study by Rahmanpour *et al.,* (2014). While the percentage composition of naphthalene was the highest in the present study in all sexes, Pyrene contributed most in the study by Rahmanpour *et al.,* (2014). It may be noted that while the current study looked at the sex

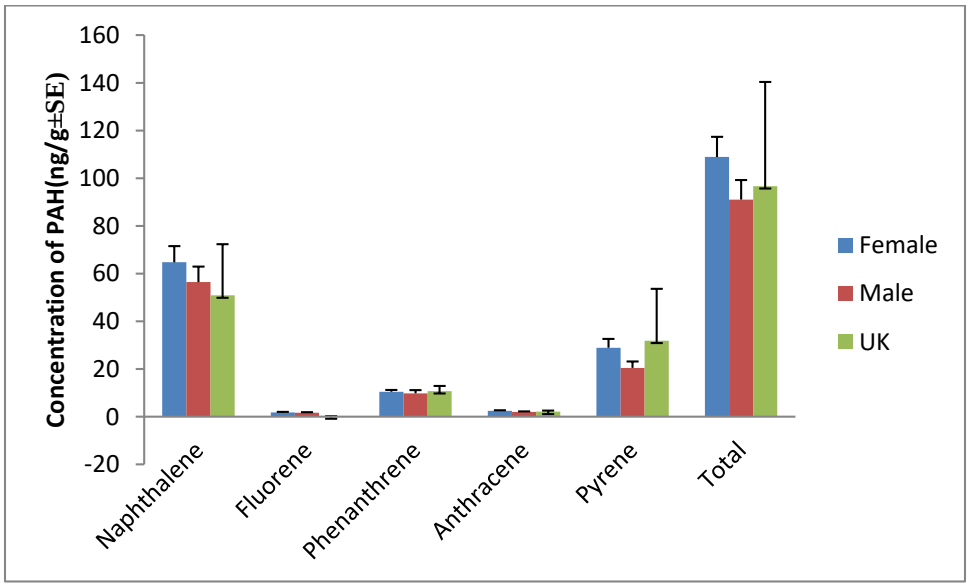
irrespective of organs and species, Rahmanpour *et al.,* 2014 looked at PAH load in the liver alone. The pattern observed in both total PAH and individual PAH levels in the present study was found to be in stark contrast to the levels reported by Ramalhosa *et al.,* (2012) in all except fluorene and phenanthrene.

While the reason for this pattern observed in the current study is unclear, it can perhaps be related to the elimination of contaminant by spawning or procreating, biotic factors, including active metabolism and excretion of the chemical, lipid content, and behaviour, play significant role in the variation of contaminants between male and female (Whipple 1979). During smoltification, juvenile salmonids, particularly in wild fish, lose significant amount of lipid (between 1 and 3% wet weight) (Brett 1995; Beckman *et al.*, 2000; Meador *et al.,* 2002).

Table 8: Concentration of PAH in fish tissue of different sexes (ng/g±SD) collected from Pallikaranai wetland, Chennai, between 2017 and 2018

	Naphthalene	Fluorene	Phenanthrene	Anthracene	Pyrene	Total
Female	64.7±6.74	1.79±0.22	10.45±0.74	2.50±0.23	28.93±3.72	108.92±8.44
Male	56.461±6.49	1.68±0.22	9.80±1.38	2.01±0.22	20.46±2.69	91.05±8.22
Unknown	50.89±21.42	0.12±0.08	10.73±2.14	2.12±0.43	31.85±21.7	96.68±43.65

Figure 17: Sex-wise variation in PAH contamination in fishes collected from Pallikaranai wetland, Chennai (2017-2018)



4.6. Levels of PAH in fins of fish from Pallikaranai Wetland, Chennai (2017-2018); Use of fins as indicators of external environment contamination

Fish fins are in continuous exposure to the external environment, the exposure to the contaminants in the environment increase too. In areas like Pallikaranai where there is influx of a cocktail of contaminants. While the levels of PAH in the internal organs are indicative of the accumulation over time, fins could serve as indicators of contamination of their surrounding environment.

Fin erosion has been fairly commonly observed phenomenon upon exposure to external stressors. There have been many studies which reported fin erosion on exposure to PAH (Tuvikene *et al.,* 1995, Steyermark *et al.,* 1999, Kaushik *et al.,* 2006). It has been reported that integumental lesions were observed within 8 days of exposure and later severe fin erosion in bottom feeding fishes when experimentally exposed to contaminated sediment (Tuvikene *et al.,* 1995). It is not clear whether dissolved chemicals alone are sufficient to cause the observed skin and fin erosion and lesions or chemical suspensoids or particles of contaminated sediment are involved (Hargis Jr *et al.,* 1993).

Worldover, there is no information available on the levels of PAHs in fish fins and whether the levels in fins could correlate with the external exposure albeit being in direct exposure to the external environment consistently. In a maiden attempt to assess the magnitude of external environmental contamination, fins of 34 fishes comprising of 8 species were analysed.

From the table 9, it can be inferred that fins had very high levels of most priority PAHs, particularly the carcinogenic PAH4 and High molecular weight PAHs. The levels were much higher than the levels of PAH detected in organs. The percentage of detection of naphthalene was 100% in fin samples. Naphthalene was also the maximum in comparison to other PAH. While benzo(b)flouranthene, indeno (1,2,3-cd) pyrene, benzo (g,h,i)perylene and dibenz (a,h) anthracene were not detected in any of the fin samples, carcinogenic PAH4 has been detected in levels higher than what is considered safe for humans (2 ng/g, SCF, EU, 2002) but no such limit has been prescribed for fish.

Levels of total PAH in fins ranged between BDL and 1589±67.29 ng/g in *Oreochromis aureus.* This was closely followed by 1453±67.29 ng/g in *Oreochromis niloticus*.

Incidentally, there is no information available in literature on the levels of PAH in fins to compare with the data generated in the present study.

Table 9: Levels of PAH in the fins of fishes collected from Pallikaranai Wetland, Chennai, between 2017 and 2018

PAH	Mean±SD	Median	Range
Naphthalene	185.97±64.8	31.23	BDL-1541.30
Acenaphthene	2.71±2.46	BDL	BDL-83.97
Fluorene	3.26±1.98	BDL	BDL-64.63
Phenanthrene	10.18±2.42	4.39	BDL-52.70
Anthracene	1.32±0.21	1.04	BDL-4.06
Fluoranthene	1.28±0.5	0.47	BDL-16.30
Pyrene	7.35±6.83	BDL	BDL-232.57
Benz (a) anthracene	1.29±1.06	BDL	BDL-17.19
Chrysene	1.23±0.60	BDL	BDL-36.44
Benzo (b) fluoranthene	BDL	BDL	BDL
Benzo (k) Fluoranthene	0.042±0.03	BDL	BDL-1.00
Benzo (a) pyrene	0.05±0.03	BDL	BDL-1.12
Benzo (g,h,i) perylene	BDL	BDL	BDL
Indeno (1,2,3-cd) pyrene	BDL	BDL	BDL
Dibenz (a,h) anthracene	BDL	BDL	BDL
Total	214.68±67.29	35.73	BDL-1589.93
PAH4	2.57±1.59	BDL	BDL-53.63
HMW	2.61±1.59	BDL	BDL-53.63

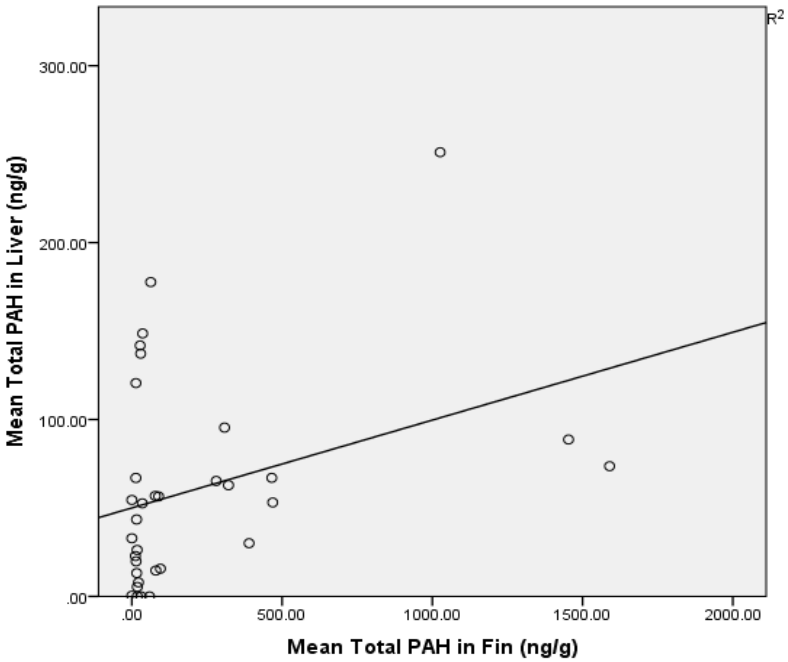
In an attempt to assess if the levels in the fins were better tools to assess the status of contamination than internal tissue, the levels of mean total PAH in the fins were correlated with the levels of mean total PAH in liver and kidney which are the primary target organs. While negative correlations (table 10) were observed for both liver and kidney (figure18), only the correlation between fins and liver was statistically significant. Since there is no previous literature available for comparison, the values observed in the current study may be treated as baseline information.

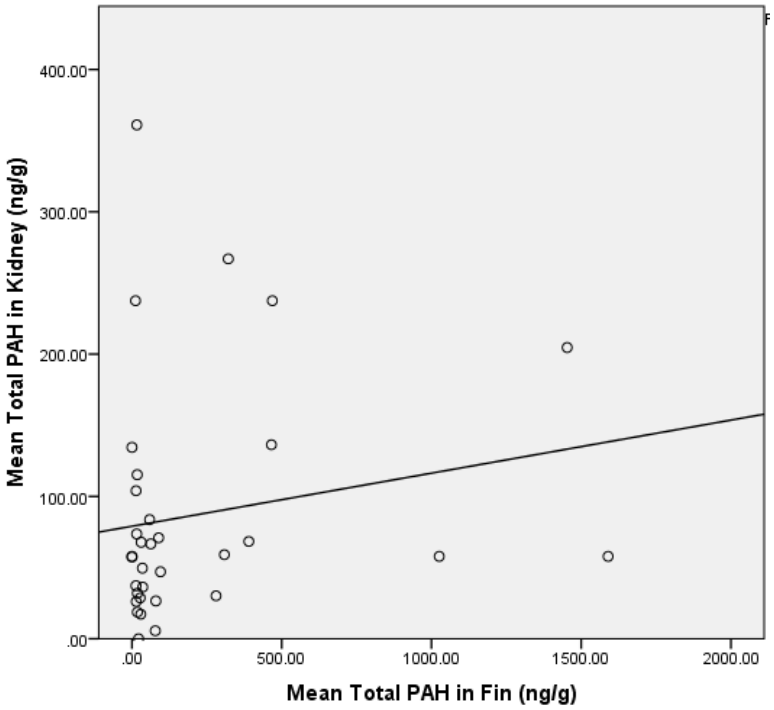
Table 10: Pearson correlation r values obtained on correlating levels of PAH in fins with liver tissue of fishes collected from Pallikaranai Wetland, Chennai (2017-2018)

Matrix 1	Matrix 2	R value	Significance
Liver	Fins	0.338	0.05
Kidney	Fins	0.176	0.326

Potential effects on fish are also of interest because these animals occupy key positions in aquatic and terrestrial food webs. They are preyed upon by numerous species including other fish, humans and other mammals, piscivorous birds, and various scavengers. Therefore, changes in fish populations can translate into changes in other populations dependent on them for food (Logan *et al.*, 2007). As such, they accumulate petroleum hydrocarbons more rapidly and eliminate them more slowly, or less completely, than organisms with better metabolic capability. The levels detected in fins could be a problem to other higher organisms such as piscivorous birds as they consume the whole fish unlike humans who consume only the muscle tissue. Hence, if the guidelines prescribed by European union for humans can be considered carcinogenic for birds too, then the levels recorded in the present study can be considered unsafe for fish-eating-birds.

Figure 18: Scatter plots of correlating levels of PAH in liver and kidney with levels of PAH in fins of fishes from Pallikaranai Wetland, Chennai (2017-2018)





4.7. Levels of PAH in gut-content of fish from Pallikaranai Wetland, Chennai (2017-2018); A major source of contamination.

Physical and chemical analyses of environmental compartments provide some of the information required for but they cannot provide adequate information on the bioavailability of environmental pollutants (EC Directive., 2000). Therefore, indicators such as fish and other aquatic biota have been used in environmental risk, and impact, assessments. Individual indicators seldom provide all information (Forbes *et al.,* 2006) required so typically a suite of indicators is preferred (JAMP., 1998). While the fins could give us an outline on the extent of contamination of the environment, it is still not adequate to infer that the fish was exposed to all of the contaminants in the surrounding environment. A potential mode of uptake of PAH is through the gastrointestinal track (Van Veld *et al.,* 1988a). Uptake processes of PAH include chemical absorption across the gills, skin, and gut (Nichols., 2007). Earlier studies of PAH in the gut showed induction of cytochrome enzyme CYP1A in the gut in fish fed with benzo(a)pyrene (Van Veld *et al.,* 1988b). A great deal of evidence indicates that the intestine may play an important role in the metabolism of dietary PAH (Van Veld *et al.,* 1988a). In an attempt to assess the extent of contamination of fish feed and measure the actual exposure, the levels of PAH in the gut-contents was measured.

Two hundred and thirty three samples comprising 21 species of gut contents were analysed to get a clear idea on the extent of PAH contamination of food (table 11). The levels of total PAH in the food ranged between BDL and 1162±12.01 ng/g in *Oreochromis mossambicus*. This was followed by 1062 ng/g and 1043 ng/g in *Trichopodus trichopterus* and *Anabas testudineus* respectively. Of all the PAH, naphthalene was the most prevalent and it accounted for most of the PAH load and this was followed by pyrene. The concentration recorded in this study is much lesser than the levels of PAH detected in stomach contents of fishes of Bahaio Parasu, Antartica and comparable to the levels in Palmer station in Antartic region (McDonald *et al.,* 1992). Varanasi *et al.,* (1993) reported a median total concentration of 23.8 ng/g of PAH in the stomach contents of *Oncorhynchus tshawytscha* collected from Puget sound estuary, USA, while the median total concentration of PAH in the gut content in the current study is 71.93 ng/g ww.

Table 11: Levels of Total PAH (ng/g ww) in gut-contents of fishes collected from Pallikaranai Wetland, Chennai between 2017 and 2018

PAH	Mean	SD	Median	Range
Naphthalene	69.17	8.08	30.53	BDL-1033.47
Acenaphthene	1.00	0.33	BDL	BDL-62.53
Fluorene	2.47	0.62	1.00	BDL-67.67
Phenanthrene	9.89	1.07	6.91	BDL-185.47
Anthracene	1.97	0.29	0.86	BDL-56.17
Fluoranthene	1.16	0.29	BDL	BDL-51.04
Pyrene	44.16	6.62	11.17	BDL-959.11
Benz (a) anthracene	0.75	0.32	BDL	BDL-56.57
Chrysene	0.47	0.13	BDL	BDL-18.83
Benzo (b) fluoranthene	0.31	0.18	BDL	BDL-39.45
Benzo (k) Fluoranthene	0.23	0.11	BDL	BDL-20.79
Benzo (a) pyrene	BDL	BDL	BDL	BDL-15.59
Benzo (g,h,i) pyrene	BDL	BDL	BDL	BDL-7.54
Indeno (1,2,3-cd)pyrene	BDL	BDL	BDL	BDL-15.43
Dibenz (a,h) anthracene	BDL	BDL	BDL	BDL-17.89
Total	131.72	12.01	71.93	BDL-1162.06

In an attempt to assess if the levels in the gut-contents were better tools to assess status of contamination than internal tissue, the levels of mean total PAH in the gut-contents were correlated with the levels of mean total PAH in liver and kidney, the primary target organs. While no correlation (figure 19) was observed between PAH in liver and gut-content, a negative correlation was observed between kidney and gut content (figure 20). The Pearson correlation coefficient was not significant, $p>0.05$

(table 12). The lack of correlation does not rule out the obvious relationship but the data generated in the current study is inadequate to justify this.

Mammals, birds, fishes, and many invertebrates efficiently metabolize and excrete aromatic hydrocarbons ingested during feeding, reproduction, grooming, and respiration (Lee, 1977; McEwan and Whitehead, 1980; Engelhardt, 1983; Eisler, 2000). While the PAHs may get metabolized and excreted, they continues to stay in the food web, one way or the other (Eisler, 2000). Liang *et al.*, (2007) reported Polycyclic aromatic hydrocarbons levels in fish collected from eight shrimp shallow ponds from July 2003 to January 2004. The results showed that significantly higher PAHs were accumulated in fish viscera (505–854ng/g) than in muscle (184–194ng/g). They also observed that viscera appeared to be a promising tissue for indicating PAH levels in Tilapia species, because it contained much higher concentrations than the muscle and the levels recorded are comparable to that of the present study.

No information is available in literature is available on the levels of PAH in gut-content. Our data suggests that gut contents are the appropriate tool to understand if the levels detected are indicative of contamination of feed. As there is no available literature on the levels of PAH in gut-contents of fish, the data obtained from this study may serve as baseline data for any future studies.

Table 12: Pearson correlation r values obtained on correlating levels of PAH in gut contents with liver and kidney tissue of fishes collected from Pallikaranai Wetland, Chennai (2017-2018)

Matrix 1	Matrix 2	R Value	Significance
Liver	Gut-content	0.007	0.930
Kidney	Gut-content	-0.120	0.406

Figure 19: Scatter plot correlating levels of PAH in liver with levels of PAH in gut-contents of fishes from Pallikaranai wetland, Chennai (2017-2018).

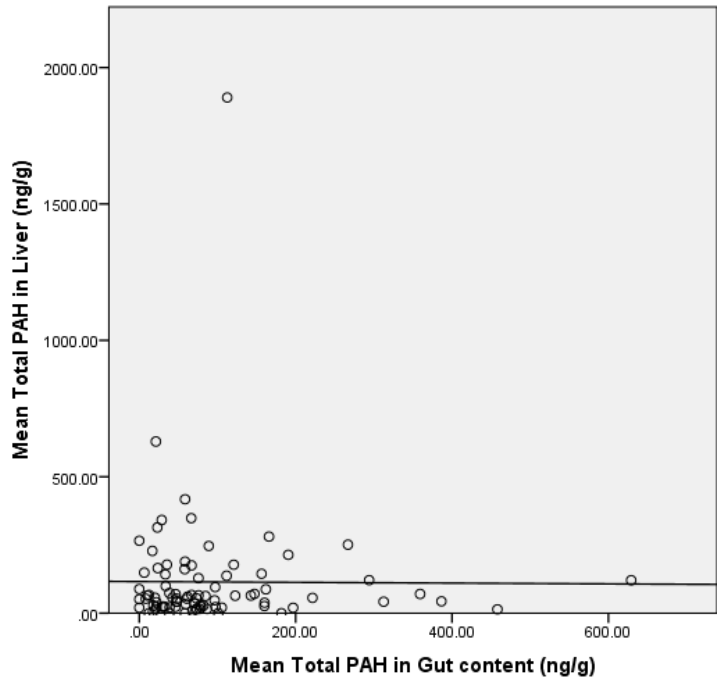
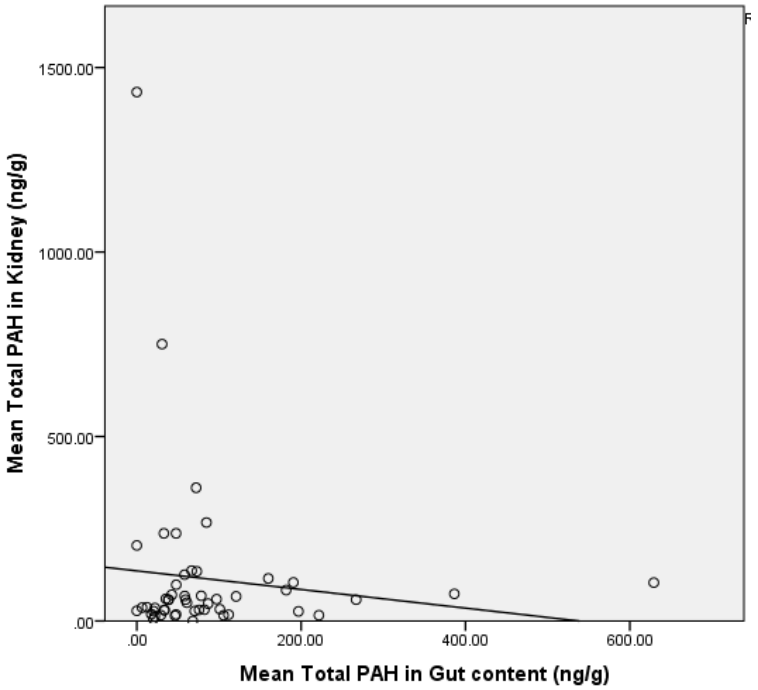


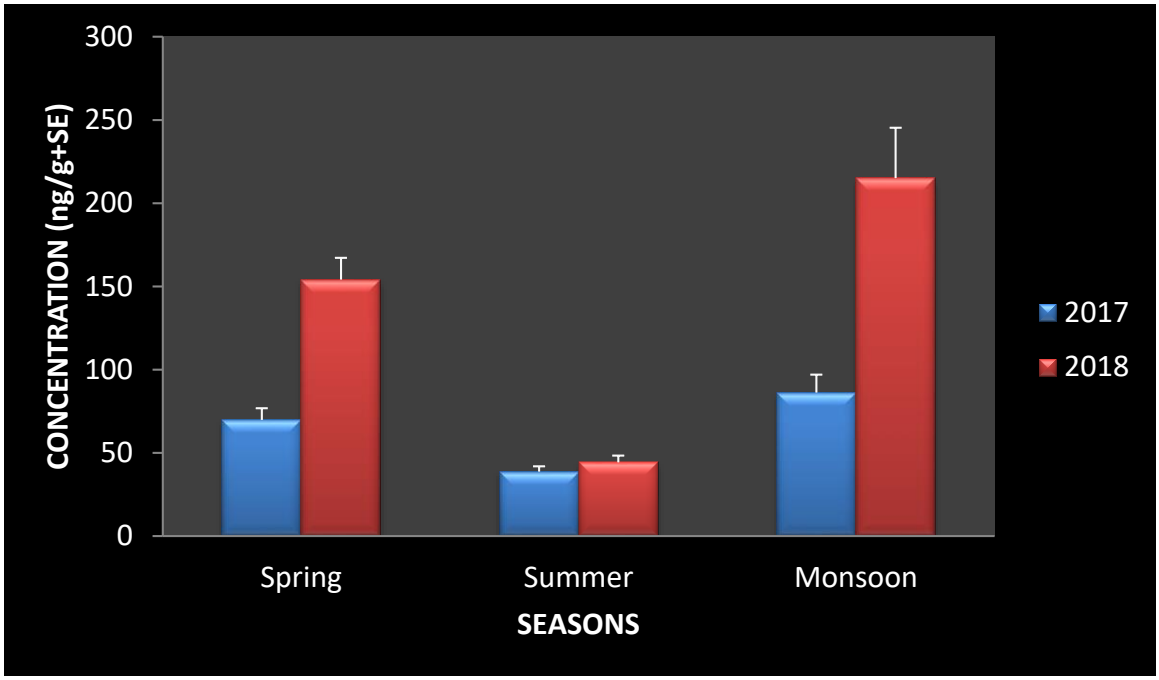
Figure 20: Scatter plot correlating levels of PAH in kidney with levels of PAH in gut-contents of fishes from Pallikaranai Wetland, Chennai (2017-2018).



4.8 Seasonal variation of PAH contamination in fishes of Pallikaranai wetland, Chennai, between January 2017 and December 2018

In order to assess if there was any seasonal variation in PAH contamination, samples which were collected in three seasons, namely Spring (January- April), Summer (May- August) and monsoon (September to December) for two years were analysed for the USEPA suggested 15 PAH. The number of months included per season does not conform to the Indian Meteorology Department and was chosen for the sake of logistical convenience. This information may be valuable in a public health protection perspective helping to select the less PAHs contaminated fish to eat in the different seasons (Ramalhosa *et al.*, 2012). Some studies demonstrated that the elimination rate of PAHs can be affected by several factors, such as, the water temperature, changes in the behavior of organism, nutritional quality, stress and oxygen consumption (Roche *et al.*, 2002; Peruguni *et al.*, 2007)

Figure 21: Seasonal variation in levels of PAH in fish collected from Pallikaranai Wetland, Chennai (2017-2018)



The data has been analysed in two angles. One was to assess if there was variation between seasons in both 2017 and 2018. The next was to assess if there was any variation between the two years. While Monsoon recorded the highest mean total PAH, Summer recorded least mean total PAH (figure 21). The variation between seasons was statistically significant for all the PAH. The data sets were subjected to Kruskal-Wallis test. Total PAH (H= 160.68, p>0.05); naphthalene (H= 38.25, p>0.050); fluorene (H=38.57, p>0.05); phenanthrene (H= 127.93, p>0.05); anthracene (H=31.38, p>0.05) and pyrene (H=151.15, p>0.05). The pattern of contamination was the same in both the years with the 2018 having higher detection than 2017, but the levels in 2017 was much lesser than that of 2018. The variation between 2017 and 2018 was statistically significant (Kruskal wallis test, H= 6.446, p>0.05) for total PAH as well as Naphthalene (Kruskal wallis test, H= 110.951, p>0.05) and Phenanthrene (Kruskal wallis test, H= 21.173, p>0.05). The seasonal variation in contamination observed in the current study was similar to that observed by Ramalhosa *et al.,* 2012 in Portugal while it was the reverse with findings of Perugini *et al.,* (2007) in the Adratic sea in Italy. Roche *et al.,* (2002) reported that there was a strong seasonal variation observed in eels (*Anguilla Anguilla*) from Camargue Biosphere Reserve in France where the pattern (2.37 ng/g in spring and 67.1 ng/g in summer) was similar to the current study. While the patterns observed by Roche *et al.,* 2002 were similar to the levels recorded in the present study during 2017, it may be noted that the level of contamination observed during 2018 was many folds higher than 2017. This is indicative of the exponentially growing magnitude of contamination. While Chennai received 554.2 mm of rainfall (74% of its average rainfall) in the North-east monsoon, South-west monsoon was a failure in 2017. In 2018, Chennai recorded 343.7mm of rainfall as against an average 757.6mm (around 50 % deficit) in North east monsoon and South-west monsoon failed again (Indian Meteorological Data, 2017 and 2018). The levels of contamination being the highest in the monsoon followed by spring and being the least in summer could be accounted for by the fact that with the influx of rainwater, the leachates could carry more contaminants into the water body making the contaminants more readily available for uptake by the fish. An oil spill had occurred near the Ennore port along the Chennai coast just 53 Km away from Pallikaranai wetland due to a crash between two oil tanker ships carrying

Petroleum on 28th January 2017. There was a spillage of 251.46 tonnes of oil spillage directly affecting 35 Km of Chennai's coast (News report, India Today 3rd February 2017). There was a second spill on 18th November 2018 when fuel was being discharged into the port at the Marine liquid Terminal 1 due to a hose failure. This resulted in a direct discharge of upwards of 2 tonnes of oil into the coast line (News report, India Today, 18th November 2018). During the north-east monsoon, there is influx of sea water from Bay of Bengal to Pallikaranai (Jayaprakash *et al.,* 2010) and this might be contributing to the high levels of PAH in the fish by bringing in contaminants during the influx.

4.9. Variation in PAH contamination level between Native and Introduced Species of Pallikaranai Wetland, Chennai (2017-2018)

Introduced species in any ecosystem are globally considered as pests. While this may be justified due to the general characteristics of most of the introduced species, and particularly the threats they pose to endemic species and the ecosystem as such, it may not be particularly true for all. In aquatic environments, fish are the group with the highest number of introduced species in the world (i.e. 624 species; Gozlan, 2008), which is also one of the most threatened taxa (approximately 3500 threatened species with population dwindling in 1958 species in 2020; IUCN, 2020).

One of the characteristics considered to make a non-native species as invasive, is a higher tolerance to environmental stressors, both natural and human induced, compared to native species (Karatayev *et al.* 2009; Leuven *et al.* 2011; Verbrugge *et al.* 2012). Geographic origin is an important predictor of species performance (Buckley and Catford., 2016) as introduced species are often more abundant, possess novel defences (Kleunen *et al.* 2010), lack natural enemies (Keane and Crawley., 2002), and have unique evolutionary histories (Strauss *et al*. 2006, Ordonez., 2010) when compared to native species. There is lack of comparative and quantitative assessment of tolerance of native and introduced species to stressors such as contaminants (Bahls, 1992; King *et al.,* 1996; Tiner *et al.,* 2002).

The biodiversity and ecosystem functioning are interconnected through an assemblage of species, habitat nature, environmental conditions, nutrient cycle, seasonal fluctuations and number of percipient invasive species with detonated frequency

(Kolar and Lodge, 2000). Exotic fishes have been intentionally introduced throughout the world for sport fishing, ornamental purposes, and as biological control agents (Taylor *et al*., 1984). Introduced fish may spread to additional waters and have been known to eliminate or greatly reduce native fauna through competition or predation (Moyle *et al.,* 1986). Despite biodiversity concerns, however, intentional fish stocking is still increasing globally (Kolar and Lodge, 2000). Biological invasions have long been recognized as a threat to the biodiversity of freshwater systems (Elton, 1958). Introduced species have historically been presumed to be evolutionarily novel and 'different' from native species (Lemoine *et al.,* 2016). Dwivedi *et al.* (2017) reported the process of species introduction as a problem and the pressure that the species have on the ecosystem are when species are introduced either for commercial or ornamental purposes, they have adapted or their own developed phenomena. There is pressure for survival, food and space. Once the introduced species maintains stable stock, there are changes in the food web structure, changes in the water quality and changes in ecosystem functioning. This leads to threatened biodiversity. However, recent evidence suggests that many introduced species perform similarly in their native and introduced ranges, and many native species can exhibit 'invasive' behavior when freed from constraints on population size (Kolar and Lodge, 2000). The opinion on this has been divided.

Hybridization among closely related species can be one of the most immediate consequences of introductions, particularly among Salmonidae, Cichlidae and Cyprinidae. It reduces the diversity of fish communities and the fitness of the native species (Dowling & Childs, 1992; Meffe, 1992).

Research on relative tolerances of non-native species to contamination is severely limited for three main reasons. Firstly, most of the studies focus on a single non-native species, usually an invertebrate (Piola and Johnston 2006; Karatayev *et al*. 2009b; Weir and Salice 2012). Secondly, most attention is given to a single physico-chemical parameter at a time such as temperature, organic waste, salinity, single chemical compounds, or nutrient pollution (Menke *et al.* 2010; Früh *et al.* 2012). Finally, there is a lack in comparative and quantitative assessment of tolerance of native and non-native species to contaminants (Scott *et al.* 2007; Alonso and Castro-Diez., 2008). A comparison of the potential impact of water pollution on native and non-native fish species has not yet been performed (Weir and Salice 2012).

Long-term studies on the impact and fate of introduced species are the need of the hour and need to be looked at in greater detail (Tiner *et al.,* 2002). In an attempt to understand the variation in contamination between native and introduced species, data collected on the fishes of Pallikaranai wetland, Tamil Nadu, has been used. The study site assumes tremendous significance in the local context as the once beautiful pristine marsh has been slowly converted into an official corporation garbage disposal site to handle municipal waste water, industrial effluents, among others. Rampant encroachment and construction activities have shrunk the size of the wetland over a period of time.

Five species of introduced and endemic fish respectively (table 13) comprising 745 tissue samples collected from Pallikaranai wetland were analysed for 15 USEPA listed Polycyclic Aromatic Hydrocarbons.

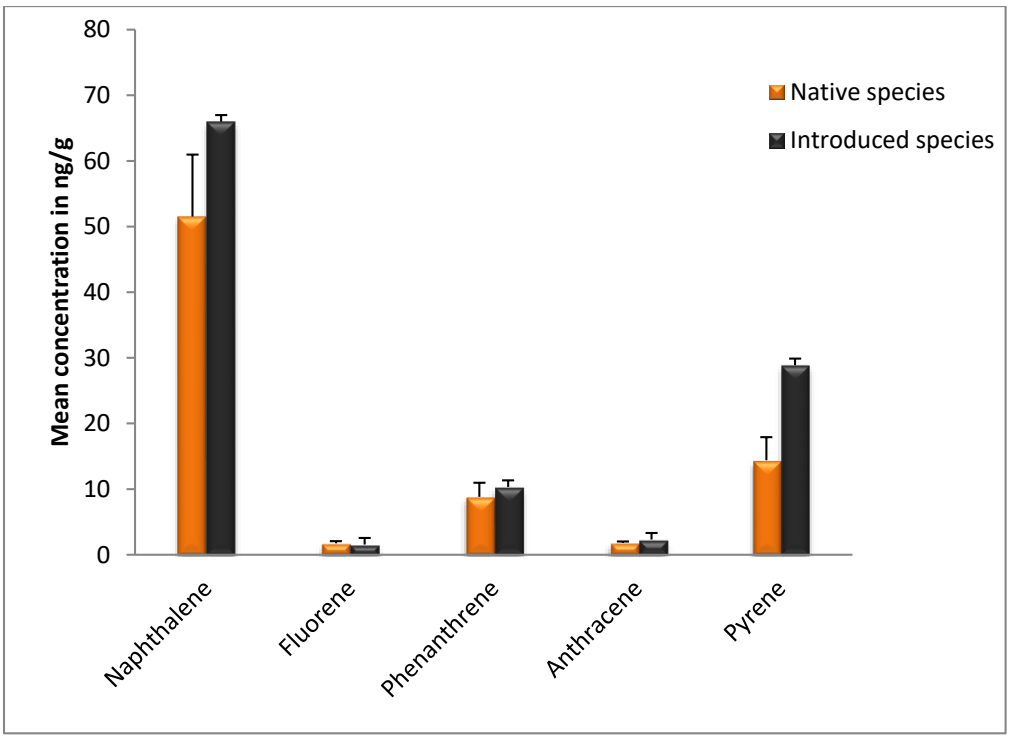
Table 13: Species included for the analyses of difference in levels of PAH between endemic and introduced species

Native	**Introduced**
Anabas testudineus	*Clarias Gariepinus*
Channa punctate	*Oreochromis aureus*
Clarias batrachus	*Oreochromis mossambicus*
Systomus sarana	*Oreochromis niloticus*
Trichopodus trichopterus	*Puntius chola*

In an attempt to assess if the endemic species were more adapted than the introduced species to the anthropogenic pressures on the wetland, the mean levels of PAH in tissues of the respective categories were compared (figure 22). Mean levels of Naphthalene, fluorene, phenanthrene, anthracene, pyrene and total PAH in samples of introduced species was much higher compared to native species. The data was also analysed to assess if covariates including species, organs, seasons and sex contributed to the high levels of PAH in the introduced species and it was observed that none of the covariates contributed significantly. Introduced species have historically been presumed to be novel evolutionarily and much different from native species. While recent evidence

suggests that many introduced species perform similarly in their native and introduced environments, geographic origin is thought to be an important predictor of species performance because introduced species are often more abundant, lack natural enemies, possess novel defences, and have unique evolutionary histories compared with native species. The high levels of PAH observed in the introduced species may be attributed to the better adaptability of endemic species to the anthropogenic stressors. One of the most common effects of introductions is that of predation on native species by an introduced predator. Hence, biomagnification may contribute to PAH load of the fish.

Figure 22: Variations in individual PAH concentration between Native and Introduced species of fishes in Pallikaranai Wetland, Chennai, during 2017- 2018

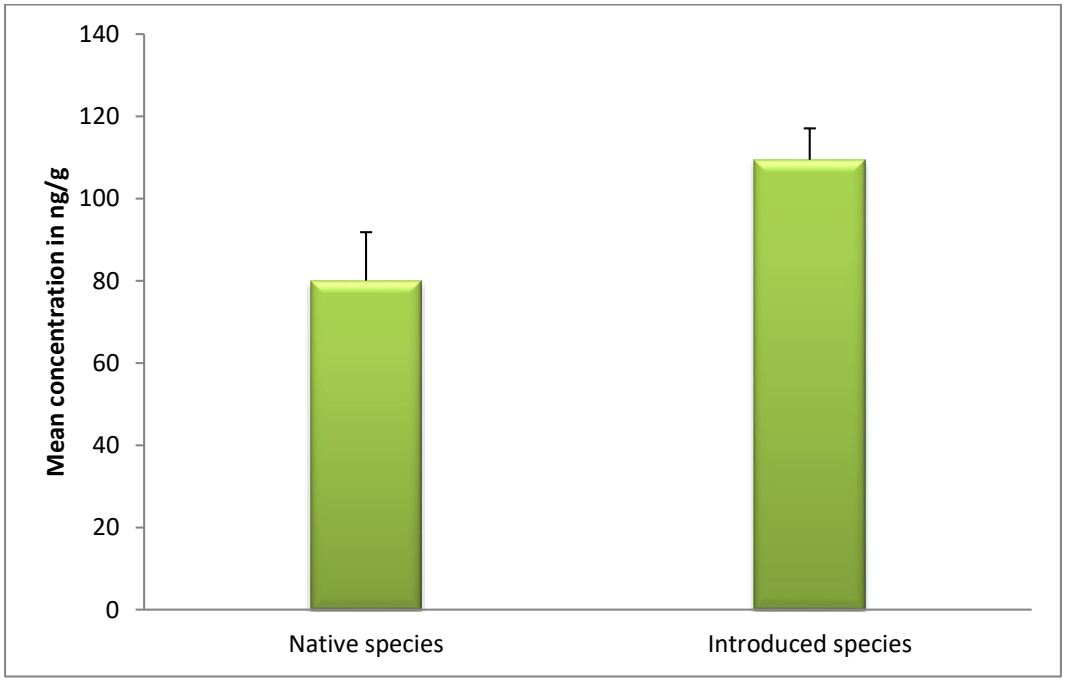


The variations in the concentrations of PAH between endemic and introduced species (figure 23)were significant. naphthalene ($H=17.409$, $p=0.043$, $p<0.05$); fluorene ($H=17.110$, $p=0.047$, $p<0.05$); phenanthrene ($H=46.319$, $p=0.00$,$p<0.05$); anthracene ($H=37.859$, $p=0.00$, $p<0.05$); pyrene ($H=75.52$, $p=0.00$, $p<0.05$) and total PAH ($H=38.59$, $p=0.00$,$p<0.05$). When compared to the present study, the levels of mean toat PAH in native fishes collected from River Elbe reported by Speer *et al.,* 1990 was very less

(>5 ng/g). In a study by Black (1983), the mean total PAH in native Nigerian fishes from Smokes creek (16,927 ng/g), Union canal ship (64,264 ng/g), Buffalo river (76,374 ng/g) and in Black Rock Canal (18,061 ng/g) were several folds greater than the native fishes in our study (100.63 ng/g, range: 0 to 1890.26 ng/g).

The concentration of PAH in of introduced fishes in the current study (109.44 ng/g ranging between BDL and 1699 ng/g; figure) were much lower than the levels of introduced zebra mussels sampled in Hamilton Harbour (between 351 and 10,039 ng/g). One of the characteristics considered to make a non-native species become invasive is a higher tolerance to environmental stressors (Bahl *et al.,* 1992) but the results obtained in this study contradict this. While information available in literature world-over on the introduced species is scarce, no information is available comparing the levels of contaminants, particularly PAH between native and introduced species. While it is not possible to conclude on the basis of the available information, there is scope to study if the native fishes in these waters with abundance of nutrition (sewage entry and waste leachate) tend to be more adaptive. The data generated in this could serve as baseline information.

Figure 23: Variation in mean±SD between endemic and introduced species of fish in Pallikaranai Wetland, Chennai (2017-2018)



4.9.1. Influence of covariates on the levels of PAHs in endemic and introduced species in Pallikaranai Wetland, Chennai (2017-2018)

To assess if any of the co-variates such as species, organs, year, season and sex, played a role in the variation of PAH concentration between native and introduced species, the data was subjected to ANCOVA and it was found that while organ ($F= 13.02$, $p=0.00$, $p<0.05$), sex ($F= 7.817$, $p=0.005$, $p<0.05$) and year ($F=27.95$, $p=0.00$, $p<0.05$) contributed significantly, the role played by the species ($F=0.013$, $p=0.909$, $p>0.05$) and seasons ($F=1.288$, $p=0.257$, $p>0.05$) considered in this study did not significantly affect the levels of PAH in endemic and introduced species. There have not been any studies available in the literature comparing the levels of PAH in introduced with endemic species and assessment on the role of co-variates on PAHs in introduced or native species. Hence, it is not possible to compare and substantiate the data generated in the current study.

4.10. Variation in PAH contamination among fishes belonging to different feeding guilds in Pallikaranai wetland, Chennai (2017-2018).

Every organism has a place to live in nature, a functional role in that place, and a complex set of adaptations for reproducing its kind. A food chain is the sequence of who eats whom in a biological community (an ecosystem) to survive and obtain necessary nutrition. Pollutants get bio-accumulated in the tissues of fauna which are final consumers (Bilandzic *et al.*, 2011).

There are four basic eating groups among fish: carnivores (interchangeably used with Piscivore), herbivores, omnivores and limnivores. Carnivores are meat-eating fish. Herbivorous fish are those that will eat only plants. These fish need to graze very often, and whether they feed regularly or not, they will nip on plant life. Omnivorous fish voraciously eat both plants and also other fish. Limnivores are also known as mud-eaters. Limnivorous fish feed mainly on mud, algae and on the microorganism.

The food items may transmit high doses of contaminants (Beyerbach *et al.* 1987; Purchart and Kula 2007). Pollutants get bio-accumulated in the tissues of animals that are final consumers (Bilandzic *et al.* 2011).

Mammals, birds, fish, and many invertebrates efficiently metabolize and excrete aromatic and non-aromatic hydrocarbons ingested during feeding, grooming, and respiration (Lee, 1977; McEwan and Whitehead, 1980; Engelhardt, 1983; Jenssen *et al.*, 1990; Eisler, 2000). Accumulation of PAH by organisms is associated with poor metabolic capabilities, high lipid content, and activity patterns or distributions that coincide with high concentrations of PAH (McElroy *et al.,* 1989; Eisler, 2000).

Although guilds were used in describing community organization in these studies, the use of feeding or foraging guilds for assessing environmental problems on marine fishes has not yet been fully explored. Feeding or foraging guilds have been used in developing tools for assessing environmental effects on demersal fish assemblages (Allen, 1982; Allen *et al.,* 1998, 2001). However, the use of guilds in assessing bioaccumulation have been little studied, if at all.

Many studies have been conducted and published about the accumulation of cadmium, lead, arsenic and mercury in freshwater fish with reference to their feeding guilds in Europe (Has-Schön *et al.,* 2006, 2008; Celechovská *et al.,* 2007; Karadede-Akin and Ünlü, 2007; Mendil and Uluozlu, 2007; Storelli *et al.,* 2007; Voigt, 2007; Maes *et al.,* 2008; Popek *et al.,* 2009; Yilmaz, 2009; Boscher *et al.,* 2010; Kensova *et al.,* 2010; Mazej *et al.,* 2010; Matasin *et al.,* 2011) and some studies in France (Chevreuil *et al.,* 1995; Durrieu *et al.,* 2005; Ribeiro *et al.,* 2005; Arleny *et al.,* 2007; Shin *et al.,* 2009; Tabouret *et al.,* 2011) while there are none in India. Data from the literature indicate that the contents of these elements in fish varied as a function of the different localities but depended on species and feeding behaviour (Berninger and Pennanen 1995; Andres *et al.,* 2000; Canli and Atli, 2003; Farkas *et al.* 2003; Peakall and Burger 2003; Has-Schön *et al.,* 2006).

In order to find if there was any difference in contamination levels between the different feeding guilds, the data was classified into three feeding guilds namely carnivore (*Channa striata, Clarias batrachus, Anabas testudineus, Sperata aor, Channa gachua and Channa punctata*) herbivore (*Labeo fimbriatis, Puntius dorsalis, Systomus sarana and Salmostoma acinaces*) and omnivore (*Clarias gareipinus, Oreochromis aureus, Oreochromis mossambicus, Oreochromis niloticus, Oreochromis aureus, Puntius chola, Puntius sophore, Trichopodus trichopterus*) ; and subjected to Kruskal Wallis test.

Naphthalene was highest in omnivore (figure 24) and was closely trailed by carnivore while herbivore was least (H=3.75, p>0.05). Although there were variations in contamination levels, they were insignificant. Similar to naphthalene, the variations in fluorene and phenanthrene among the feeding guilds were not significant. Fluorene was the highest in carnivore followed by omnivore while the least was in herbivore (H=5.374; p>0.05). Phenanthrene was very similar in all three guilds (H=0.239; p>0.05). Anthracene was highest in herbivore followed by carnivore and was least in omnivore (H=14.030, p<0.05). Pyrene was highest in omnivore followed by herbivore and least in carnivore (H=10.184, p<0.05). The variation in anthracene and pyrene were significant. Total PAH was highest in omnivore (107.27±7.29 ng/g ranging between BDL and 1890 ng/g). Pattern being Omnivore>Carnivore> Herbivore While herbivore had the least total PAH there wasn't a significant difference (H=5.547, p>0.05) between carnivore and herbivore.

The levels of PAHs in omnivorous *Oreochromis sp*. ranged from 15.1 to 92.5 with an average of 63.3 ng g−1 wet wt in study by Kong *et al.,* (2005b). The levels of PAH in the same species by Kong *et al*., (2005a) ranged between 1.91–62.1 ng g−1 wet wt. The authors had reported that according to the US EPA 2000 guidelines, the fishes were far below the limit of concern. Although, the values in the current study were much higher in omnivorous fish and indicative of problem, the values are still below the limit of serious concern with respect to human consumption (potency weighed PAH< 0.67 ng/g). Ahmed *et al.,* 2014 reported that the levels of total PAH in omnivorous fish collected in Lake Temsa, Egypt was 115±44 ng/g, herbivorous species was 191±23 ng/g while for carnivorous species it was 315±87 ng/g. The pattern followed in their study was Carnivore>Herbivore>Omnivore based on total PAH. The pattern observed in their study is very different from the pattern observed in the present study. This result could be explained in light of of Chen (2002) publication which suggested that fish harbour hydrocarbons from a number of sources that include sediment, food, and water, though it is impossible to determine the relative uptake of these contaminants and their bioavailability in each pathway for each fish species. Thus, the distribution of hydrocarbons may differ considerably between species and this might explain the different affinities of the fish species in this study to accumulate hydrocarbons. Food does not appear to be a major

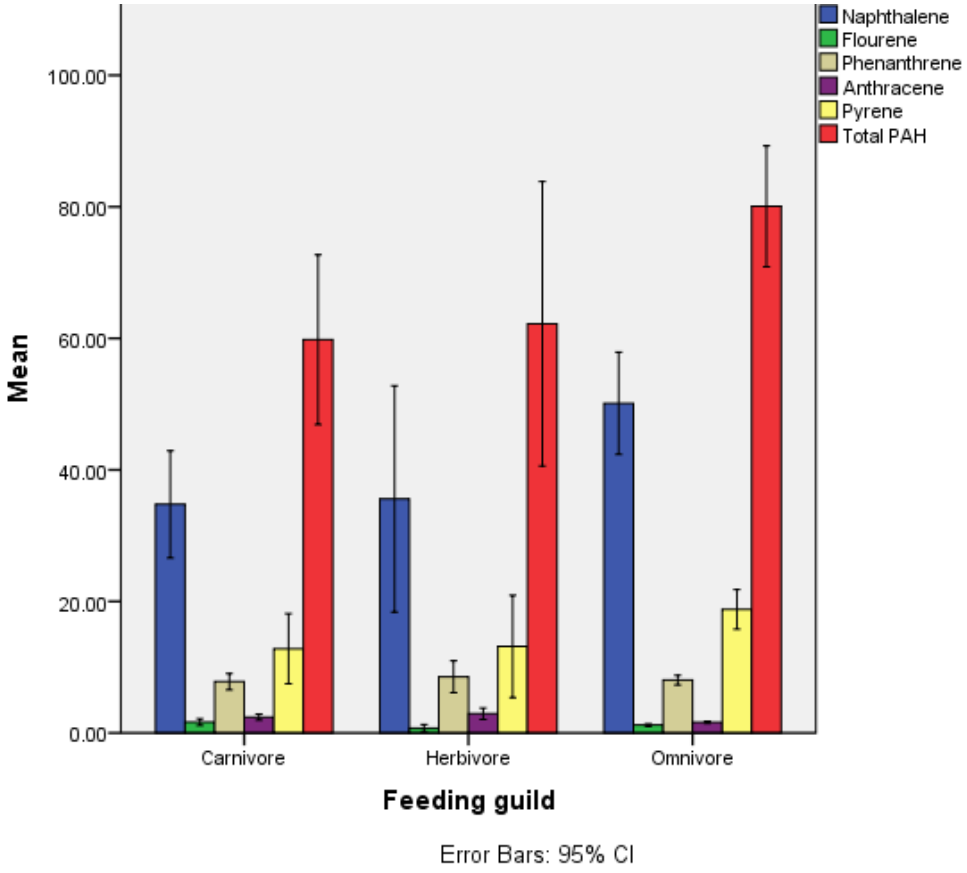
source of low molecular weight PAHs. While Rahmanpour *et al.*, (2014) report that top predatory fish generally contain lower tissue residues of PAHs than do other fish, such as omnivorous and phytoplanktivores fish that occupy lower trophic positions, the data collected in the present study contradict this. According to Broman *et al.* (1990), carnivorous species are prone to have higher PAHs concentrations than herbivorous, omnivorous, or planktivorous species.

Of all the fish samples collected from Pallikaranai wetland, 15% of the omnivore samples had detectable levels of high molecular weight PAH. While the number of samples with high molecular weight PAH above the guideline limits was 3.5%, the samples with detectable levels of carcinogenic PAH4 was 13.3% with 2 % being alarmingly high. The long term consumption of these fish by other organisms namely birds and humans could be cause for serious concern.

While 25% of the herbivore samples had detection of high molecular weight PAH, 8% of the samples had levels above permissible limits and 20% of the samples had detection of carcinogenic PAH4 with 5% of the samples having levels which are greater than permissible levels.25% of the carnivore samples had detectable levels of HMW and 7.4% of the total carnivore samples detected levels higher than the permissible limits. 23% of the carnivores had detectable levels of PAH4 with 4.5% of these being several manifolds higher than permissible limits. Top-level carnivores are liable to consume prey containing high level of pollutants and hence, can accumulate higher levels of contaminants than organisms that are placed lower on the food chain (Burger and Gochfeld 2002). Nevertheless, fish are not specifically considered a sound bioindicators for HMW PAH bioaccumulation because of their ability to degrade these compounds. However, levels of PAHs in fish may indicate the level of temporal exposure of the lake to discharges of hydrocarbon pollutants, and also as indicators for fish consumers about fish quality and their content of hydrocarbons (Ololade and Lajide 2010).

While it is true that, because of their low water solubility and hydrophobicity PAH tend to adsorb to particulate material and be deposited in the sediments, where the degradation of PAHs with high molecular weights is particularly slow (Readman *et al.*, 1982), limnivores have not been considered because overlaying of species in 2 categories.

Figure 24: Accumulation pattern of PAH in different feeding guilds of fish tissue collected from Pallikaranai Wetland, Chennai (2017-2018)



Chapter 5

CHAPTER 5

SUITABILITY OF FISHES OF PALLIKARANAI FOR HUMAN CONSUMPTION IN TERMS OF THEIR PAH CONCENTRATION

There has been considerable and sustained interest on research pertaining to fishes across the globe. This is largely because of its direct link to humans as food. The global supply of fish for human consumption has outpaced the growth of human population over the past five decades (FAO, 2016). There were around 4.6 million fishing vessels in the world in 2014, 90 percent of which are in Asia and Africa, and only 64,000 of which were 24 meters or longer (SOFIA, 2016). Fish provide 6.7 per cent of all protein consumed by humans, as well as offering a rich source of long-chain omega-3 fatty acids, vitamins, calcium, zinc and iron (Perello *et al.,* 2010). While on the one hand, fish consumption has been known to reduce risk of coronary heart disease, cardiac arrhythmia and hyper-tension (Perello *et al.,* 2010), on the other hand, consumption of fish has been reported as an important route of exposure to chemical contaminants (Saeed *et al.,* 1995; Kulkarni *et al.,* 2007; Guo *et al.,* 2008; Zhang *et al.,* 2010). Fishes bioaccumulate chemical contaminants and transfer them to humans from water (Dorea., 2006)

Although the vital organs such as gill, kidney and liver are sensitive to PAHs accumulation, muscle forms the major edible portion in the fish. Therefore data on muscle tissues alone was analysed in order to ascertain the dietary intake by the human (Alaguraj., 2011, Navamani., 2011, Jayanthi., 2012)

5.1. Toxicity of PAH to humans

PAHs are classified as carcinogenic; The Scientific Committee on Food in 2002 assessed the toxicity of 34 PAHs and concluded that 15 of them are potentially genotoxic and carcinogenic to humans**.** Screening value for Benz [a] pyrene (BaP SVs) were determined to assess the toxicity of individual PAHs (USEPA, 1993).

SV is defined as the concentration of chemical in edible tissue that is of potential public health concern (USEPA, 2000). The SVs for PAHs were calculated by the following equation (Cheung *et al*., 2007).

$$SV = \frac{(RL/SF) \times BW}{CR} \quad (1)$$

Where SV is screening value (µg/g) that it is used as threshold value; SF is oral slope factor (µg/g/day); RL is maximum acceptable risk level (dimensionless); BW and CR are body weight (kg) and consumption rate (g/day), respectively (USEPA, 2000). According USEPA, body weight (BW) was chosen at 70 kg which is the average body weight for adult population; consumption rate (CR) for fishes and oral slope factors (SF) of PAHs were chosen as 142.2 g/day and 7.30 (µg/g/day), respectively; maximum acceptable risk level (RL) was chosen at 10^{-5}, which means that the increased risk would be at most one additional cancer death per 100,000 persons if a person weighing 70 kg consumed 142.2 g of fish per day with the same concentration of contaminant for 70 years (USEPA, 1989).

According to USEPA (1993), the potency equivalent concentration (PEC) of total PAHs was calculated for individual PAHs for comparison with the screening value for Benz [a] pyrene (BaP SVs) and it can be calculated using the following equation (Nisbet and Rasmussen, 1992);

$$PEC = \Sigma (RP \times C) \quad (2)$$

Where RP is equal to relative potency for each PAHs and C is equal to concentration of each PAH. Values given by Nisbet and Rasmussen are given in table 4.7. Also, the actual risk level (ARL) of total PAHs could be calculated accurately by the following expression deduced from Eq. (3).

$$ARL = \frac{PEC \times SF \times CR}{BW} \quad (3)$$

Where ARL in total PAHs through fish consumptions (dimensionless); the meanings for the SF, BW and CR are the same as in the Eq. (1); calculated potency equivalent concentrations and actual risk level were depicted in fig.4.6, and 4.7 respectively.

Based on the data for RL, SF, BW and CR set by USEPA, it was recommended that the guideline concentration (SV) is 0.67 ng BaP/g or potency equivalent concentration

(PEC) of total PAHs/g wet wt for human fish consumption (USEPA, 2000). The health risk level of total PAHs though fish consumptions would be estimated through the comparison of the PEC of total PAHs with the BaP SVs. If the PEC of total PAHs in fish samples exceeds the screening value (0.67 ng/g wet wt), the health risk of total PAHs through fish consumptions would be higher than the maximum acceptable risk level (RL) (10^{-5}); otherwise, it would be lower than 10^{-5}.

5.2. Actual Risk Limit

Based on a questionnaire survey conducted in 45 families living around the Palliakaranai wetland, the average per capita consumption of fishes was estimated at 144 g/day. This consumption pattern is much higher than that of the South Korean population (Moon *et al.,* 2009). However it was comparable to the fish consumption rate (142.2 g/day) set by USEPA (2000). The dietary intake concentrations of PAH were calculated by multiplying PAH concentration which were measured in each species of fish with the per capita consumption and the levels were compared with various ADI limits (table 14).

Table 14: Detection frequency and Actual Risk Level of PAH in the muscle tissue of fishes in Pallikaranai wetland, Chennai. (2017-2018)

PAH	Detection frequency in muscle samples (%)	Actual risk level computed (ng/g)	Tolerable daily intake according to EU (ng/g) (SCF, 2001)
Benz (a) anthracene	9.27	0.05	0.002
Chrysene	5.85	0.0002	0.002
Benzo(b) flouranthene	2.44	0.04	0.002
Benzo(k) flouranthene	5.37	0.002	0.002
Benzo(a) pyrene	4.39	0.19	0.002
Benzo (g,h,i) perylene	2.44	0	0.002
Indeno (1,2,3-cd) pyrene	0.00	0	0.002
Dibenz (a,h) anthracene	0.00	0	0.002
Total		**0.28**	0.002

Table 14. shows that the fishes collected from Pallikaranai had levels which are 100 times the maximum risk limit permitted by European union according to SCF, 2001 which allows tolerable daily intake of 2 pg TEQ/kg body weight/day as recommended by the United and European Commission. At the same time the fishes collected from Pallikaranai wetland, Chennai had levels not exceeding the statutory limits prescribed by Minnesota Department of health which classifies nine (benzo[a]anthracene, benzo [a]pyrene, benzo[b]flouranthene, benzo[g,h,i]perylene, benzo[k]flouranthene, chrysene, dibenz[a,h]anthracene, flouranthene and indeno[1,2,3-cd]pyrene) of the 15 analysed PAH as carcinogenic to humans. The levels obtained have been compared with acute, chronic and subchronic non-carcinogenic health risk limit where data on human carcinogenicity is not available for an individual PAH.

Carcinogenic action of PAHs and EPA Classification are given in Table 15 and 16.

Table 15: Carcinogenic and Genotoxic potential of PAH listed by US EPA., 2002

PAH	ARL ng/g	Carcinogenicity (IARC 2010, US EPA., 2003)
Naphthalene	70	C (inhalation)
Acenaphthene	100	-
Fluorene	80	-
Phenanthrene	70	C (low carcinogenic potential)
Anthracene	1000	-
Fluoranthene	70	-
Pyrene	90	-
Benz (a) anthracene	0.1	C/G
Chrysene	1	C/G
Benzo (b) fluoranthene	0.1	C/G
Benzo (k) fluoranthene	0.1	C/G
Benzo (a) pyrene	0.1	C/G
Benzo (g,h,i) perylene	1	G
Indeno (1,2,3-cd) pyrene	1	C/G
Dibenz (a,h) anthracene	1	C/G

C- Carcinogenic G- Genotoxic - Not applicable

Table 16: Carcinogenic action of PAHs (Nikolaou *et al.*, 2009)

PAH	Indicator of Carcinogenesis (a)	Total estimation (b)	EPA Classification (c)
Acenaphthene			D
Fluorene	I	3	D
Phenanthrene	I	3	D
Anthracene	I	3	D
Fluoranthene	I	3	D
Pyrene	I	3	D
Benz (a) anthracene	S	2A	B2
Chrysene	L	3	B2
Benzo (b) fluoranthene	S	2B	B2
Benzo (k) fluoranthene	S	2B	B2
Benzo (a) pyrene	S	2A	B2
Benzo (g,h,i) perylene	I	3	D
Indeno (1,2,3-cd) pyrene	S	2B	B2
Dibenz (a,h) anthracene	S	2A	B2

a-No adequate data for humans: I-insufficient data; L-limited data, S-sufficient data

b-Carcinogen for humans:

2A- probable carcinogen for humans, 2B-possible carcinogen for humans, 3- not classified regarding carcinogenicity for humans.

D- not classifiable as human carcinogen; B2- Probable human carcinogen

Table 17: Intervals of PAH concentrations with acute or sub acute[1] effects on animals and algae (Knutzen, 1995).

S.No	Name of the PAHs	Concentration (mg/L)	
		Animals	Algae
1.	**Acenaphthene**	0.66	-
2.	**Fluorene**	0.3-5.8	0.5-1.2
3.	**Phenanthrene**	0.03-1.1	0.2-1.0
4.	**Anthracene**	-	0.2
5.	**Fluoranthene**	0.024-0.5	0.02
6.	**Benz(a)anthracene**	0.01-1.0	0.005
7.	**Chrysene**	>1.0	-
8.	**Benzo(a)pyrene**	0.005->1.0	-

[1]In animals mostly short-term LC_{50} values or concentrations resulting in immobilization; in algae reduced photosynthesis or growth.

-Not available

Since the data is not normally distributed, the median and range have been indicated in table 18. But mean has been computed for the sake of comparison with literature available elsewhere.

Table 18 : Levels of individual PAH in muscle tissues of fishes collected from Pallikaranai Wetland, Chennai (2017-2018).

PAH	Mean±SD	Median	Range
Naphthalene	34.57±4.31	15.07	BDL-488.84
Acenaphthene	0.45±0.10	BDL	BDL-12.67
Fluorene	0.87±0.13	BDL	BDL-17.26
Phenanthrene	5.76±0.41	3.94	BDL-42.34
Anthracene	1.83±0.21	1	BDL-37.42
Fluoranthene	1±0.65	BDL	BDL-154.09
Pyrene	5.29±1.24	BDL	BDL-207.98
Benz[a]anthracene	0.27±0.10	BDL	BDL-21.23
Chrysene	0.11±0.05	BDL	BDL-12.33
Benzo[b]fluoranthene	0.25±0.17	BDL	BDL-40.05
Benzo[k]fluoranthene	0.12±0.07	BDL	BDL-18.03
Benzo[a]pyrene	0.11±0.07	BDL	BDL-16.60
Benzo[g,h,i]perylene	0.02±0.014	BDL	BDL-2.25
Indeno[1,2,3-cd]pyrene	BDL	BDL	BDL
Dibenz[a,h]anthracene	BDL	BDL	BDL
	50.32±5.15	26.0158	BDL-601.50

Food appears to be the main source of PAH to human beings. Many countries such as Germany and Italy formulated restrictions for PAH residues especially for benzo[a]pyrene in food and meat products (1mg/kg) and beverages (30ng/kg) (Jacob and Seidel, 2002).

Fishes living in PAH-contaminated environments can absorb these compounds through their body surface and gills or by ingestion of contaminated food or sediment or particles and then transfer contamination through the aquatic food web (Wang *et al*., 2009). The results of the present study indicated variation in total PAHs among different species of fishes. Accumulation and depuration of PAHs in fish can be influenced by various factors including route and duration of exposure, lipid content of tissues, environmental factors (e.g., salinity, temperature, etc.), differences in species, age and sex and exposure to other xenobiotics (Varanasi *et al*., 1990).

Factors influencing bioconcentration of PAHs in fish include rate of dietary intake (food chain hierarchy), efficiency of assimilation, degree and nature of substance biotransformation, rate of depuration, and fish-growth rate (Dórea, 2008). Fishes can bioaccumulate PAHs more than shell fishes (Siddiqi *et al*., 2009). Fish tissues can be classified in to four categories based on the contamination level (table 19)

Table 19: Fish tissue contamination criteria (Varanasi *et al*., 1993)

Classification	PAH Concentration(ng/g)
Not contaminated	<10
Mildly Contaminated	10-99
Moderately contaminated	100-1000
Highly contaminated	>1000

Table 20: Species-wise variation of levels of total PAH, PAH4 and contamination status of the fishes in Pallikaranai wetland, Chennai. (2017-2018)

Species	Mean total PAH (ng/g±SD)	PAH4(ng/g ±SD)	Contamination status as proposed by Varanasi *et al.*, 1993
Anabas testudineus	102.45±14.24	6.75±5.56	Moderately contaminated
Channa gachua	37.53±10.45	0.54±0.49	Mildly Contaminated
Channa punctate	30.60±17.01	1±2.38	Mildly Contaminated
Channa striata	46.44±35.04	BDL	Mildly Contaminated
Clarias batrachus	39.8±37.69	1.0±2.58	Mildly Contaminated
Clarias gariepinus	31.92±15.79	1.18±2.37	Mildly Contaminated
Heteropneustes fossilis	19.70±10.94	BDL	Mildly Contaminated
Labeo fimbriatis	23.38±12.85	BDL	Mildly Contaminated
Oreochromis aureus	86.97±32.76	BDL	Mildly Contaminated
Oreochromis mossambicus	65.39±99.5	1.0±0.72	Mildly Contaminated
Oreochromis niloticus	45.94±53.83	BDL	Mildly Contaminated
Pseudotroplis maculata	16.17	BDL	Mildly Contaminated
Puntius chola	23.87±4.94	1±1.36	Mildly Contaminated
Puntius dorsalis	26.68	11.36	Mildly Contaminated
Puntius sophore	40.20±48.61	BDL	Mildly Contaminated
Puntius ticto	8.84±8.24	BDL	Not contaminated
Salmostoma acinases	96.26±152.33	BDL	Mildly Contaminated
Sperata aor	16.54±6.9	BDL	Mildly Contaminated
Systomus sarana	19.60±13.80	BDL	Mildly Contaminated
Trichopodus trichopterus	48.70±65.51	1.03±1.60	Mildly Contaminated

The concentration of total PAHs (PAH (the sum of 15 PAHs)) and carcinogenic PAH4 are presented in table 20. The levels of mean total PAH and PAH4 ranged from 8.24 to 102.45 ng/g wet wt. and BDL to 11.36 ng/g wet wt, respectively. The maximum concentration of total PAH was found in *Oreochromis mossambicus* (1162 ng/g). In the

present study, Benzo(a)anthracene was maximum (21 ng/g) in *Oreochromis mossambicus* followed by 7 ng/g in *Anabas testudineus*, Chrysene was 12 ng/g in Anabas testudineus followed by 4 ng/g in *Clarias batrachus*, benzo(b)flouranthene was 40 ng/g in *A.testudineus* followed by 5 ng/g in Puntius dorsalis, Benzo(k) flouranthene and Benz(a) pyrene were 18 ng/g and 16 ng/g respectively in *A. testudineus*. Benzo (ghi) perylene was 2.12 in *Oreochromis niloticus* while indeno (1,2,3-cd) pyrene and dibenzo (a,h) anthracene were not detected in any of the muscle tissues analyzed. It may be noted that *Anabas testudineus* which detected maximum levels of most carcinogenic PAH is carnivorous in feeding nature. According to Broman *et al.* (1990), carnivorous species are prone to have higher PAHs concentrations.

Studies on PAHs in Indian waters are limited. Chouksey *et al.*, 2004 studied PAHs in the marine environment of Mumbai. They reported the concentration of PAHs in the water of Mumbai to vary widely from 2.9 to 39.2µg/L. Ingole *et al.*, (1995) studied the concentration of PAHs in inter tidal ecosystem along the Bombay coast. A study by Singh *et al*., 2008, on PAH levels in Indian children revealed significantly elevated carcinogenic blood PAHs levels (125.55ng/g and 23.96ng/g in rural and urban regions of Lucknow respectively). A study by Tsang *et al.*, 2011, documented PAHs concentration in human milk, maternal and cord serum to be 1981 ng/g lipid wt, 1461 ng/g, and 1158 ng/g respectively in Hong Kong residents.

Uno *et al*., (2010) investigated the concentrations of total PAHs in fishes following oil spill accident in Philippines, immediately and after one month of the accident. While the levels ranged between 11.9 and 52.3 ng/g dw, surprisingly there was no variation after one month.Varying levels of PAHs concentrations in edible fishes were documented by many researchers. Cheung *et al*., (2007) found high PAHs concentration in marine fishes (118ng/g) when compared to fresh water fishes (24.8ng/g) collected from Hong Kong market. Kong *et al*., (2005) examined *Oreochromis mossambicus*, a freshwater fish purchased from the markets showed higher concentrations of PAHs (76.5ng/g ww) than those collected from fish ponds (60.1ng/g ww) in Hong Kong. Xu *et al*., (2011) studied the residual levels of PAHs in different tissues of four common edible freshwater fishes collected from Lake Small Bai-Yang-Dian in northern China. The levels of total PAHs concentration were 28.031, 7.503, 62.379 and 14.536ng/g w/w for grass carp, snakehead fish, crucian carp and silver carp respectively.

Muscle concentrations of PAHs were determined in the fish *Siganus oramin* collected from Victoria harbour, Hong Kong by Fang *et al*., (2009). Arias *et al*., (2009) documented mean concentration of 1095 ng/g d.w. in whole tissues of the fish *Odontesthes sp.* from Bahı´a Blanca Estuary, Argentina. Total PAHs levels of 64.8, 23.8, 26.6, 22.8, 24.2, 16.4 and 25.0 ng/g dw were documented in different species of fish namely Carp, Allice, Bleak, Chub, Tench, Perch and Pike respectively from Lake Iseo by Binelli and Provini in 2004. Sole *et al.,* (2001) documented high levels of 6 ring PAHs in NW Mediterranean deep sea fish *Moro moro*. PAH exposure was documented in fish tissue (154ng/g dw) from Arthur Harbor, Antarctica by Mcdonald *et al*., in 1992.

Malik *et al*., (2008) documented ΣPAHs concentration in fish samples of the Gomti River, India, to have ranged between 12.85 and 34.89 ng/g ww in fish muscles. The PAH residues in five fish species from three places of Mumbai were documented by Chouksey *et al*., (2004). Average concentrations were found to be 2.6, 4.8, 3.3, 2.9, 7.4µg/g ww in Bombay duck, Catfish, Indian mackerel, Anchovy and Dhoma respectively. Mean PAHs levels of 3.34, 1.76, 1.35, and 3.49µg/g were documented in different fishes namely *Chlorophthalamus agassizi, Sardinella longiceps, Mystus singhalis* and *Nemipterus japonicus* from three sites namely Mahul, Sewri and Nhava by Mahendran, (2007). Jehosheba (2004) studied the biochemical effects of PAHs on the tropical teleost *Oreochromis mossambicus*. Jasmine *et al.,* (2003) studied PAHs in seafood. Investigations on the stress effects of PAHs on *Metapenaeus dobsoni* were done by Miriam (2002). Studies on PAHs 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 PAHs in six species of fish and prawn ranged from 0.2 to 102µg/g w/w. Ribeiro *et al*., (2005) documented total PAHs in bile of eel *Anguilla anguilla* from two sites in France. PAH pollution in coastal areas of the NW Mediterranean had been assessed by measuring bile PAH metabolites in two fish species, namely *Mullus barbatus* and *Serranus cabrilla* by Escartín and Porte (1999). *Mullus barbatus* (1259ng/ml) was found to be more contaminated than *Serranus cabrilla* (224 ng/ml). The preceding review of literature on PAHs indicates various studies made mainly outside India and also explains the scarcity of data on PAHs levels in edible fishes. In view of the great concern in the safety of food fishes, the current investigation was aimed to generate base line data on PAHs levels in edible fishes.

The biomagnification of PAH has previously been reported in lower links of the food chain (Nett, 1993; Adamo *et al.,* 1997). It has also been shown that trout exposed to PAH through the diet bioaccumulate increasing levels of contaminants in tissues with time (Hellou *et al.,* 2002; Hellou and Leonard, 2004).

Gomes *et al*., (2010) suggested that the low values observed in fishes, regardless of their diet, may be associated with their mobility or to the fact that most occur in the water column rather than the air–water interface, where most of the oil slick was located. Nonetheless, present study does not show consistent patterns of accumulation relative to the different diets of the fishes collected.

Average total PAHs concentration from the present study (131.72 ng/g) is higher than the levels documented by Al-Shwafi, (2008) in Red sea and Gulf of Aden (90 ng/g). Singh, (1992) also recorded similar levels (0.76 ug/g) in *Scomberomorus* sp. from Trinidad. Al-Shwafi (2008) also recorded similar level of contamination in *Rastrelliger kanagurta* (1.0μg/g) and *Sphyraena* sp (0.6μg/g). Total PAHs concentrations documented in this study is higher than in *Nemipterus japonicus* by Shriadah (2001) (0.43μg/g), while *Rastrelliger kanagurta* (41ng/g) showed less contamination than the present study. Chouksey, 2004 recorded much higher amount of total PAHs in *Rastrelliger kanagurta* (4μg/g) and *Stolephorus species* (3.9μg/g). A study in Bay of Bengal by Veerasingam *et al.,* (2010) showed much higher concentration in *Stolephorus indicus* (1.2μg/g) when compared to the present study.

5.3. Estimation of cancer risk to humans due to consumption of fishes of Pallikaranai Wetland, Chennai in terms of their PAH concentration.

The mean dietary intakes of the seven PAHs are considered probable human carcinogens by the US EPA, and hence they are considered. The general equation for estimating exposure, through ingestion of fish is as follows:

$$\text{Excess cancer risk} = \frac{EI * ED * CSF}{BW * AT}$$

where EI is estimated intake (mg/kg/d), ED is exposure duration (years; adults = 30 years), CSF is the oral cancer slope factor ($(mg/kg/d)^{-1}$), BW is human body weight (assuming 60 kg weight), and AT is the average time for carcinogens (years, assuming 70 years for

adults). The CSF data for individual PAHs, obtained from the integrated risk information system reported by the USEPA (2004), are BaA (0.73), Chr (0.0073), Bbf (0.73), BkF (0.073), BaP (7.3), InP (0.73), and DbA.

To estimate the carcinogenic potency of analyzed PAHs, TEF values proposed by Nisbet and LaGoy (1992) were applied. TEFs are widely used to calculate toxicities of PAH mixtures based on the toxicity of a specific PAH compound relative to the toxicity of the reference compound—benzo(a)pyrene. The potential cancer risk associated with exposure to PAH mixtures is calculated by summing up the concentrations of individual PAH compounds, multiplied by their TEF values (table 21).

Table 21: Toxic Equivalence Factors as proposed by Nisbet and Rasmussen, EPA (1993)

PAH	TEF (Nisbet and Rasmussen, EPA 1993)	PEC (Nisbet and Rasmussen, EPA 1993)	Cancer Slope Factor USEPA (2004)
Naphthalene	0.001	0.000001	-
Acenaphthene	0.001	0.000017	-
Fluorene	0.001	0.000065	-
Phenanthrene	0.001	0.000089	-
Anthracene	0.001	0.000003	-
Fluoranthene	0.001	0.000003	-
Pyrene	0.001	0.000108	-
Benz (a) anthracene	0	0	0.73
Chrysene	0.01	0.000063	0.0073
Benzo (b) fluoranthene	0.1	0.000104	0.73
Benzo (k) fluoranthene	0.1	0.001145	0.073
Benzo (a) pyrene	1	0.557120	7.3
Benzo (g,h,i) perylene	0.01	0.000453	0.73
Indeno (1,2,3-cd) pyrene	0.1	0.017438	0.73
Dibenz (a,h) anthracene	5	0.005081	7.3

It has to be emphasized that TEFs assigned based on analytical criteria described above may not adequately reflect the real toxicological properties of non-EPA PAHs. Since to our knowledge, there are no toxicological studies on these compounds in India, this approach was used to estimate the relative importance of the non-EPA PAHs and selected those that deserved a more thorough toxicological characterization. In order to evaluate the adequacy of the TEFs, the expected results using this TEFs were compared with the empirical results of the Schmahl *et al.* (1977) and Pfeiffer (1977) studies. It should be also noted that 75% of the assigned TEFs had the lowest TEF value (0.001), which makes overestimation of their toxicity less likely unlike Schmahl *et al.* (1977) and Pfeiffer (1977) studies whose results are are likely to overestimate cancer potency for groups of non-carcinogenic PAHs. However, both data sets are somewhat limited in that the two most potent PAHs, namely B[a]P and DBA, are responsible for much of the observed response.

5.4. Contribution of PAHs to Total Toxic Equivalence (TEQs)

The estimated intake of TEQ-PAHs from fish consumption for the general population ranged between BDL and 0.58 ng/g; these levels were higher than the levels reported by Moon *et al.,* (2009) in Korea through consumption of seafood. PAHs concentration was proposed as one of the toxic chemicals in food, consumer products, and the environment (COT 2001) for estimating the tolerable daily intake of 2 pg TEQ/kg body weight/day by the United and European Commission (SCF, 2001). Based on these results, TEQPAHs estimated in the present study was the highest contributor to total TEQ intake from fish consumption, indicating that PAHs in fish should be considered in risk assessment along with PCDD/Fs and dioxin-like PCBs.

5.5. Dietary Intake of PAHs through Fish Consumption

The average intake of PAHs through fish consumption was estimated to be 26.94 ng/Kg bodyweight/ day. Among species of fish analysed, *Anabas testudineus* contributed to the highest intake of total PAHs. Estimated intakes through consumption of all the species analysed are greater than 7 ng/kg body weight/d. Only a few studies have examined dietary intakes of PAHs through fish consumption worldwide. The amount of average dietary intake of PAHs in humans through fishes estimated in

India is far less compared to that in other countries. The estimated intake of PAHs from fish consumption reported for the general populations in other counties such as Spain (626–712 ng/d) (Falco *et al.,* 2005), Kuwait (231 ng/d) (Saeed *et al.,* 1995), and Korea (13.8–16.7 ng/kg body weight/d (Moon *et al.,* 2009). This result seems to be associated with the consumption rate and accumulation level of PAHs in the fishes in Pallikaranai wetland.

Table 22: Limits for monthly meals of fish concerning the carcinogenic effects of the PAHs (Binelli and Provini, 2003)

Number of fish meals consumed per month per adult	**Cancer health endpoints**
	Fish tissue concentrations (mg/g w.w.)
>16	0–0.0004
16	>0.0004–0.0008
12	>0.0008–0.0011
8	>0.0011–0.0016
4	>0.0016–0.0032
3	>0.0032–0.0043
2	>0.0043–0.0064
1	>0.0064–0.013

Based on the present study, it is clear that the concentration of PAH in all fishes except *Channa gachua* and *Puntius ticto* from Pallikaranai were highly unsafe for human consumption. The results must be viewed with concern as they may cause ill-effects not just only to humans but many other organism, particularly birds. Further, the levels reported in this study are much higher than levels reported in many countries. There is very little information available in the Indian context on the safety for human consumption, hence, more detailed investigations need to be carried out.

Chapter 6

CHAPTER 6

POLYCYCLIC AROMATIC HYDROCARBON RESIDUES IN SEDIMENTS COLLECTED FROM PALLIKARANAI WETLAND, CHENNAI (2017-2018).

Fresh and marine water sediments in many parts of the world are polluted with PAHs, possibly due to increased urbanization, industrialization, incessant discharge of untreated or partially treated effluents into the water bodies and intense shipping activities, amongst many other inputs (Adeniji *et al.,* 2018). Sediments act as sink and release PAHs back into water as an intrinsic source, which harms aquatic organisms, and eventually human health. PAH, being hydrophobic, tends to rapidly adsorb on particles (Landrum & Robbins, 1990). PAH pollution was reported to be more pronounced in the Asian and African continents than other parts of the globe (Adeniji *et al.,* 2018).

Seventy eight sediment samples were collected from Pallikaranai wetland seasonally. Spring (January –April), summer (May – August) and monsoon (September to December). The samples were analysed for the 15 US EPA priority listed PAHs as mentioned elsewhere in the thesis, and the data sets were subjected to Kruskal-Wallis test to understand whether they were statistically significant. The data sets were analysed for the levels of PAH in the sediment samples, seasonal variation of PAH in the sediment samples, frequency of occurrence of individual PAH and percentage composition of the individual PAH.

6.1. Levels of PAHs in sediments from Pallikaranai Wetland, Chennai, between January 2017 and December 2018

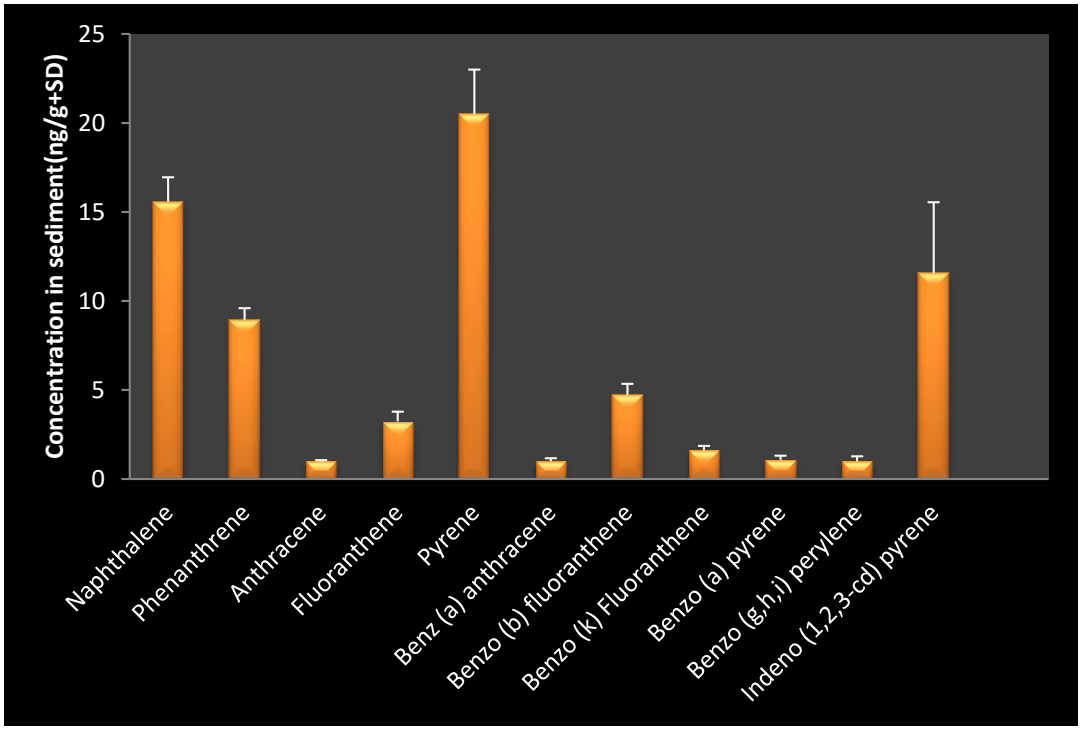
The contamination of aquatic sediments by PAHs is evaluated by comparing the PAH concentrations in such sediment sample with the effects-based guideline values such as the Effects Range Low (ERL) and Effects Range Median (ERM) established by the United States National Oceanic and Atmospheric Administration (USNOAA) (Kim *et al.,* 1999 and MacDonald *et al.,* 2000). While higher ERL values (table 23) will suggest a likelihood of serious biological effects on aquatic creatures (Adeniji *et al.,* 2018), a lesser PAH concentration than the ERL would guarantee no adverse effects on the

benthic invertebrates (Hussein *et al.*, 2016). However, any concentration between the ERL and ERM might suggest the occurrence of such effects occasionally in the aquatic milieu (Kim *et al.*, 1999). Meanwhile, higher PAHs concentrations above the ERM value would cause adverse effects on the organisms from time to time (EPAI, 2001).

Table 23: Individual PAH and their corresponding ERL levels as proposed by Adeniji *et al.*, (2018)

PAH	ERL (ng/g) (Adeniji *et al.*, 2018)	Calculated ERL values (ng/g±SD)
Naphthalene	160	15.58±1.42
Acenaphthene	16	BDL
Fluorene	19	BDL
Phenanthrene	240	8.93 ±0.69
Anthracene	843	1 ±0.6
Fluoranthene	600	20.49± 2.59
Pyrene	665	4.72 ±0.64
Benzo(a) anthracene	261	1.61± 0.26
Chrysene	384	1.07 ±0.24
Benzo(b)flouranthene	NA	1 ±0.29
Benzo(k)flouranthene	NA	11.56 ±4.12
Benzo(a)pyrene	430	2.68±0.54
Dibenzo (a,h) anthracene	63.4	BDL
Indeno (1,2,3-cd)pyrene	NA	6.52±3.53

Figure 25 : Levels of individual PAH in the sediments collected from Pallikaranai wetland, Chennai (2017-2018)



The concentration of individual PAH (in ng/g±SD) are plotted in figure 25. The total PAH concentration in sediment of the present study (4- 69.07 ng/g) appear to be higher than the concentration reported in sediments in Gomti River, Lucknow, India (0.207- 3.365 ng/g) (Malik *et al.*, 2008). The total PAH concentration in the current study is comparable to the levels reported in a study conducted in coastal lagoons (54 ng/g) of Veracruz state, Gulf of Mexico (Botello *et al.*, 2001) and much lesser than levels reported by Benlahcen *et al.*, 1997 (48090 ng/g) and comparable with the concentration reported in sediment from Soda lake, Casper, Wyoming. The levels reported by Black (1983) in sediments collected from Smokes Creek (16,920 ng/g), Union canal ship (65,263 ng/g), Buffalo river (76,374 ng/g) and in Black Rock Canal (18,061 ng/g) in Nigeria are several manifolds greater than the levels observed in the current study. Although, there is undoubtedly some contribution of these compounds through urban run-off via combined sanitary/storm sewer overflow, limited data available from studies conducted relative to this point suggest these contributions are less important sources than some industrial discharges (Black 1983).

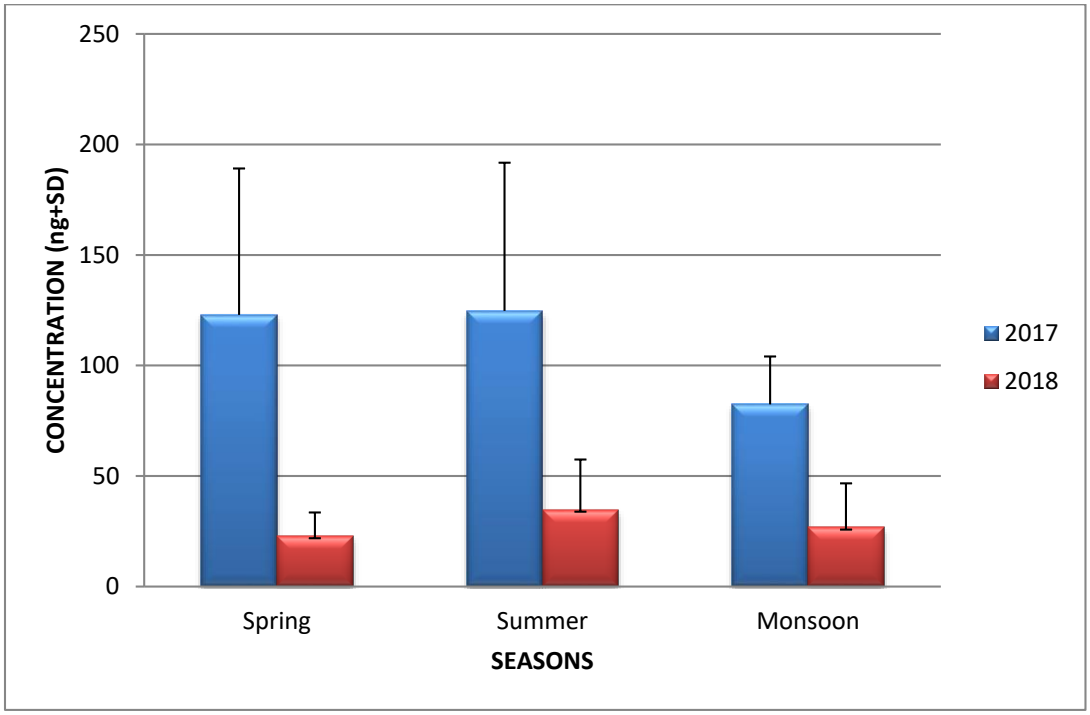
The levels of mean total PAH reported in Ebro river basin (Lacorte *et al.*, 2006) was 6.28 ng/g which is much less compared to the levels recorded in the present study. The levels in this study are also much higher than the levels reported in Nadal *et al.,* 2004; Bartolome´ *et al.,* 2005; Olivella *et al.,* 2006 and is similar to that of Baumard *et al.,* 1999 (between 25 and 208 ng/g).

While the levels reported in sediment in the current study may not be indicative of problems to aquatic biota immediately, it still cannot be considered safe and hence, there is a need to continuously monitor levels of PAH in sediments and strictly adhere to environmental laws in order to achieve a conducive environment for humans and other biota.

6.2. Seasonal variation of PAH residues in sediments of Pallikaranai Wetland, Chennai (2017-2018)

The pattern of contamination in season-wise variation of levels of PAH in sediment samples was in the following order; Summer>Spring>Monsoon (figure 26) with no significant difference statistically. However, there is a significant difference between the years ($H=-.923$, $p=0.002$, $p<0.05$). Literature reveals that variable temperatures tend to cause an increase in air concentrations in turn could lead to strong seasonal fluctuations (Wania and Mackay, 1995). A higher concentration of total PAHs in the spring results from a higher contribution due to coal combustion and petrogenic sources (Liu *et al.,* 2009). As for petrogenic PAHs, the lower temperature in the winter and spring is an important factor, because low temperature decreases the evaporation of petrogenic PAHs (Feng *et al.,* 2006). Meanwhile, rainfall in the monsoon brings particles with petrogenic and coal combustion PAHs into the water body (Liu *et al.,* 2009).

Figure 26: Seasonal variation in levels of total PAH in sediments of Pallikaranai Wetland, Chennai (2017-2018)



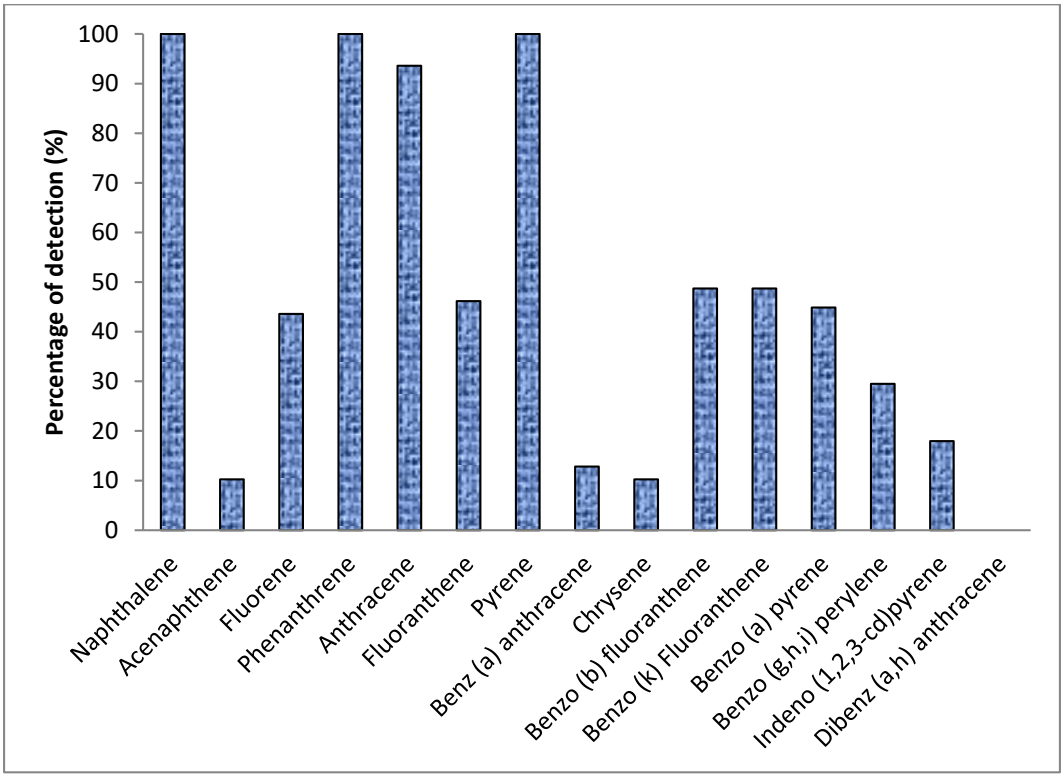
In a study by Liu *et al.*, 2009, there was a distinct seasonal variation with the concentration of PAH in sediment in Spring being significantly higher than other seasons. Levels of phenanthrene and pyrene were relatively high in sediment in spring while Benzo(a)pyrene reached peak in fall in a study by Lang *et al.*, 2007. The authors justified by the difference in deposition rate from air, which was higher in Spring and Summer than the other seasons. The level of total PAH in sediment collected in 3 different seasons, namely Spring (January to April), Summer (May to August) and Monsoon (September to December) between January 2017 and December 2018 have been determined and presented in figure. The mean total PAH was 69.07 ng/g. The levels of individual PAH in all the sediment samples were less than the ERL mentioned in table reported by Adeniji., *et al.*, (2018) and although may not cause problem to the ecosystem immediately, it still doesn't fall in the safety bracket and hence can have harmful consequences in the future.

Although there are many studies available in the international context on the seasonal variation of the concentration of PAH, there is no study in the Indian context. Given that Indian climatic conditions do not match other countries, particularly the lack of a significant drop in temperature (winter) in locations like Pallikaranai, the information gathered in this study could certainly serve as baseline data.

6.3 Frequency of occurrence of individual PAH in sediment samples collected from Pallikaranai (2017-2018)

As given in figure 27, naphthalene, phenanthrene and pyrene were detected in all the sediment samples analysed and dibenz (a,h)anthracene was detected in none of the samples. Anthracene in 93%, benzo (b) flouranthene and benzo(k)flouranthene in 48%, fluoranthene in 46%, benzo(a)pyrene in 44%, fluorene in 43%, benzo(g,h,i)perylene in 29%, indeno(1,2,3-cd)pyrene in 17%, Benz (a) anthracene in 12%, acenaphthene and chrysene was detected in 10% of the samples. The pattern is Low Molecular Weight PAH>High Molecular Weight PAH (figure 27). The higher levels of low molecular weight PAH may be due to their high volatilization potential and increased solubility in water(Albers, 2006). PAH solubility decreases with increasing molecular weight (Porte & Albaiges, 1993; Djomo *et al.,* 1996). Because of this, the lower molecular weight PAHs are preferentially dissolved while the heavier molecular weight compounds are preferentially absorbed onto or associated with particles (Landrum & Robbins, 1990). Consequently, the uptake of a contaminant is governed by its bioavailability, and organisms are often enriched in the lower molecular weight PAHs relative to the sediment (Porte & AlbaigeÂs, 1993; Djomo *et al.,* 1996; Baumard *et al.,* 1998).

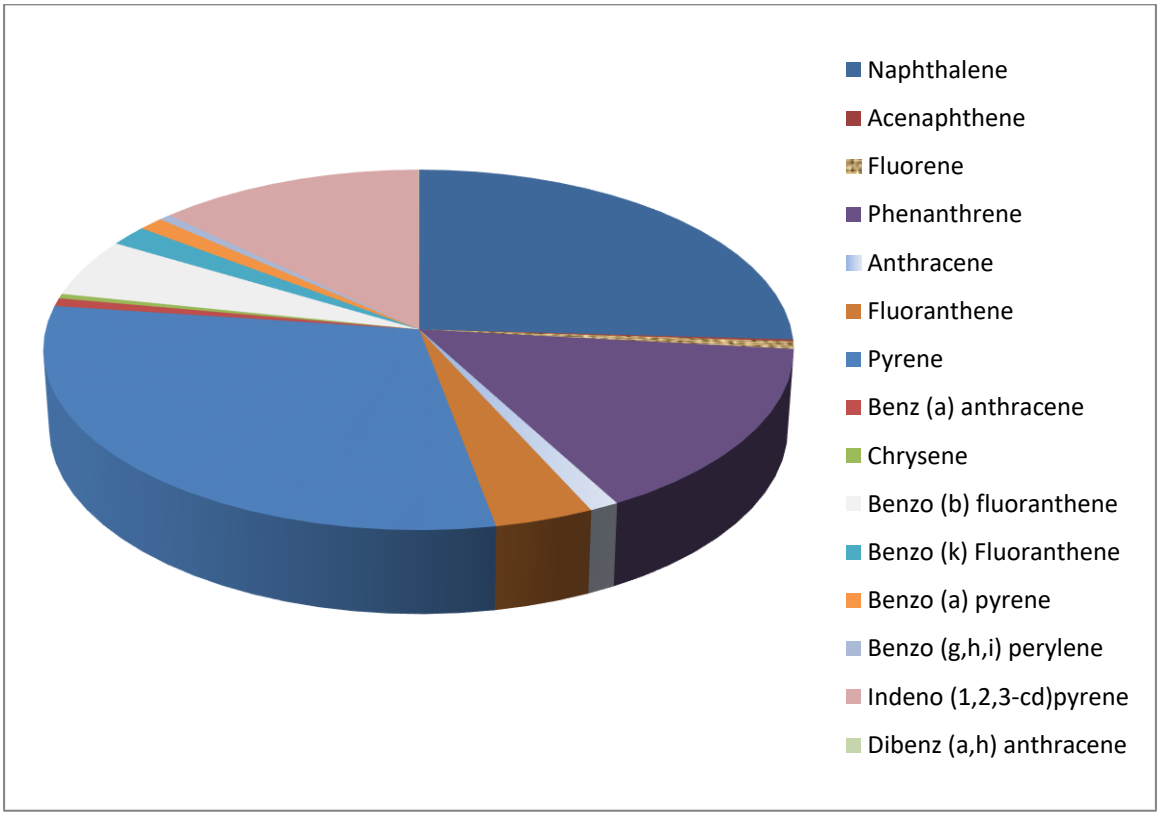
Figure 27: Frequency of occurrence of individual PAH in sediments from Pallikaranai Wetland, Chennai, between 2017 and 2018



6.4 Percentage Composition of individual PAH in sediments of Pallikaranai Wetland, Chennai (2017-2018)

As seen in the figure 28, pyrene contributed 30.06% to the total PAH load, naphthalene 25.9%, Phenanthrene 15.36%, indeno(1,2,3-cd) pyrene12.81%, benzo(b)flouranthene 5.27%, flouranthene 3.84%, benzo(k)flouranthene 1.20%, anthracene 1.13%, benzo (a)anthracene 0.72%, fluorene 0.67%, benzo (g,h,i)perylene 0.56%, chrysene 0.41%, and acenaphthene contributed 0.16% to the total PAH load. The relatively high contribution of pyrene may be due to high particulate deposition and due to the high volatilization potential of pyrene (ATSDR, 1990), pyrene becomes loosely bound to sediment.

Figure 28: Percentage composition of individual PAH in sediment samples collected from Pallikaranai Wetland, Chennai, between 2017 and 2018



6.5. Source identification of PAH in Pallikaranai Wetland, Chennai (2017-2018)

The acquirement of the ratio phenanthrene/anthracene and fluoranthene/pyrene allows us to appreciate the source of contamination (Raoux, 1991; Mille *et al.*, 1982). Hence, a ratio of phenanthrene/anthracene < 10 and fluoranthene/pyrene > 1 indicated that the contamination by PAHs was due to combustion processes: pyrolytic origin; (Colombo *et al.*, 1989; Garrigues *et al.*, 1991; Raoux, 1991)

For identification of sources of PAHs Phenanthrene(Phe)/Anthracene(Ant), Fluoranthene (Flu)/Pyrene(Pyr) and Chrysene(Chry)/Benz[a]anthracene (Benz-a) ratios were determined. In most of the samples, Phe/Ant ratio was higher than 15. Flu/Pyr and Chry/Benz-a ratios for all the fish species were detected less than 1. The data in the present study indicates that PAHs in all sediments seem to be of pyrolytic origin.

Chapter 7

CHAPTER 7

CORRELATION BETWEEN LEVELS OF PAH IN SEDIMENT AND THAT OF FISHES OF PALLIKARANAI WETLAND, CHENNAI, (2017 AND 2018)

The mean PAH concentration in fish was higher than the level in sediment (Figure 29). The LMW PAH (figure 30) in fish was much higher than the sediment but HMW PAH was higher in sediment. LMW PAH being dominant in the fish tissue could reflect on their presence in the environment. They are loosely bound to sediment and readily available for uptake by organisms including fish (ATSDR, 1990). The low levels of HMW PAH in the fish tissue may indicate the faster depuration of these compounds in fish systems.

Figure 29: Levels of mean total PAH (ng/g±SD) in sediment and fish samples collected from Pallikaranai Wetland, Chennai between 2017 and 2018

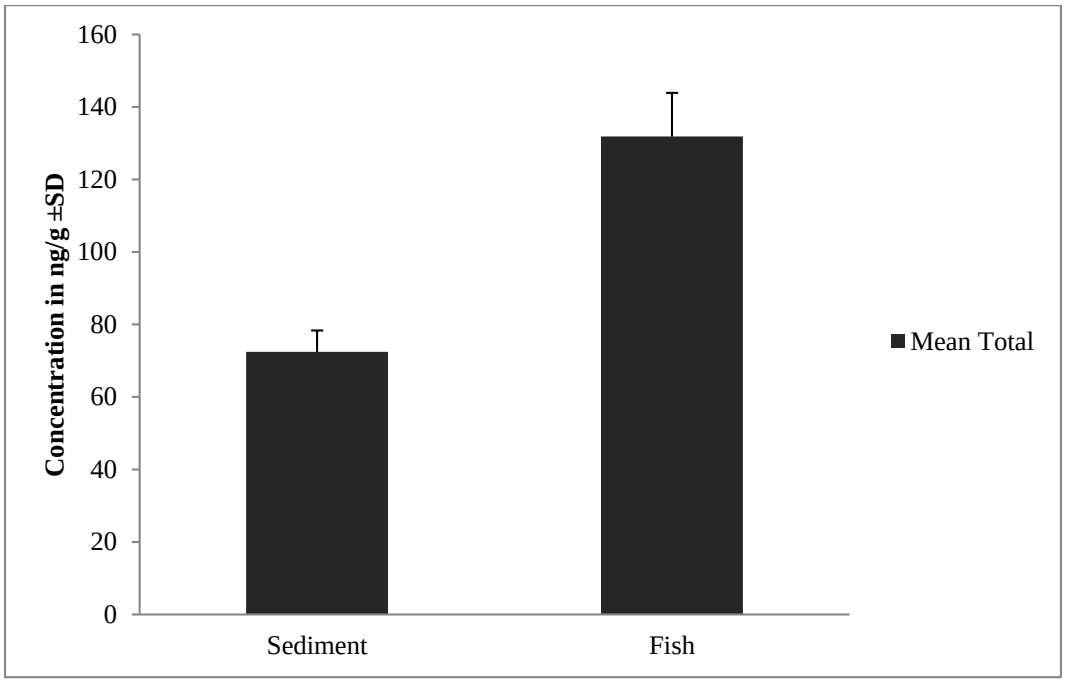
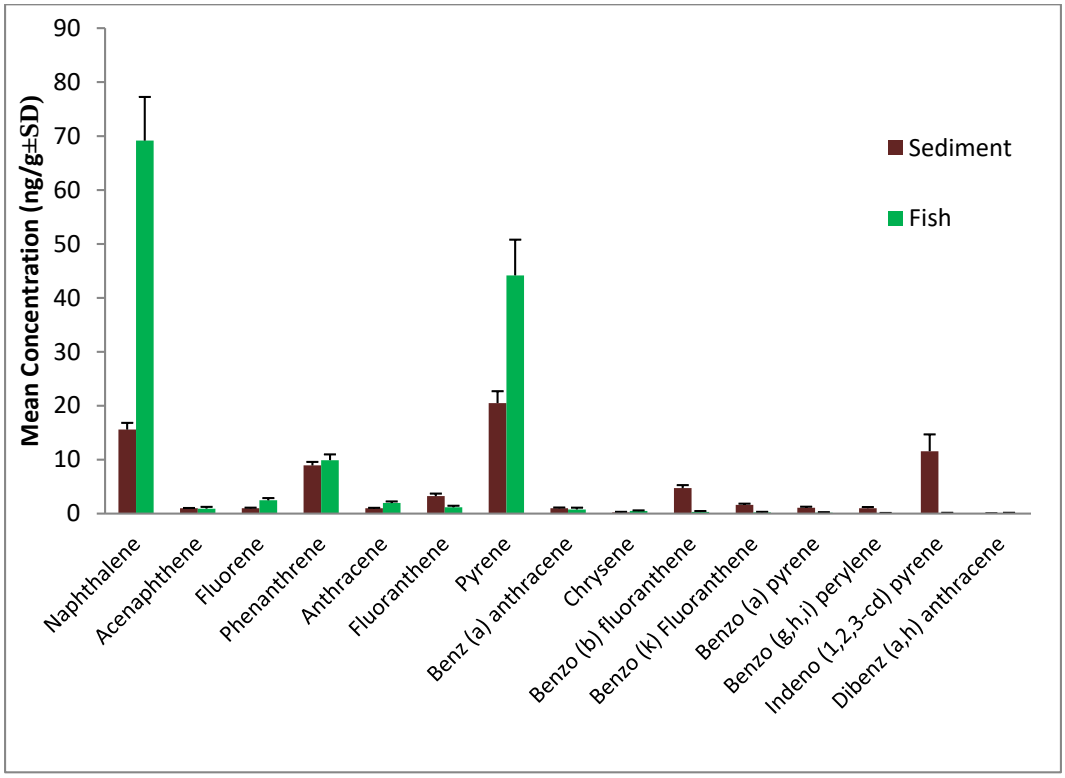


Figure 30: Levels of individual PAH (ng/g±SD) in sediment and fish samples collected from Pallikaranai Wetland, Chennai between 2017 and 2018



A negative correlation (figure 31) was observed while correlating mean total PAH in fish with that of sediment and this was significant (table 24). Negative correlations were observed on correlating naphthalene and phenanthrene between fish and sediment (figure 31). A mild positive correlation was observed for fluorene but was not significant. No correlations were observed for anthracene and pyrene. The concentrations of PAHs found in fish are expected to be much higher than in the environment from which they were taken (Shafy and Mansour., 2016).

Figure 31: Correlation curves of levels of PAH between sediment and fish collected from Pallikaranai Wetland, Chennai. (2017-2018)

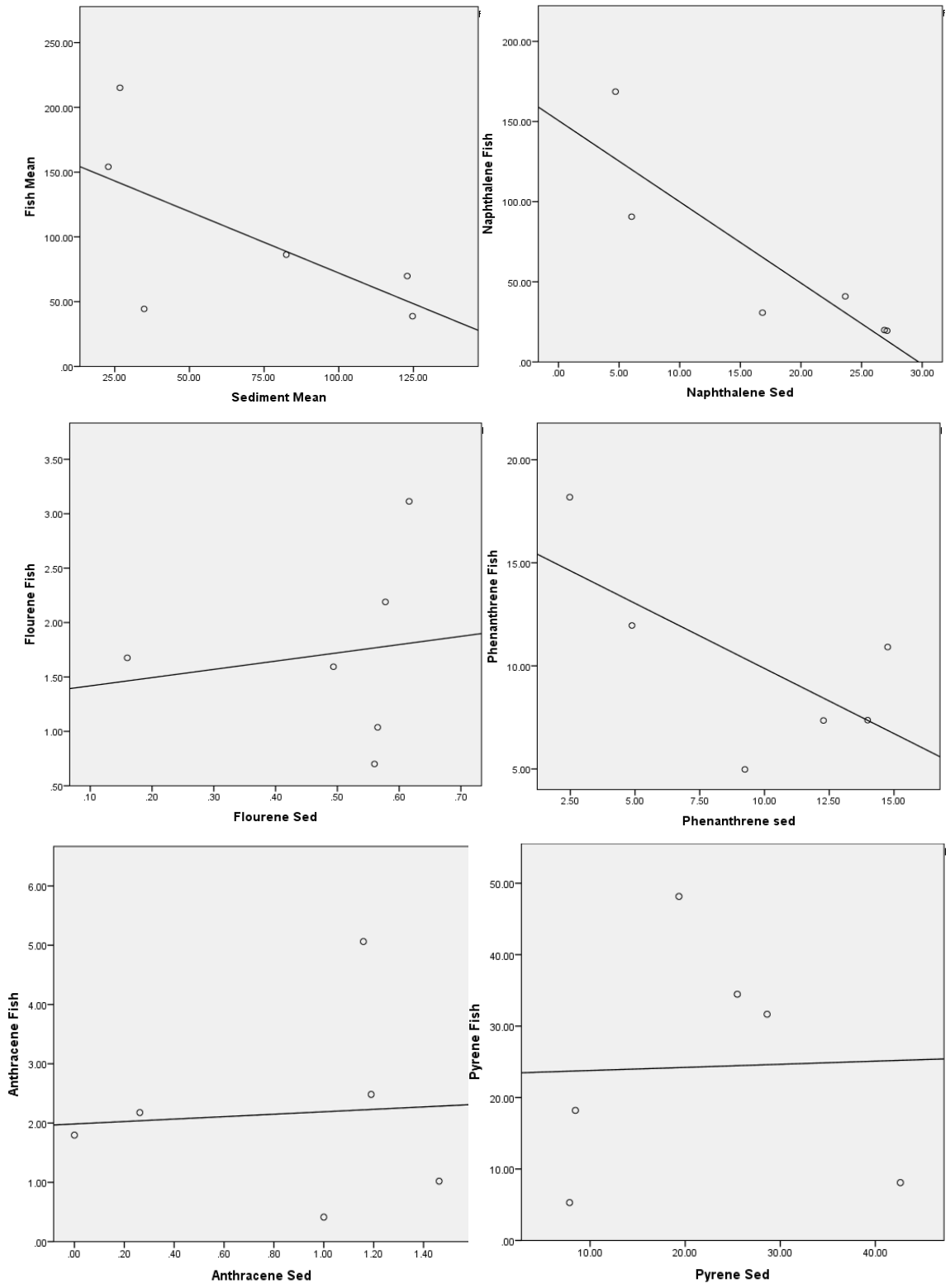


Table 24: Spearman Correlation of levels of PAH between sediment and fish collected from Pallikaranai wetland, Chennai between 2017 and 2018.

Matrix 1	PAH	Matrix 2	PAH	Rho	Significance
Sediment	Naphthalene	Fish	Naphthalene	-.943	**0.002**
Sediment	Fluorene	Fish	Fluorene	.486	0.164
Sediment	Phenanthrene	Fish	Phenanthrene	-.429	0.198
Sediment	Anthracene	Fish	Anthracene	.086	0.436
Sediment	Pyrene	Fish	Pyrene	-.829	**0.021**
Sediment	Mean Total	Fish	Mean Total	-.771	**0.036**

Connor *et al.,* 1984 reported a significant positive correlation between concentration in sediment and fish with higher concentration in sediment than in fish. Similarly, Brown *et al.,* 1998 reported a statistically significant (0.01 level) positive (Spearman) correlation in the levels between sediment and stomach contents and liver tissue with higher concentration in sediment than in fish was reported. Studies correlating the levels of Fish biliary EROD activity and levels of PAH in sediment (Garrigues *et al.*, 1990; Wang *et al.*, 2008) have been reported but not many studies correlate the levels of PAH in sediment with levels of PAH in fish tissue particularly. Information available in the Indian context on the levels of PAH in sediment and its relation with fish is less. A negative correlation between levels of PAHs in fish and sediment points towards the inverse relationship between the two. In an area like Pallikaranai wetland as there are various factors continuously contributing and the cocktail of contaminants in the water body are in continuous interaction. In the present study, only the 15 parent compounds listed as priority pollutants (mentioned elsewhere in this thesis) have been assessed. Since the metabolites have not been assessed, it is difficult to justify the inverse relationship between sediment and fish.

Chapter 8

CHAPTER 8

SUMMARY AND CONCLUSION

Enormous quantities of man-made chemicals are being used every day to meet the ever increasing food, energy, transport and industrial demands. During the past few decades, there has only been a steady increase in the use of these chemicals leading to their excessive presence throughout the global environment. Wetlands, being the most fragile ecosystems have suffered the maximum. Extensive stretches of wetland habitats have been altered and destroyed. Among the gazillion types of chemicals, Persistent Organic Pollutants (POPs) are significant because they are resistant to degradation in the environment and biota. Due to their fat soluble nature and resistance to degradation, ingestion of certain POPs by fauna leads to bioaccumulation throughout their lives, generally in fat tissues, and biomagnification through food chain. Among the POPs, Polycyclic Aromatic Hydrocarbons (PAHs) are highly prevalent in vertebrates, particularly fishes. Polycyclic Aromatic Hydrocarbons (PAHs) are a unique class of POPs constituted by hundreds of individual organic compounds. These ubiquitous environmental contaminants are wide-spread in the air, bonded to particulate matter and readily bioavailable. Studies have demonstrated the carcinogenic, mutagenic and teratogenic behaviour of many of the PAHs. Based on their properties, 16 PAHs are listed as “priority pollutants” by the U.S. Environmental Protection Agency (EPA). These 16 PAHs are being measured and monitored in different environmental components in the ecosystem throughout the globe.

Due to their toxic properties, PAHs are considered to be potential threats to wildlife. Fishes, one of the important components of a wetland ecosystem, occupy different positions in different food chains. Fishes have shorter life cycles when compared to other vertebrates, their biology is well studied and hence, they serve as versatile ecotoxicological tools. Pallikaranai, a wetland in south Chennai, is of tremendous importance in an urban setting. The mismanagement of the wetland has led to the wetland being used as a wasteland and garbage dump. The present study documents the levels of PAHs in fishes and sediments collected from Pallikaranai. In an attempt to understand the factors those influenced the PAH load in fishes, the data

sets have been analyzed on the basis of frequency of occurrence of individual PAH, percentage composition, variation among species, organs, sexes, seasons, Use of fins as indicators of contamination and gut contents as indicators of contamination of feed, variations between endemic and introduced species of fishes and the suitability of these fishes for human consumption have been assessed. In terms of sediment, the levels of PAHs, season-wise variation and correlation between levels in sediment and fishes have been assessed. While information available on all these aspects are adequate in North American and European countries, in Indian context, it is very poor. Hence a study has been conducted with the following objectives;

1. To check the usefulness of fish as an indicator to understand the magnitude of PAHs contamination in Pallikaranai Wetland, Chennai by estimating the PAH residues in sediment and select species of fishes in the Pallikaranai wetland.
2. Assess suitability of these fishes for human consumption.

Fish and sediment samples were collected following standard procedures from Pallikaranai wetland between January 2017 and December 2018 seasonally. Organs, namely muscle, liver, kidney, gills and reproductive organs of 358 fishes comprising 21 species, namely *Anabas tetudineus, Clarias batrachus, Clarias gariepinus, Chana gachua, Chana punctata, Chana striata, Labeo fimbriatus, Heteropneustes fossilis, Oreochromis aureus, Oreochromis mossambicus, Oreochromis niloticus, Pseudotropis maculatis, Puntius chola, Puntius dorsalis, Puntius sophore, Puntius sarana, Puntius ticto, Sperata aor, Stolephorus indicus Salmostoma acinaces* and *Trichopodus trichopterus* from Pallikaranai, Chennai, Tamil Nadu, India were analysed for PAH residues. In addition to the above referred organs, fins, scales and gut contents were considered. Totally 886 tissues from 358 individuals were analysed. Among the USEPA listed 16 PAHs, 15 of them, namely Naphthalene, Acenaphthene, Phenanthrene, Anthracene, Fluorene, Fluoranthene, Pyrene, Chrysene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenzo[a,h]anthracene, Benzo[g,h,i]perylene and Indeno[1,2,3-cd]pyrene were estimated in the tissues of fishes included in the study. QuEChERS multi residue extraction procedure as described by Ramalhosa *et al*. (2003) was followed to extract all the 15 PAHs from the tissue samples.

Analyses of PAH residues were performed on a Agilent 1100 High Performance Liquid Chromatograph (HPLC) equipped with programmable Florescence Detector (FLD) and controlled by Chemstation software. The chromatographic separation was achieved on Agilent Zorbax Eclipse PAHs (4.6*150mm, 3.5µm) column by gradient elution with a binary system of water and acetonitrile. Out of the above-mentioned 21 species, 14 of them (*Clarias batrachus, Clarias gariepinus, Anabas testudineus, Labeo fimbriatus, Sperata aor, Oreochromis aureus, Oreochromis mossambicus, Oreochromis niloticus, Puntius chola, Puntius dorsalis, Puntius ticto, salmophasia acinases, Channa striata* and *Trichopodus trichopterus*) which had more than 10 individuals were chosen for assessing species–wise variation in PAH contamination.

Polycyclic Aromatic Hydrocarbon (PAH) residues in tissues of select species of fishes collected from Pallikaranai Wetland, Chennai, (2017-2018)

Mean concentrations of total PAH recorded ranged from below detection limits (BDL) to 1890 ±286 ng/g. The individual PAH levels ranged from below detection limit (BDL) to 1396 ±242 ng/g of naphthalene in *Oreochromis niloticus*, 64 ±10 ng/g of flourene in *Anabas testudineus*, 510 ±57.10 ng/g of Phenanthrene in *Trichopodus trichopterus*, 71± 7.61 ng/g of anthracene in *Oreochromis mossambicus* and 959 ± 10.78 ng/g of pyrene in *Oreochromis mossambicus*. High molecular weight PAH ranged between BDL and 158.29±10.46 ng/g in *O.mossambicus*, carcinogenic PAH4 (Sum of Chrysene, Benzo(a)anthracene, Benzo(b)flouranthene and Benzo (a)pyrene) ranged between BDL and 76.12± 6.43 ng/g in *Anabas testudineus* and Benzo(a) pyrene ranged between BDL and 16.6±0.8 ng/g in *Anabas testudineus*. *Anabas testudineus* appears to have the most unsafe levels of carcinogenic PAH, though *Orecochromis niloticus* had the highest levels of mean total PAH. The variation in the level of PAH among the species may be due to the difference in their metabolic rate, difference in feeding habits, levels of exposure and quality of the habitat they live in.

While only 7.76% of the fishes fell into the bracket of being not contaminated, 65.57% were mildly contaminated and 1.34% were highly contaminated. Distribution profile of individual PAHs and their relative contribution to the total concentration were

studied. Low molecular weight PAHs were dominant in the tissues of the fishes studied. This reflects their abundance in the environment which may be due to their high volatilization potential and increased solubility of low molecular weight PAHs. The same pattern was observed in many of the studies carried out elsewhere. The source of PAHs detected in the tissue of fishes included in the current study suggest it be pyrogenic in nature.

Accumulation pattern of PAHs among the organs of various species of fishes collected from Pallikaranai Wetland, Chennai, (2017-2018)

While naphthalene, flourene, phenanthrene and pyrene followed a similar pattern in their residue levels to that of mean total PAH (liver > kidney > gills > reproductive organ >muscle), it was slightly different for anthracene (kidney>liver>reproductive organs> gills>muscle). The variation among organs with respect to individual PAH concentration were significant (n=886, H= 47.35, 51.92, 46.85, 20.69, 130.11 for naphthalene, flourene, phenanthrene, anthracene and pyrene respectively; $p<0.05$). Similarly, accumulation pattern of total PAHs residues among the organs were in the order of liver (113.33 ± 23.87 ng/g) > kidney (107.67 ± 28.95 ng/g) > gills (102.18 ± 13.20 ng/g)> reproductive organ (87.52±14.85 ng/g)> muscle (48.43± 5.03 ng/g). The high levels in the liver and kidney may be due to the fact that they are the primary target organs and contain high fat.

Frequency of occurrence, percentage composition and concentration of individual PAHs in the tissues of fishes studied.

Naphthalene was detected in 94% the samples, and it was the most frequently detected PAH in the study, followed by phenanthrene (89%) and anthracene (89%), flourene (54%) and pyrene (44%). While dibenzo(a,h)anthracene was detected in only 0.5% of the samples and the least detected followed by indeno(1,2,3-cd)pyrene was detected in 1% of the samples. The levels recorded in the current study are indicative of consistent exposure and hence, regular monitoring of PAHs in the environment is suggested.

Significant differences were observed in percentage composition of individual PAHs to total PAH load. Naphthalene (58.81%) contributed the maximum to the total

PAH load followed by pyrene (24.23%) and Phenanthrene (9.86%). Similarly, dibenzo(a,h)anthracene (0.02%) contributed the least to the total PAH load followed by indeno(1,2,3-cd)pyrene (0.03%). In general, the distribution profile of individual PAHs and their relative contribution to the total concentration, that is low molecular weight PAHs being dominant in the tissues of the fishes studied, reflect their abundance in the environment.

Sex-wise variation in levels of PAH in fishes collected from Pallikaranai wetland, Chennai, between 2017 and 2018

With the aim to characterize a possible influence of sex, a comparison of the PAH concentrations was made among all the fish species studied. Towards this, fishes were categorized into three groups. One being distinctly male with only testis as its reproductive organ, one being distinctly female with only ovary as its reproductive organ and the third being unknown. Individuals whose gender could not be identified and individuals who had reproductive organs of both sexes were grouped under unknown category.

While the pattern was Female>Male>Unknown for naphthalene, fluorene and pyrene, phenanthrene, anthracene, total PAH followed the pattern of Female>Unknown>Male. The high levels in females could perhaps be justified by spawning, procreation, biotic factor, active metabolism and lipid content.

Use of fins as indicators of external environment contamination

Since the fish fins are in continuous exposure to the external environment, the exposure to the contaminants in the environment increases too. In areas like Pallikaranai where there is an influx of a cocktail of contaminants, fins could serve as indicators of contamination of their surrounding environment.

World over, there is no available information on the levels of PAH in fish fins and whether the levels in fins could correlate to the constant external exposure. In a maiden attempt to assess the external environmental contamination, fins of 34 fishes were analysed. Fins had very high levels of the most priority PAH, particularly the carcinogenic PAH4 and High molecular weight PAH. The levels were much higher than

the levels of PAH detected in organs. Percentage of detection of naphthalene was 100% in fin samples. Naphthalene was also the maximum in comparison to other PAH. While benzo(b)flouranthene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene and dibenzo(a,h)anthracene were not detected in any of the fin samples, carcinogenic PAH4 were detected in levels higher than what were detected in the tissues of the fishes. While PAHs are known to cause fin erosion, there is no information available in literature to understand the levels at which this could happen, and how this would affect organisms like piscivorous birds which consume the whole fish.

Levels of total PAH in fins ranged between BDL and 1589±67.29 ng/g in Oreochromis aureus. This was closely followed by 1453±67.29 ng/g in Oreochromis niloticus.

Levels of PAH in gut-content as indicators of contaminants in feed of the fish.

Two hundred and thirty three samples of gut contents were analysed to get an idea on the extent of exposure to PAH through food. Total PAH ranged between BDL and 1162±12.01 ng/g in *Oreochromis mossambicus*. This was followed by 1062 ng/g and 1043 ng/g in *Trichopodus trichopterus* and *Anabas testudineus* respectively. Naphthalene was the most prevalent, and it accounted for most of the PAHs studied. Levels of PAH in gut contents were correlated with the levels detected in liver and kidney. Although, no relation could be perceived, it could be explained that the continued exposure through food leads to eventual accumulation in organs. The findings of this study could be considered as a snap-shot.

Seasonal variation in PAH contamination in fishes collected from Pallikaranai Wetland, Chennai, between January 2017 and December 2018.

In order to assess if there was any seasonal variation in PAH contamination, samples which were collected in three seasons, namely Spring (January to April), Summer (May to August) and Monsoon (September to December) for two years, were analysed for the USEPA-suggested 15 PAH. This information may be valuable in a public health protection perspective to help people in selecting the less PAHs contaminated fishes for consumption. The data has been analysed in two angles. One was to assess if there was variation between seasons in both 2017 and 2018.

The next was to assess if there was any variation between the two years. While Monsoon recorded the highest mean total PAH, Summer recorded the least. The variation between seasons was statistically significant for all the PAH. The pattern of contamination was the same in both the years with the 2018 having higher contamination than 2017. The variation between 2017 and 2018 was statistically significant. This is indicative of the exponentially growing magnitude of contamination.

Variation in PAH contamination level between native and introduced species of fishes in Pallikaranai Wetland, Chennai, (2017-2018).

In an attempt to assess if the endemic species were more adapted than the introduced species to the anthropogenic pressures on the wetland, the mean levels of PAH in tissues of the respective categories were compared. Mean levels of Naphthalene (25.15±5.86 ng/g), Fluorene (1.58±0.15 ng/g), Phenanthrene (10.35±0.65 ng/g), Anthracene (2.31±0.24 ng/g), Pyrene (31.4±3.36 ng/g) and total PAH (110.87±7.56 ng/g) in samples of introduced species was much higher compared to native species (52.16±9.35, 1.73±0.32, 8.83±2.13, 1.82±0.21, 14.53±3.48, 79.74±11.63 ng/g respectively). The data were also analysed to assess if covariates such as species, organ, season and sex influenced the levels of PAH in the introduced species, and it was observed that none of the covariates contributed significantly.

Variation in PAH contamination levels on the basis of feeding guilds of fishes in Pallikaranai Wetland, Chennai, (2017-2018).

In order to understand if there was any difference in contamination levels among the different feeding guilds, the data were classified into three feeding guilds, namely carnivores, omnivores and herbivores. Naphthalene was the highest in omnivore and was closely trailed by carnivore while herbivore was the least (variation was not significant; $H=3.75$, $p>0.05$). Flourene was the highest in carnivore followed by omnivore, while least in herbivore (variation was not significant; $H=5.374$; $p>0.05$). Phenanthrene was very similar in all three guilds with no significant difference statistically ($H=0.239$; $p>0.05$). Anthracene was the highest in herbivore followed by carnivore and was the least in omnivore. Pyrene was the highest in omnivore followed by herbivore and least in carnivore. Total PAH was the highest in omnivore (107.27±7.29 ng/g ranged between

BDL and 1890 ng/g). The pattern was Omnivore>Carnivore> Herbivore. While herbivore had the least total PAH, the difference was not significant between carnivores and herbivores. Omnivores had the highest PAH contamination and this could be justified by their voracious eating of both plants and other organisms including fishes.

Suitability of fishes collected from Pallikaranai Wetland, Chennai, for human consumption

In an attempt to assess if the fishes collected from Pallikaranai wetland were safe for human consumption, the data were computed and compared with guidelines prescribed by different statutory agencies world over. The data showed that the fishes collected from Pallikaranai wetland are 100 times more contaminated than the maximum risk limit permitted by European Union according to SCF, 2001 which allows tolerable daily intake of only 2 pg TEQ/kg body weight/day by the United and European Commission. When the species were considered, *Anabas testudieneus* was the most contaminated species, while *Puntius ticto* and *Pseudotroplis maculata* were the least. It may be safe to say that with regard to PAHs, consumption of *Puntius ticto* and *Pseudotroplis maculata* collected from Pallikaranai wetland, could be considered safe There was no season-wise variation that was observed in terms of suitability for consumption. The fishes of Pallikaranai also have higher cancer risk as per the guidelines by different statutory agencies world over making them unsuitable for human consumption.

Polycyclic Aromatic Hydrocarbon residues in sediment samples collected from Pallikaranai Wetland, Chennai, (2017-2018).

Sediment acts as sink, and release PAHs back into water as an intrinsic source, and harm aquatic organisms, and eventually human health. PAHs, being hydrophobic, tend to rapidly adsorb on particles.

Seventy eight sediment samples were collected from Pallikaranai wetland seasonally; Spring (January –April), Summer (May – August) and Monsoon (September to December). The samples were analysed for the 15 US EPA priority listed PAHs as mentioned elsewhere in the thesis, and the data sets were subjected to statistical tests to understand whether they were statistically significant.

Levels of PAH in sediment samples collected from Pallikaranai Wetland, Chennai, between January 2017 and December 2018

The levels of contamination documented in sediments in the current study may not be harmful to aquatic biota immediately. However, it still cannot be considered safe and hence, it is suggested that the contamination be monitored regularly so as to strictly adhere to environmental laws in order to achieve a conducive environment for humans and other biota. The levels of PAH in all the sediment samples were less than the ERL reported by Adeniji., *et al*., (2018). Although the levels may not be harmful to the ecosystem components immediately, it still does not fall in safety bracket and might have harmful consequences in the near future.

Seasonal variation of PAH residues in sediment samples collected from Pallikaranai Wetland, Chennai, (2017-2018).

The seasonal pattern of contamination observed in sediments was Summer>Spring>Monsoon with no significant difference statistically. Season-wise variation need not be expected in sediments, unless there is a very high sedimentation rate in terms of fluvial sediments. The variation between the years was significant.

Frequency of occurrence of individual PAH in sediment samples collected from Pallikaranai Wetland, Chennai, (2017-2018).

Naphthalene, phenanthrene and pyrene were detected in all the samples, while dibenzo(a,h)anthracene was detected in none of the samples. Anthracene in 93%, benzo (b) fluoranthene and benzo(k)fluoranthene in 48%, fluoranthene in 46%, benzo(a)pyrene in 44%, flourene in 43%, benzo(g,h,i)perylene in 29%, indeno(1,2,3-cd) pyrene in 17%, benzo(a)anthracene in 12%, acenaphthene and chrysene were in 10% of the samples. The pattern is Low Molecular Weight PAH>High Molecular Weight PAH. Higher levels of low molecular weight PAHs may be due to their high volatilization potential and increased solubility in water.

Percentage composition of individual PAH in sediment samples collected from Pallikaranai Wetland, Chennai, between 2017 and 2018.

Pyrene contributed 30.06%, the maximum, to the total PAH load, while naphthalene 25.9%, phenanthrene 15.36%, indeno(1,2,3-cd)pyrene 12.81%, benzo(b)flouranthene 5.27%, flouranthene 3.84%, benzo(k)flouranthene 1.20%, anthracene 1.13%, benzo(a)anthracene 0.72%, fluorene 0.67%, benzo(g,h,i)perylene 0.56%, chrysene 0.41%, and acenaphthene contributed 0.16% to the total PAH load. The pattern is Low Molecular Weight PAH>High Molecular Weight PAH. The higher levels of LMW PAH may be due to their high volatilization potential.

Correlation between levels of PAH in sediment and that of fish collected from Pallikaranai Wetland, Chennai, between 2017 and 2018.

The mean PAH concentration in fish was higher than the levels recorded in sediment. The LMW PAH in fish was much higher than the sediment but HMW PAH was higher in sediment than fish. The dominant presence of LMW PAH in the fish tissue could reflect on their abundant presence in the environment. The low levels of HMW PAH in the fish tissues, may indicate the faster depuration of these compounds in fish systems.

A significant negative correlation was observed in the levels of mean total PAH in fish with that of sediment. A significant negative correlation was observed in naphthalene and phenanthrene between fish and sediment. A mild positive correlation was observed for flourene level between fish and sediment. But it was not significant. No correlations were observed for anthracene and pyrene.

While the levels of PAH in fishes and sediments in Pallikaranai wetland are not dangerously high, they are not completely safe. Moreover, if the indiscriminate dumping of waste continues to be the same, we will have no option but to completely loose this invaluable wetland of national importance sooner than later.

In India, while there are studies carried out on the levels of PAH, they are sporadic and are not oriented towards assessing the impact on wetland ecosystem with special reference to fishes and their consumers. Considering the fact that PAHs can

accumulate and magnify through the food chain, the birds, particularly fish-eating birds of the wetland might be at very high risk. Equally important is the health impact on the people consuming the fishes from Pallikaranai wetland. The current study has highlighted the magnitude of PAH contamination in Pallikaranai wetland and the need for continued monitoring and formulation of guidelines. A framework of guidelines and statutory limits in Indian context to ensure human food safety is imperative.

"You cannot get through a single day without having an impact on the world around you. What you do makes a difference, and you have to decide what kind of difference you want to make".

Jame Goodall

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